

RESISTANCE OF THE LIMPET *CELLANA RADIATA* (BORN) TO VARIATIONS IN TEMPERATURE, SALINITY AND DESICCATION

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Tolerance limits of *Cellana radiata* to high and low temperatures, to salinities above and below the ambient conditions and to desiccation were studied with a view to ascertain the influence of these parameters on the vertical distribution of the animal.

The experimental studies have shown that the limpet is temporarily tolerant to both high and low temperatures and to salinities above and below the ambient conditions. The lethal temperature of the limpet was found to be 42°C. Salinities above 37‰ and below 20‰ appear to be critical to the animals. No marked differences were observed in the tolerance of the limpets collected from the high and low water zones.

The desiccation experiments showed that the animals living at the higher levels are more tolerant to dry air than those living at the lower levels. It has also been shown that the limpets living at the higher levels are more resistant to higher temperatures in dry air than those at lower levels. The rate at which water is lost is related to the weight and the vertical position of the limpets. In both the high and low water limpets the total water-loss in the smaller animals is less than that of the larger ones, but the weight specific water loss in the smaller animals is always greater. When compared at the same body weight, the low water limpets are found to lose water at a greater rate than those at higher levels. Further, the limpets living at the higher levels are found to withstand greater water-loss than those living at lower levels.

The results are discussed and compared with those of the other workers on the limpets from temperate regions.

INTRODUCTION

The important physical factors which influence the distribution of the animals in the intertidal region are temperature, salinity and desiccation. Colgan (1910) and Broekhuysen (1940) studied the tolerance of some common shore molluscs to variations in these physical factors. Thermal resistance of many intertidal molluscs was studied by several investigators among whom may be mentioned Evans (1948), Orr (1955), Southward (1958) and Fraenkel (1960, 1961, 1966, 1968). Studies on the tolerance of the limpets to variations in these physical factors are few. Evans (1948), while studying the lethal temperature of some common British littoral molluscs made some observations on the heat resistance of the limpets *Patella vulgata*, *P. depressa* and *P. athletica*. Arnold (1957, 1959) studied the response of *Patella vulgata* to different salinity concentrations. Recently, Davies (1969) studied the desiccation effects in *Patella*. Sukumaran and Krishnaswamy (1962) made some observations on the response of *Cellana radiata* to changes in salinity and made an attempt to compare their observations with those in the allied limpet *Patella vulgata*. In the present study an attempt has been made to study the tolerance of *Cellana radiata* collected from the high and low-water levels to varying temperatures and salinities and to desiccation.

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MATERIALS AND METHODS

Experiments were performed on the limpets collected at the high and low-water zones. The animals were collected from rocks with even surfaces. Only healthy and active animals were selected for the experiments. The animals which extended their cephalic tentacles and mantle papillae beyond the margins of the shell were considered as healthy and active. They were used shortly after collection. Experiments were performed on animals of all sizes above 10 mm shell length. Tolerance of the animals was tested at temperatures and salinities above and below the ambient conditions, and to desiccation. The details in the methods employed are given in the relevant places.

In all the temperature, salinity and desiccation experiments the animals were considered as 'active' and fully recovered if they extended their cephalic tentacles and mantle papillae beyond the margins of the shell, and movements of the foot and head were observed when turned on the ventral surface. The animals were considered dead if they failed to revive even after 24 hr when brought back to the normal conditions.

RESULTS

Resistance to high temperatures

To study the resistance of the animals to higher temperatures Fraenkel's (1960, 1961, 1966, 1968) method of exposing the animals for exactly one hour, to different higher temperatures was followed. These experiments were carried out at temperatures between 32° and 40°C, at two degree intervals. Five animals were used in each experiment. They were placed directly into the experimental temperatures and were maintained for exactly one hour. 500 ml of sea-water of ambient salinity (about 30‰) was used in each experiment. After exposure for exactly one hour the animals were brought to room temperature ($27^{\circ} \pm 1^{\circ}\text{C}$) and the number of animals recovering was recorded. The results are presented in Table I. It may be seen from the Table that all the animals which were subjected to 36°C and below recovered when returned to room temperature. At 38°C only 60 per cent and at 40°C none of the animals recovered. No marked differences were observed in the tolerance of the animals collected at the high and low-water zones to higher temperatures.

