



EFFECT OF EYESTALK EXTIRPATION ON HAEMOLYMPH IONIC CONCENTRATION OF *Metapenaeus monoceros*

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ABSTRACT

Metapenaeus monoceros in the size range 65-70mm was subjected to the evaluation of sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) ionic concentration in the haemolymph after unilateral (UEA) and bilateral eyestalk ablation (BEA) in 6 (low), 16 (medium) and 35 (high) PSU salinity levels. The present investigation suggests the possible presence of a Na^+ regulating factor in the eyestalks of *M. monoceros* as the regulation of these ions was observed to be affected in destalked prawns ($P < 0.05$). The K^+ concentration showed differences among the ablated and control prawns ($P < 0.05$). The BEA prawns registered an increased concentration of K^+ by 11.7%, 13.1% and 16.0% at the three salinities compared to controls. Among the UEA prawns the differences in the average concentration of K^+ compared to control were 1.5, 0.33 and 11.3% at low, medium and high salinity levels. The concentration of Ca^{2+} in the BEA prawns registered a decrease by 21% at low, 19% at medium and 22% at high salinity whereas in the UEA prawns, an increase by 5.5%, 0.7% and 5.2% at these three salinity levels was observed compared to control prawns. Thus it can be inferred that, UEA prawns at 35 PSU could be recommended for better growth of *M. monoceros* in culture conditions.

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1 Introduction

Body fluids of most of the marine invertebrates generally have the same ionic concentration as in the surrounding seawater but when they migrate to brackish water they have to maintain their body fluids at levels higher than that of dilute medium (Panikkar, 1968). The efficient osmoregulatory functioning in prawn is essential for the successful completion of its life cycle in the estuary and the sea. The calcium ion has considerable role in the moulting process (Sarda et al., 1989; Parado et al., 1989) and its concentration in the haemolymph is also evaluated. The effect of eyestalk ablation on haemolymph osmotic and ionic concentrations during acute salinity exposure in the fresh water shrimp (*wiegmann*) was studied by John et al. (1990).

Metapenaeus monoceros is very common in the brackish and estuarine waters of Cochin. It is sturdy, large and fast growing. It is euryhaline and spends its post larval and juvenile life in brackish water and migrates to the sea for maturation and reproduction. Due to its high commercial value, many experiments have been designed to study growth, metabolism, reproduction and behavior of penaeid prawns. Eyestalk ablation is frequently performed for inducing maturation of gonads and spawning in prawns. Extirpation of eyestalk removes the neuro-endocrine system of prawn located in the eyestalk which influences growth, reproduction and other metabolic activities. Effect of eyestalk ablation on the growth of *M. dobsoni* and *M. monoceros* has been described by Venkitaraman et al. (2004) and Venkitaraman et al. (2010). The concentration of ions in the haemolymph of crustaceans have been attempted by O'Brien (1972) in the crab *Hemigrapsus edwardsi* and Greenway (1976) in *Carcinus maenas*. Guderley (1977) related the changes in the ionic concentration in *Cancer magister* to the changing osmotic conditions of the environment. Kiron and Diwan (1984a) estimated the haemolymph sodium level in eyestalk ablated *Penaeus indicus* for a period of 24 hours. Ziegler et al. (2000) analysed the ion composition and volume of the haemolymph of *Ligia pallasii* in 4 different stages of the molt cycle. Pillard et al. (2002) studied the potassium deficiency relative to marine water in mysid shrimp *Americmysis baliia*. Arcos et al. (2003) studied the haemolymph metabolic variables in relation to eyestalk ablation and gonad development of Pacific white shrimp *Litopenaeus vannamei*, whereas, Molenoek and Lovett et al. (2006) studied in green crab, *Carcinusmaenas Limnaeus*. David & Ravi (2006) studied the effect of sudden salinity change on *Penaeus latissulcatus* kishinouye osmoregulation, ion regulation. The present work has been undertaken to collect useful information on the haemolymph ionic balance of sodium, potassium which have significance in osmoregulation and calcium ion which has considerable role in the moulting process in *M. monoceros* and the effect of single and double eyestalk ablation on the ions under three salinity levels: 6 (low), 16 (medium) and 35 (high) PSU when their hormonal supply is manipulated over a period of 15 days.

2 Materials and methods

2.1 Collection of prawns

M. monoceros was collected from the Cochin Backwaters and from the nearby prawn culture fields. Throughout the year the smaller size (35-40mm) was available. The prawns collected in large plastic buckets were sorted and transferred to rearing tanks of the main recycling system (Nair et al., 2002).

2.2 Recycling system

A closed system of sea water aquarium was set up for maintaining the prawns at the desired conditions during the experimental period (Nair et al., 2002). The system consisted of a plastic stock tank of size 80 X 80 X 80 cm³. The bottom of the tank was provided with a biological filter which served as an efficient physical filter and also as an ideal substratum for the growth of bacterial flora which essentially degrade the organic matter and purifies water thereby maintaining the water quality. From the bottom of the biological filter, water was pumped into experimental polyvinyl chloride (PVC) trough of size 61X36X22 cm³. Water flows through the interconnections within the tanks and finally empties into another filter provided inside the stock tank. This filter was a porous plastic jar of 15 cm diameter and 30 cm depth in which activated charcoal, glass wool and stone pebbles were layered alternatively. This filter removes suspended particles from the system and the activated charcoal absorbs dissolved gases. This filter was changed every week while the biological filter was changed once in three months. The stock tank was aerated continuously and uniformly with 3 cm air stones. The PVC troughs were also aerated individually. Continuous 24 hour illumination was provided by florescent tubes.

For conducting the experiments another closed recycling system of similar model was arranged. The prawns were maintained in individual compartments of about 4 liter capacity kept inside the rectangular trough and they form part of the experimental system. This was to facilitate individual observation and also to prevent cannibalism among the prawns. The compartments were interconnected by polythene pipes which are provided with meshes to prevent test animal, food, excreta or moult from passing into the next compartment. Eight such compartments were placed inside the large rectangular PVC trough and filled with sea water. This was to provide stable temperature and also to maintain the level of water in the compartments.

Sea water was collected from the near shore area of Fort Cochin. The water was filtered on collection and transported in large carboys of 25 and 50 liter capacity. Before transferring to the recycling system the carboys were kept undisturbed for two days for the particulate matter if any to settle and thereafter filtered once again. Salinity was adjusted to the desired level by adding aerated fresh water. Salinity was

determined by salinometer (DIGI-AUTO, model 3-G, Tsurumi Seiki make with ± 0.01 PSU accuracy). Salinity of sea water in the recycling system was periodically monitored and changes in salinity compensated by adding fresh water. Water was kept fully saturated with oxygen by providing continuous aeration (Venkitaraman, 1991). The temperature of the system was recorded both in the morning and in the evening and the mean value was taken because the system was not a temperature controlled one. The average temperature recorded was 27°C throughout the experimental period. Three sets of experiments were conducted under three different salinities mentioned earlier. The prawns collected from wild were acclimatized to the desired salinity for 7 days. Each set had 21 animals consisting of 7 BEA, 7 UEA and 7 intact prawns. 15 days was the duration of each experiment. The vernier calipers were used to measure the length of the prawn. From the base of the rostrum to the base of the telson the measurement was taken.

2.3 Feeding

Fresh calm meat washed and stored in refrigerator was thawed and used as feed for the prawns throughout the experiment. Prawns were fed twice per day approximately equivalent to 20% of its body weight. The unused food was removed after 6 to 8 hrs. The individual compartments and PVC tanks were cleaned daily and the moults, excreta and unused food were blotted and weighed separately for future studies. They were dried for sufficient length of time at 55°C until a constant weight was achieved and stored in the desiccators for further analysis (Venkitaraman et al., 2010).

