OAM TEO BEHAVIOUR
OSMOTIC BEHAVIOUR

Fresh-water, because of its extremely low osmotic pressure, is a more difficult medium for an animal to survive than sea water. Hence all fresh water animals are hyper-tonic to the medium in which they live and they have to maintain their blood composition much different from that of surrounding water. The maintenance of hypertonicity needs the operation of a regulatory mechanism.

There have been several contributions on the osmo-regulatory mechanism among the decapod crustaceans (Harms, 1932; Krogh, 1939; Jones, 1941; Gross, 1955, 1957; Williams, 1960; Born, 1963; Haefner, 1969). Panikkar (1941) investigated osmoregulation in Palaemonetes varians, Palaemon serratus and P. alekana. He showed that these animals could maintain hypotonocity in waters of high salinity while remaining hypertonic in brackish water. Identical results were obtained by Broekema (1941) in the shrimp Crangon crangon. Parry (1957) studied osmoregulation in the prawn, Palaemonetes antennarius and duplicated the findings of Panikkar (1941) on P. varians. Dobkin and Manning (1964) made determination of osmotic concentrations in the blood samples of Palaemonetes paludosus and P. intermedius. Lofst (1956) in the prawn, Palaemonetes varians, Parry (1957) on fresh water prawn, Palaemon antennarius and Pamapathirao in Metapenaeus monoceros studied the metabolic responses in
relation to osmotic stress. They showed increased oxygen consumption with osmotic stress. Sukumaran (1961) studied osmoregulation of *Gibanarius padarensis* under heterosmotic condition.

Salt and water balance in various invertebrate groups which have been studied appears to be under the regulation of neurosecretory factors as in most vertebrate groups. Among the Crustacea, most of the work on neurosecretion and osmoregulation has been concerned with the changes in weights and water contents of the animal in relation to moult cycle. Scudamore (1947) demonstrated that the disturbed ecdysial water metabolism of eyestalkless crayfish was attributed to the absence of sinus glands. Since then a neuroendocrine regulation of ecdysial water balance has been suggested by a number of investigators (Bauchau, 1948; GuyseLMAN, 1953; Carlisle, 1956; Passano and Jyssum, 1963; Rangarao, 1965). Bliss et al. (1966) have proposed that in the crab *Gecarcinus lateralis* ecdysial water volume is controlled by non-nervous antidiuretic hormone and a diuretic hormone from the brain and ventral ganglionic mass. Kamamoto et al. (1966) provided an evidence for a neuroendocrine regulation of salt and water 'homeostasis' in two species of crustaceans, the crayfish, *Procambarus clarkii* and the grapsoid crab, *Metapograpsus messor*. 
In the present investigation, the freshwater prawn, *Caridina rajadhari* was used to study the osmotic behaviour and the role of neurosecretion on osmoregulation as no work was done on this decapod crustacean in this direction.

**MATERIAL AND METHODS**

*Caridina rajadhari* were collected from Khan river near Aurangabad. On arrival in the laboratory, prawns were kept in shallow water tanks containing one fourth water. Both males and females were used in the experiments. Different salt concentrations (0.05, 0.2, 0.3, 0.5, 0.7 and 1 % NaCl) were prepared by dissolving pure sodium chloride in distilled water. In each experiment the animals were kept in finger bowls containing 500 mls. of salt water. All experiments were repeated ten times to get constant results. The blood samples were collected by insertion of the finely drawn out end of the glass capillary of 0.6 to 0.8 mm. internal diameter through the membrane at the posteriordorsal surface of the carapace of the carefully dried prawn. The blood chlorides were estimated using the method employed by Kato and Kamemoto (1969).

To estimate the influx of water under varying experimental conditions both the nephropores were sealed with araldite. In all cases the adhesive provided a permanent seal. The changes in the weight were then determined at desired intervals after the animals were put back into the water.
The eyestalk extracts were made after removing the retinal pigments as completely as possible. The tissues were homogenised in physiological saline and then centrifuged for five minutes. The supernatant fluid (2 eyestalks/0.1 ml/prawn) was injected to each prawn on the ventral side of the abdomen.

