STUDIES ON OSMOTIC ADAPTATIONS WITH RESPECT TO HAEMOLYMPH OSMOLALITY AND CHANGES IN GILL STRUCTURE IN *METAPENAEUS DOBSONI* (MIERS)

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CERTIFICATE

This is to certify that this Dissertation is a bonafide record of work carried out by Kumari P. Mini under my supervision and that no part thereof has been presented before for any other degree.

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CONTENTS

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PREFACE

The development of osmoregulatory powers is closely dependent on the adaptation of the marine animals to one of the most stressful and exacting aquatic biotopes, the brackishwater environment. A major problem encountered by the estuarine organisms is salinity change due to the tidal cycle and climatic conditions.

Salinity affects aquatic organisms both directly and indirectly. Direct effects are related to the organismic and molecular capability of animals at any stage of their life cycle, which ensure proper water balance in their internal fluids either intracellular or extracellular. Indirect effects are, essentially of two types: osmotic shocks may produce modifications of the biocoenotic composition of an ecosystem, which will result in changes in the biotic background for the remaining forms; salinity stress may become a limiting factor when acting together with one or several other stressful conditions.

Among crustaceans, Hyper-hyporegulation represents the most elaborate adaptation to salinity stress. They are able to hyperregulate their blood osmolality in dilute media and to hyporegulate it in concentrated ones. Thus, such crustaceans are extremely powerful osmoregulators, which can cope with any fluctuation of the salinity of the external medium that normally occurs in nature.

By maintaining a hyperosmotic internal concentration in a dilute medium, osmoregulators place less of a burden on their internal tissues. The problem of osmotic influx of water can be overcome by reducing permeability to water, increasing efflux of water via the urine and increasing the uptake of salts from the dilute medium. Similarly hypoosmotic regulation of haemolymph by animals in media of high salinity presents, less of a burden of shrinkage on the tissues, than would osmoconformity, but it results in influx of salts and osmotic loss of water. In both the situations, hyper-and hypoosmotic regulation, specialized boundary epithelia, notably gills, gut and excretory organs are responsible for active transport of salts to or from the medium. The presence of lime encrusted exoskeleton covering the body of the crustaceans as a whole, allows only restricted volume changes exchanged through the gills, primarily. A perusal of the literature reveals that the gill tissue plays an important role in blood ionic regulation in crustaceans.

The osmoregulatory capabilities of the penaeid prawns are reflected in their migratory pattern in the course of their life cycle. The highly fecund females liberate the fertilized eggs demersally in offshore continental shelf waters. After 3-4 weeks of larval existence, the postlarvae settle in shallow inshore waters, open estuaries or in some cases, penetrate considerable distances up river systems to regions of very low salinity. As growth progresses, the juvenile prawns tend to move into deeper waters and sexual maturity is attained usually in waters of oceanic salinity. Similar life cycle pattern is also met with in the penaeid prawn, Metapenaeus dobsoni taken up for the present study.

Though M. dobsoni is one of the common species of the Cochin backwaters and highly euryhaline in nature, not much work has been carried out on its osmoregulation. The earliest of such works was by Panikkar (1951) who described osmoregulation as an adaptation to the environment in M. dobsoni. Kalpana (1987) compared the osmoregulatory capabilities of juveniles and adults, M. dobsoni. Work on the lines of effect of abrupt salinity changes on the osmolality and ionic concentration (Na^+, Cl^- and K^+) has not been worked out so far in this species. The histological changes in gills as a result of the abrupt salinity changes has also so far not been worked out in this species. However, in the context of the rapidly developing coastal aquaculture in the country, describing the response of this species to fluctuations in external salinity provides a good indication of its environmental limitations as a potentially important aquaculture species, and hence the present investigation was taken up.

I wish to express my deepest sense of gratitude to Dr.P.S.B.R.James Director, Central Marine Fisheries Research Institute, under whose very valuable and scholarly guidance, this work has been completed. I am deeply grateful to Dr.A.D.Diwan, Scientist S3 and Dr.K.C.George, Scientist, PNP Division for their valuable advice and help at different stages of the work. I express my gratitude to Shri.K.Narayana Kurup, Head of FRA Division for his help in the statistical analysis. I have great pleasure in acknowledging my deep sense of gratitude to Prof.K.T.Damodaran, Head of Marine Geology Department, School of Marine Sciences and Research Scholars, Shri.K.Abdulla Bava and Shri.Padmalal for their timely help with the chemical

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INTRODUCTION

The regulation of total particle concentration of the body fluids at levels different from those of the external medium, constitutes osmotic regulation (Robertson, 1957). Maintenance of the concentration of ions in the blood plasma, differing from those of a passive equilibrium with the external medium is universal in crustaceans. Euryhaline crustaceans withstanding wide variations in the environmental salinity may behave as osmo-conformers, hyper-osmoregulators or hyper-hypo-osmoregulators,exhibiting almost any of the possible known patterns of blood osmotic regulation (Gilles and Pequeux, 1983; Mantel and Farmer, 1983). The osmolality of the crustacean blood mostly results from a control of its NaCl concentration. This control implicates active and passive movements of both sodium and chloride between the extracellular fluid and the environmental aquatic medium (Gilles and Pequeux, 1985).

Most of the crustaceans are osmoconformers. Hence, only a rather limited number of groups among the crustaceans are able to maintain their internal osmotic concentrations, relatively different, either higher or lower, from that of the medium over part, or all of their ecological salinity range, despite the steep concentration gradients generating important diffusive forces (Pequeux and Gilles, 1984).

The wide range of osmoregulatory powers in the euryhaline crustaceans account for the successful completion of their life history in the widely differing aqua-regions, viz., the sea and the estuary. Euryhaline crustaceans come across wide fluctuations in the salinity in the medium, during migration from the estuary to the sea or vice versa. Many penaeid prawns of euryhaline habitat have been found to have a common migratory behaviour returning to high saline conditions for maturation and spawning (George and Vedavyasa Rao, 1968; Castille and Lawrence, 1981) and Metapenaeus dobsoni is no exception to it. This migratory pattern to higher salinities during maturation process can, possibly be well correlated with osmoregulatory capabilities (Panikkar, 1968). Panikkar (1951) emphasised the role of osmoregulation as an adaptation to the estuarine environment in M. dobsoni, Penaeus carcinatus and P. indicus.

Crustaceans have been studied with respect to osmotic and ionic regulation for many years. Early investigations by Nagel and Krough (1939) revealed the euryhaline capabilities of many crustaceans and the detailed studies by Shaw (1960), Robertson (1949, 1953, 1957) and Gross (1959 and 1964) provided information on the actual changes in the composition of haemolymph and tissue of animals exposed to varying salinities. Dall (1970) studied the osmoregulation in the lobster, Homarus americanus. The regulation of the intracellular concentration of ions, Na^+ and Cl^- in the haemolymph of the shore crab, Hemigrapsus edwardsi, exposed to different salinities was investigated by Bedford and Leader (1977). Sharp and Neff (1980) studied the responses of Clibanarius vittatus to salinity fluctations

by measuring haemolymph osmotic pressure and chloride ion concentration. Studies on the effect of salinity acclimation on the osmoregulatory abilities of Crangon crangon and Praunus flexuosus were conducted by McLusky et al. (1982). Wright et al. (1984) conducted a comparative study on the regulation of the major ionic haemolymph components, viz., Sodium, Chloride, Potassium, Calcium, Sulphate and Magnesium in the species of Uca.

Campbell and Jones (1989), in their study dealt with the effects of temperature upon the salinity tolerance and also the blood osmolality and blood chloride concentration in the brackishwater shrimp Palaemonetes varians. A quantitative comparison of the osmoregulatory capability in the larvae, postlarvae and adult stages of Macrobrachium petersi was worked out by Read (1984). The osmoregulatory performance of juvenile M. rosenbergii adapted to varying salinities and ionic concentrations in terms of haemolymph osmolality and regulation of key ions (Cl^-, Na^+, K^+, Ca^{++} and Mg^{++}) was carried out by Stern et al. (1987). Moreira et al. (1988) studied the effects of salinity on haemolymph osmotic, Ca^{++}, K^+, Na^+ regulation in Macrobrachium carcinus. Hagerman et al. (1983) studied the influence of temperature on the osmoregulation of the brackish-water shrimp Palaemonetes varians. Castille and Lawrence (1981) studied the effect of salinity on the osmotic, sodium and chloride concentration in the haemolymph of M. ohione and M. rosenbergii.

Certain aspects of osmoregulatory capabilities of some penaeid species have been well characterised in the recent years. McFarland and

Lee (1963) have shown that adult P. setiferus and P. aztecus, whose juveniles are euryhaline, showed well developed osmotic regulation of body fluid, whereas adults of the stenohaline species Trachypenaeus similis and Sicyonia dorsalis have limited osmoregulatory ability. Adults of the euryhaline species M. monoceros and M. bennettae have even better osmoregulatory abilities than P. setiferus and P. aztecus (Panikkar and Viswanathan, 1948; Dall, 1964).

Panikkar and Viswanathan (1948) worked on the Chloride regulation in M. monoceros. McFarland and Lee (1963) studied the osmotic and ionic regulation in general on P. aztecus and T. similis and by Bursey and Lane (1971) on Penaeus duorarum. Castille and Lawrence (1981) compared the capabilities of juvenile and adult P. setiferus and P. stylirostris to regulate the osmotic, sodium and chloride concentration in the haemolymph. An investigation was made on the osmoregulation over a salinity range 3-50‰ of early juvenile and adult penaeid prawns by Dall (1968). Studies on the regulation of sodium, potassium, calcium, magnesium, chloride and sulphate ions in the adults of Australian penaeid prawn over a salinity range of 10-50‰ were carried out by Dall and Smith (1981).

Ferraris et al. (1986) studied the effect of salinity on the osmotic and chloride concentrations in the haemolymph of P. monodon as a function of time after moult. Parado Estepa et al. (1987) worked on the responses of intermoult P. indicus to large fluctuations in environ-

mental salinity. Ferraris et al. (1987) determined the response of P. monodon in various moult stages to a wide range of salinities (8-44‰) and compared the osmotic and chloride regulatory abilities of one moult stage to another. Kalpana (1987) compared the osmotic pressure of the haemolymph of preadult and adult Metapenaeus dobsoni acclimated to different salinities. Diwan and Laxminarayana (1989) studied the osmoregulatory ability in adult P. indicus in relation to varying salinities while Diwan et al. (1989) conducted a similar study in P. monodon.

The active role played by crustacean branchiae in the regulation of internal salt and water balance is well documented (Robertson, 1960; Lockwood, 1962, 1968; Croghan, 1976; Krischner, 1979; Towle, 1984). Ultrastructural studies have been conducted on the gills of several decapod crabs such as Callinectes (Copeland and Fitzjarrell, 1968); Gecarcinus (Copeland, 1968), Hemigrapsus and Pachygrapsus (Wright, 1964); Holothrisiana (Taylor and Greenaway, 1979). Penaeus (Talbot et al., 1972) and Uca (Finol and Croghan, 1983).

Gill epithelium has been identified as the primary site of blood ionic regulation (Barra et al., 1983; Copeland, 1968; Krogh, 1934). The fine structure of arthrobranch of Pacifastacus leniusculus with reference to active ion uptake was studied by Morse et al. (1970). Kümmel (1981) studied the fine structural indications of an osmoregulatory function of the gills in terrestrial isopods. Kikuchi (1983) studied the fine structure of the gill epithelium of a freshwater flea Daphnia magna and changes associated with acclimation to various salinities.

The penaeid prawn, M. dobsoni was selected for the present study, since it is one of the most common species of prawns cultured extensively in the prawn culture fields of Kerala. In the course of its migration from the estuaries to the sea for breeding, the prawns face the problem of osmoregulation due to exposure to the fluctuating salinities in the medium. The present study was conducted to throw light on the osmoregulatory capacity and ionic regulation (Na^+, K^+ and Cl^-) in the prawn, when exposed to abrupt changes in salinity. The associated structural changes at cellular level in the gills of the prawns with respect to the salinity changes have also been examined.

MATERIALS AND METHODS

Adult specimens of Metapenaeus dobsoni required for the present study were collected usually from the perennial fields, Edavanakkad and at times also from Matsyafed farm, Narakkal. They were collected by using filtration nets and also cast nets. The size of the selected prawns for the experimental purpose ranged between 70-90 mm total length. Normal and healthy prawns were selected and transported in transportation bags of 10 l capacity to the laboratory. In the laboratory, the prawns were maintained at the same salinity as that of the collection site for a period of 24 hours.

Experimental set up

Two different experiments were conducted, the first for the osmo-ionic studies and the second for histological studies. The first experiment was designed to find out the changes in osmolal and ionic levels in haemolymph in relation to varying salinities.

Around two hundred prawns maintained in the laboratory were divided into two groups, A and B of one hundred prawns each;

a) Prawns belonging to group A were acclimated at a low salinity of 5‰ and

b) Prawns belonging to group B were acclimated at a high salinity of 35‰.

PLATE-I. Adult prawn, M. dobsoni.

Prawns of both group A and B were maintained in two separate pools of one tonne capacity for a period of seven days. The acclimation pools were filled with previously well aerated water of the required salinity, namely 5‰ and 35‰ respectively.

The seawater required for the experimental purpose was collected by the research vessel Cadalmin from offshore areas of Cochin. Experimental salinities required below 30‰ were prepared by dilution of seawater with freshwater in the required proportions. Seawater of higher salinities was prepared by partial freezing and removal of the ice formed thereby (Shapiro, 1961). The salinity of the water was determined by the Mohr's method (Strickland and Parson, 1968). The range of prepared salinities varied from 5‰ to 35‰, at the interval of 5‰.

During the period of acclimation, the prawns were fed ad libitum with fresh clam meat once a day. The left over feed and faecal matter was siphoned out daily. The water temperature during the course of acclimation was 28 ± 1°C. At the end of the acclimation period, the intermoult prawns were selected for the experimental study, without regard to sex.

Prawns from group A numbering about seventy were selected and were further subdivided into seven sets of ten prawns each. One of the seven sets was maintained in the salinity of 5‰ to serve as the control for the experiment. The remaining six sets were exposed to the respective ascending salinities of 10‰, 15‰, 20‰, 25‰, 30‰ and 35‰ in six

PLATE-II. Experimental set up.

tubs for a period of 84 hours each. Each tub had a capacity of 40 l and was filled with previously prepared well aerated seawater of the particular salinity. No feeding was carried out during the period of exposure.

A similar set up was done for the Group B prawns as well. They were exposed to the respective descending salinities of 30‰, 25‰, 15‰, 10‰ and 5‰, with 35‰ serving as the control, in this case. No feeding was carried out during exposure as in the above.

After the start of the experiment, one prawn from each of the seven tubs of both Groups A and B were taken and the haemolymph collected at 0 hours and thereafter at 12, 24, 36, 48, 60, 72 and 84 hours.