2.4 Eyestalk ablation

Eyestalks were removed using sterile surgical blades to reduce mortality to the minimum. Prawns in the inter-moult stage were chosen for eyestalk ablation. During ablation, the prawns were held in pre-cooled sea water at temperature of 15°C to 18°C to reduce the heart beat rate and loss of haemolymph through amputation (Venkitaraman et al. 2004). For bilateral eyestalk ablation (BEA), the second eyestalk was removed after a 6 hours time delay. By adopting this procedure zero mortality was achieved. The ablated prawns were maintained in separate troughs. The large size group (65-70 mm) of *M. monoceros* was subjected to the experimental conditions. Throughout the study, data was obtained from 7 individual prawns of the same size (65-70 mm length) and age in order to reduce variation.

Haemolymph was collected using a micro-syringe (Hamilton model) which is provided with large detachable needles. The syringe is calibrated at 0.1μ liter interval and has the capacity of 10μ liter. For collection of haemolymph the prawn was held firmly with fingers extended and surrounding the anterior abdominal region bringing the animal to a dug in posture. The needle of the micro syringe was then inserted under the posterior – dorsal edge of the carapace directly into the heart

and 0.5μ liter of haemolymph was syringed out. The whole process could be completed in 20 – 30 seconds.

The withdrawn haemolymph was collected in corning glass tubes containing anticoagulants. The glass tubes were previously acid washed repeatedly and passed through many changes of the distilled water and finally in triple distilled water and air dried. The haemolymph samples were stored in refrigerator in frozen conditions. Haemolymph samples from individual prawns were collected on every second day. The moulting days were recorded, only just to compare the ionic concentration changes associated with the moulting. Feeding pattern adopted here was similar to the one followed for growth studies (Venkitaraman 1991; Venkitaraman et al., 2004; Venkitaraman et al., 2010). Samples of the medium, the rearing water, were also taken for the estimation of ions along with haemolymph samples.

2.5 Estimation of ions

The blood samples were analysed for Na^+ , K^+ and Ca^{2+} ions. The ionic concentration was determined by using atomic absorption spectrophotometer. Three standard solutions of 0.5 ppm, 1ppm and 2ppm of Na^+ , K^+ and Ca^{2+} were used. At each estimation, instrument was zeroed using the blank. Sodium is read on an absorption wave length of 589 nm, potassium at 766 nm and calcium at 422 nm. In the determination of calcium, lanthanum chloride was used to avoid interference. The concentration of ions in the haemolymph was expressed as mille equivalent (mEq/L) per litre.

2.6. Statistical analysis

Ionic concentration of Na^+ , K^+ and Ca^{2+} of ablated and control prawns were subjected to various statistical techniques, such as

- (1) 3way ANOVA for testing the significance of the differences between salinity levels, animals (treatments) and days, and to test the significance of the first order interaction effects of these main effects (Snedecor & Cochran 1967),
- (2) Having found that treatment (animals) wise difference was less significant than that over days, Students 't' test was applied to test the significance of the differences between treatment means of independent samples at different ablation levels at different salinities (Federer, 1967).
- (3) Regression analysis has been applied to study the trend of the change in the ionic concentration with respect to days (Rholf & Sokal 1981). A set of 12 curves (Snedecor & Cochran 1967; Jayalakshmy 1998) were fitted and the best trend was selected based on the variability explained. Higher degree polynomial selection over lower degree polynomial is based on the significance of the reduction in the deviation sum of squares due to addition of a

higher degree independent variable (Rhoif & Sokal 1981).

3 Results and discussion

Three way analysis of variance showed that the sodium, potassium and calcium ion concentrations varied significantly among salinity levels for bilaterally ablated, unilaterally ablated and for the control prawns ($P < 0.001$, Table 1). Student's *t* test ($P < 0.001$, Table 2) applied, delineated that salinity levels contribute substantially on controlling the concentration of monovalent and divalent ion concentrations in the haemolymph of prawns. The bilaterally and unilaterally eyestalk ablated and control prawns maintained hyper ionic concentration of sodium in their haemolymph when compared to that of the medium at low and medium salinity and hypo ionic at high salinity (Figure 1) whereas hyper ionic concentration was maintained in the case of potassium (Figure 2) and calcium ions (Figure 3) at all salinity levels compared to the rearing water or the medium.

3.1 Concentration of sodium ions

3.1.1 Salinity 6 ± 1 PSU

The sodium ion concentration remained low in ablated and control prawns on moulting days (Figure 1). Significant difference was found between BEA and control prawns ($t = 18.83$, $P < 0.05$). The haemolymph of the BEA prawns contained 10% less sodium ions than the intact prawns while that of UEA prawns showed almost the same concentration as that of control prawns (Table 1, $P > 0.05$).

The average sodium concentration on the moulting days in the BEA prawns was 203.38mEq/l, in the UEA, 219.17mEq/l and in the control prawns, 226.75mEq/l. For the medium, the concentration is reciprocally related to days (Figure 1, Table 3) and it is increasing with days. For BEA prawns it is increasing up to the 10th day and thereafter it shows a tendency to diminish. For UEA prawns, a similar trend is obtained and this trend leads to a steady decrease with experimental progress even though the rate of decrease is not so steep as in the case of BEA prawns, reaching a minimum on the moulting days, increases during the post moulting period and subsequently decreases with the approach of the subsequent moulting. No such definite trend could be obtained in the control. This implies that the variation in the sodium ion concentration is being controlled by some factors other than moulting/intermoulting days.