The effect of the response of the animals to gradually rising temperatures was also studied by the method adopted by Evans (1948) by raising the temperature by 1°C after every 5 min. Twenty-five animals were used at a time for each experiment. They were placed in a one-litre beaker containing sea water of ambient salinity (about 30‰), with the foot uppermost to facilitate easy observation. The beaker containing the animals was gently heated on a water bath. Care was taken that the rise in temperature was 1°C every minutes. The condition of the animals at different temperatures was noted and at each higher temperature five animals were brought to room temperature ($27^{\circ} \pm 1^{\circ}\text{C}$) and the number of animals recovering was noted. The results of these experiments are summarized in Table II. The animals maintained their normal activity with extended mantle and pallial tentacles, and with the movements of foot, head and cephalic tentacles with a rise in temperature up to 35°C.

TABLE I

Resistance of Cellana radiata to high temperatures after exposure for one hour

Temperature °C±0.1°C	Numbers in experiment	Numbers recovered	Remarks
32	10	10	All recovered within 15 min
34	15	15	
36	20	20	All recovered within one hour
38	15	9	5 reactive after treatment up to six hours, but did not recover
40	15	—	Not recovered

TABLE II

Tolerance limits of Cellana radiata to rising temperatures

Temperature at which spontaneous movement ceases	37°–38°C
Point of heat coma (Temperature at which irritability is temporarily lost)	39°–40°C
Lethal point (50%)	41°C
Lethal point (100%)	42°C

TABLE III

Time required for 50% and 100% mortalities at different higher temperatures

Temperature °C	Exposure period for mortalities (min)	
	50%	100%
34	210–240	300
36	150–210	240
38	60–90	210
39	45–60	120
40	20–30	60

Their active movement slows down when 36°C is reached and between 37° and 38°C the spontaneous movement of the animals ceased. At temperatures above 39°C the mantle margin often tends to curl away from the shell. The animals respond only to strong stimuli between 40°C and 41°C. All the animals subjected to 39°C and below recovered when returned to room temperature. At 40°C only 80 per cent and at 41°C 50 per cent of the animals recovered. None of the animals subjected to

42°C recovered. No marked differences were observed in the tolerance of the animals collected at the high and low-water zones to rising temperatures.

To determine the time required for 50 and 100 per cent mortalities at different higher temperatures the animals were maintained at various higher temperatures for varying periods in an electrically heated and thermostatically controlled bath. Fifteen animals were used in each experiment. Sets of five animals were taken out at regular intervals after they showed signs of inactivity and the number of animals recovering was noted. These experiments were performed at temperatures between 34°C and 40°C, at two degree intervals and the results are presented in Table III and Fig. 1. It may be seen from the Figure that the time required for 100 per cent mortality decreased with an increase in temperature, and that the decrease is progressive from 34°C to 38°C and steep from 38°C to 40°C.