The prawn was blotted dry with a blotting paper. The haemolymph was collected through the pericardial cavity with the help of a 1 ml Tuberculin syringe equipped with a 26 gauge needle. Everytime before the extraction of the haemolymph, the syringe was rinsed with an anticoagulant (5% Sodium citrate).

The collected haemolymph was transferred to osmomat cuvettes and maintained in frozen condition until use. Simultaneously the water samples from the seven tubs of both groups A and B were also collected at the respective time intervals and stored in the osmomat cuvettes and maintained in frozen condition until use. The experiment was replicated three times.

The haemolymph osmolality was determined by taking 50 micro-litre of the haemolymph sample collected, into a osmomat cuvette and taking the reading on 030 Osmomat directly. The osmolality of the medium was also similarly determined.

Chemical analysis

Sodium and Potassium ions in the haemolymph and in the medium were also determined simultaneously in all experimental and control prawns using Systronics digital Flame Photometer. Chloride ion concentration in the haemolymph and medium was determined colorimetrically (Schoenfeld and Lewellen, 1964). The colour measurement was made on an ECIL Senior Spectrophotometer.

The second experiment was designed to study the histological changes in the gill structure in relation to osmotic stress. Twenty laboratory maintained prawns were divided into two groups of ten each namely, Groups A and B. In Group A, prawns were maintained at a low salinity of 5‰ for a week and in Group B, the prawns were maintained at high salinity of 35‰ for a week. Five of the prawns from Group A were exposed to 35‰ saline water for five days and remaining five were maintained at 5‰ saline water to serve as control. Similarly in the Group B prawns, five were maintained at 35‰ saline water, serving as control, while five were exposed to 5‰ saline water.

At the end of the five days, the gills were collected from the

control and exposed prawns of both groups A and B. The entire gill tissue was removed and fixed in Bouin's fixative for 48 hours. The fixed tissue were washed and dehydrated in ascending grades of alcohol (70-100%) and then cleared in xylene. Hot impregnation in molten wax was carried out for one hour before embedding the tissue in Paraffin wax (Merck 58-60°C). Sections of 5-7 microns were cut using a manual rotary microtome. Staining was done after deparaffinising with xylene, using Haematoxylin and Eosin and the sections were mounted in DPX. Photomicrographs of the histological preparations were taken using Olympus Research Microscope.

Statistical analysis

Preliminary computations included determination of mean and standard deviations of the osmolality, Sodium, Potassium and Chloride ion concentrations of haemolymph and medium. All the statistical analysis were carried out according to Snedecor and Cochran (1967) and data were processed on WIPRO Computer with suitable programmes. A two way classification of ANOVA was adopted. The variance due to salinity changes and also due to the time intervals of exposure was verified for statistical significance in both the haemolymph and the medium in the case of each of the parameters studied.

RESULTS

1. OSMO-IONIC STUDIES

1.1. Osmolality

The osmotic concentrations of the haemolymph and the medium at each salinity are shown for both groups A (Table 1, Fig. 1) and B (Table 2, Fig. 2), as a function of time. In group A prawns, which were acclimated to 5‰ saline water, the haemolymph was hyperosmotic to the medium. Similar behaviour was also seen on exposure of these prawns to the salinity of 10‰, and 15‰ to some extent. At 20‰ salinity exposure, the haemolymph and medium osmolalities were almost isosmotic to each other, although at a later stage (after 60 hours) of exposure, the prawns became hypoosmotic with the medium. At 25‰ salinity, the exposed prawns were hypoosmotic with the medium. So also, at the higher salinities of 30‰ and 35‰, this behaviour was more pronounced. When the prawns were introduced from 5‰ to 35‰, the haemolymph osmolality showed a steep fall initially upto 12 hours of exposure after which it rose to about 866.5 m Osm/kg water. After 48 hours of exposure, the prawn adjusted to the new medium and behaved according to the external medium either as a hyper-regulator or hypo-regulator (Table 1, Fig. 1).

In Group B prawns which were acclimated at 35‰ saline water and then exposed to descending grades of salinity, a similar trend was

TABLE-1. VARIATIONS IN THE OSMOTIC PRESSURE (MILLI OSMOLES/KG WATER) OF HAEMOLYMPH(HL) AND MEDIUM (M) AS A FUNCTION OF SALINITY AND EXPOSURE TIME (F)*

SALINITY		TIME (HOURS) 0	12	24	36	48	60	72	84
5‰ (Control)	HL	269 ± 4.6	281 ± 2.5	269 ± 3.2	364 ± 3.6	454.5 ± 7.1	279 ± 7.3	437 ± 6.1	292.5 ± 8.7
	M	186 ± 5.1	290.5 ± 2.0	326.5 ± 3.8	273 ± 7.2	82 ± 5.4	136 ± 5.6	155.5 ± 6.3	407.5 ± 10.6
10‰	HL	290.5 ± 3.8	348.5 ± 4.9	243 ± 3.2	305 ± 5.3	242 ± 4.8	268 ± 4.9	392 ± 5.1	380 ± 3.4
	M	327 ± 2.5	303.5 ± 4.8	360.5 ± 5.0	378.5 ± 4.1	259 ± 4.9	243 ± 4.8	240 ± 4.8	479 ± 5.1
15‰	HL	395 ± 3.9	403.5 ± 5.7	394 ± 10.6	424.5 ± 6.9	465 ± 4.9	469.5 ± 4.8	407 ± 2.9	436 ± 2.7
	M	423 ± 3.4	411.5 ± 2.8	412 ± 4.2	437.5 ± 4.4	356.3 ± 3.9	357.3 ± 3.5	373 ± 3.6	315 ± 4.6
20‰	HL	492.5 ± 4.9	483 ± 5.1	487 ± 8.1	497.5 ± 4.3	496 ± 5.0	483.5 ± 4.9	474.5 ± 4.1	434.5 ± 3.9
	M	453.5 ± 3.1	489.5 ± 5.0	481 ± 5.1	537.5 ± 4.9	421.5 ± 4.6	477 ± 4.1	490 ± 4.6	467.5 ± 4.6
25‰	HL	515.5 ± 5.0	509.5 ± 3.4	545.5 ± 2.1	580 ± 2.6	520.5 ± 2.7	506 ± 3.7	452.5 ± 3.6	486 ± 6.0
	M	486 ± 3.1	514.5 ± 3.4	503 ± 3.1	489 ± 3.2	584 ± 7.1	566.5 ± 3.8	597 ± 3.8	641.5 ± 3.7
30‰	HL	657.5 ± 2.5	594.5 ± 4.9	585.5 ± 3.1	602.5 ± 2.9	513 ± 3.2	525 ± 4.9	525 ± 6.1	570.5 ± 1.3
	M	587.5 ± 2.5	630 ± 2.6	650.5 ± 4.0	629.5 ± 3.6	707.5 ± 4.9	641 ± 5.1	794.5 ± 4.9	804.5 ± 4.9
35‰	HL	815 ± 5.5	336.5 ± 9.9	821 ± 4.9	866.5 ± 9.7	638 ± 2.6	592.5 ± 7.4	635.5 ± 7.5	708.5 ± 1.3
	M	540 ± 3.3	545 ± 3.7	550 ± 1.5	636.5 ± 4.0	792 ± 4.5	840.5 ± 3.4	840 ± 2.5	868.5 ± 2.4

All values are mean of 3 replications and figures represent $\bar{X}$ ± S D

ANOVA Table for Osmotic Pressure of Haemolymph

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	399395.000	66565.840	16.46	HI.SIG(1%)
Replication	7	49750.000	7107.143	1.76	N.S
Error	42	169826.000	4043.476		

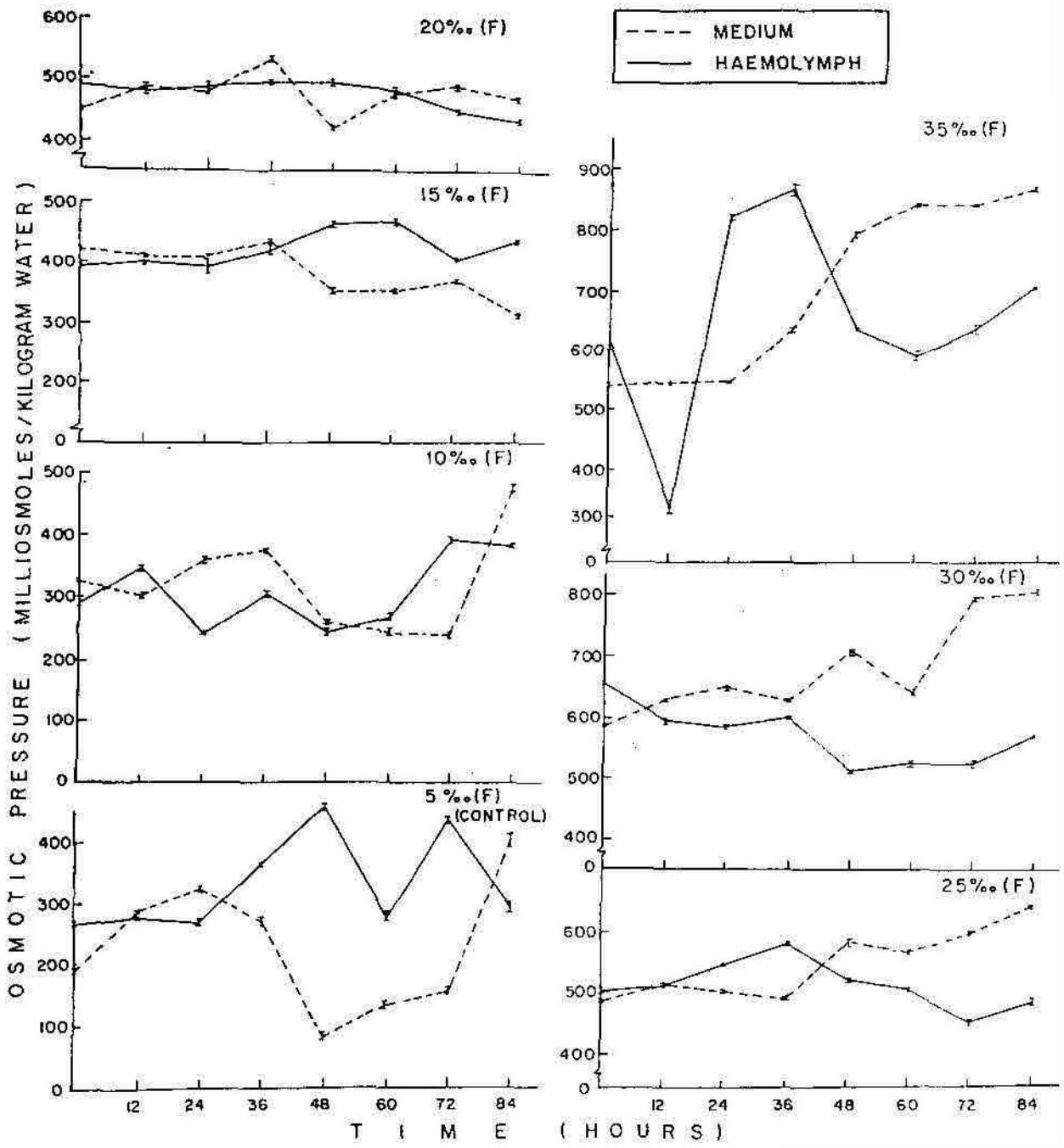
ANOVA Table for Osmotic pressure of medium

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	1508842.000	251473.700	36.95	HI.SIG(1%)
Replication	7	86257.000	12322.430	1.81	N.S.
Error	42	285821.000	6805.262		

*F =Forward exposure from low to high salinities

Fig. 1. Changes in the osmotic pressure of the haemolymph and medium in the control and exposed prawns of Group A, as a function of salinity and time.

F = Forward Exposure from low to high salinities.


MEDIUM
HAEMOLYMPH
20‰ (F)
15‰ (F)
10‰ (F)
5‰ (F)
(CONTROL)
35‰ (F)
30‰ (F)
25‰ (F)
OSMOTIC PRESSURE (MILLIOSMOLES/KILOGRAM WATER)
TIME (HOURS)
12
24
36
48
60
72
84

noticed (Table 2, Fig. 2). The prawns behaved as a hyperosmoregulator at lower salinities (15‰ and below) and as hypoosmotic at higher salinities (20‰ and above) and isosmotic condition was found at intermediate salinities of 15‰ and 20‰ initially and after 48 hours, the trend of hyperosmotic and hypoosmotic regulation to medium became obvious at the two salinities respectively. When the 35‰ acclimated prawns were exposed to 5‰ saline water, the time taken for adjustment was much less when compared to the reverse case. The variations in the osmolality of haemolymph as a function of time was also less when compared to the reverse case (Fig.2) as described earlier. In both the cases, it has been revealed that a minimum of 48 hours was taken by the prawn to adjust itself to its new environment, before which, the prawn behaves as an osmo conformer by adjusting the haemolymph osmolality according to its medium.

Analysis of variance revealed that no significance was associated with the changes in osmolality in the haemolymph and medium as a result of time but the salinity of the medium had significant effect on the haemolymph and medium osmolality (ANOVA $P = 0.01$) in both the cases.

