TEMPERATURE TOLERANCE
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Temperature is one of the environmental factors, which limits the distribution of the animals and determines their activity. In temperature zone, extremes of mid-winter and late summer may result in a shift of metabolic processes of animals tending to compensate for these extremes. However, tropical forms living in a relatively constant thermal environment do not have to face with such extremes and their metabolic processes may not show a compensatory shift (Vernberg, 1962). Thus the temperature effects on an animal may be reflected in its physiology. It is to be expected that the ability to exist at an environmental temperature is expressed in the physiological and biochemical responses of the animal. Much has been written on the adaptation of poikilothermic animals to temperature of their surroundings (Bullock, 1955; Prosser and Brown, 1961; Mcwinnie, 1967; Vernberg and Vernberg, 1970). Evidence also exists that adaptation to highest temperature implies an upward shifting of the lethal temperature.

Acclimation to low and high temperatures has been demonstrated in many animals on the basis of laboratory acclimation. Edwards and Irving (1943) showed seasonal acclimation in *Emerita talpoida*. Redney (1961, 1962) found an upper limit of thermal tolerance in a
variety of species of the fiddler crab _Uca_. Loss of heat tolerance in crayfish, _Cragonesta rusticus_ was not completed by the end of sixteenth day, when the crayfish were transferred from 22°C to 4°C (Spoor, 1955). Finne (1956) found that the upper thermal limits may be modified by decrease in salinity and for many marine animals, the upper lethal temperature decreases, as salinity decreases. Brockema (1941) found that the shrimp _Cragonon cragonon_ could endure better low salinity when temperatures were high during the period of acclima-
tion. A decrease in salinity with the acclimation temperature held constant, resulted in a lowering of thermal resistance (McLeese, 1956). Todd and Dehnel (1960) studied in detail the effect of temperature and salinity on heat tolerance in two grapsid crabs, _Hemigrapsus nudus_ and _H. oregonensis_.

Spaargaren (1973) determined the effect of salinity and temperature on the heart rate of hyperregulating _Cragonon cragonon_ and hypo-regulating _Palaemon serratus_. He stated that heartbeat frequency may entail a certain blood pressure. For water transport this hydrostatic pressure is equivalent to osmotic pressure. With hypertonic regulation an increase in heart rate may play a role in low salinities by suppressing the water emerging by osmosis.
Variations in chemical constitution have been associated with differences in environmental temperature. Heilbrunn (1943) found that in plants and poikilotherms maintained at lower temperatures, have low melting point of fats than the members of the same species living at higher temperature. Hoar et al. (1952) working on a goldfish observed decrease in lipid content with the rise in temperature. Dean and Vernberg (1965) reported effects of temperature on carbohydrate metabolism in decapods.

In view of its convenient size, longevity and availability, the temperature relations of Caridina rajadhari are of considerable interest not only to the ecologist but also to the physiologist concerned with the problem of explaining the mechanism of acclimatization to temperature. In the present chapter, effect of temperature and salinity on heat tolerance, effect of temperature on heart beat and changes in organic parameters were studied.

**Materials and Methods**

Caridina rajadhari were collected from Kham river near Aurangabad. The temperature of river water varied between 20°C and 29°C. On arrival to the laboratory, the prawns were kept in shallow water tanks containing water. Both males and females were used in the experiments, when preliminary experiments showed no
difference in the resistance of sexes. Married females or individuals missing any appendages were discarded. The animals were not fed during the experimental period.

In the present study, holding experiments refer to those in which the temperature tolerance of the animals was determined without any previous laboratory acclimation. The animals were tested after keeping for 24 hours in the laboratory. This time period allowed the gut to be cleared partially so that at the high test tolerance temperature, deposition of faeces did not foul the water. In acclimation experiments, on the other hand, animals were held at previously determined acclimation conditions which differed from holding experimental conditions in one factor. In these experiments, the prawns were acclimated for at least one week in the laboratory.

In cases where acclimation temperature differed from the holding temperature, the animals were gradually warmed or cooled until the desired temperature was reached; this usually took about two hours. Holding or acclimation temperatures were either 16°C or 31°C. The salinity used was 0.35 % NaCl. Four experimental combinations were used. Ten animals were placed in each container.
The water was changed usually once per 24 hours, to the medium of appropriate temperature over the acclimation or holding period. The animals were brought to the test temperatures over a period of 2 - 4 hours at which time the experiment was begun and was conducted for 24 hours. Animals were considered dead only when all movements were ceased. Graphic representations of the data were made for all experiments after repeating the same once.