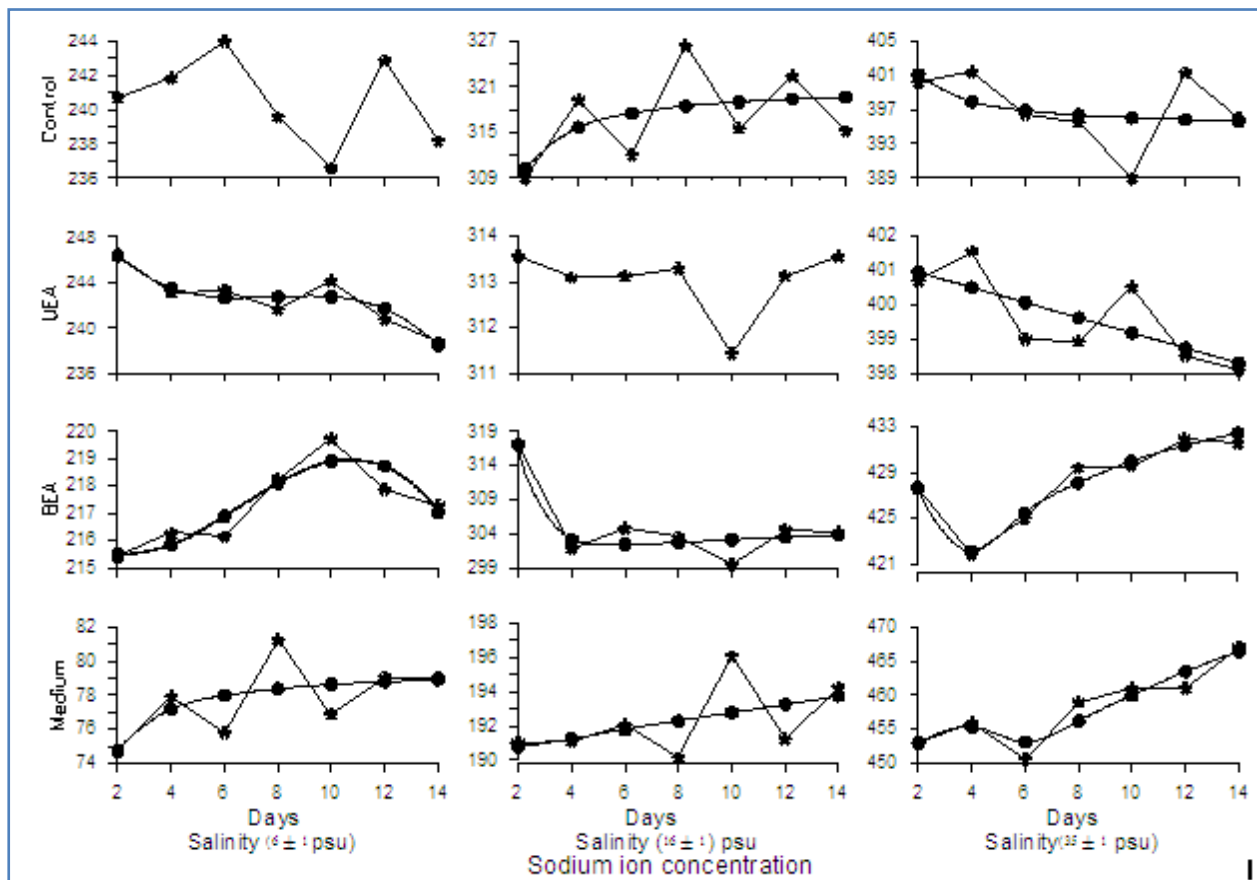


Figure 1 Variation in sodium ion concentration in the medium and in the three salinity levels.

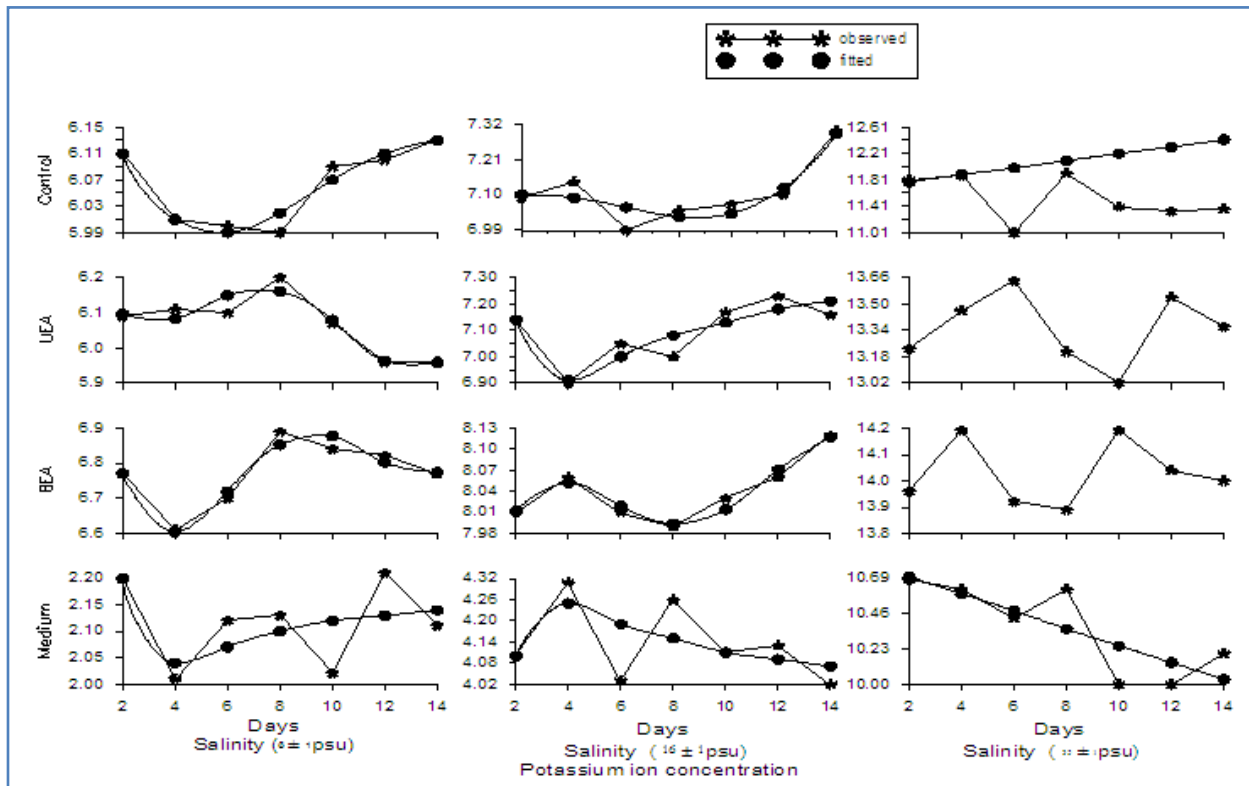


Figure 2 Variation in potassium ion concentration in the medium and in the three salinity levels.

Table 1: 3 way ANOVA showing the significance of the difference in the ionic concentration among BEA, UEA and control prawns at Salinities 6± 1PSU, 16± 1PSU, 35±1 PSU.

Source	DOF of F ratio	F-Ratio BEA	F-Ratio UEA	F-Ratio Control
Sodium ion concentration				
(A) Between salinity levels	(2, 72)	15361.63**	47853.69**	1789.84**
(B) Between animals/treatments	(6, 72)	4.56*	7.69**	13.05**
(C) Between Days	(6, 72)	7.89**	33.50**	7.32**
AB interaction	(12, 72)	7.20**	9.35**	4.71**
BC interaction	(36, 72)	1.10	0.92	1.38
AC interaction	(12, 72)	4.80**	20.00**	13.79**
Potassium ion concentration				
(A) Between salinity levels	(2, 72)	15710.01**	7767.50**	13964.11**
(B) Between animals/treatments	(6, 72)	6.84**	3.38*	3.69*
(C) Between Days	(6, 72)	15.98**	5.98**	17.80**
AB interaction	(12, 72)	7.62**	3.75*	5.99**
BC interaction	(36, 72)	0.91	0.54	0.88
AC interaction	(12, 72)	16.53**	16.23**	13.62**
Calcium ion concentration				
(A) Between salinity levels	(2, 72)	1789.84**	1414.69**	991.18**
(B) Between animals/treatments	(6, 72)	15.18**	0.87	2.72
(C) Between Days	(6, 72)	12.71**	3.56*	2.51
AB interaction	(12, 72)	22.33**	4.46**	2.39*
BC interaction	(36, 72)	0.90	0.48	0.54
AC interaction	(12, 72)	4.75**	2.84*	1.65*

* Calculated F statistic is significant at 5% level, P<0.05, ** Calculated F statistic is significant at 1% level, P<0.01, *** Calculated F statistic is significant at 0.1% level, P<0.001.

Table 2 Showing the mean (X), standard deviation σ , t statistic and percentage increase in the ionic concentration in BEA, UEA and control prawns at different salinities.