1.2. Ionic regulation

1.2.1. Sodium ion regulation:

The values of the haemolymph and medium sodium ion concentration are shown as a function of time at each salinity in both groups A (Table 3, Fig. 3) and B (Table 4, Fig. 4). In group A prawns, the control animals at 5‰ saline water showed a sharp increase initially in the blood Na^+

TABLE-2. VARIATIONS IN THE OSMOTIC PRESSURE (MILLI OSMOLES/KG WATER) OF HAEMOLYMPH(HL) AND MEDIUM (M) AS A FUNCTION OF SALINITY AND EXPOSURE TIME (R)*

TIME (HOURS)

SALINITY		0	12	24	36	48	60	72	84
5‰	HL	482 ± 5.1	481 ± 9.6	446 ± 12.5	453.5 ± 6.3	439.5 ± 7.5	298 ± 7.6	438 ± 9.4	397.5 ± 5.4
	M	169 ± 4.8	201 ± 2.5	269.5 ± 2.7	204 ± 4.8	177.5 ± 6.8	179 ± 5.3	180.5 ± 7.3	264.5 ± 3.6
10‰	HL	457 ± 2.5	444 ± 7.5	428 ± 7.3	493 ± 10.2	531.5 ± 12.1	489 ± 6.8	500 ± 8.6	506 ± 5.3
	M	284 ± 2.7	263.5 ± 4.6	295 ± 6.3	278 ± 3.8	263.5 ± 6.4	261 ± 5.8	274 ± 5.2	220 ± 4.8
15‰	HL	594 ± 2.8	474 ± 6.4	411.5 ± 3.3	509.5 ± 4.6	526.5 ± 9.7	474 ± 5.1	500.5 ± 7.4	436.5 ± 7.8
	M	331 ± 2.6	402 ± 5.5	431 ± 3.8	348.5 ± 6.7	356 ± 4.3	332.5 ± 4.5	389.5 ± 3.7	337 ± 6.3
20‰	HL	568.5 ± 5.4	526.5 ± 3.8	486 ± 6.4	531.5 ± 8.1	494 ± 7.2	407.5 ± 10.3	583 ± 3.1	538 ± 9.9
	M	478.5 ± 3.9	429 ± 1.3	533 ± 4.3	494 ± 7.8	530.5 ± 2.3	530.5 ± 9.4	505 ± 6.3	475 ± 2.8
25‰	HL	551 ± 3.6	610 ± 3.4	470 ± 7.4	524.5 ± 7.6	620.5 ± 8.3	533 ± 4.1	485.5 ± 5.7	504.5 ± 4.6
	M	542.5 ± 2.6	668 ± 3.5	685.5 ± 5.3	618.5 ± 6.3	590 ± 4.2	611.5 ± 3.9	646 ± 5.5	590.5 ± 5.1
30‰	HL	526 ± 7.3	598.5 ± 4.6	650 ± 4.3	627 ± 2.4	615 ± 5.5	593 ± 4.6	590.5 ± 4.5	569.5 ± 7.3
	M	656 ± 3.2	703 ± 7.4	890.5 ± 7.6	713 ± 2.1	696.5 ± 3.1	722.5 ± 5.0	746 ± 5.6	740.5 ± 5.1
35‰ (Control)	HL	486 ± 7.4	603.5 ± 3.1	489 ± 6.8	582 ± 2.6	616 ± 6.2	630.5 ± 6.3	641 ± 6.1	525 ± 9.9
	M	805.5 ± 4.9	829 ± 2.3	949 ± 2.1	865 ± 1.2	861 ± 3.5	883.5 ± 4.4	826 ± 4.5	868.5 ± 3.0

All values are mean of 3 replications and figures represent $\bar{X}$ ± S D

ANOVA Table for Osmotic Pressure of Haemolymph

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F-Val	Remarks
Treatment	6	155224.000	25870.670	11.10	HI.SIG(1%)
Replication	7	25269.000	3609.857	1.56	N.S.
Error	42	97227.000	2314.929		

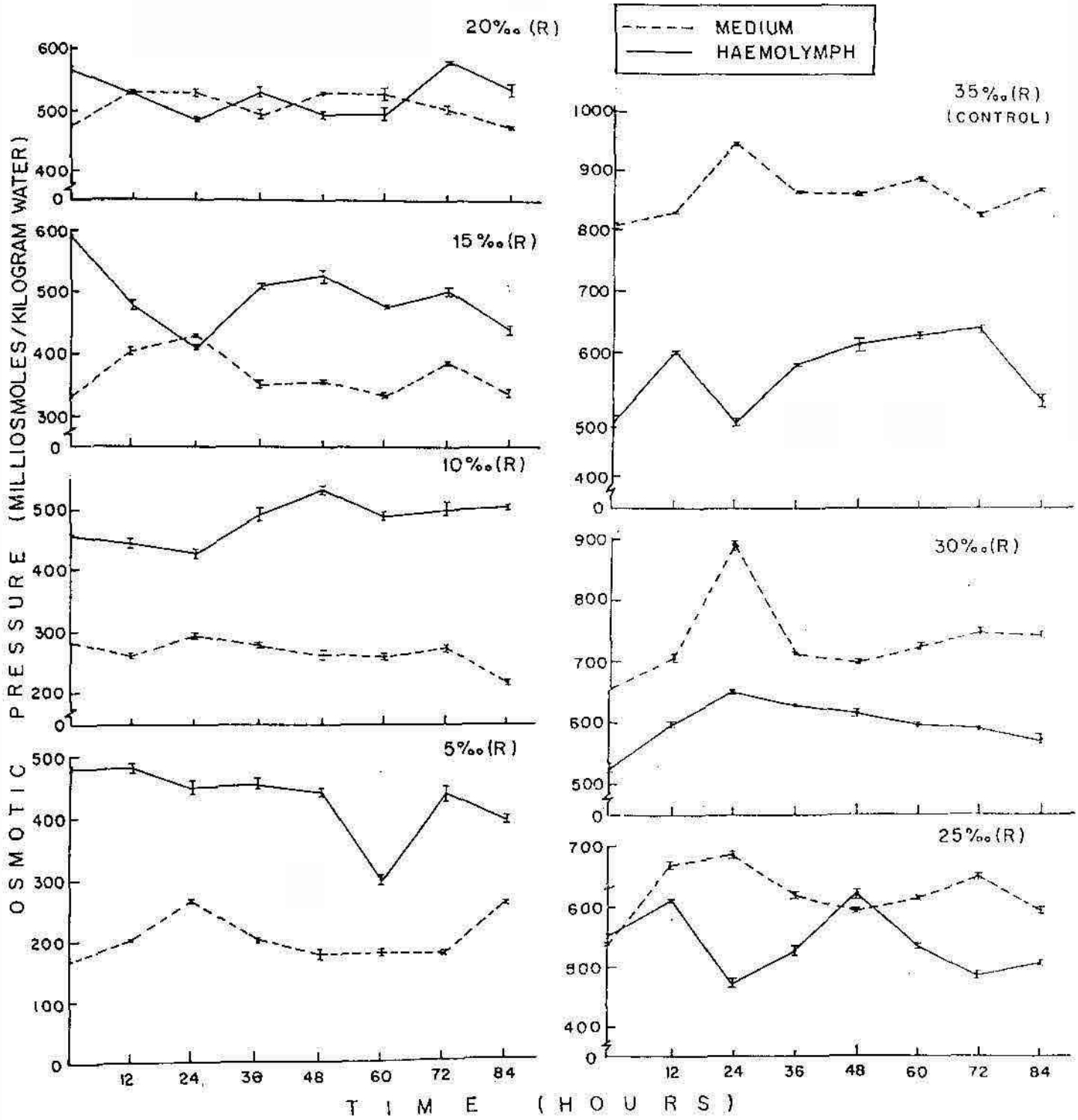
ANOVA Table for Osmotic Pressure of Medium

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	2858080.000	476346.700	481.22	HI.SIG(1%)
Replication	7	49337.000	7048.143	7.12	HI.SIG(1%)
Error	42	41575.000	989.881		

*R = Reverse exposure from high to low salinities

Fig. 2. Changes in the osmotic pressure of the haemolymph and medium in the control and exposed prawns of Group B, as a function of salinity and time.

R = Reverse exposure from high to low salinities.


MEDIUM
HAEMOLYMPH
20‰ (R)
15‰ (R)
10‰ (R)
5‰ (R)
35‰ (R)
(CONTROL)
30‰ (R)
25‰ (R)
OSMOTIC PRESSURE (MILLIOSMOLES/KILOGRAM WATER)
TIME (HOURS)
12
24
36
48
60
72
84

concentration in the 12 hours of exposure and slight increase further upto 36 hours and fall in concentration at 48 hours of exposure. After 48 hours the blood sodium ion concentration stabilised in the range of 293-313 meq/l. When the 5‰ salinity acclimated prawns were exposed to 10‰ saline water, the haemolymph Na^+ concentration in the medium remained hyperionic to medium. At 15‰ and 25‰ salinity also, the blood Na^+ concentration remained hyperionic to the medium. At 25‰ saline water exposure, the prawns showed hyperionic blood Na^+ concentration upto 72 hours of exposure, after which hypoionic condition began. At 30‰ and 35‰ salinity exposure, the haemolymph Na^+ concentration was hypoionic to the medium very distinctly.

Anova revealed that the salinity changes had a highly significant effect on the blood Na^+ concentration and on medium Na^+ concentration (ANOVA P = 0.01), whereas the time of exposure had a significant effect on medium Na^+ concentration at (ANOVA P = 0.05) and highly significant effect (ANOVA P = 0.01) in the blood Na^+ concentration (Table 3, Fig. 3).

In the reverse case, where the 35‰ acclimated prawns were exposed to the descending grades of salinity abruptly, the haemolymph Na^+ concentration behaved hypoionic to medium Na^+ concentration, initially at 25‰ 30‰ and 35‰ upto 12 hours and then became hyperionic to the medium Na^+ concentration. While in the 5‰, 10‰, 15‰ and 20‰ exposed prawns, blood Na^+ concentration remained hyperionic to the medium right from the start of the exposure (Table 4, Fig. 4).

TABLE-3. VARIATIONS IN THE SODIUM ION CONCENTRATION (MILLIEQUIVALENTS/LITRE) OF HAEMOLYMPH(HL) AND MEDIUM (M) AS A FUNCTION OF SALINITY AND EXPOSURE TIME (F)*

SALINITY		TIME (HOURS) 0	12	24	36	48	60	72	84
5‰ (Control)	HL	185.1 ± 2.4	342.9 ± 3.0	373.5 ± 0.9	384.5 ± 3.9	313.0 ± 1.9	306.3 ± 4.1	293.0 ± 1.9	304.2 ± 2.3
	M	67.9 ± 4.1	79.7 ± 1.3	131.0 ± 2.2	108.1 ± 2.3	94.5 ± 1.8	165.1 ± 5.4	110.0 ± 0	85.4 ± 1.9
10‰	HL	226.5 ± 1.6	219.7 ± 1.5	378.0 ± 0.9	479.8 ± 0.7	252.1 ± 0.6	235.0 ± 0.8	333.3 ± 0.9	283.7 ± 0.4
	M	135.1 ± 3.4	196.1 ± 2.3	162.2 ± 1.6	276.3 ± 0.8	153.5 ± 1.5	93.9 ± 1.4	162.2 ± 1.3	148.6 ± 1.0
15‰	HL	282.5 ± 0	408.5 ± 1.1	356.9 ± 0.8	257.3 ± 1.5	338.6 ± 1.4	249.8 ± 1.3	88.3 ± 0	210.0 ± 1.4
	M	219.6 ± 1.4	237.3 ± 1.6	188.8 ± 1.7	224.4 ± 1.5	188.4 ± 1.3	184.0 ± 0.8	120.5 ± 1.1	126.6 ± 1.4
20‰	HL	339.7 ± 0.7	370.5 ± 1.1	415.9 ± 0.8	439.8 ± 0.6	331.3 ± 0.7	238.0 ± 0.3	320.8 ± 0.9	383.7 ± 0.7
	M	238.9 ± 1.9	285.6 ± 1.4	296.0 ± 0.8	255.9 ± 0.9	274.0 ± 1.1	141.4 ± 1.6	280.3 ± 0.7	248.9 ± 0.8
25‰	HL	360.4 ± 0	362.8 ± 2.3	421.8 ± 2.1	437.6 ± 6.5	304.3 ± 6.8	323.2 ± 6.1	378.6 ± 4.3	316.5 ± 5.7
	M	336.02 ± 0	359.9 ± 1.6	326.5 ± 2.4	337.9 ± 2.3	311.5 ± 0	282.3 ± 5.4	362.4 ± 1.6	331.3 ± 2.3
30‰	HL	277.4 ± 2.1	389.9 ± 1.7	341.0 ± 1.9	450.2 ± 2.0	345.3 ± 7.1	271.2 ± 3.6	212.8 ± 0.1	269.7 ± 0.8
	M	397.6 ± 2.8	432.4 ± 3.3	468.8 ± 2.6	383.6 ± 2.1	349.5 ± 3.7	350.7 ± 4.6	258.4 ± 1.2	296.7 ± 1.0
35‰	HL	306.6 ± 1.8	332.2 ± 1.9	570.1 ± 4.7	468.3 ± 2.9	502.8 ± 1.3	437.8 ± 3.1	449.7 ± 5.0	412.6 ± 0.9
	M	454.5 ± 2.1	5227 ± 1.4	478.0 ± 3.6	489.8 ± 4.1	510.8 ± 2.8	475.3 ± 2.6	480.7 ± 2.7	480.7 ± 1.1

All values are mean of 3 replications and figures represent $\bar{X}$ ± S D

ANOVA Table for Na^+ concentration of Haemolymph

SOURCE	d.f	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	134079.000	22346.500	5.86	HI.SIG(1%)
Replication	7	130087.500	18583.930	4.87	HI.SIG(1%)
Error	42	160170.500	3813.583		

ANOVA Table for Na^+ concentration of Medium

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	842935.500	140489.300	84.60	HI.SIG(1%)
Replication	7	27707.750	3958.250	2.38	SIG(5%)
Error	42	69745.250	1660.601		

* F = Forward exposure from low to high salinities

Fig. 3. Changes in the sodium ion concentration of the haemolymph in the control and exposed prawns of Group A, as a function of salinity and time.

F = Forward exposure from low to high salinities.

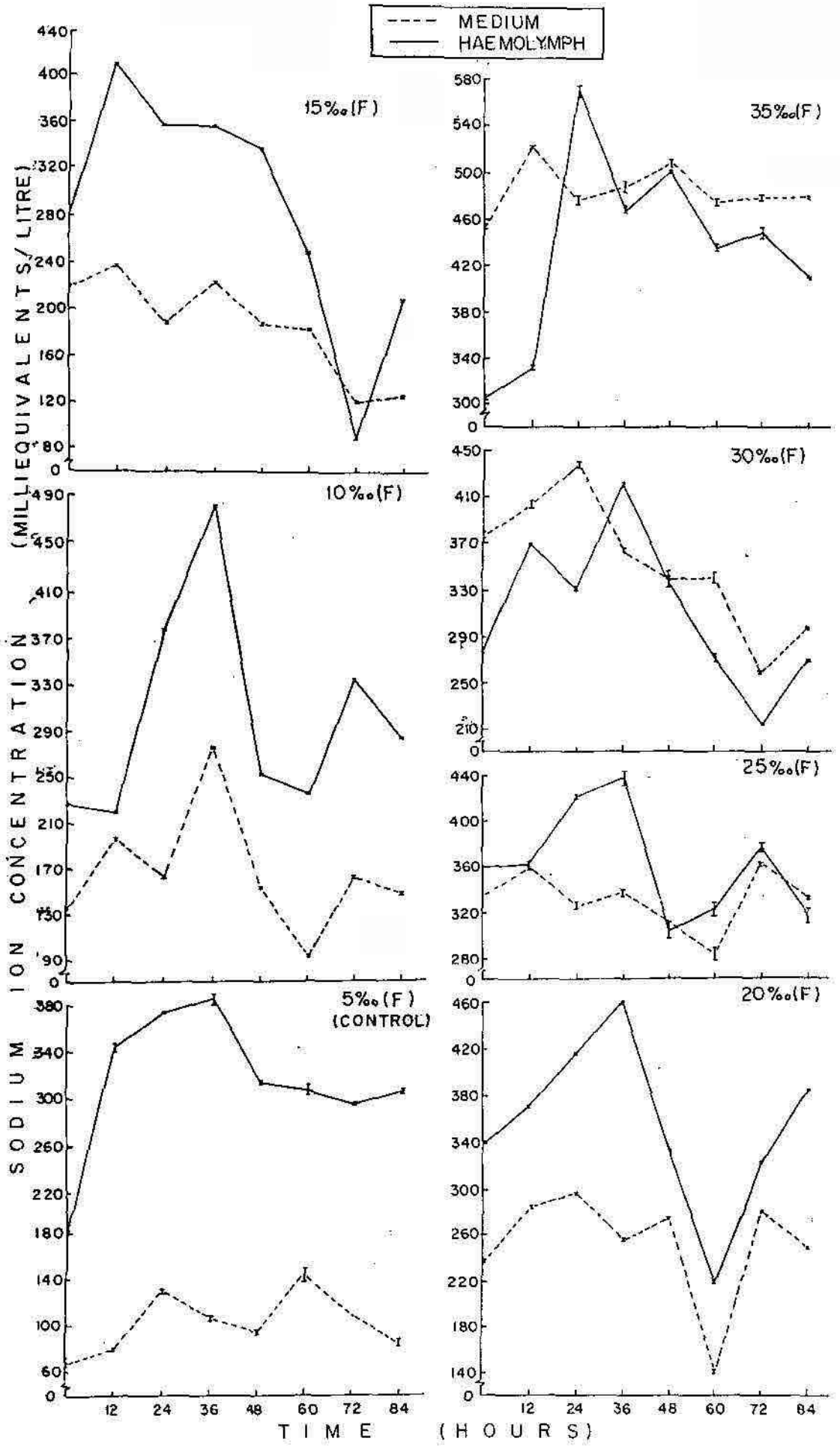

MEDIUM
HAEMOLYMPH
15‰(F)
35‰(F)
10‰(F)
30‰(F)
25‰(F)
5‰(F)
(CONTROL)
20‰(F)
SODIUM ION CONCENTRATION (MILLIEQUIVALENTS/LITRE)
TIME (HOURS)
0
12
24
36
48
60
72
84