The heart-beats were registered after measuring the beats per minute through a convex lens. Biochemical changes after acclimation to low temperature (18 ± 1°C) and high temperature (33 ± 1°C) for eight days along-with the control prawns were also studied. Estimations were made on whole animals. Water content was obtained by exposing the animals for 24 hours to a temperature of 100°C in an oven. Total nitrogen was measured by Micro-Kjeldal method (Hawk et. al. 1954). The amount of protein was calculated by multiplying the nitrogen value by the factor 6.25. Total fat was extracted from dried and finely powdered meat in a Soxhlet apparatus. Glycogen was estimated by the method recommended by Kemp et.al. (1954). All results are expressed on dry weight basis.

RESULTS

Average conditions taken into consideration for the first series of experiments were 31°C and fresh water.
Survival values of the animals kept at the above mentioned temperature with no laboratory acclimation (holding experiments) were considered as baseline curves. These curves were compared with heat tolerance of animals acclimated to (i) 31°C and fresh water, (ii) 31°C and 0.35 % NaCl, (iii) 16°C and fresh water and (iv) 16°C and 0.35 % NaCl. Temperature at which 50 % survival occurred, for 24 hours was used as the basis for comparison.

**Holding Experiments: 31°C and fresh water.**

Results of holding experiments showed that the lethal temperature was found to be 35°C for 24 hours (fig. 1).

**Acclimation Experiments: 31°C and fresh water.**

Increase in heat tolerance was noted after acclimation to 31°C and fresh water for one week. The 50 % survival was found to be 39°C for 24 hours (fig. 2).

11) 31°C and 0.35 % NaCl.

This combination of temperature and salinity differed from the baseline condition (fig. 3) only in the salinity, having changed from fresh water to 0.35 % NaCl. The lethal temperature was 38.5°C.
iii) $16^\circ C$ and fresh water.

Results obtained in these experiments showed that the tolerance was lowered, compared to the previous one. The 50% survival temperature was $36^\circ C$ for 24 hours (fig. 4).

iv) $16^\circ C$ and 0.35 % NaCl.

Low temperature and salinity combinations appeared to be highly unfavourable for survival of *Caridina*. The 50% survival temperature recorded was $33^\circ C$ (fig. 5).

**Experiment II:**

Average conditions taken into consideration for the second series of experiments were $16^\circ C$ and fresh water. The survival value obtained from the animals which were kept at the above mentioned temperature without any laboratory acclimation (holding experiments) were considered as base line curves (fig. 6). The animals were acclimated to the following combinations: (i) $16^\circ C$ and fresh water (ii) $16^\circ C$ and 0.35 % NaCl (iii) $31^\circ C$ and fresh water (iv) $31^\circ C$ and 0.35 % NaCl.

**Holding Experiments:** $16^\circ C$ and fresh water.

The 50% survival for this holding experiment was $34.0^\circ C$ (fig. 6).
Acclimation Experiments: 16°C and fresh water.

The prawns acclimated to this combination have better survival value, compared to the holding experiments. The 50% survival temperature is 36°C (fig. 7) which is comparable to the earlier series of experiment.

(ii) 16°C and 0.35% NaCl.

The results obtained with this combination showed considerable agreement from the counterpart of the first series of experiments. The 50% survival value for the prawns was 33°C for 24 hours (fig. 8).

(iii) 31°C and fresh water.

This combination proved most favourable for prawns in the second series of experiments. There was a marked increase in temperature tolerance and 50% mortality observed was at temperature 39°C for 24 hours (fig. 9).

(iv) 31°C and 0.35% NaCl.

The lethal temperature of the animals in this combination was 37.5°C for 24 hours (fig. 10).

Heart Rate:

Change in temperature exerted a remarkable effect on the heart rate of Caridina. At elevated temperature, (32 ± 1°C) O10 value was 1.106; the average heart
### Table 1

$Q_{10}$ of heart beat of *Cardina rajadhari* in relation to temperature.

<table>
<thead>
<tr>
<th>Temperature $^\circ$C.</th>
<th>Average No. of heart beats</th>
<th>$Q_{10}$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$32 \pm 1$</td>
<td>150</td>
<td>1.108</td>
</tr>
<tr>
<td>$18 \pm 1$</td>
<td>70</td>
<td>2.142</td>
</tr>
</tbody>
</table>

### Table 2

$Q_{10}$ of heart beat of *G. rajadhari* in relation to temperature and salinity of the medium.