RESULTS

1) Survival in different media: Lethal salinity.

To study the survival of *Cardina* in different media, ten healthy specimens of approximately equal size and weight were transferred from tap water to experimental solutions kept in glass troughs. It was found that *Cardina rajadhari* was able to tolerate salt concentrations from 0.05 to 0.3 % NaCl (isotonic with 1.43 to 14.29 % sea water) (Fig. 1). Between the salt concentrations of 0.05 % and 0.30 % NaCl (isotonic with 1.43 and 3.57 % sea water), the survival rate was 100 %. Between 0.50 and 0.05 % NaCl, the rate of survival of the prawns increased with decrease of salt concentration. The 50 % survival salinity was found to be 0.6 % NaCl (Fig. 1).

2) Blood chloride changes.

Blood was collected from the prawns after keeping them for one day in each of the media. From the data given in Table 1, it is clear that in distilled water there was a
### Table 1

Blood chloride concentration of *Caridina rajadhari* in different salt concentrations.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Cl⁻ in blood (mg./ml.)</th>
<th>Cl⁻ in medium (mg./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1.680 ± 0.014</td>
<td>-</td>
</tr>
<tr>
<td>Tap water</td>
<td>2.981 ± 0.181</td>
<td>0.824 ± 0.003</td>
</tr>
<tr>
<td>0.1 % NaCl</td>
<td>2.999 ± 0.013</td>
<td>0.824 ± 0.004</td>
</tr>
<tr>
<td>0.2 % NaCl</td>
<td>3.741 ± 0.008</td>
<td>3.235 ± 0.004</td>
</tr>
<tr>
<td>0.3 % NaCl</td>
<td>4.211 ± 0.021</td>
<td>4.273 ± 0.008</td>
</tr>
<tr>
<td>0.5 % NaCl</td>
<td>4.004 ± 0.063</td>
<td>5.102 ± 0.012</td>
</tr>
</tbody>
</table>
loss of chloride ions from the body. When the prawns were transferred to tap water and different concentrations of salt solutions, there was increase in the uptake of chloride ions. The animals gained Cl\(^-\) ions in 0.1% and 0.2% salt solutions and maintained hypertonicity. In 0.3% and 0.5% salt concentrations, the blood remained distinctly hypotonic (Table 1).

Weight changes in different media.

The above results show a definite change in blood chloride level, following immersion in different salinities. They, however, give no indication whether the changes are due to the movements of chloride ions or the movements of water across the body to the outside.

It is generally observed that the weight changes occur due to the influx and efflux of ions in the body. This can be seen by studying the weight changes in the animals in the media used. The animals were weighed at the commencement of the experiment, then they were immersed in the appropriate media and weighed after every 24 hours, till 72 hours. It can be seen from the Fig. 2 that there was gradual loss of weight in higher salt concentrations. In distilled water there was a gradual increase in weight.
Neuroendocrine control of osmoregulation.

Experiment I:

The aim of the experiment was to study the effect of eyestalk ablation on the weight changes of the prawns. Forty animals were selected and divided into two groups A and B, of 20 each. The prawns of A group were treated as intact controls, whereas eyestalks of the B group prawns were ablated and served as experimental prawns. After 24 hours of eyestalk ablation, both the control as well as experimental animals were weighed at the intervals of 1, 3, 6, 24 and 48 hours, to study changes in the body weights of the animals. The results are presented in Fig. 3. From the figure, it is seen that there is a little change in weight of the normal animals over the forty eight hour period of the experiment. However, in destalked animals there was an initial drop in the weight followed by a slight recovery over the period of 48 hours. Except for this initial decrease in weight of destalked prawns there appears to be a little difference between experimental and control animals. Further observations on the weight changes could not be followed due to the approach of the premoult condition which influences the water content in the animals (Fig. 3).

Experiment II:

In this experiment changes in blood chloride concentration were studied in control as well as eyestalk ablated
prawns at 3, 6 and 24 hours after eyestalk ablation. Twenty animals were used in each group. The normal blood chloride concentration of prawns was found to be 2. 981 mg/ml. The results are presented in Table 2. From the table, it is evident that there is significantly a greater increase in the concentration of blood chloride after eyestalk extract injection in ablated specimens as compared to the destalked prawns.