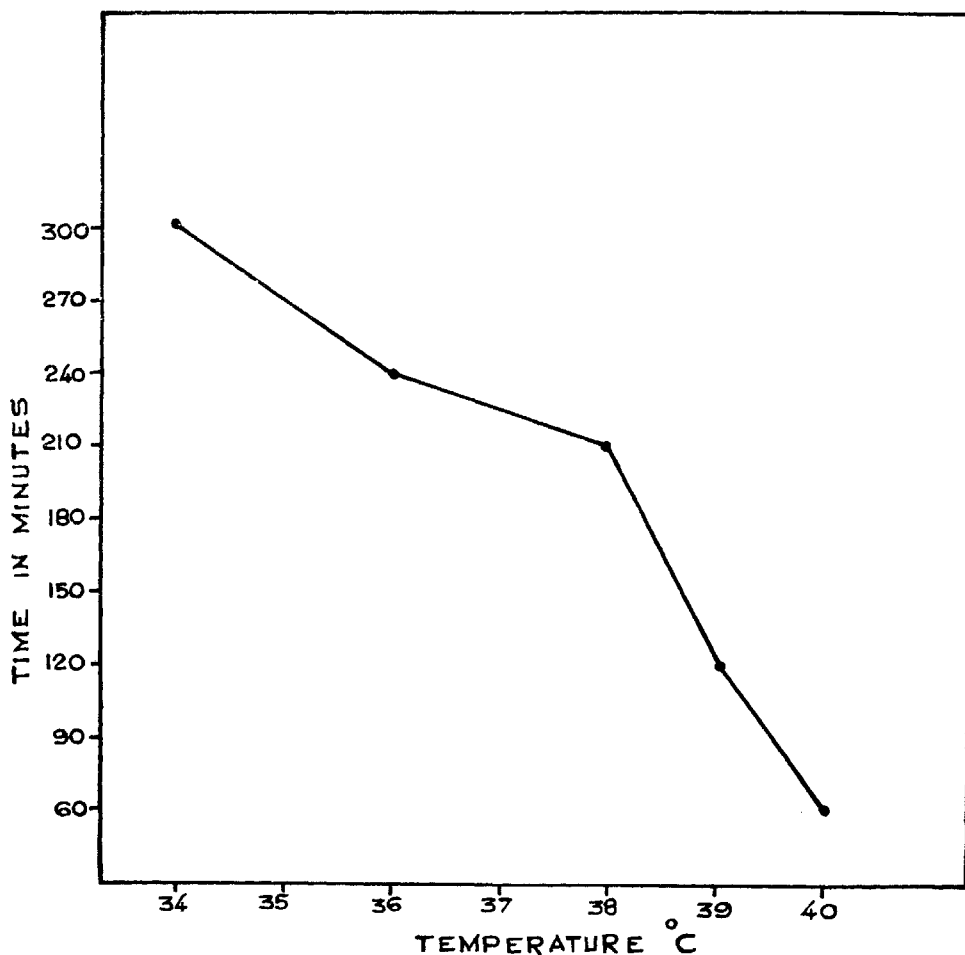


FIG. 1. Time required for 100 per cent mortality at higher temperatures.

Resistance to low temperatures

To study the tolerance of the limpet to lower temperatures, the animals were exposed to different lower temperatures for a period of one hour. These experiments were carried out in a B.O.D. incubator, and at temperatures between 16°C and 8°C, at two degree intervals. Five animals were used at a time for each experiment. They were placed directly in the sea-water which was maintained at the required temperature in the B.O.D incubator. After exposure for exactly one hour, the animals were brought to room temperature and the number of animals recovering was recorded. The results of these experiments are summarized in Table IV. It may be seen from the Table that all the animals subjected to lower temperatures ranging from 16°C to 8°C for one hour recovered when returned to room temperature. No marked differences were observed in the activity of the animals collected at the high and low-water zones when subjected to different lower temperatures.

TABLE IV

Resistance of Cellana radiata to different low temperatures after exposure for one hour

Temperature °C±0.1°C	Numbers in experiment	Numbers recovered	Remarks
16	10	10	All recovered within 5 min
14	10	10	All recovered within 15 min
12	15	15	
10	15	15	All recovered within one hour
8	10	10	Recovered after 24 hr

Resistance to lower and higher salinities

Experiments were carried out at lower and higher salinities above and below the habitat range of salinity (24‰–34‰). The lower range of salinity studied was between 5‰ and 21‰ and the higher range between 37‰ and 51‰. The lower salinity concentrations were obtained by diluting sea-water of salinity about 30‰ with appropriate volumes of distilled water. Higher salinity concentrations were prepared by adding saturated solution of sea salt to normal sea-water. The salinity of the experimental solutions thus prepared was checked by standard Knudsen's method. Fifteen animals were used in each experiment. They were placed in 500 ml beakers containing experimental salinity solutions. The condition of the animals and also their response to stimulus in different salinity concentrations was noted at hourly intervals for the first twelve hours and later every six hours. Sets of three animals were brought to normal sea-water at regular intervals, after they showed signs of inactivity, and the number of animals recovering was noted. The beakers were maintained at a constant temperature of 25°±1°C. The sea-water was frequently aerated and changed every 24 hr. Preliminary experiments have shown no marked difference in the activity and resistance of the animals collected at the high and low-water zones to different lower and higher salinities. Experiments were performed

at four lower and four higher salinity concentrations below and above the habitat salinity range, and the results of these experiments are presented in Tables V and VI and Figs. 2 and 3. It may be seen from the figures that the time required for 100 per cent mortality decreased with a decrease and also with an increase in the salinity concentration below and above the habitat conditions respectively. Further it may also be seen from the figures that the time required for 100 per cent mortality decreased steeply from 20.84‰ to 15.07‰ (Fig. 2) and 37.39‰ to 40.48‰ (Fig. 3) and progressively from 15.07‰ to 5.52‰ and 40.48‰ to 50.66‰.