Salinity level	Statistical constants	BEA	UEA	Control
Sodium ion concentration				
6±1%	$X_1(\text{Mean}) \pm \sigma_1(\text{std.})$	217.30 ± 1.37	242.53 ± 2.37	240.53 ± 2.44
16±1%	$X_2 \pm \sigma_2$	305.17 ± 5.19	313.02 ± 0.68	318.41 ± 5.28
35±1%	$X_3 \pm \sigma_3$	428.16 ± 3.38	399.62 ± 1.20	397.12 ± 4.06
t statistic	t_{12} (6 ±1% and 16±1%)	37.14**	55.61**	30.36**
	t_{13} (6 ±1% and 35±1%)	131.17**	133.91**	75.05**
	T_{23} (16 ±1% and 35±1%)	45.07**	158.40**	26.81**
% increase	(L_2 / L_1) (6 ±1% and 16±1%)	40.44	24.94	32.38
	(L_3 / L_1) (6 ±1% and 35±1%)	40.30	31.88	24.72
	(L_3 / L_2) (16 ±1% and 35±1%)	97.04	64.77	65.70
Potassium ion concentration				
6±1%	$X_1(\text{Mean}) \pm \sigma_1(\text{std.})$	6.77±0.08	6.07±0.08	6.05±0.05
16±1%	$X_2 \pm \sigma_2$	8.04±0.04	7.08±0.10	7.11±0.09
35±1%	$X_3 \pm \sigma_3$	14.03±0.11	13.35±0.20	12.11±0.23
t statistic	t_{12} (6 ±1% and 16±1%)	30.10**	17.60**	22.46**
	t_{13} (6 ±1% and 35±1%)	116.87**	77.29**	57.48**
	t_{23} (16 ±1% and 35±1%)	114.51**	63.68**	45.56**
% increase	(L_2 / L_1) (6 ±1% and 16±1%)	18.74	16.62	17.23
	(L_3 / L_1) (6 ±1% and 35±1%)	74.49	88.62	70.41
	(L_3 / L_2) (16 ±1% and 35±1%)	107.18	119.96	99.77
Calcium ion concentration				
6±1%	$X_1(\text{Mean}) \pm \sigma_1(\text{std.})$	15.72±0.40	21.11±0.15	19.72±0.37
16±1%	$X_2 \pm \sigma_2$	19.37±0.29	23.94±0.36	23.78±1.74
35±1%	$X_3 \pm \sigma_3$	25.15±1.59	34.70±0.69	32.38±0.83
t statistic	t_{12} (6 ±1% and 16±1%)	16.78**	16.35**	5.18**
	t_{13} (6 ±1% and 35±1%)	13.04**	43.65**	16.16**
	t_{23} (16 ±1% and 35±1%)	8.11**	31.43**	10.13**
% increase	(L_2 / L_1) (6 ±1% and 16±1%)	23.18	13.35	20.56
	(L_3 / L_1) (6 ±1% and 35±1%)	29.80	44.96	36.17
	(L_3 / L_2) (16 ±1% and 35±1%)	59.80	64.32	64.17

**_Calculated t statistic for 12 degrees of freedom is significant at 1% level of significance, ($P < 0.01$).

3.1.2 Salinity 16±1PSU

The haemolymph of BEA prawns showed a decrease by 4.8% in the concentration of sodium ions compared to that of the control. In the UEA prawns it was less by 1.5% than that of intact prawns (Table 2). Significant difference was observed between BEA and control prawns ($t=4.06$, $P < 0.05$). On the moult days, the same trend as in low salinity was observed, remaining low on moult days when compared to the intermoult days at this salinity level also. The average values for BEA, UEA and control prawns on moult days were 287.2mEq/l, 302.3mEq/l and 306.1mEq/l respectively. The trend (Figure 1, Table 3) in the sodium ion concentration in medium is a linear function with time. For BEA prawns a reciprocal quadratic function is obtained and it indicated that only at the initial stages a decreasing tendency is reflected. Thereafter the concentration remains more or less steady, moving to a saturation level. In the case of UEA prawns no definite trend

could be obtained theoretically implying that sodium ion concentration is not controlled by moult process alone. In the case of control prawns, the trend is defined by a reciprocal linear function. The sodium ion concentration shows an increase up to the 4th day and after that the rate of increase is highly steady but very low. In all these cases a higher degree polynomial is not better than the lower degree ($P > 0.05$).

3.1.3 Salinity 35±1 PSU

As in the previous two salinities the sodium ionic concentration in the haemolymph of ablated and control prawns remained low on the moult days when compared to that of intermoult days (Fig. 1). The average values for BEA prawns on moult days was 401.92mEq/l, that of UEA prawns, 395.40mEq/l and in the intact prawns, 391.12mEq/l. The concentration showed significant differences between BEA and control prawns ($t=13.34$, $P < 0.05$).

Table 3 Showing the best trend equation in each level of ablation variability explained and test of significance.

Salinity level	Trend equation	V.E.	F ratio ^a
Sodium ion			
Salinity 6±1 psu medium	$Y = 79.595 - 9.622 / X$	32.02	(1,5), 3.823
Salinity 6±1 psu BEA	$Y = 216.404 - 0.859 X + 0.227 X^2 - 0.0116 X^3$	64.89	(3,3), 4.696
Salinity 6±1 psu UEA	$Y = 252.167 - 3.839 X + 0.503 X^2 - 0.0213 X^3$	74.70	(3,3), 6.904
Salinity 6±1 psu control	No significant trend	-----	
Salinity 16±1 psu medium	$Y = 190.278 + 0.250 X$	11.16	(1,5), 1.754
Salinity 16±1 psu BEA	$Y = 306.557 - 47.304 / X + 136.526 / X^2$	81.94	(2,4), 14.614
Salinity 16±1 psu UEA	No significant trend	-----	
Salinity 16±1 psu control	$Y = 321.240 - 22.233 / X$	17.55	(1,5), 1.798
High- medium	$Y = 494.574 - 531.850 / X + 2102.437 / X^2 - 2409.84 / X^3$	75.88	(3,3), 7.292
Salinity 35±1 psu BEA	$Y = 439.930 - 118.083 / X + 187.107 / X^2$	93.65	(2,4), 45.255
Salinity 35±1 psu UEA	$Y = 401.397 - 0.222 X$	44.45	(1,5), 5.801
Salinity 35±1 psu control	$Y = 394.798 + 12.554 / X$	2.62	(1,5), 1.161
Potassium ion			
Salinity 6±1 psu medium	$Y = 2.232 - 1.426 / X + 2.710 / X^2$	11.95	(2,4), 1.407
Salinity 6±1 psu BEA	$Y = 7.610 - 0.672 / X + 0.150 / X^2 - 0.0125 / X^3 + 0.000352 / X^4$	79.88	(4,2), 6.954
Salinity 6±1 psu UEA	$Y = 6.413 - 0.284 X + 0.0769 X^2 - 0.00762 X^3 + 0.000244 X^4$	67.28	(4,2), 4.084
Salinity 6±1 psu control	$Y = 6.313 - 0.132 X + 0.0162 X^2 - 0.000555 X^3$	85.36	(3,3), 12.664
Salinity 16±1 psu medium	$Y = 3.928 + 2.212 / X - 3.720 / X^2$	14.97	(2,4), 1.046
Salinity 16±1 psu BEA	$Y = 7.779 + 0.191 X - 0.0445 X^2 + 0.00390 X^3 + 0.000112 X^4$	85.23	(4,2), 9.654
Salinity 16±1 psu UEA	$Y = 7.430 - 3.552 / X + 5.941 / X^2$	71.48	(2,4), 8.519
Salinity 16±1 psu control	$Y = 7.070 + 0.0333 X - 0.00949 X^2 + 0.000590 X^3$	69.71	(3,3), 5.602
Salinity 35±1 psu medium	$Y = 10.809 - 0.0555 X$	58.64	(1,5), 9.506
Salinity 35±1 psu BEA	No significant trend	-----	
Salinity 35±1 psu UEA	No significant trend	-----	
Salinity 35±1 psu control	$Y = 11.673 + 0.0534 X$	76.51	(1,5), 20.534
Calcium ion			
Salinity 6±1 psu medium	$Y = 6.769 + 18.160 / X - 83.450 / X^2 + 104.263 / X^3$	12.29	(3,3), 1.280
Salinity 6±1 psu BEA	$Y = 16.267 - 7.980/X + 17.406/X^2$	96.42	(2,4), 81.734
Salinity 6±1 psu UEA	$Y = 21.264 - 0.779 / X$	38.63	(1,5), 4.777
Salinity 6±1 psu control	$Y = 17.682 + 1.912 X - 0.603 X^2 + 0.069 X^3 - 0.00249 X^4$	22.11	(4,2), 1.441
Salinity 16±1 psu medium	$Y = 10.571 - 0.446 X + 0.180 X^2 - 0.0203 X^3 + 0.000701 X^4$	65.07	(4,2), 3.795
Salinity 16±1 psu BEA	$Y = 18.468 + 9.605 / X - 16.153 / X^2$	69.61	(2,4), 7.871
Salinity 16±1 psu UEA	$Y = 23.365 + -.554 X - 0.0864 X^2 + 0.00340 X^3$	98.07	(3,3), 102.97
Salinity 16±1 psu control	$Y = 19.009 - 0.033 X + 0.610 X^2 - 0.093 X^3 + 0.00367 X^4$	56.83	(4,2), 2.974
Salinity 35±1 psu medium	$Y = 319.917 + 29.757 / X - 174.820 / X^2 + 231.279 / X^3$	66.38	(3,3), 4.949
Salinity 35±1 psu BEA	$Y = 1.479 - 0.0957 \log_{10}(X)$	95.71	(1,5), 134.84
Salinity 35±1 psu UEA	$Y = 26.026 + 6.433 X - 1.514 X^2 + 0.141 X^3 + 0.00448 X^4$	95.20	(4,2), 30.718
Salinity 35±1 psu control	$Y = 29.510 + 0.716 X - 0.0352 X^2$	83.46	(4,2), 16.139