Experiment III:

In this experiment the nephropores of normal and eyestalkless animals were sealed, thereby preventing the elimination of urine. The weights of the animals were taken after keeping them in tap water for 24 hours period. The results are presented in Fig. 4. From the figure it can be concluded that there was a significantly greater increase in the weights of the animals after eyestalk removal as compared to the normal prawns. This greater water influx was probably through the gill or gut into the prawns (Fig. 4).

Experiment IV:

This experiment was conducted to observe the effect of brain and thoracic ganglia extract injections on blood chloride concentration in Caridina rajahart. Injections of 0.02 ml. of distilled water, 0.02 ml/2 brain homogenate and 0.02 ml/2 thoracic ganglia extract were given into the prawns of first, second and third groups.
### Table 2

Effect of eyestalk extract injection on the Cl⁻ concentration of blood of normal and destalked *C. raiadhari*.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Cl⁻ concentration (mg./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>normal</td>
<td>2.981 ± 0.196</td>
</tr>
<tr>
<td>2</td>
<td>destalked</td>
<td>1.654 ± 0.348</td>
</tr>
<tr>
<td>3</td>
<td>destalked prawns receiving eyestalk extract injection</td>
<td>3.603 ± 0.603</td>
</tr>
</tbody>
</table>

### Table 3

Effect of brain and thoracic ganglia extract injections on blood chloride concentration in *C. raiadhari*.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Volume (ml.)</th>
<th>Chloride concentration (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>distilled water</td>
<td>0.02</td>
<td>1.680 ± 0.471</td>
</tr>
<tr>
<td>2</td>
<td>Brain extract injection</td>
<td>0.02/2 brains</td>
<td>3.008 ± 0.833</td>
</tr>
<tr>
<td>3</td>
<td>Thoracic ganglia extract injection</td>
<td>0.02/2 ganglia</td>
<td>2.475 ± 0.536</td>
</tr>
</tbody>
</table>
respectively. The results are presented in Table 3. It is evident from the table that the brain factor increases the concentration of the chloride in blood. Extracts of the thoracic ganglia also increase the blood chloride concentration although they were less effective than brain homogenates.

**DISCUSSION**

It emerges from the present investigation that *Caridina rajadhari* appears to possess good osmoregulatory ability at very low salt concentrations. The animals maintained their body fluids hypertonic to the medium in 0.2% NaCl concentration and further low concentrations. In salt concentrations 0.3% and above the body fluids were slightly hypotonic to the medium.

Hyperosmotic regulation which involves active absorption of ions against concentration gradient is common in freshwater animals and it has been shown to occur in several aquatic animals including crustaceans (Krogh, 1939). As far as crustaceans are concerned, Verwey (1956) suggested that hyper regulation is "must" in freshwater and lower salinities whereas in higher salinities hypo-regulation may or may not occur. Identical results were obtained in the present study with *Caridina rajadhari*, where the animal was hyper-regulator in 0.2% NaCl and further low concentrations.
Panikkar (1941) found that *P. serratus*, *P. alegana* and *P. variana* maintained blood hypotonic to high salinity and hypertonic to brackish water. Panikkar and Vishwanathan (1943) found the same ability shown by *Metapanaeus monoceros*. To lesser extent, *M. dobsoni*, *Panacus indicus* and *P. carinatus* also maintained their internal concentration hypotonic in high salinity and hypertonic in brackish water (Panikkar, 1951). Dobkin and Manning (1964) found the same condition in *Palaemonetes intermedius*. On the other hand, *P. paludosus* was found to be hypertonic to its medium in a variety of concentrations (Dobkin and Manning, 1964). The behaviour of *Caridina rajadhari* was found to be similar to that of *Palaemonetes paludosus* (Dobkin and Manning, 1964).

It was evident from present investigation that *C. rajadhari* was a hyperosmoregulator in 0.2% NaCl, became isosmotic to 0.3% NaCl while further high concentration the prawn showed hypo-osmoregulation.