TABLE-4. VARIATIONS IN THE SODIUM ION CONCENTRATION (MILLIEQUIVALENTS/LITRE) OF HAEMOLYMPH(HL) AND MEDIUM (M) AS A FUNCTION OF SALINITY AND EXPOSURE TIME (R)*

SALINITY		TIME (HOURS) 0	12	24	36	48	60	72	84
5‰	HL	309.7 ± 1.4	300.0 ± 2.7	269 ± 2.8	313.2 ± 3.9	509.5 ± 5.9	239.1 ± 3.8	281.9 ± 2.8	357.8 ± 2.9
	M	111.4 ± 1.5	118.3 ± 2.4	125.3 ± 4.8	135.7 ± 1.8	111.4 ± 2.1	121.8 ± 2.6	121.8 ± 2.7	157.3 ± 3.6
10‰	HL	247.1 ± 0	357.4 ± 3.9	250.6 ± 2.1	357.7 ± 1.9	425.3 ± 3.8	306.9 ± 2.0	425.9 ± 1.9	354.9 ± 1.4
	M	146.2 ± 3.0	111.4 ± 1.8	139.2 ± 2.8	139.2 ± 2.7	149.6 ± 2.8	139.2 ± 2.9	146.2 ± 1.9	161.5 ± 2.1
15‰	HL	302.8 ± 1.4	431.5 ± 7.9	309.7 ± 6.9	378.6 ± 6.3	397.4 ± 3.6	423.5 ± 8.9	423.5 ± 0.9	296.2 ± 9.8
	M	177.5 ± 1.4	170.5 ± 1.2	177.5 ± 1.3	184.3 ± 2.1	177.5 ± 2.2	184.4 ± 1.6	184.4 ± 1.9	177.5 ± 2.0
20‰	HL	268.0 ± 0.1	381.4 ± 6.8	438.1 ± 1.6	316.7 ± 6.4	440.6 ± 7.4	338.6 ± 8.2	431.5 ± 1.3	419.7 ± 1.1
	M	184.4 ± 0	229.7 ± 1.9	236.6 ± 2.1	226.2 ± 8.2	222.7 ± 7.3	219.2 ± 9.7	226.2 ± 1.7	238.3 ± 1.1
25‰	HL	97.4 ± 0.4	284.4 ± 1.1	400.2 ± 2.5	365.4 ± 7.6	386.3 ± 7.7	469.8 ± 3.2	384.9 ± 4.8	397.4 ± 1.2
	M	292.3 ± 0.1	271.4 ± 0.8	306.2 ± 1.5	264.5 ± 1.7	261.0 ± 1.3	250.6 ± 3.2	268.0 ± 3.8	282.6 ± 2.1
30‰	HL	292.3 ± 2.1	306.2 ± 2.8	316.7 ± 9.8	466.7 ± 4.8	349.4 ± 1.6	425.6 ± 1.1	451.2 ± 0.9	396. ± 3.9
	M	313.2 ± 2.0	313.2 ± 2.4	316.7 ± 0	306.2 ± 2.0	299.3 ± 0.8	285 ± 0.9	299.3 ± 3.6	300.7 ± 1.9
35‰ (Control)	HL	313.2 ± 0.1	417.2 ± 0.9	316.3 ± 1.1	434.4 ± 0.8	442.1 ± 0.7	375.9 ± 0.6	529.4 ± 1.0	417.3 ± 0.8
	M	348.0 ± 2.0	358.4± 0.6	382.8 ± 0.8	372.4 ± 0.7	527.4 ± 1.9	327.1 ± 0.8	320.2 ± 0.9	352.2 ± 1.0

All values are mean of 3 replications and figures represent $\bar{X}$ ± S D.

ANOVA Table for Na^+ concentration of haemolymph

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F.Val.	Remarks
Treatment	6	41029.500	6838.250	1.67	N.S.
Replication	7	127940.000	18277.140	4.48	HI.SIG(1%)
Error	42	171476.000	4082.762		

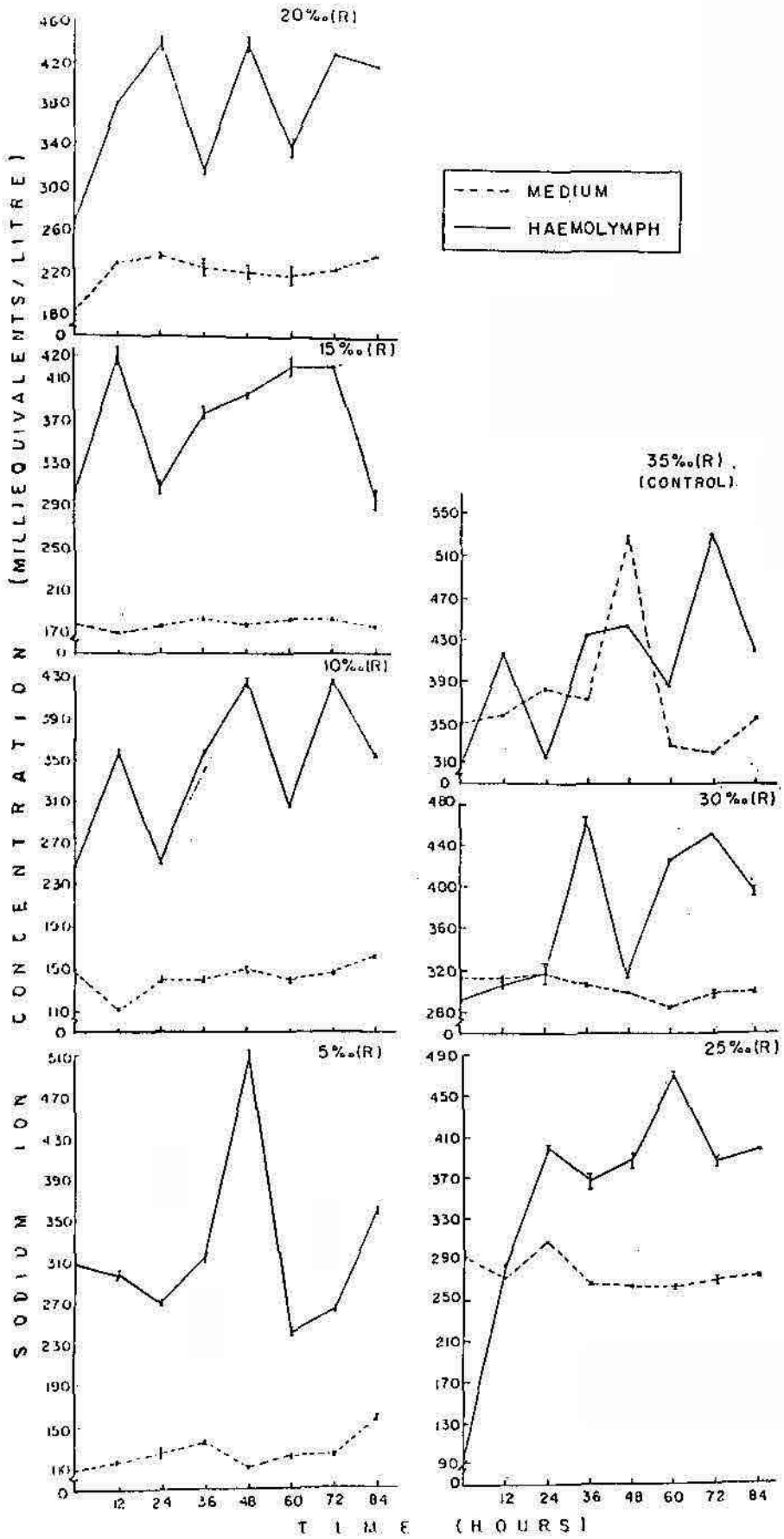
ANOVA Table for Na^+ concentration of medium

SOURCE	d.f.	Sum.Sqr.	Mean Sqr.	F.Val.	Remarks
Treatment	6	399327.500	66554.890	77.45	HI.SIG(1%)
Replication	7	6047.750	863.964	1.01	N.S.
Error	42	36089.500	859.274		

*R = Reverse exposure from high to low salinities

Fig. 4. Changes in the sodium ion concentration of the haemolymph in the control and exposed prawns of Group B, as a function of salinity and time.

R = Reverse exposure from high to low salinities.


20‰(R)
15‰(R)
10‰(R)
5‰(R)
35‰(R).
(CONTROL)
30‰(R)
25‰(R)
MEDIUM
HAEMOLYMPH
SODIUM ION CONCENTRATION (MILLIEQUIVALENTS/LITRE)
TIME (HOURS)
0
12
24
36
48
60
72
84

The salinity grades did not have any significant effect on the Na^+ concentration while the time of exposure was found to be highly significant (ANOVA P = 0.01) in the blood and the salinity changes had high significance to medium Na^+ concentration (ANOVA P = 0.01) and no significance with the time of exposure in the case of medium was observed.

1.2.2. Chloride ion regulation:

The values of the chloride ion concentration in the haemolymph and medium at each salinity as a function of time in both groups A (Table 5, Fig. 5) and B (Table 6, Fig. 6) are respectively represented.

In group A prawns, the chloride ion concentration in the blood was found to be hyperionic to the medium in 5‰ and 10‰ saline water. But in 15‰ and above, till 35‰ salinity, the blood Cl^- concentration became hypoionic to the medium Cl^- concentration (Table 5, Fig. 5). In the group B prawns, the blood Cl^- concentration of the control animals at 35‰ salinity initially, was hypoionic to medium and at 12 hours became hyperionic to medium. With slight variations according to the time, the blood Cl^- concentration ultimately became hyperionic to the medium, whereas in the prawns exposed to 30‰, 20‰ and 15‰, the blood Cl^- concentration showed the hypoionic trend with respect to the medium Cl^- concentration. In the 5‰ and 10‰ salinity maintained prawns, however, blood Cl^- concentration remained hyperionic to that of the medium (Table 6, Fig. 6).

Analysis of variance showed no significance in the blood Cl concentration between the prawns exposed to different salinities, while

TABLE-5. VARIATIONS IN THE CHLORIDE ION CONCENTRATION (MILLIEQUIVALENTS/LITRE) OF HAEMOLYMPH(HL) AND MEDIUM (M) AS A FUNCTION OF SALINITY AND EXPOSURE TIME (F)*

SALINITY		TIME (HOURS) 0	12	24	36	48	60	72	84
5‰ (Control)	HL	61.4 ± 6.2	96.9 ± 3.4	120.8 ± 3.6	181.4 ± 4.1	161.1 ± 2.7	135.5 ± 2.6	159.6 ± 2.9	174.7 ± 5.6
	M	31.3 ± 6.1	63.3 ± 2.1	24.6 ± 0.8	83.8 ± 0.9	41.4 ± 2.6	22.4 ± 2.2	61.9 ± 4.9	82.4 ± 2.3
10‰	HL	65.5 ± 0.9	11.6 ± 2.1	130.0 ± 1.9	145.1 ± 2.8	154.8 ± 4.2	184.4 ± 1.8	89.3 ± 0.9	196.9 ± 1.0
	M	59.7 ± 0.6	42.2 ± 1.1	92.5 ± 1.4	35.2 ± 2.0	107.0 ± 0.6	108.5 ± 2.1	139.9 ± 1.8	141.0 ± 1.4
15‰	HL	86.8 ± 1.0	105.0 ± 0.7	179.0 ± 1.8	131.7 ± 1.2	179.8 ± 2.9	160.8 ± 2.5	153.5 ± 1.4	160.0 ± 0.8
	M	111.0 ± 0.8	190.2 ± 0.9	151.2 ± 1.1	163.4 ± 2.4	132.1 ± 1.2	128.1 ± 2.4	212.2 ± 3.7	160.7 ± 0.7
20‰	HL	69.2 ± 1.4	156.4 ± 4.3	171.6 ± 1.0	161.7 ± 2.0	144.1 ± 1.5	121.6 ± 1.7	224.3 ± 0.8	176.9 ± 3.6
	M	148.4 ± 1.5	229.6 ± 2.0	253.5 ± 2.4	165.2 ± 2.1	200.5 ± 2.1	115.6 ± 1.0	228.4 ± 1.1	195.3 ± 0.9
25‰	HL	113.4 ± 1.6	90.9 ± 3.6	139.1 ± 2.1	148.3 ± 2.2	133.1 ± 2.7	162.2 ± 2.8	159.7 ± 1.8	206.6 ± 1.9
	M	254.0 ± 0.9	114.5 ± 3.0	348.9 ± 9.1	220.7 ± 2.2	240.6 ± 4.3	194.7 ± 2.5	209.4 ± 2.0	205.9 ± 1.9
30‰	HL	116.0 ± 3.0	170.2 ± 2.0	172.8 ± 2.5	162.9 ± 2.6	282.2 ± 3.1	163.9 ± 3.1	153.3 ± 5.2	145.7 ± 2.2
	M	210.9 ± 0.9	162.7 ± 3.0	221.9 ± 2.1	242.0 ± 2.9	285.3 ± 6.1	303.6 ± 1.5	308.3 ± 2.6	262.7 ± 3.1
35‰	HL	123.4 ± 1.5	114.7 ± 2.1	140.2 ± 2.6	178.3 ± 2.8	82.8 ± 2.9	112.6 ± 1.9	164.3 ± 1.5	83.3 ± 1.4
	M	205.4 ± 1.6	237.2 ± 2.4	204.4 ± 3.9	255.9 ± 2.1	141.6 ± 1.9	218.5 ± 2.7	173.7 ± 2.9	276.0 ± 4.9

All values are mean of 3 replications and figures represent $\bar{X}$ ±S D

ANOVA Table for Cl^- concentration of haemolymph

SOURCE	d.f	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	10521.830	1753.646	1.47	N.S.
Replication	7	31852.630	4550.375	3.82	HI.SIG(1%)
Error	42	50007.500	1190.655		

ANOVA Table for Cl^- concentration of Medium

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	253122.300	42187.050	21.84	HI.SIG(1%)
Replication	7	18022.880	2574.697	1.33	N.S.
Error	42	81112.750	1931.256		

*F = Forward exposure from low to high salinities

Fig. 5. Changes in the chloride ion concentration of the haemolymph in the control and exposed prawns of Group A, as a function of a salinity and time.