<table>
<thead>
<tr>
<th>% Nacl in medium</th>
<th>Temperature $^\circ$C. of medium</th>
<th>Average number of beats</th>
<th>$Q_{10}$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>26 - 32</td>
<td>162 - 159</td>
<td>1.236</td>
</tr>
<tr>
<td>0.3</td>
<td>26 - 32</td>
<td>137 - 159</td>
<td>1.186</td>
</tr>
<tr>
<td>0.5</td>
<td>26 - 32</td>
<td>144 - 159</td>
<td>1.070</td>
</tr>
</tbody>
</table>
beats were 150/min. while at lower temperature (18 ± 1°C) $Q_{10}$ value was 2.142 (Table 1), the average heart beats were 70/min.

Temperature and NaCl combination decreased the heart rate. In 0.1 % NaCl and 26 - 31°C temperature, the $Q_{10}$ value was 1.236 while at 0.3 % NaCl and 0.5 % NaCl at same temperatures the $Q_{10}$ value was 1.186 and 1.070 respectively (Table 2).

Changes in the biochemical components in relation to temperature

Acclimation to high and low temperatures has an influence on the biochemical components. At room temperature (25 ± 1°C) the water percentage was 75.51 ± 1.66 %. It was increased to 82.55 ± 0.98 % at 33 ± 1°C while at 18 ± 1°C a decrease in the percentage of water was noticed (70.00 ± 1.001 %). Glycogen and fat contents also increased under cold acclimated prawns while proteins did not show any changes (Table 3).

When the animals were acclimated to higher temperature the glycogen content was noted to have decreased while fat content was increased very slightly (Table 3).
<table>
<thead>
<tr>
<th>Organic constituents</th>
<th>Acclimation Temperature°C</th>
<th>33 ± 1</th>
<th>25 ± 1</th>
<th>18 ± 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>82.55</td>
<td>75.51</td>
<td>70.00</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>1.66</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteins</td>
<td>80.00</td>
<td>60.63</td>
<td>60.66</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>3.91</td>
<td>4.27</td>
<td>4.87</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipids</td>
<td>4.85</td>
<td>4.10</td>
<td>5.78</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Effect of temperature acclimation on the organic components of *C. rajadhari* (expressed in percentage ± S.D.)
DISCUSSION

Acclimation to high temperature with resulting increase in the high temperature tolerance of the species has been demonstrated many times (Summer and Doudoroff, 1936; Brett, 1946; Mellanby, 1954; Spoor, 1955 and McLeese 1956). The freshwater prawn Caridina rajadhari would seem to be no exception to this theme. The combined effect of temperature and salt concentration on the heat tolerance had been studied less extensively and it was of interest to find whether the salinity had a marked effect on temperature tolerance of the species.

It is evident from the present study on Caridina rajadhari that the prawn could regain its tolerance very rapidly after it was returned to the high temperature acclimation. This rapid gain of heat tolerance had been demonstrated many times. Mellanby (1954) reported that the heat coma point is shifted with experimental acclimation to the upper end of the tolerable range in the mealworm, Tenebrio molitor. Spoor (1955) found complete gain of heat tolerance in the crayfish in less than 24 hours with an approximate 18°C change in temperature (4°C to 22°C).

A rapid gain at heat tolerance would be of extreme advantage to most intertidal animals. For intertidal animals, the increase in temperature may be great over
a period of few hours in the tidal rhythm. Probable increase in salinity aids the animals in resisting the high temperatures, as experimental results have indicated that high salinity provides the most favourable environment.

With the low salinity and low temperature combination, loss in heat tolerance is much greater in Caridina. In nearly all instances low salinity resulted in a lower value for the 50% survival temperature with the salinity as found in the present study with Caridina was due to which osmotic stress imposed by the medium. The lethal or near lethal temperatures in the test tolerance experiments presumably alone are causing a marked strain on the metabolic activity of the prawns and the additional strain of maintaining a large gradient between the blood and the external medium in the salinity results in the animal dying at a lower temperature. Similar observations were reported in terms of oxygen consumption in certain crustaceans by Lofts (1956) and Pampapatirao (1958) who noticed decreased oxygen consumption where there was osmotic stress. A similar relation between salinity and temperature was found by Broekema (1941) in Crangon crangon and Hemigrapsus by Todd and Dehnel (1960).
The heart rate was chosen to evaluate energy consumption in *Caridina rajadhari*. It was found that rapid changes in temperature result in the fluctuations in the heart rate. The prawns also showed considerable influence of salinity and temperature on the heart rate. At hypotonic regulation of blood concentration in saline media, a decrease in the heart rate of *Caridina* with increase in salinity was observed. This hyporegulation in *Caridina* may lead to the fact that heart rate may be connected with an increasing energy consumption in the process of salt excretion. Spaargren (1973), while working on marine shrimps stated that, 'the measurement of heart rate in the animals used in the experiments may be summed up in a simple rule: the physiological adaptation in the less permeable osmoregulating species exposed to fluctuations in salinity and temperature is compensated in osmo-confirming species by an ecological adaptation: the choice of habitat with possibly fewer fluctuations in salinity and temperature.'