^aThe values given in brackets are the degrees of freedom and the value of F statistic.

It was observed that the ionic concentration in the BEA prawns was more by 8.1% over that of the control prawns while in the UEA prawns it was almost the same as that of the control prawns (397.1 mEq/l , $P > 0.05$) (Table 1).

The trend (Figure.1, Table 3) studied for medium has been delineated by a reciprocal cubic polynomial function. An increasing trend with a fall on the 6th day has been observed with a steady rate of increase from the 8th day onwards. In the case of BEA prawns also a similar quadratic reciprocal trend is obtained increasing up to the 12th day with a fall on 4th day in between. Thereafter it shows a tendency to be stable or to decrease, the reason for which is to be considered in the future research. On the other hand, in the case of UEA prawns a steadily linearly decreasing trend is obtained. In the case of control prawns, no significant definite trend other than a reciprocal linear could be observed (Table 3, Figure 1) with a higher decrease at the initial period. From the 6th day onwards,

the rate of decrease is not very conspicuous, it being inversely proportional to the day of moult/ intermoult.

it was found that in the BEA prawns between low and medium salinities, the increase in concentration was by 40.43%, between low and high salinities, the increase was by 97.04% and between medium and high salinities, the increase was by 40.31% whereas for UEA prawns the corresponding values were 29.06%, 64.77% and 27.66% and for the intact prawns the values were 32.38%, 65.70% and 24.72% respectively.

However, when compared to control prawns, the BEA prawns showed a decrease in their haemolymph concentration of Na^+ by 10% at low salinity, by 4.8% at medium and an increase of 8.5% at high salinity. The UEA prawns showed almost the same Na^+ concentration in their haemolymph as that of control prawns except at medium salinity where decrease by 1.5% was noticed.

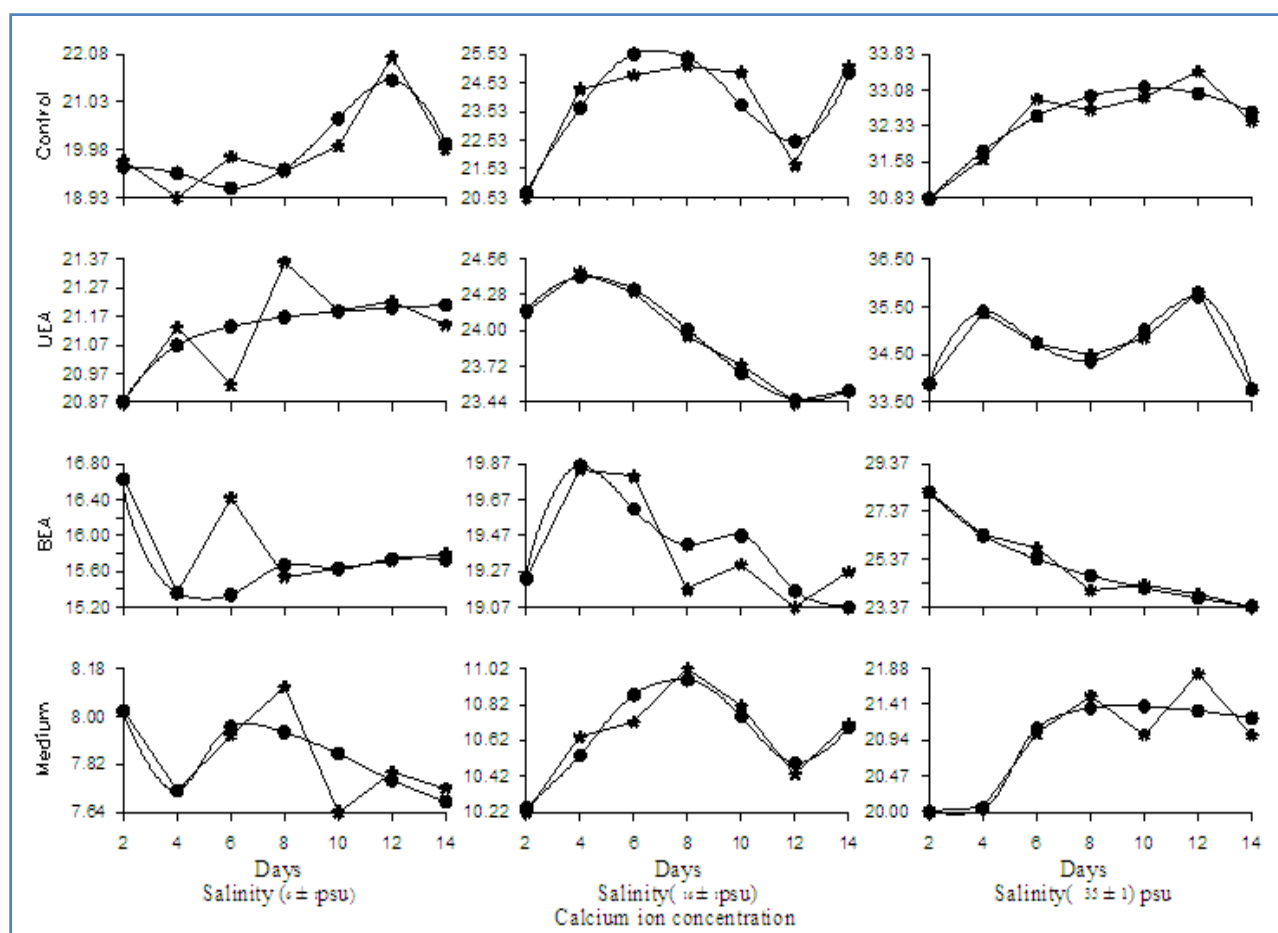


Figure 3 Variation in calcium ion concentration in the medium and in the three salinity levels.