F = Forward exposure from low to high salinities

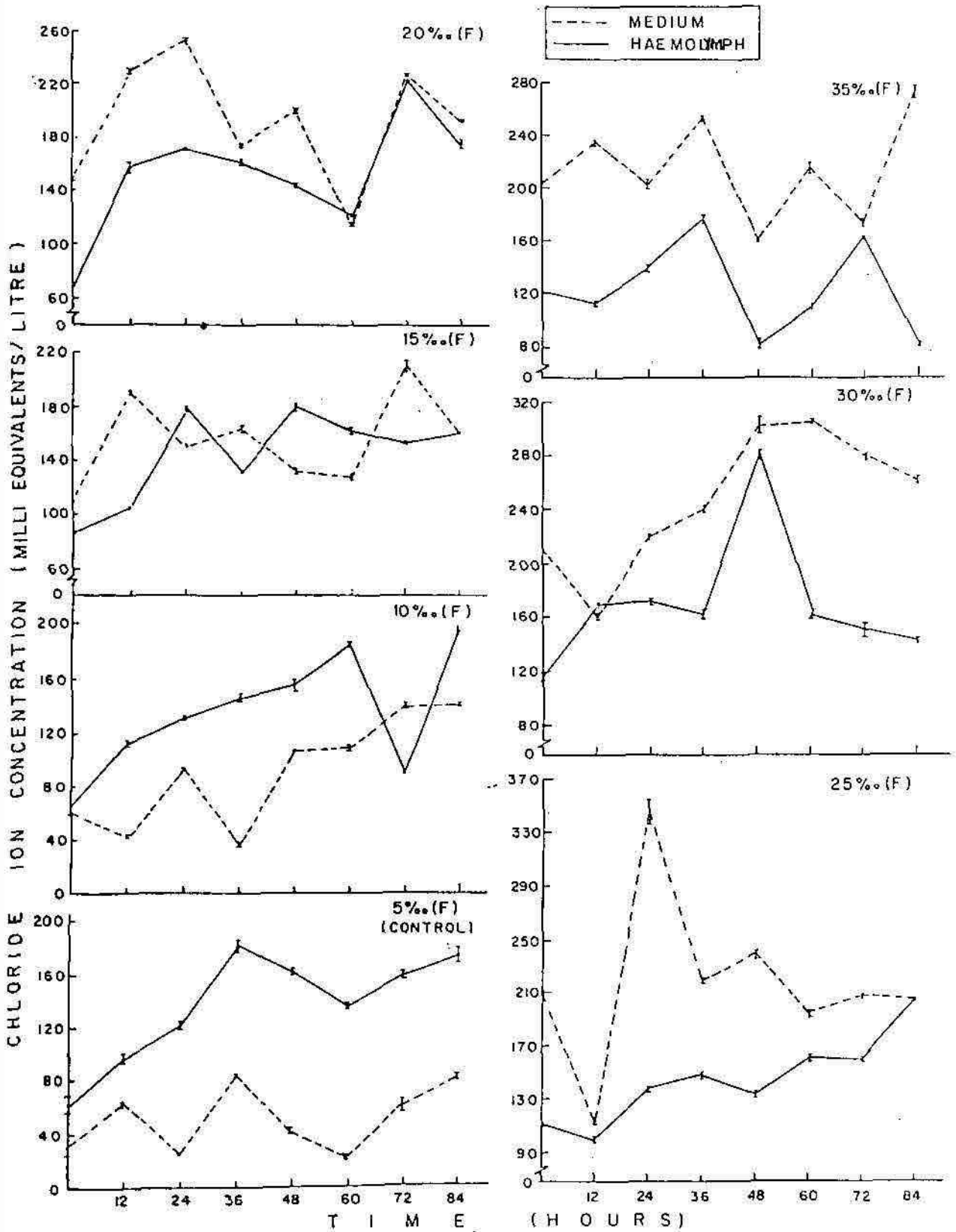

MEDIUM
HAEMOLYMPH
20‰(F)
15‰(F)
10‰(F)
5‰(F)
(CONTROL)
35‰(F)
30‰(F)
25‰(F)
CHLORIDE ION CONCENTRATION (MILLI EQUIVALENTS/LITRE)
TIME (HOURS)

TABLE-6. VARIATIONS IN THE CHLORIDE ION CONCENTRATION (MILLIEQUIVALENTS/LITRE) OF HAEMOLYMPH(HL) AND MEDIUM (M) AS A FUNCTION OF SALINITY AND EXPOSURE TIME (R)*

SALINITY		TIME (HOURS) 0	12	24	36	48	60	72	84
5‰	HL	188.1 ± 0.7	170.2 ± 0.8	125.9 ± 0.3	66.2 ± 0.2	118.1 ± 0.4	134.5 ± 0.3	108.6 ± 0.9	218.9 ± 0.1
	M	19.4 ± 0.6	78.8 ± 0.5	49.3 ± 0.3	55.5 ± 0.4	34.3 ± 0.2	85.9 ± 0.1	70.7 ± 0.1	58.1 ± 0.2
10‰	HL	99.3 ± 0.2	95.0 ± 0.4	276.6 ± 0.9	237.8 ± 1.0	120.4 ± 0.2	111.6 ± 0.7	141.8 ± 0.4	148.3 ± 0.4
	M	54.7 ± 0.2	102.3 ± 0.4	152.2 ± 0.4	102.5 ± 0.2	114.9 ± 0.4	290.0 ± 0.5	147.8 ± 0.4	136.5 ± 0.3
15‰	HL	48.3 ± 0.2	116.0 ± 0.5	155.5 ± 0.08	214.1 ± 0.5	121.2 ± 0.9	133.0 ± 0.7	211.0 ± 0.6	100.8 ± 0.4
	M	72.1 ± 0.4	187.6 ± 0.7	235.0 ± 0.2	148.9 ± 0.6	248.9 ± 0.3	464.0 ± 0.1	379.7 ± 0.1	310.3 ± 1.2
20‰	HL	28.1 ± 0.5	93.6 ± 0.3	60.7 ± 0.2	205.5 ± 0.6	130.2 ± 0.3	140.5 ± 0.4	146.4 ± 0.1	124.2 ± 0.4
	M	102.4 ± 0.2	294.5 ± 0.6	381.5 ± 0.3	203.2 ± 0.6	313.8 ± 0.6	373.1 ± 0.2	314.8 ± 0.2	440.3 ± 0.3
25‰	HL	134.7 ± 0.1	57.0 ± 0.7	137.5 ± 0.8	143.1 ± 0.9	169.0 ± 0.4	160.9 ± 0.1	253.0 ± 0.2	46.9 ± 0.9
	M	86.0 ± 0.1	350.4 ± 0.2	384.4 ± 0.4	245.4 ± 1.0	337.5 ± 0.2	507.0 ± 0.7	341.2 ± 0.5	284.8 ± 0.9
30‰	HL	153.1 ± 0.2	67.2 ± 0.2	210.0 ± 1.3	253.6 ± 0.5	208.3 ± 0.1	171.2 ± 0.4	201.4 ± 0.4	96.8 ± 0.4
	M	120.0 ± 0.1	284.9 ± 0.1	243.4 ± 0.5	312.5 ± 1.0	461.4 ± 0.2	669.9 ± 0.7	299.3 ± 0.2	551.1 ± 0.2
35‰ (Control)	HL	98.9 ± 0.4	328.7 ± 0.2	386.0 ± 0.2	118.8 ± 0.2	155.8 ± 0.9	143.9 ± 0.2	247.6 ± 0.7	284.2 ± 0.2
	M	147.7 ± 0.2	462.9 ± 1.0	400.0 ± 0.2	252.3 ± 0.2	785.7 ± 0.9	820.1 ± 0.5	558.8 ± 0.2	525.2 ± 0.4

All values are mean of 3 replications and figures represent $\bar{X} \pm S D$

ANOVA Table for Chloride ion concentration of Haemolymph

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	52383.250	8731.375	2.09	N.S.
Replication	7	42152.380	6021.768	1.44	N.S.
Error	42	175283.100	4173.527		

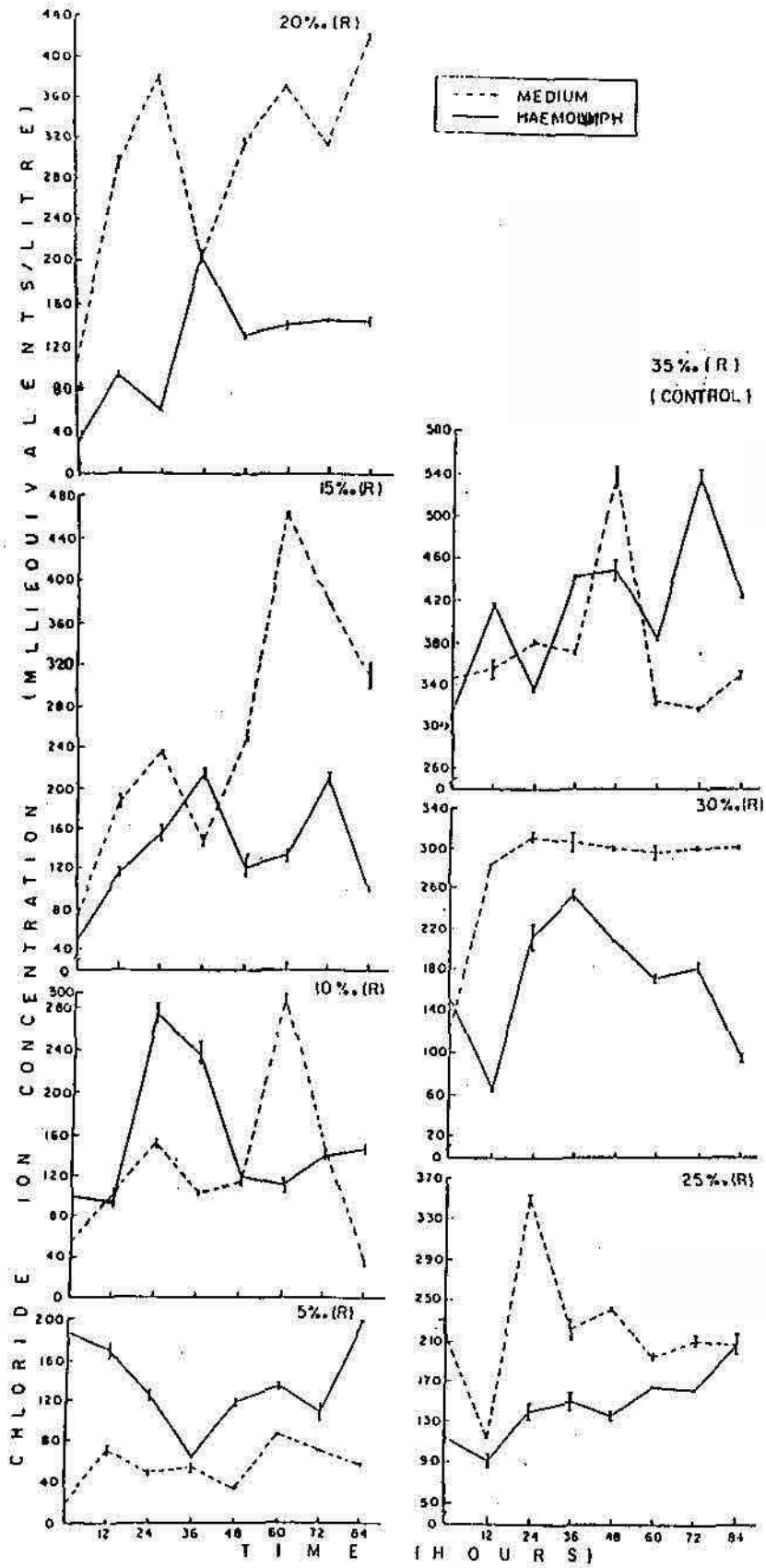
ANOVA Table for Chloride ion concentration of Medium

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	1051920.00	17532 .000	20.34	HI.SIG(1%)
Replication	7	578920.500	82702.930	9.59	HI.SIG(1%)
Error	42	362088.500	8621.154		

* R = Reverse exposure from high to low salinities.

Fig. 6. Changes in the chloride ion concentration of the haemolymph in the control and exposed prawns of Group B, as a function of salinity and time.

R = Reverse exposure from high to low salinities.


20‰ (R)
MEDIUM
HAEMOLYMPH
35‰ (R)
(CONTROL)
15‰ (R)
30‰ (R)
10‰ (R)
25‰ (R)
5‰ (R)
CHLORIDE ION CONCENTRATION (MILLIEQUIVALENTS/LITRE)
TIME (HOURS)
0
12
24
36
48
60
72
84

the medium chloride concentration had significant variation between salinities (ANOVA P = 0.01). The time of exposure showed significant variation on both the blood and medium Cl^- concentration (ANOVA P = 0.01).

1.2.3. Potassium ion regulation:

The values of the potassium ion concentration in the haemolymph and medium as a function of time at each salinity in both groups A (Table 7, Fig. 7) and B (Table 8, Fig. 8) are respectively represented.

In group A prawns, the blood K^+ concentration remained hyperionic to the medium K^+ concentration at all salinities from 5-35‰ (Table 7, Fig. 7). While in group B prawns, the blood K^+ concentration in the control salinity of 35‰ was hypoionic to the medium. In 30‰ salinity also, the blood K^+ concentration was hypoionic to medium, although initially, the concentration of K^+ in the blood was high, the prawns adjusted to the environmental K^+ concentration by 48-72 hours exposure. In all the other salinities from 25‰- 5‰, the prawns have their blood K^+ concentration hyperionic to the medium K^+ concentration. Another important feature that is notable is K^+ concentration in the medium was found to be increasing with salinities (i.e.) from 5-35‰ (Table 8, Fig. 8).

Analysis of variance revealed no significance in the blood K^+ concentration in both group A and B prawns with salinity changes and significance (ANOVA P = 0.05) for the blood K^+ concentration variation with the time of exposure in the group A prawns and highly significant variation in group B prawns (ANOVA P = 0.01) (Tables 7 & 8).