The weight changes in different media suggest that there is some degree of water permeability from hypotonic media resulting in increase in weight and loss in weight by the animal when placed in hypertonic media. Hiscock (1953) found that *Hyridella australis* regulates weight in the media of low salinities. There was an increase in weight in hypotonic and decrease in weight in hypertonic media; and concluded that some part of body surface must
be permeable to both salts and water. Nagel (1934) observed that the weight increase in *Carcinus* in 50% sea water was ten times greater with the excretory pores open than with them closed. Nagabhushanam and Lomte (1971) showed that *Pararvadia corrugata* regulates its water content in hypotonic media, but in hypertonic media, the mussel loses weight.

Considerable attention has been paid by numerous investigators on the osmoregulation and ionic regulation among invertebrates and vertebrates (Prosser and Brown, 1961; Potts and Parry, 1964). On the other hand, information on the control of hydromineral regulation is rather very rare. Among Crustacea, most of the work on the neurosecretion in relation to hydromineral regulation has been concerned with the changes in the body weights and water content.

Scudamore (1947) observed in the crayfish that removal of the eyestalk or sinus gland resulted in an increase in weights with greater water content and these changes were prevented by implantation of sinus gland. In *Carcinus maenas*, Carlisle (1955) obtained similar results. In *Uca pugilator*, a possible role of sinus gland hormone in regulating the diurnal rhythmic fluctuations in the weights was observed which was presumably due to the changes in their water content (Guyselman, 1953).

Kamemoto et al. (1966) had observed that in destalked animals an initial sharp drop in the body weight followed
by a recovery period as compared to very little change in the weight of normal animals over the four day period. The results obtained in the present investigation followed same pattern as had been observed by Kamemoto et al. (1966) over a period of 48 hours. The bilateral eyestalk ablation affects the osmotic concentration of the blood in Caridina rajadhari. After eyestalk ablation, there was a relatively greater fall in the concentration of blood chloride as compared to the normal ones. Physiologically, a decrease in the blood osmotic concentration of the ablated animals may result from an alteration of the permeability characteristics of the animals resulting in a greater influx and/or greater loss of salts, a decrease in active uptake of salts and/or an increase in urine production resulting in a greater net loss of salts. This greater drop was prevented by the eyestalk extract injections into the destalked animals.

According to Dehnel and Stone (1964) the antennal glands of Hemigrapsus nudus and H. oregonensis serve an osmoregulatory function only in wintering condition. However, it seems that the antennal glands of brackish water species function in ionic regulation, removal of waste matter and possibly the control of body fluid volume. They reported that there was no clear evidence for an osmotic function of the antennal glands except for the specific situation. The osmotic concentration of the urine is slightly higher than the osmotic concentration of the
blood but follows the same trend with either the concentration or dilution of the medium. The amount of urine produced in *Caridina rajadhari* was not determined. However, the amount of urine production in *Caridina* can be calculated from the data presented in Fig. 2. The increase in weight due to the influx of water, after sealing the nephropores might represent the amount of urine normally eliminated by the animal in as much as there is decrease in weight of prawns if the nephropores are not sealed. In normal animals, there was approximately 2.3% increase in weight in the first 6 hours (Fig. 3). This might be interpreted as the amount of urine produced by the prawn in six hours in terms of percentage of body weight. However, in the eyestalkless animals under similar conditions, there was increase in the urine formation perhaps in response to the increased influx of water as was suggested by Kamemoto et al. (1966) for *Procambarus alarkii* and in the crab, *Matanograpthus messor* (Kato and Kamemoto, 1969). In most species of crabs, the urine is isosmotic with blood (Nagel, 1934; Prosser et al. 1955; Beadle, 1957; Gross, 1963, 1964; Potts and Parry, 1964 and Gross et al. 1966). McWhinnie (1962) injected extracts of the neurosecretory sites into normal crayfish and observed significant changes in the free calcium levels of the blood. The injection of the brain extract caused an increase in the free calcium while eyestalk extracts caused a decrease. She observed these
results two hours after injections and stated that such results are highly suggestive of the relatively rapid responses of animals to the environmental stresses which are necessary for the maintenance of homeostasis (McWhinnie, 1962).