Table 4 The level of Na⁺, K⁺ and Ca²⁺ (mEq/L) in the haemolymph of ablated and intact penaeid prawn, *M. monoceros* during moult days.

Na ⁺ ion concentration			
Level of ablation	Low salinity	Medium salinity	High salinity
BEA	203.38	287.2	401.92
UEA	219.17	302.3	395.40
control	226.75	306.1	391.12
K ⁺ ion concentration			
Level of ablation	Low salinity	Medium salinity	High salinity
BEA	6.89	8.14	14.13
UEA	6.15	7.12	13.78
Control	6.09	7.57	12.57
Ca ²⁺ ion concentration			
Level of ablation	Low salinity	Medium salinity	High salinity
BEA	15.85	20.66	26.10
UEA	20.52	24.77	33.15
Control	20.61	26.10	35.60

It is evident from the above that in the BEA prawns the ionic concentration of sodium in the haemolymph fell below that of normal prawns when in low saline medium and increased above that of the normal when in high saline medium. On the whole a steady decrease in Na⁺ ion concentration with respect to time is observed at low level and a steady state at medium level and an increasing trend at high level of salinity is observed for ablated prawns. This implies that the optimum level of salinity for a constant level of Na⁺ ions is 16PSU for this species. For a decreasing sodium ion concentration, the salinity is to be decreased to 6PSU and for higher level of Na⁺ ions salinity is to be increased to 35PSU for this species.

3.2 Concentration of potassium ions

3.2.1 Salinity 6±1 PSU

The BEA prawns recorded on an average, 6.89mEq/l of potassium ion concentration in the haemolymph on moult days, the UEA prawns and control prawns recorded 6.15 and 6.09mEq/l respectively. A significant difference was observed in the concentration of potassium ion in the haemolymph of BEA and control prawns ($t=15.74$, $P<0.05$). In the BEA prawns the haemolymph contained about 11.8% more potassium than that of the intact prawns. However, in the UEA prawns it remained almost equal to that of intact prawns (6.08mEq/l, $P>0.05$) (Table 2). It was noticed that the concentration of this ion slightly increased with the approach of the moult in all the three cases. The trend functions (Figure. 2, Table 3) studied revealed that for medium the trend is defined by a reciprocal quadratic polynomial. In this case the general trend is to increase from the 4th day onwards where the ion concentration falls to a value, 2.05 from 2.20 on the 2nd day. In the case of BEA prawns, a high complexity is observed, the trend being defined by a reciprocal 4th degree polynomial. In the initial period the concentration decreases up to the 4th day and then increases steadily up to the 10th day and

then again falls down up to the 14th day. In the case of UEA prawns a trend reverse to that of BEA prawns was obtained, the best trend being defined by a 4th degree polynomial function. In the case of the control prawns, the trend function is similar to that of UEA prawns, a 3rd degree polynomial, but more similar to that of BEA prawns up to the 8th day. In this case even after the 8th day, a tendency to increase in the ionic concentration is observed whereas in the case of BEA prawns it decreases (Table 3, Figure. 2), Hence the complexity and the higher order trend.

3.2.2 Salinity 16±1 PSU

Significant differences between the K⁺ concentration of BEA and control prawns ($t=21.42$, $P<0.05$) was observed. The increase was by 13.5% in BEA prawns compared to that of control prawns and in the UEA prawns, the observed K⁺ was almost at the same value as that of control prawns (7.08mEq/L, $P>0.05$), (Table 2). As in the low salinity level, the haemolymph of ablated and control prawns showed an elevated level of K⁺ on moult days. Also, an increasing trend was observed with the approach of moult in all the three cases (Figure. 2). For BEA, UEA and control prawns average values on moult days (Table 4) were 8.14, 7.12 and 7.57mEq/L respectively. The trend equation (Figure.2, Table 3) obtained for medium is a reciprocal quadratic function. In the case of BEA prawns, a 4th degree polynomial has been found to be the best fit. In this case an increase is observed up to the 4th day, followed by a fall up to the 8th day and thereafter a steady increase is obtained. In the case of UEA prawns, a reciprocal quadratic polynomial is obtained as the best trend function. The trend shows a reverse pattern of that of BEA, up to the 10th day, even though the trend is similar to that of BEA prawns after the 10th day. In the case of control prawns, a moderate increase followed by a minimum concentration on the 8th day and thereafter a steady increase is observed (Figure. 2, Table 2).

3.2.3 Salinity 35±1PSU

On the moult days the K^+ was slightly higher than that during the intermoult days in ablated and control prawns. The average K^+ in the haemolymph of BEA, UEA and the control prawns were 14.03, 13.35 and 12.11mEq/L respectively (Table 4). Between BEA and control prawns significant difference in the K^+ concentration existed ($t=16.92$, $P<0.05$). The BEA prawns maintained at this salinity has about 7.0% more K^+ in their haemolymph than the control prawns. Another significant observation was that UEA prawns which maintained almost the same concentration of potassium as that of the control prawns in the previous two salinity levels, contrastingly showed 10.3% increase in the haemolymph K^+ over that of the control prawns at this higher salinity. A decreasing trend (Figure 2) is observed for the K^+ concentration in the medium, estimated by the linear equation (Table 3). In the case of BEA and UEA no well-defined pattern could be obtained at this higher salinity. In the case of control prawns, marginally increasing linear trend could be observed (Figure 2, Table 3). Since the significance of the trend function for all cases of ablation is feeble it indicates that other than moulting process some other physiological process also contributes significantly in controlling the K^+ ion concentration in the haemolymph at this high saline condition which is to be sought out

Among the BEA prawns, the percentage of increase of potassium concentration between low and medium salinity was by 18.74%, between low and high, by 107.18%. and between medium and high, by 74.49%. At the same time, the corresponding increase of the ion in the UEA prawns were 16.62%, 119.96% and 88.62% and for the control prawns, it being, 17.53%, 99.89% and 70.41%. These differences are augmented by the different trend equations for the ablated and non-ablated prawns at different salinity levels given above.

The K^+ ion concentration in the haemolymph of *M. monoceros* (65-70 mm) subjected to three different salinities showed differences among the ablated and control prawns. The BEA prawns registered an increased concentration of K^+ in the haemolymph by 11.7%, 13.1% and 16.0% at the low medium and high salinities when compared to that of controls. Among the UEA prawns the corresponding differences when compared to that of control were 0.33%, -0.42% and 10.24%. From the results it can be comprehended that the ligation of eyestalks could have caused substantial increase in K^+ in the haemolymph of BEA prawns.

3.3 Concentration of calcium ions

3.3.1 Salinity 6±1PSU

In the haemolymph of BEA prawns Ca^{2+} was found to be on the average 20.25% less than that of control prawns while for the UEA prawns it was more by 7.08% than the control prawns (Table 2). Between BEA and control prawns ($t=16.67$, $P<0.05$)

and between UEA and control prawns ($t=7.91$, $P<0.05$) significant difference was observed.

A definite trend was observed in the Ca^{2+} ion concentration of haemolymph of prawns studied over the days (Figure 3, Table 3). During the intermoult days immediately following the moult, the concentration decreased, whereas on the days immediately preceding a moult the concentration gradually increased reaching a maximum on the day of the moult (Figure 3). The BEA prawns recorded on an average 15.85 mEq/l on the moult days (Table 4) while the UEA and control prawns showed 20.52 and 20.61mEq/l of Ca^{2+} in the haemolymph on moult days. The trend equations (Table 3) fitted is similar to that in the case of sodium and potassium. At this low salinity level, for the medium the temporal change in the calcium ion concentration is described by the reciprocal cubic function (Figure 3). In this case a steady decrease after the 6th day is observed, with an initial fall on the 4th day. In the case of BEA prawns a reciprocal quadratic function has been obtained as the best prediction equation, in a manner similar to that of medium but with an increase of low gradient after the 8th day whereas it is a decrease of low gradient in the case of medium after the 8th day. In the case of UEA prawns, a reciprocal linear function explains the trend implying a slowly and steadily increasing trend throughout the period of study. In the case of control prawns, a 4th degree polynomial has been delineated as the best trend equation. This trend shows a minimum concentration on the 6th day followed by another minimum on the 14th day (Figure 3).