TABLE-7. VARIATIONS IN THE POTASSIUM ION CONCENTRATION (MILLIEQUIVALENTS/LITRE) OF HAEMOLYMPH(HL) AND MEDIUM (M) AS A FUNCTION OF SALINITY AND EXPOSURE TIME (F)*

SALINITY		TIME (HOURS) 0	12	24	36	48	60	72	84
5‰ (Control)	HL	12.4 ± 0.1	8.5 ± 0.18	8.4 ± 0.18	11.7 ± 0.08	10.4 ± 0.03	9.0 ± 0.13	8.5 ± 0.2	7.9 ± 0.1
	M	1.2 ± 0.08	2.1 ± 0.05	2.5 ± 0.13	1.8 ± 0.26	3.1 ± 0.06	0.9 ± 0.05	1.5 ± 0.08	0.6 ± 0.2
10‰	HL	4.6 ± 0.13	6.7 ± 0.05	10.8 ± 1.18	12.6 ± 0.1	7.2 ± 0.05	11.1 ± 0.2	8.0 ± 0.2	8.4 ± 0.1
	M	1.8 ± 0.15	5.1 ± 0.15	4.2 ± 0.16	2.5 ± 0.12	2.5 ± 0.1	1.6 ± 0.13	1.7 ± 0.15	1.4 ± 0.1
15‰	HL	6.9 ± 0.1	13.5 ± 0.2	8.4 ± 0.08	10.8 ±0.1	10.7 ± 0.15	10.5 ± 0.18	8.9 ± 0.1	5.0 ± 0.09
	M	4.0 ± 0.12	4.2 ± 0.08	3.9 ± 0.15	5.1 ± 0.1	2.4 ± 0.15	3.8 ± 0.01	3.9 ± 0.2	3.04 ± 0.01
20‰	HL	8.0 ± 0.1	12.9 ± 0.13	8.9 ± 0.16	12.6 ± 0.15	11.8 ± 0.1	10.5 ± 0.1	6.5 ± 0.05	8.2 ± 0.11
	M	4.6 ± 0.08	4.5 ± 0.05	4.2 ± 0.15	4.9 ± 0.1	4.1 ± 0.02	5.1 ± 0.13	7.1 ± 0.05	8.2 ± 0.03
25‰	HL	6.6 ± 0.06	10.5 ± 0.08	14.6 ± 0.06	7.0 ± 0.01	9.6 ± 0.1	10.6 ± 0.15	9.5 ± 0.1	7.6 ± 0.1
	M	7.3 ± 0.06	9.0 ± 0.05	5.8 ± 0.1	7.3 ± 0.1	4.4 ± 0.2	5.5 ± 0.05	5.9 ± 0.05	4.1 ± 0.1
30‰	HL	7.5 ± 0.1	11.6 ± 0.2	12.9 ± 0.3	12.3 ± 0.15	11.5 ± 0.15	8.5 ± 0.05	5.5 ± 0.09	10.4 ± 0.1
	M	6.6 ± 0.1	8.3 ± 0.1	6.6 ± 0.08	9.4 ± 0.15	4.9 ± 0.2	7.0 ± 0.08	6.2 ± 0.08	8.0 ± 0.15
35‰	HL	11.1 ± 0.25	9.8 ± 0.2	8.44 ± 0.21	14.9 ± 0.1	12.4 ± 0.1	12.5 ± 0.07	12.5 ± 0.18	9.4 ± 0.1
	M	8.8 ± 0.15	11.3 ± 0.6	7.7 ± 0.18	14.9 ± 0.1	13.1 ± 0.08	10.3 ± 0.1	9.4 ± 0.06	8.9 ± 0.13

All values are mean of 3 replications and figures represent $\bar{X}$ ± S D

ANOVA Tables for K^+ concentration of Haemolymph

SOURCE	d.f	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	33.414	5.569	1.21	N.S.
Replication	7	86.753	12.393	2.68	SIG(5%)
Error	42	194.286	4.621		

ANOVA Table for K^+ concentration of Medium

SOURCE	d.f	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	431.949	71.991	35.33	HI.SIG(1%)
Replication	7	24.018	3.431	1.68	N.S.
Error	42	85.590	2.038		

*F = Forward exposure from low to high salinities.

Fig. 7. Changes in the potassium ion concentration of the haemolymph in the control and exposed prawns of Group A as a function of salinity and time.

F = Forward exposure from low to high salinities.

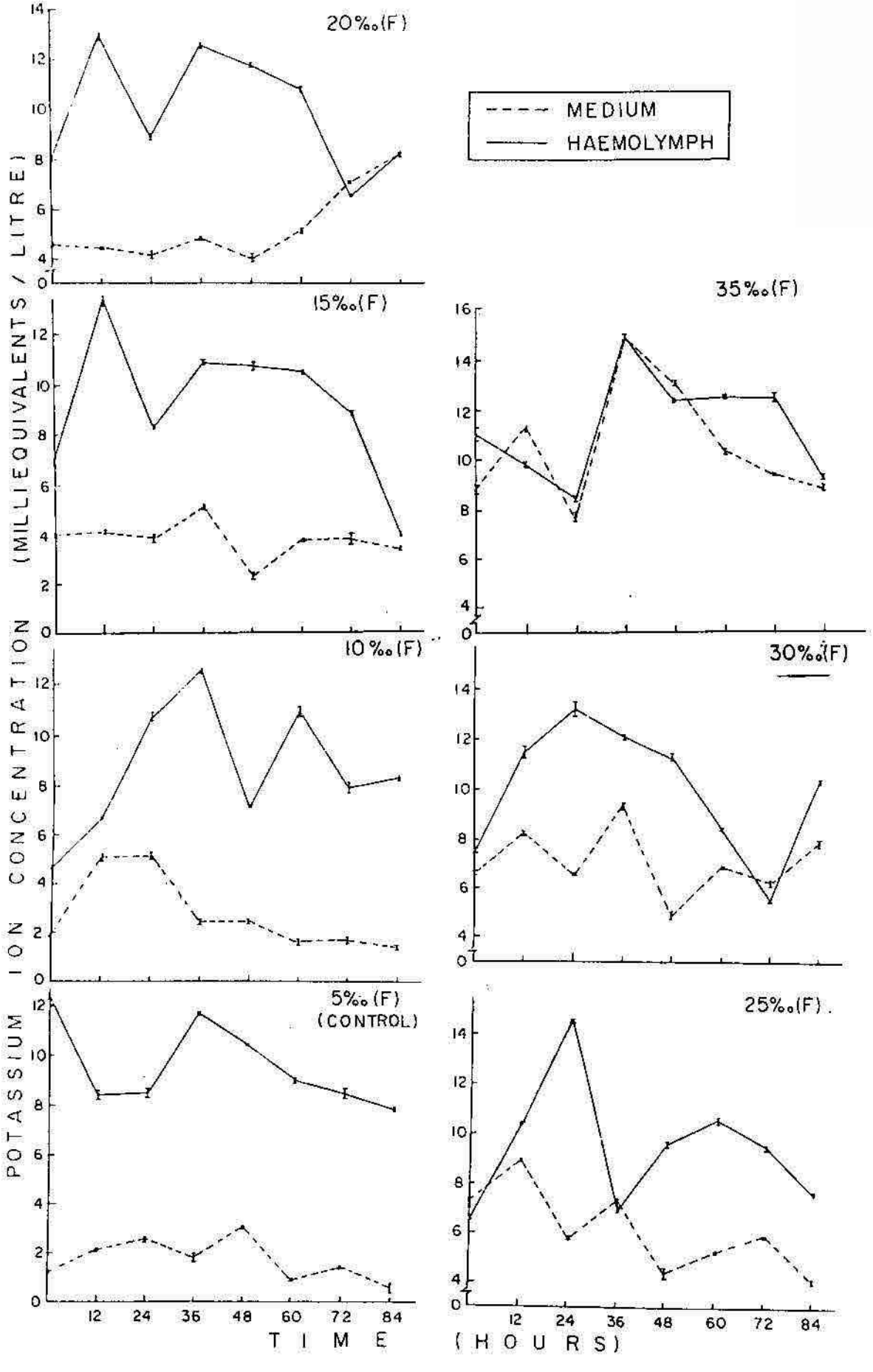

20‰(F)
MEDIUM
HAEMOLYMPH
15‰(F)
35‰(F)
10‰(F)
30‰(F)
5‰(F)
(CONTROL)
25‰(F).
POTASSIUM ION CONCENTRATION (MILLIEQUIVALENTS / LITRE)
TIME (HOURS)
0
2
4
6
8
10
12
14
16
12
24
36
48
60
72
84

TABLE-8. VARIATIONS IN THE POTASSIUM ION CONCENTRATION (MILLIEQUIVALENTS/LITRE) OF HAEMOLYMPH(HL) AND MEDIUM (M) AS A FUNCTION OF SALINITY AND EXPOSURE TIME(R)*

SALINITY		TIME (HOURS) 0	12	24	36	48	60	72	84
5‰	HL	7.6 ± 0.2	10.8 ± 0.21	4.5 ± 0.38	10.4 ± 0.45	8.4 ± 0.20	2.4 ± 0.11	4.9 ± 0.21	5.2 ± 0.27
	M	0.6 ± 0.0	2.6 ± 0.2	3.8 ± 0.05	3.7 ± 0.28	2.5 ± 0.13	1.8 ± 0.08	2.6 ± 0.08	2.8 ± 0.08
10‰	HL	13.2 ± 0.75	5.9 ± 0.2	8.1 ± 0.22	8.0 ± 0.19	8.4 ± 0.55	5.4 ± 0.28	8.4 ± 0.8	6.3 ± 0.2
	M	1.0 ± 0.28	2.4 ± 0.25	4.4 ± 0.35	4.4 ± 0.25	3.8 ± 0.38	3.7 ± 0.08	3.6 ± 0.28	1.8 ± 0.08
15‰	HL	13.8 ± 0	4.6 ± 0.5	8.8 ± 0.35	8.8 ± 0.53	0.4 ± 0.2	2.6 ± 0.08	5.4 ± 0.3	3.6 ± 0.25
	M	2.0 ± 0.4	4.0 ± 0.18	4.8 ± 0.08	4.8 ± 0.18	3.7 ± 0.1	5.9 ± 0.15	3.1 ± 0.18	1.6 ± 0.06
20‰	HL	12.6 ± 0.01	5.1 ± 0.05	13.1 ± 0.3	13.1 ± 0.1	11.4 ± 0.1	4.3 ± 0.45	6.1 ± 0.05	7.1 ± 0.18
	M	2.4 ± 0.28	5.8 ± 0.1	6.3 ± 0.3	6.3 ± 0.1	5.5 ± 0.18	6.07 ± 0.45	4.1 ± 0.05	3.3 ± 0.09
25‰	HL	6.9 ± 0.3	6.1 ± 0.2	8.6 ± 0.18	8.6 ± 0.5	10.7 ± 0.13	8.6 ± 0.4	2.4 ± 0.1	6.7 ± 0.13
	M	5.9 ± 0.05	6.8 ± 0.18	7.3 ± 0.12	7.3 ± 0.1	8.4 ± 0.1	8.1 ± 0.15	4.8 ± 0.01	3.7 ± 0.14
30‰	HL	11.8 ± 0.5	8.4 ± 0.4	5.5 ± 0.15	12.1 ± 0.26	7.6 ± 0.25	8.9 ± 0.3	7.7 ± 0.3	4.4 ± 0.55
	M	6.7 ± 0.48	8.0 ± 0.2	9.1 ± 0. 1	9.2 ± 0.15	8.9 ± 0.4	8.9 ± 1.5	8.2 ± 0.1	6.9 ± 0.1
35‰ (Control)	HL	10.7 ± 0.13	6.9 ± 0.05	2.3 ± 0.28	11.7 ± 0.1	10.2 ± 0.5	5.6 ± 0.48	7.1 ± 0.28	6.7 ± 0.5
	M	8.8 ± 0.28	10.4 ± 0.31	11.9 ± 0.2	12.1 ± 0.4	10.8 ± 0.06	9.4 ± 0.09	17.7 ± 0.1	6.8 ± 0.05

All values are mean of 3 replications and figures represent $\bar{X}$ ± S D

ANOVA Table for K^+ concentration of Haemolymph

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	37.335	6.223	0.48	N.S.
Replication	7	435.793	62.256	4.78	HI.SIG(1%)
Error	42	546.478	13.011		

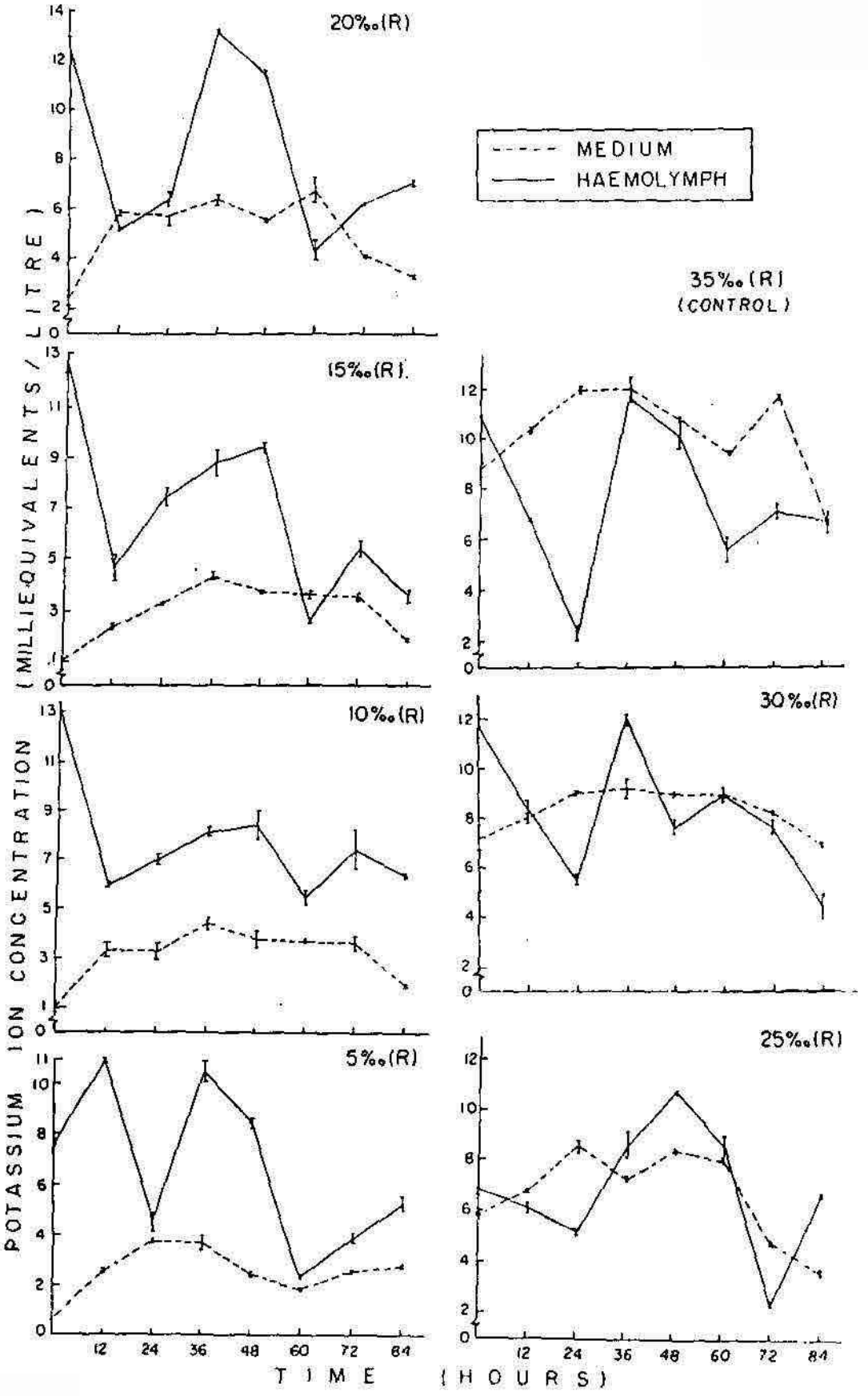
ANOVA Table for K^+ concentration of medium

SOURCE	d.f.	Sum Sqr.	Mean Sqr.	F-Val.	Remarks
Treatment	6	399.62	66.604	85.87	HI.SIG(1%)
Replication	7	66.999	9.571	12.34	HI.SIG(1%)
Error	42	32.577	0.776		

* R = Reverse exposure from high to low salinities.