The experiments on *Caridina* proved the above connection between stability of environmental temperature and salinity and stability of heart rate is a general rule.

One of the factors accounting for metabolic compensation to thermal stress was free-water/bound-water ratio (Precht, 1958). In most cases including the
present one, tissue water was found to be directly proportional to the acclimatization temperature (Roar and Cottle, 1952; Suhrmann, 1955; Vanungo and Prosser, 1959; Saroja and Pampapathirao, 1965). The increase in the water content on warm acclimatization might lead to a decrease in the metabolic rate through reducing the proportion of respiring tissue. In the present study, the water content of _Caridina_ acclimatized to 18 ± 1°C was 70.00% and prawns held at 35°C was 62.55%. Two factors may be responsible for the increased water content at higher temperatures. In the first place, prawns unlike reptiles, are unable to regulate their body temperatures by behaviour, thus all of the metabolic compensation permitting escape from the severity of environmental change was due to acclimatization. In the second place, increased oxidation of fat produced more metabolic water. McWinnie (1967) stated that biochemical changes provide a molecular basis for the thermogenesis essential to account for the activity and synthesis at suboptimum temperature as well as survival and homeostasis at superoptimum temperature. Raghupathirami Reddy and Rao (1963) while working on annelid _Lampito mauritii_ considered the decreased amino acid levels in the body fluids after cold acclimation, to induce increase in protein synthesis. Dean and Vernberg (1965) found and increase in the blood glucose content with increasing temperature acclimation from 2°C to 30°C in decapod Crustacea.
In the present study on Caridina rajadhari it was found that the glycogen content decreased in warm acclimated prawns and increased in cold acclimated animals. The fat content was increased in cold acclimated prawns, while no change was observed in protein composition.
SUMMARY

1. The influence of laboratory acclimation on heat tolerance was determined in fresh water prawn, Caridina rajadhari at various temperatures and salinity combinations. There was a change in 50% survival of the animals.

2. Acclimation to high temperature generally increased resistance to lethal temperature whereas acclimation to salinity either at high or low temperatures generally decreased the resistance. High temperatures and normal fresh water combination was the most favourable to withstand the high test tolerance temperature.

3. After acclimation to low temperature, gain in heat tolerance both at high temperature and fresh water as well as at high temperature and 0.35 d NaCl combinations was rapid.

4. Measurements of heart rate yielded indications for a considerable energy consumption during temperature and osmotic process in two different ways. At lower temperatures $Q_{10}$ was increased than at higher temperatures, while increase in salinity at two temperatures decreased the $Q_{10}$ value.
5. Water percentage was increased in warm acclimated prawns while glycogen and fat contents increased during cold acclimation. No changes were noticed in protein content under different acclimation temperatures.
Fig. 1. 50% survival value of animals kept at 31°C, and fresh water without acclimation (Holding experiment) - Base line experiment.

Lethal temperature = 35°C.
Fig. 2: The influence of laboratory acclimation to $31^\circ$C. and freshwater on 50\% survival value for 24 hours.

Lethal temperature = $39.0^\circ$C.
Fig. 3.  The influence of laboratory acclimation to 31°C, and 0.35 % NaCl on 50 % survival value for 24 hours.

Lethal temperature = 38.5°C.
Fig. 4. The influence of laboratory acclimation to 16°C. and freshwater on 50% survival value for 24 hours.

Lethal temperature = 36.0°C.
Fig. 5. The influence of laboratory acclimation to 16°C, and 0.35 % NaCl on 50 % survival value for 24 hours.

Lethal temperature = 33.0°C.
Fig. 6. 50% survival value of animals kept at 16°C and freshwater without acclimation (Holding experiment) - Baseline experiment.

Lethal temperature = 34.0°C.
Fig. 3. 50% survival value of animals kept at 16°C, and freshwater for 24 hours after laboratory acclimation.

Lethal temperature = 36.0°C.
Fig. 8. 50% survival value of animals kept at 16°C and 0.35% NaCl for 24 hours after laboratory acclimation.

Lethal temperature = 33.0°C.
Fig. 9. 50% survival value of animals kept at 31°C, and freshwater for 24 hours after laboratory acclimation.

Lethal temperature = 39.0°C.
Fig. 10. 50% survival value of animals kept at 31°C. and 0.35% NaCl for 24 hours after laboratory acclimation.

Lethal temperature = 37.5°C.