3.3.2 Salinity 16±1 PSU

At this salinity level, Ca^{2+} ion concentration in the haemolymph of BEA prawns was found to be 18.5% less than that of control prawns. However, the concentration of this ion in the UEA prawns (23.94mEq/l) remained almost the same as that of the intact prawns (23.78mEq/l), (Table 2). Between BEA and control prawns significant differences existed ($t=5.67$, $P<0.05$). As in the case of lower salinity a definite trend was visible when the calcium ion concentration in the haemolymph was studied over the days. In all the three cases the concentration reached maximum on moult days, then decreased soon after moulting and the values again increased with the approach of the next moult. The average ion concentrations on moult days in BEA, UEA and control prawns were 20.06, 24.77 and 26.10mEq/l respectively. The variation in the calcium ion concentration in the medium is described by a 4th degree polynomial, which shows a mixed trend as in the lower salinity level with an increasing gradient up to the 8th day followed by similar decreasing gradient up to the 12th day (Figure 3). For the BEA prawns, a reciprocal quadratic function explains the trend. The estimated trend shows a general steady decrease from the 4th day onwards. In the case of UEA prawns, a cubic polynomial explains the trend. In this case a very smooth decreasing gradient is observed from the 4th day onwards unlike that in the case of

BEA prawns. In the case of control prawns, a 4th degree polynomial has been obtained as the optimal trend equation implying the complexity and sensitivity in the variation (Figure 3, Table 3).

3.3.3 Salinity 35±1PSU

Significant differences in the ionic concentration between BEA and control prawns ($t=9.15$, $P<0.05$) and between the UEA and control prawns ($t=7.65$, $P<0.05$) was observed. The haemolymph concentration of calcium in BEA prawns was less by 22.30% than that of control while the UEA prawns showed an increase in the concentration of this ion in their haemolymph by 7.20% than the control prawns.

The trend for variation in the ion concentration of calcium observed in the previous two salinity levels on intermoult and moult days was observed here also. The average values for the BEA, UEA and control prawns on moult days were 26.1, 33.15 and 35.6 mEq/L respectively. At this higher salinity level, the trend equation (Figure 3) for medium is a reciprocal cubic function (Table 3). The trend is an increasing curve up to the 6th day followed by almost a static situation thereafter with not much appreciable variation. In the case of BEA prawns, a semi log function is obtained for the variability in the calcium ion concentration. The estimated trend has a steadily decreasing gradient. In the case of UEA prawns also an exactly fitting trend has been obtained defined by a 4th degree polynomial. This function gives a mixed trend with a minimum concentration on the 8th day and maximum being on the 4th and 12th day (Figure 3, Table 3). Similar to the case of UEA, a quadratic polynomial with a steady increasing trend up to 10th day followed by almost a static concentration for the remaining period has been obtained for the intact prawns.

In the BEA prawns between the lower two salinities there was increase in calcium concentration by 23.21% and between low and high salinity, by 59.98% and between the medium and high salinity, by 29.83%. In the case of UEA prawns the corresponding increase in concentration of Ca^{2+} are 13.35%, 64.32% and 44.96% where as in the case of control prawns the increase was by 20.56%, 64.2% and 36.17% respectively (Table 2).

BEA prawns showed decreased concentration of Ca^{2+} in their haemolymph when compared to that of control prawns by 21%, 19% and 22% in low, medium and high salinity levels while the UEA prawns, it remained slightly higher by 5.5%, 0.7% and 5.2%. than that of the control prawns. A steady state trend at low salinity with a steadily decreasing trend at medium salinity level where as a gradually decreasing trend at high salinity is observed for Ca^{2+} concentration in the eyestalkless prawns.

Evaluation of the results of the present investigation suggest the possible presence of a Na^+ regulating factor in the eyestalks

of *M. monoceros* as the regulation of these ions was observed to be affected in destalked prawns. The decreased concentration of Na^+ in the haemolymph when in dilute saline medium and the increased concentration of Na^+ when in higher saline medium in the eyestalk less prawns when compared to the control prawns is possibly due to the passive crossing of the exchange barrier by sodium ions as the regulatory process appears to be affected.

Taylor et al. (1987) suggested that extrusion of ammonia is a physiological process in the gills of crustaceans and these ion effluxes for the active influx of Na^+ from the medium. The variations in the haemolymph concentration of Na^+ observed in the eyestalkless penaeid prawns here and the already discussed (Venkitaraman & Jayalakshmy 2009) enhanced ammonia excretion rate therefore are closely interconnected.

The Na^+ concentration in the haemolymph of BEA prawns differed on moulting days when compared to that of control ones. On the moult days the average Na^+ concentration recorded for BEA, UEA and control prawns showed a 2 fold, 1.8 fold and 1.7 fold increase from low to high salinity levels showing a positive correlation between ionic concentration and salinity regardless of the level of ablation. It further leads to the conclusion that moulting rather than the salinity of the medium influences the Na^+ levels and that the rate of change is more in ablated ones.

There seems to be no study on the changes in the Na^+ concentration during the period between two successive moults in penaeid prawns. In the present study as haemolymph samples were collected from the same animal on every second day for the whole experimental period, the changes in Na^+ concentration between the periods of two successive moults could be evaluated. It was observed that both in ablated and control prawns the Na^+ concentration in the haemolymph showed a definite trend. The concentration remained low on the moult days thereafter slowly increased in the post moult period and began to decrease with the approach of the next remount. Further, this study suggests, for higher sodium concentration bilateral eyestalk ablation is the optimal at the higher salinity level and unilateral eyestalk ablation at lower salinity level and that the variation in the Na^+ ion concentration is being controlled by some factors other than moulting for control and UEA prawns particularly at 16PSU and 35PSU salinity level, since the explained variability is <45%.

Potassium ion has received lesser attention from the investigators of crustacean physiology. Quinn & Lane (1966) suggested that active uptake of Na^+ across the gill is promoted by stimulating Na^+ - K^+ activated ATPase in *Cardisoma quanzhumi*. Sodium and chloride ions are considered to be the major cat ions concerned with the osmoregulation in crustaceans. The low concentration of K^+ when compared to Na^+ concentration observed in the haemolymph of both ablated and control prawns may be because the K^+ are in the bound

state with proteins. In the haemolymph of BEA prawns the increased concentration of K^+ observed in all the three salinities may be attributed to the neuroendocrine imbalance brought about in eyestalkless prawns. In this study it is obtained that potassium ions increase to 7-15% in all salinity levels in the BEA prawns whereas only in the high saline medium UEA prawns show some significant increase indicating bilateral ablation is optimal for higher potassium concentrations in the haemolymph at all the three salinity levels. This study further suggests that since higher degree polynomials/ reciprocal polynomials explains well the trend (variability explained >70%) in the variation of K^+ ions with respect to days at 6psu and 16 psu salinity levels, only at 35 PSU salinity level, factors other than moulting control the changes in the potassium ion concentration in the haemolymph of prawns particularly in the ablated ones.

The K^+ concentration in the haemolymph of BEA, UEA and control prawns remained elevated on moult days. This observation is important as the corresponding concentration of Na^+ in the haemolymph remained low on the moult days. It can be observed from the above findings that the behavior of K^+ and Na^+ concentration in the haemolymph is almost opposite (Table 4).