It was evident from the present investigation on Cardina rajadhari that the brain factor increased the blood chloride concentration although they were less effective than the brain homogenates. Thus the present observation strongly supports the suggestion that a neuro-endocrine factor is present in the brain, which effects hydromineral regulation. Similar findings were reported in freshwater crayfish, Procambarus clarkii (Kamemoto and Tullis, 1972) who further stated that the blood chloride concentration of marine crabs placed in dilute media decreased after eyestalk ablation or thoracic ganglia extract injection.

The present investigation supports the hypothesis of Kamemoto et al. (1966). From the results obtained it can be concluded on the hypothetical view that in prawns, a substance is secreted in the brain and transported to the eyestalk for release, presumably by the sinus gland. The substance maintains a normal permeability of the body surfaces to water. Such a neurosecretory pathway has been described by Bliss et al. (1954) Eyestalk removal in these prawns result in the removal of
the release site, causing an influx of water and a decrease in the salt concentration of the blood. The substance also causes an increase in the salt and osmotic concentration of the blood as demonstrated by the increased concentration following a injection of the brain homogenates. Thompson (1967) has found that in freshwater crabs, *Pseudephelphusa jouyi* the eyestalk ablation resulted in an increased permeability to water. Mantel (1967) studied in vitro that the effect of the ventral ganglion on the permeability of the foregut of *Gecarcinus lateralis*. She has noticed that in vitro preparation of the foregut, the addition of the ventral ganglionic extract to the haemolymph resulted in an increased permeability of the foregut to water and salts. Kamemoto and Ono (1967) by continuous collection of urine in the crayfish found that the rate of urine flow increased by twofold after bilateral eyestalk ablation. Leersnyder (1967) obtained similar results in the crab *Eriocheir sinensis*.

Although the data fills within the eyestalk ventral ganglion pathway scheme (Kamemoto and Ono, 1967; Leersnyder, 1967) it does not preclude the possibility that the eyestalk component function directly as an antagonistic to the ventral ganglionic factor. Probably, that the eyestalk might also contain a substance which acts direct on the animal body surfaces in decreasing permeability. If such is the case, the permeability characteristic of the animal may be determined by the factor which has the highest titer in the blood.
SUMMARY

1. A study was made on *Caridina rajadhari* to see the survival and osmotic regulation following the transfer of animals from distilled water to different concentrations of salt solutions.

2. Fifty percent mortality was observed in salt concentration of 0.6 % NaCl.

3. It was found that there was loss of chloride ions in distilled water whereas the prawns gained chloride in different salt concentrations. The gain of chloride ions was found to increase with the increasing salt concentration up to 0.3 % NaCl.

4. The weight changes showed that there was increase in weight when placed in distilled water. In tap water the prawns showed little fluctuations in weight, whereas the weight increased with decreasing concentrations, thus suggesting that in hypotonic media *Caridina* could regulate the water content, but in hypertonic media the animals lost considerable weight due to loss of water.

5. The role of neurosecretion in osmoregulation in *Caridina rajadhari* was also studied. The eyestalk ablation caused a rapid fall in the blood osmotic concentration and this fall was prevented by the injection of an eyestalk extract.
6. The decrease in the blood osmotic concentration in eyestalkless animals is presumably due to increased water influx.

7. It was found that injections of brain homogenate increased the blood chloride concentration. The extracts of thoracic ganglia also increased the blood chloride concentration although they were less effective than brain homogenates.
Fig. 1 Showing the relationship between salinity and percentage survival of Caridina.

F. W. = Freshwater
Fig. 2  Weight changes in *Caridina rajadhari* in different salt concentrations.

A = distilled water.
B = Tap water.
C = 0.1% NaCl.
D = 0.2% NaCl.
E = 0.3% NaCl.
F = 0.5% NaCl.
Fig. 3 Effect of eyestalk ablation on weight changes in *Cardina rajadhari*.

Normal
Destalked
Fig. 4 Effect of sealing the nephropores on the weight changes in *Cardina rajadhar*.

Normal
Destalked