Fig. 8. Changes in the potassium ion concentration of the haemolymph in the control and exposed prawns of Group B, as a function of salinity and time.

R = Reverse exposure from high to low salinities.


20‰(R)
MEDIUM
HAEMOLYMPH
35‰(R)
(CONTROL)
15‰(R)
30‰(R)
10‰(R)
25‰(R)
5‰(R)
POTASSIUM ION CONCENTRATION (MILLIE-QUIVALENTS / LITRE)
TIME (HOURS)
12
24
36
48
60
72
84

2. HISTOLOGICAL STUDIES

General organisation:

There are eight gills inside each gill chamber. The gills are more or less crescentic in shape. They gradually increase in size backwards, so that each gill is larger than the one in front of it. Each gill is attached to the thorax by a small connection in its middle called the gill root, through which the nerves and the blood channels enter and leave the gill.

The gills are phyllobranchiae, composed of a double row of closely spaced lamellae extending anteriorly and posteriorly from a median shaft (Plate III). Along the outer side of each gill runs the afferent branchial vessel.

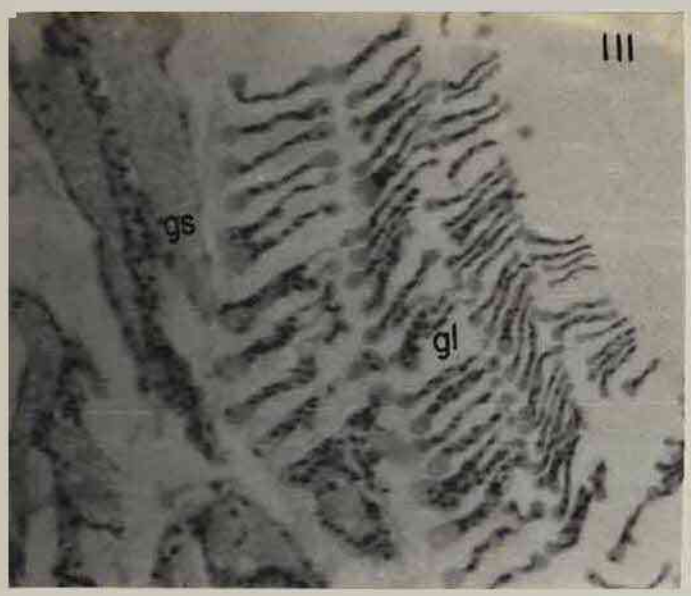
In T.S. each gill is triangular in shape. The efferent vessel is situated at the apex of the triangle and the afferent vessel lies in the middle of the base of the triangle. Stretching across from the afferent to efferent vessels is the branchial septum, which separates the anterior from the posterior lamellae. In L.S. each gill lamella is covered by a single layer of epithelial cells lining the cuticle. In each lamella there are narrow haemolymph spaces called lamellar sinuses. Each lamellar sinus is in contact with the afferent branchial sinus on the outer side and efferent branchial on the inner side and it is in this lamella that the blood is purified. These lamellar cavities are generally formed because of the inflow of the blood and thus separating the two layers of lamella.

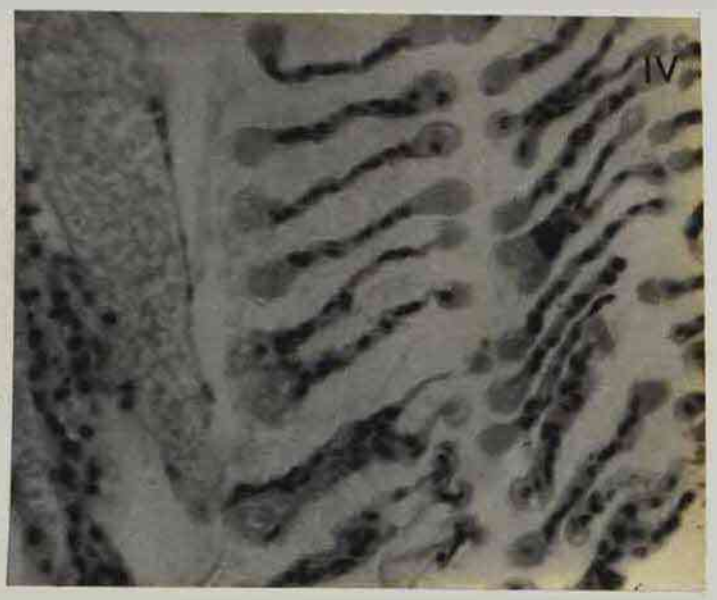
PLATE III. Longitudinal section of the gill tissue showing the normal structure. Haematoxylin & Eosin. X 100.
gs - gill shaft; gl - gill lamellae.

PLATE IV. L.S. of the gill tissue from the control prawn of group A revealing very little alteration from the normal structure. H&E. X 200.

PLATE V. L.S. of the gill tissue of the control prawn of group A under higher magnification. Note the mild dilation in some of the gill lamellae and the mild accumulation of haemocytes (h) within the lamellar blood sinuses. H&E. X 400.

PLATE VI. L.S. of the gill tissue of the control prawn acclimated to 5‰ saline water for 10 days, revealing gill lamellae as thin filaments and mild accumulation of haemocytes in the lamellar blood sinuses. H&E. X 400.


III
gs
gl


IV


V
h


VI

The epithelial cells have abundant mitochondria, many of which are associated with the infoldings of the basal cell membrane. Each cell has a nucleus containing dispersed chromatin near the periphery and is connected to the next one by septate desmosomes with a well defined 'Zonula adhaerens'. Golgi complexes, rough endoplasmic reticulum and abundant free ribosomes occurring either singly or as polyribosomes, are also present in the cells. These structures are however, not visible and distinguishable under light microscope.

Histological observations were made on the gill tissue of both the control and exposed prawns in both groups A and B. The group A prawns acclimated at 5‰ for 10 days served as the control and showed normal structure of the gill lamellae (Plate IV). There was however, slight to mild swelling of some of the gill lamellae (Plate V). The gill lamellae appeared as thin filaments with single lining of epithelial cells and lamellar blood sinuses were narrow in appearance (Plate VI). Mild accumulation of the haemocytes were seen within the blood sinuses (Plate V & VI). The group A prawns, which were exposed to 35‰ for 5 days showed various changes. The gill lamellae appeared shortened in length due to the swollen nature of the lamellar sinuses. The lamellae appear shorter and swollen, when compared to the control gill lamellae (Plate VII, VIII and IX). The outer lamellar sinus was enlarged. Other lamellar blood sinuses appeared distended due to the accumulation of albuminous fluid (Plate IX). the cells lining the lamellar sinuses appeared hypertrophic and contained large number of haemocytes, when compared to that in the control (Plate VII, VIII and X).

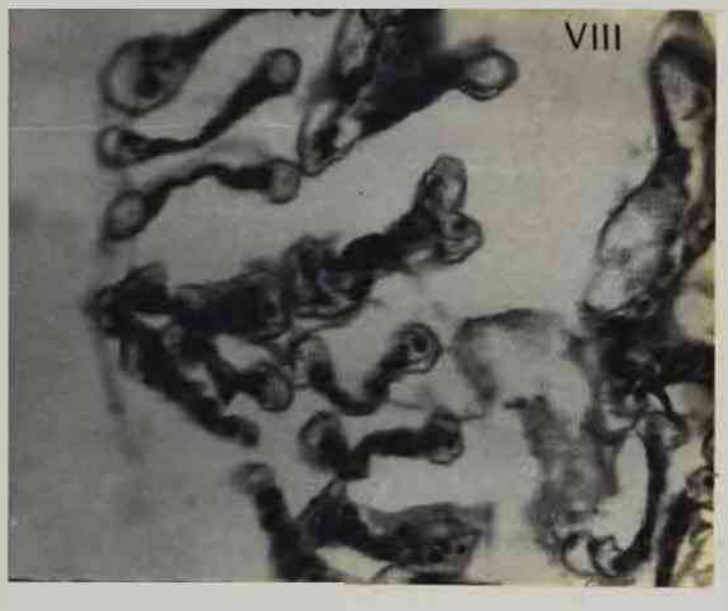
PLATE VII. L.S. of the gill tissue of the group A prawns acclimated to 5 ‰ saline water for a week and then exposed to 35‰ saline water abruptly. Note the shortened length of the gill lamellae and the enlarged outer lamellar sinus. H&E. X 200.

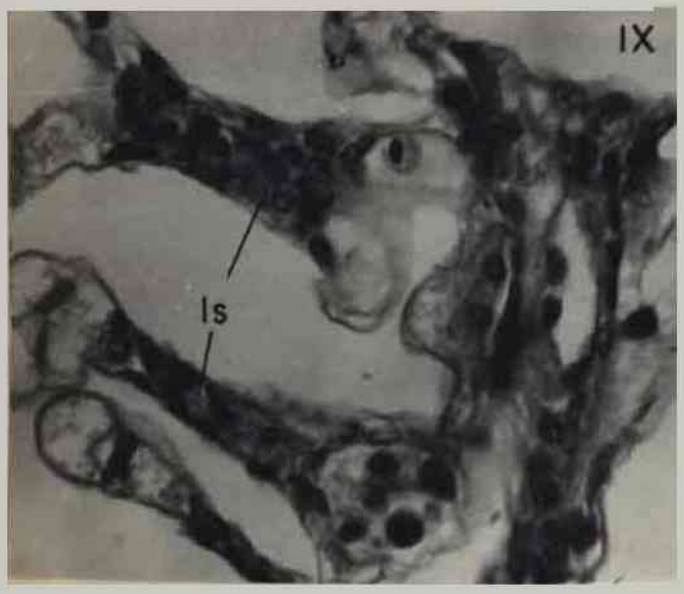
PLATE VIII. Another L.S. of the gill tissue of exposed group A prawns to 35‰ saline water for 5 days revealing the shortened gill lamellae, enlargement of gill lamellar sinus and severe accumulation of haemocytes in the lamellar sinuses. H&E. X 200.

PLATE IX. Higher magnification of the gill lamellae of the group A prawn revealing the accumulation of large number of haemocytes, enlarged gill lamellae and the hypertrophic cells lining the lamellar sinuses (ls). H&E. X400.

PLATE X. L.S. of the gill tissue of the group B control prawns acclimated to 35‰ saline water for 10 days showing enlargement of the gill lamellae with moderate accumulation of haemocytes in the lamellar blood sinus. H&E. X400.


VII


VIII


IX
ls


X

PLATE XI. L.S. of the gill tissue of the control prawn of group B revealing the enlarged *gill lamellae*. But in majority of the lamellae, the outer lamellar sinus is not swollen. H&E. X 400.

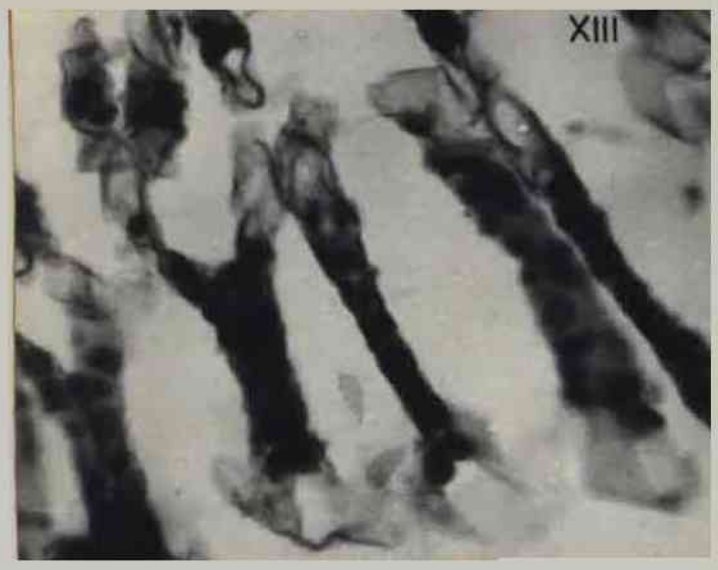
PLATE XII. L.S. of the gill tissue of the 5‰ saline water exposed group B prawns after being acclimated to 35‰ for a week. Note *the shrunken condition of gill lamellae*. H&E. X 200.

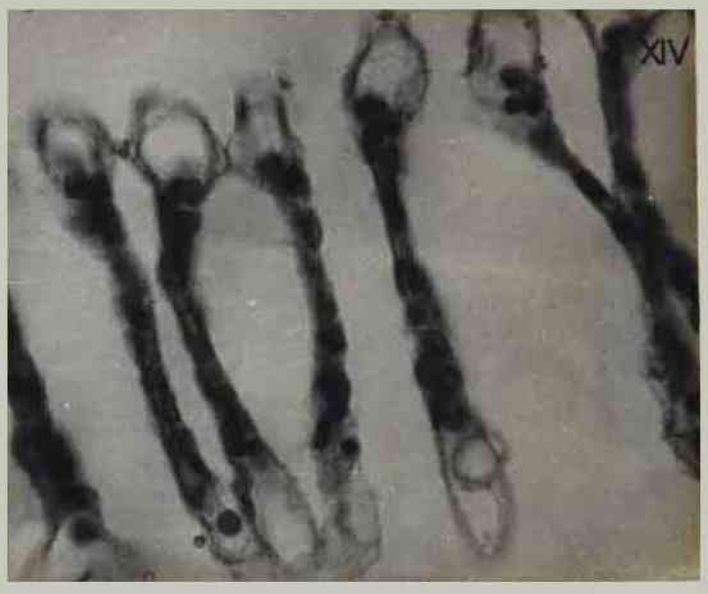
PLATE XIII. Higher magnification of the gill tissue of 5‰ salinity exposed prawn of group B revealing dense accumulation of haemocytes within the lamellar blood sinuses indicating congestion. H&E. X 400.