David & Ravi (2006) showed that prawns are stronger regulators of divalent than monovalent cations. Similar results were obtained during this study as shown in the above table. It was found that Na^+ ion concentration decreases with higher level of ablation in each salinity level and increases with salinity level at any level of ablation. The same trend is observed for calcium ions also. But an opposite trend is observed in the case of potassium with increasing trend for potassium ions with increase in the level of ablation at any salinity level even though it behaves in the same way as sodium ions with respect to salinity levels at any level of ablation. Hence this study could not support the observations of David & Ravi (2006) for all monovalent and divalent cations. A steady increase of K^+ ions at all levels of ablation in medium salinity whereas an asymptotic parabolic trend regardless of ablation level at low salinity and with no definite trend at higher salinity level is observed which is just opposite of what has been observed in the case of Na^+ ions as obtained by David & Ravi (2006).

Further the studies of David & Ravi (2006) revealed that potassium deficient inland saline water requires fortification with potassium to allow prawn survival and efficient osmoregulation. Salinity is an important environmental factor affecting euryhaline penaeid growth and survival (Chen & Lin, 1994b) as observed in this study. Several prawn species can tolerate sudden changes in salinity with haemolymph osmolality and ion concentrations stabilizing within 12-48 hours (Parado et al., 1987; Chen & Lin 1994a, 1994b; Diaz et al., 2001). The observation in this study that Intermoult penaeids are typically excellent ionic osmoregulatory supports

the observations of Mantel & Farmer, 1983 and Parado et al., 1989, where they have compared with other crustaceans.

Pillard et al. (2002) had shown that sudden changes in external osmolality can disrupt penaeid osmotic balance. Prawns may use a considerable quantity of energy to readjust their internal osmotic concentration (Chen & Lin 1994b). The observation that penaeid prawns become hyper osmoregulatory in this study supports partially the observation of Parado et al. (1987) and Chen and Lin (1994b) that one characteristic of penaeid prawns is to become hyper osmoregulatory when the ambient salinity is below the isosmotic point (29-32ppt) because in this study hyper ionic concentration is observed even at 35PSU except for sodium ions.

John et al. (1990) showed by ANOVA treatment that exposure time affected the haemolymph osmotic and Na^+ , Cl^- , K^+ , Mg^{2+} and Ca^{2+} concentrations ($P < 0.01$). However, Na^+ was affected by eyestalk ablation ($P < 0.01$). About 15-30% lower haemolymph Na^+ concentrations were observed in destalked shrimps compared to control shrimps. In this study also a similar result is observed but only 6-8% but observed only at salinity <16PSU. These observations may be due to the fact that less Na^+ enters the haemolymph of eyestalk less shrimps on exposure to sea water presumably resulting from the absence of an eyestalk located neuro factor as observed by John et al. (1990) at 21PSU. Hence a factor causing the influx of Na^+ into the haemolymph could be proposed to be present in the eyestalk of angliconic system of intact *M. monoceros* also as in *M. olfersii* (John et al. (1990)) in freshwater. Reduction in secretion of the factor may act to reduce Na^+ entry in high salt environments with <35 PSU only because in this study a 3% increase was observed at 35 PSU.

In contrast to potassium ion concentration BEA has drastically reduced the calcium ion concentration by about 18-23% compared to the control whereas an increase of 7-8% is observed in the UEA prawns. The significant fall in the Ca^{2+} concentration in the haemolymph of BEA prawns can be possibly due to deprivation of a neuroendocrine factor controlling Ca^{2+} concentration caused by eyestalk extirpation.

Menon & Sivadas (1968) have suggested the presence of hyper calcaemic factor in the eyestalk of *Scylla serrata*. They observed a decrease in the Ca^{2+} level in the blood following eyestalk extirpation. In *Orconect esvirellis* (McWhinnie et al., 1969) it was observed that hormones were involved in the regulation of Ca^{2+} in the cuticle. Mc Whinnie et al. (1972) observed that injection of crustecdysone into intact *Orconectes virilis* caused gastrolith formation and they suggested that the hormones are involved in calcium mobilization also. This study further establishes that the eyestalk controls osmotic and ionic balance in the prawn *M. monoceros* as demonstrated by Scudmore (1947) experimentally that the neuroendocrine factors are involved in the hydro mineral regulation in decapods crustaceans and later in the larvae and juveniles of

the lobster *Homarus americanus* by Charamantier et al. (1984).

On the whole, from the trend curves it could be inferred that a steady decrease in Na^+ ion concentration with respect to time is observed at low level and a steady state at medium level and an increasing trend at high level of salinity is observed for ablated prawns. This implies that the optimum level of salinity for a constant level of Na^+ ions is 16PSU for this species. For a decreasing concentration the salinity is to be decreased to 6 and for higher level of Na^+ ions, salinity is to be increased to 35 PSU for this species. A steady increase of K^+ ions at all levels of ablation in medium salinity whereas an asymptotic parabolic trend regardless of ablation level at low salinity and with no definite trend at higher salinity level is observed which is just opposite of what has been observed in the case of Na^+ ions as obtained by David & Ravi (2006). A steady state trend at low salinity with a steadily decreasing trend at medium level for ablated prawns whereas a gradually decreasing trend at high salinity is observed for Ca^{2+} concentration. This study reveals that higher the degree of the trend equation greater is the complexity in the relation and greater is the impact of moult and intermoult processes along with effect of eyestalk removal on the ionic concentrations thus leading to the conclusion that more closer observation on the changes in the level of ions is highly essential, which may be considered in the future studies.

Detailed studies on moulting and growth of penaeid prawns have already revealed that growth occurs during the intermoult period (Venkitaraman et al., 2010). Na^+ , K^+ and Ca^{2+} being essential macro ions, their concentration at a given period in the haemolymph can be counted as clear indicators of growth. Na^+ and K^+ play important roles in absorption of carbohydrate and breakdown of glucose respectively, while Ca^{2+} is instrumental in exoskeleton formation. Earlier studies on the same species have already proved the higher conversion efficiency of UEA prawns. Present studies on haemolymph ionic concentration of eyestalk ablated prawns confirms the efficiency of UEA prawns over BEA prawns with respect to growth. The BEA prawns on the whole did not show any significant particular trend in the haemolymph ionic concentrations at all the 3 salinities. This can be probably due to the physiological imbalance caused by the extirpation of both the eyestalks. The X-organ sinus gland complex in the eyestalks harbor many important hormones including MIH, GIH, ECH, LAH, HGH, NDH etc. and regulators of various enzymes like Na^+ - K^+ ATPase enzyme. Thus BEA leads to total imbalance of the enzymatic hormonal system, possibly causing the enhanced mortality and negative production (Venkitaraman 1991; Venkitaraman et al., 2004, 2009, 2010). The mortality in BEA prawns usually occurs due to exoskeleton thinning as a result of reduced Ca^{2+} deposition and loss of Ca^{2+} from the body (Figure 3) resulting from the total removal of Ca^{2+} regulating hormones. In the present study high concentrations of Na^+ and Ca^{2+} were observed during the

intermoult period at 35PSU salinity in UEA prawns. The slight decrease in K^+ concentration may be due to the osmoregulatory mechanism. Further it was noted that in UEA prawns at 35 PSU Na^+ and K^+ concentrations were inversely proportional indicating the proper functioning of the osmoregulatory system which in turn indicates a healthy condition. Thus it can be inferred from this study that UEA prawns at 35PSU could be recommended for better growth of *M. monoceros* in culture conditions.

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