PLATE XIV. Another area of the same section revealing almost normal structure of the gill lamellae as in PLATE VI but for, the more dense accumulation of haemocytes in the lamellar blood sinus in this case. H&E. X 400.


XI


XII


XIII


XIV

Group B prawns acclimated at 35‰ salinity for a period of about 10 days, showed enlarged gill lamellae with slight to moderate congestion, due to accumulation of haemocytes in the lamellar blood sinuses (Plate X). Majority of the gill lamellae were not swollen at the outer lamellar sinuses in this, when compared to the group A case (Plate XI). In the 5‰ exposed prawn gills of group B, the lamellae showed slightly shrunken condition (Plate XII). The lamellar blood sinuses were filled with large number of haemocytes indicating congestion (Plate XIII). In few areas the gill lamellae appeared normal like in group A control case, the only difference being, in this case, a greater concentration of haemocytes were seen in the lamellar blood sinuses (Plate XIV).

The type of the haemocytes involved in the haemocyte accumulation in the gills were not determined.

DISCUSSION

The penaeid prawn, Metapenaeus dobsoni is a hyper-hypo-osmoregulator, confirming that this advanced form of genetic adaptation to salinity change is similar to that possessed by all marine, estuarine and brackish water palaemonid prawns studied to date (Panikkar, 1941; Dobkin and Manning, 1964; Born, 1968; Spaargaren, 1972; Hagerman and Uglow, 1983; Kirkpatrick and Jones, 1985; Ramirez de Isla Hernandez and Taylor, 1985; Campbell and Jones, 1989). The osmolal concentrations of M. dobsoni maintained in different salinities after acclimation at a salinity of 5‰, showed that the species showed hyperosmotic regulation at low salinities (below S 15‰) and hypoosmotic regulation at high salinities (above S 25‰) while isosmotic regulation was seen at the intermediate salinity range of 15-25‰. Similar trends were obtained in the osmolal concentrations of the prawns acclimated at 35‰ and exposed to the different grades of salinity. The findings of hyperosmotic regulations of this species at low salinities and hypoosmotic behaviour at high salinities are in agreement with the earlier reports on other crustaceans (Williams, 1960; McFarland and Lee, 1963; Bursey and Lane, 1971; Hill, 1976).

Most of the penaeid species as reported earlier, are able to adapt extremely well to very low salinities during their early juvenile life but this ability appears to be reduced in adults (Dall, 1981). But it is found from the present investigation that adult prawns have extremely good capacity

to osmoregulate both at low and high salinities. Similar results were obtained when studies were conducted on the osmoregulation of the adult P. monodon (Diwan et al., 1989) and P. indicus (Diwan and Laxminarayana, 1989). There is a great diversity in the osmoregulatory ability among penaeid species and other decapod crustaceans (Kalber, 1970; Forskett, 1977). Therefore, the osmoregulatory ability appears to be purely an adaptive feature and may change according to the environmental situations.

Results of the present investigation showed that the prawn M. dobsoni required at least 48 hours for stabilising osmolal concentration of the haemolymph. Generally, when the prawns are acutely transferred to different salinities, there is a rapid change in the osmolal concentration of haemolymph and, to reach a steady equilibrium, the prawns require time. Bursey and Lane (1971) have reported that for P. duorarum, a period of about 24 hours was required to establish a new steady state of equilibrium for haemolymph concentration. Castille and Lawrence (1981) reported 3-4 days for P. setiferus to stabilise the haemolymph. A short period of 1-2 hours has been reported as the steady state of achievement for Krill and Macrobrachium sp. (Forward and Fyhn, 1983; Read, 1984). Studies on P. monodon and on P. indicus revealed that these species also required minimum period of 48 hours to stabilise the haemolymph (Diwan and Laxminarayana, 1989). Differences in the adaptation times may, in part, be due to differences in moult stage when animals are transferred to a new salinity. Acute transfer experiments in other penaeids as well, were validated when the transfers are made during the inter-moult period of the animal

(Ferraris et al., 1986). It is possible that different ions may require different equilibration times and certain ions may not achieve stable haemolymph concentration within one moult cycle, even when the animals are transferred in intermoult. Thus, it may be beneficial to establish a steady state condition in each parameter to be studied. Ferraris et al. (1986) showed that for P. monodon, 1-2 days may be required for osmolality, chloride and total protein concentrations to achieve steady state values after transfer from control to experimental salinities.

There is a speculation that aquacultured species could be best grown under isosmotic condition when osmotic work of an organism would be minimal (Panikkar, 1968). Not only would the energy saved from lowered work be channelled to the increased costs of ionic regulation but also energy spent for osmoregulation was a small component of the total metabolic rate of penaeids (Bishop et al., 1980). Other factors must also be considered for optimal growth conditions. The present investigation has revealed that the isosmotic condition for M. dobsoni ranges between 15-25‰, which will be ideal or favourable salinity range for optimal growth of the species as this range of salinity, it is under minimum stress.

Effective physiological mechanisms of osmoregulation either at the level of the extracellular fluid or at the level of intracellular medium are, of course necessary prerequisites to a successful invasion of mixohaline waters. It was shown that animals adapted to very dilute media had uptake systems with high affinity for Na^+, while those in brackishwater had lower affinities (Shaw, 1964). The first step in the invasion of dilute media required

developing mechanisms for maintaining high blood Sodium chloride (Beadle and Cragg, 1940).

The most important ions involved in the maintenance of osmotic balance are the Sodium and Chloride. The exchange of these ions result from passive diffusion in an isosmotic medium as has been shown in Carcinus maenas (Shaw, 1955, 1961; Zanders, 1980), Hemigrapsus sp. and Pachygrapsus crassipes (Dehnel and Carefoot, 1965; Rudy, 1966). Webb (1940) and Shaw (1961) have suggested that a small amount of active uptake of sodium ion is involved in its distribution between the medium and the haemolymph in crab Carcinus maenas.

The present investigation showed that both the ions, Sodium and Chloride behaved hypoionically to the medium at higher salinities and hyperionically at lower salinities. M. dobsoni was extremely efficient throughout the range of salinities tested and similar results were obtained in M. bennettae (Dall and Smith 1981) Chloride regulation in Palaemonetes varians yielded similar results (Hagerman and Uglow, 1983). Robertson (1960) suggested that the increase in osmolality of the haemolymph of the euryhaline crustacean, Carcinus maenas during moult in seawater is mainly due to increase in Na^+, Ca^+, Mg^{++}, and Cl^- concentrations over the intermoult stage. The chloride ion concentration in the haemolymph was found to be a function of external salinity in the present investigation as was seen in earlier studies by Ferraris et al. (1987).

In the case of potassium ion concentration, it has been found that its concentration increases with the increase in salinity. The

concentration of potassium was found to be higher than the medium throughout, except at 35‰ saline water. Similar results were obtained in M. rosenbergii by Stern et al. (1987). At higher salinities the blood potassium ion concentration increased, apparently the result of a decrease in the regulatory ability and at lower salinities, the high blood potassium concentration was the result of osmoregulatory mechanisms which work to conserve or minimize K^+ loss through diffusion or excretion. As there has been no evidence in the literature supporting potassium ion absorption through the gill epithelium (Waterman, 1960), blood K^+ concentration must be attributed to the ingested food and selective reabsorption of K^+ in the ultrafiltrate of the antennal gland.

Work on the histological changes in the gills of the prawns when exposed to abrupt changes in the salinity was meagre. Results of gill chamber perfusion work of Bryan (1960) and Bielawski's (1971) experiments with salt uptake in isolated gills, there is strong evidence that salt uptake does occur in the gills. The present study revealed that the gill lamellae got swollen due to the accumulation of albuminous fluid in the lamellar sinus. The cells lining the lamellar sinus appeared hyperchromatic containing large number of haemocytes, when compared to the control which was more or less with a normal structure, when the prawns acclimated at a low salinity of 5‰ was exposed to 35‰ saline water for 5 days. In the reverse experiment, 35‰ acclimated prawns on exposure to 5‰ saline water for 5 days, developed certain changes in the gill structure. The gill lamellae showed shrunken condition with lamellar blood sinuses being filled with numerous haemocytes resulting in congestion. Nash et al. (1988)

studied the pathological changes associated with adult P. monodon cultured in grow out ponds developed from potentially acid sulphate mangrove soils. Histological and ultrastructural study showed $Fe(OH)_3$ accumulation in the gills and the associated gill changes which led to hypoxic damage in other tissues. The gills exhibited oedema and separation of the opposing epithelial layers. The lamellae also exhibited hypertrophic, hyperplastic necrotic and inflammatory changes. Epithelial hyperplasia led to multifocal lamellar thickening and focal fusion. Evidences support the idea that changes in the ultrastructure of the gill epithelium, possibly correlated with changes in its physiological function that occur upon acclimation to media of different salinity. Copeland and Fitzjarrell (1968) demonstrated that silver staining patches of the gills of hyperosmotic regulators increased in size after acclimation to dilute media. As a general rule, the gills located posteriorly in the gill chamber of euryhaline hyperregulators appeared to be the main, if not the only ones responsible for active Na^+ uptake in dilute medium (Pequeux and Gilles, 1984). In a species, such as Eriocheir sinensis, adaptation from seawater to freshwater results in a decrease in blood osmolarity (Gilles, 1974). When E. sinensis, was passed from seawater to freshwater medium, there is first a swelling of the tissue, however the swelling is regulated very rapidly. After the initial phase of swelling the muscle tissue fluid comes into isosmotic equilibrium with the blood.

The fact that many cell types show no volume readjustment after shrinkage in vitro, does not mean that they lack the necessary mechanisms. It would seem rather that these mechanisms are present but are not able to bring about volume readjustment during the short period of time of

the in vitro experiments. Tissues of euryhaline crabs are unable to effect volume readjustment after hyperosmotic shocks in vitro (Lang and Gainer, 1969; Gerrard and Gilles, 1972; Gilles, 1977, 1978). It would thus appear that in most cases, volume readjustment is achieved much more rapidly after swelling than after shrinkage. Studies on the ultrastructure of the salt transporting epithelium from posterior gills of E. sinensis acclimated either to freshwater or seawater was conducted by Gilles and Pequeux (1985). A major structural difference which is immediately visible, lies in the importance in the development of the apical infolding system in the two sets of acclimation conditions. This system is almost non-existent in the gills of seawater acclimated crabs, while it is largely developed in the freshwater acclimated ones. There may be also some differences in the structural organization of the basolateral infoldings and of the associated mitochondria, more work needs, however, to be done before a complete discussion of this particular problem can be undertaken, to see if the same changes can be seen in the prawn gills as well.

The changes observed in the organisation of the apical infolding system in E. sinensis are not simply due to a rapid osmotic swelling of the intercellular spaces that could occur during direct transfer from seawater to freshwater. Formation of the apical infolding system develops only slowly after the animals have been placed in freshwater. During the first 24 hours after transfer to freshwater, a few evaginations appear at the basis of the relatively few folds present at the apical side of the cells. The number of evaginations as well as the number of folds appear to increase progressively. The overall process takes 4-5 days to be completed. It

is reasonable to assure that this increase in plasma membrane area is associated with an increase in the number of pumping sites and with the development of the transepithelial transport activity. This is in agreement with the progressive increase in (Na^+, K^+) ATPase activity shown to occur upon acclimation of different euryhaline crabs to dilute media (E. sinensis, Pequeux et al., 1984; C. sapidus, Towle et al., 1980) is that acclimation to salt concentrated media leads to the inactivation of the pumping sites and also to a progressive loss of the capability of transport actively Conversely, upon transfer of seawater crabs to a salt dilute environment the pumping activity is progressively restored within 3-8 days of acclimation (Gilles and Pequeux, 1981, 1983, 1985). Similar, epithelium of the transporting and respiratory type are present in C. maenas and Callinectes sapidus (Copeland and Fitzjarrell, 1968). So they substantiate the idea that the structural and functional organisation of gills described for E. sinensis are applicable to other euryhaline crabs.

The swelling of the gill lamellae in the present study may be due to stress or hyper activity of the prawn when exposed to the extreme salinities abruptly. When the stress is more, oxygen requirement is increased and to facilitate this, the gills might expand. The high metabolic activity may be another reason of swelling which results in the enlargement of the different cell organelles like Mitochondira. The changes in the gill lamellae observed in the present investigation could be associated with no particular cause. Hence further studies are required in this connection to give a conclusive evidence.

SUMMARY

The present study on osmoregulation of the penaeid prawn Metapenaeus dobsoni was undertaken to study the effect of abrupt changes in the salinity on the haemolymph osmolality and ions viz, Na^+, K^+, and Cl^-. The associated changes in the gill structure at the cellular level was also studied. The prawns were acclimated in the laboratory at a low salinity of 5‰ for a week and then exposed to the ascending grades of salinity namely 10‰, 15‰, 20‰, 25‰, 30‰ and 35‰. One set was maintained at 5‰ to serve as the control. The haemolymph was collected from one prawn at each salinity at an interval of every 12 hours, starting from 0, 12, 24, 36, 48, 60, 72 and 84 hours. The medium was also collected simultaneously. The haemolymph and medium osmolality, Na^+, Cl^- and K^+ concentration were determined. In the next experiment, the high saline 35‰ acclimated prawns for a week were exposed to descending grades of salinity of 30‰, 25‰, 20‰, 15‰, 10‰ and 5‰, with 35‰ serving as the control. The experiment was carried out as above.

Histological studies were carried out on the gills removed from the prawns maintained at low saline water of 5‰ for 10 days and then exposed to 35‰ (high saline water) for 5 days. In the next experimental group, the 35‰ acclimated prawn for 10 days were exposed to 5‰ saline water for 5 days. Gills were taken after sacrificing the prawns in both the control and exposed prawns of both the experimental groups.

The results obtained in the present investigation revealed the penaeid prawn, M. dobsoni is a hyper-hypo-osmoregulator. The haemolymph osmolality and ion concentration, especially Cl^- was a function of the salinity. The prawns were hyperosmotic to the medium at low salinities and hypo-osmotic at higher salinities. At the intermediate salinities, the prawns were more or less at isosmotic condition with the medium. The prawns became adapted to the exposed medium within 48 hours, initially although it behaved as an osmoconformer. The time taken by the prawn to adjust from lower salinity to higher is more when compared to the reverse. The Na^+ and Cl^- concentrations also showed a hyperionic trend at lower salinities and hypoionic trend at higher salinities. The K^+ concentration increased with increasing salinity.

The gill structure studies reveal that the prawn tries to adopt to the higher salinity by swelling of the gill lamellae and to low salinity exposure by shrinkage. A number of haemocytes are found to be accumulated in the lamellar blood sinus indicating severe congestion. No cause could however, be associated with the swelling of the gill lamella or shrinkage. This study happens to be the first of its kind in the prawn, Metapenaeus dobsoni. Much work on the histological changes of the prawn gill as an effect of abrupt salinity changes under light microscopy is available. Since literature is also meagre on such work, no definite conclusions could be drawn. Future may aim to find out the physiological functional relationships between the changes in the gill structure and salinity variations.

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