It is clear from the above observations that sea-water of salinities below 20.84‰ and above 37.39‰ are critical to the animals because there is a steep decrease in the period of their survival below and above these salinities respectively.

TABLE V

Effect of low salinity on the survival rate of Cellana radiata

Salinity (%)	Numbers in experiment	Temperature °C±1°C	Time required for mortalities (hr)	
			50%	100%
5.52	15	25	6-8	10
10.41	15	25	10-12	14
15.07	15	25	18-20	24
20.84	15	25	84-96	120

TABLE VI

Effect of high salinity on the survival rate of Cellana radiata

Salinity (%)	Numbers in experiment	Temperature °C±1°C	Time required for mortalities (hr)	
			50%	100%
37.39	15	25	96-120	168
40.48	15	25	25-37	48
45.12	15	25	23-25	30
50.66	15	25	10-23	25

Desiccation

To study the tolerance of the limpets to desiccation the animals were first allowed to settle on a glass plate and were then kept in dry air at a constant temperature of 28°C±1°C in an electrically heated and thermo-statically controlled hot air oven. First at the end of 6 and 12 hours, later at the end of every 3 hours batches of five animals were brought to sea-water and the number of animals recovering after different

periods of exposure in dry air was recorded, and the results are summarized in Table VII. It is clear from these experiments that the high-water limpets are more tolerant to dry air than those living at the lower levels.

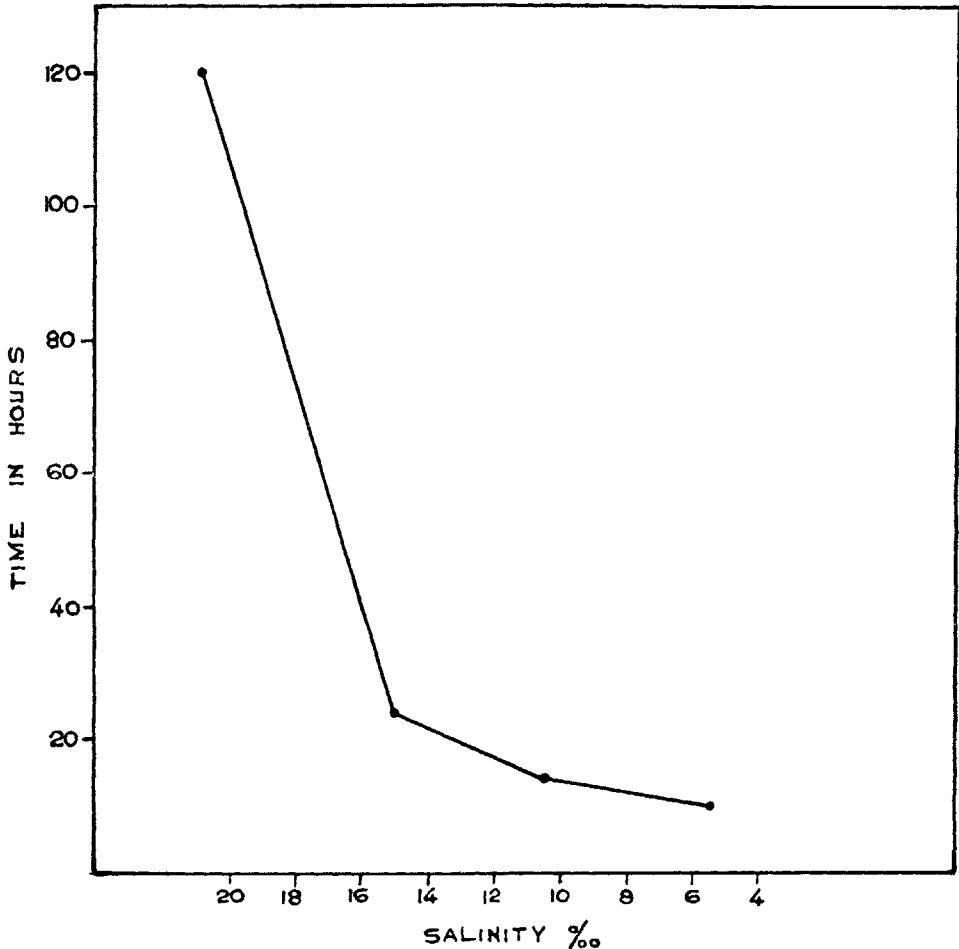


FIG. 2. Time required for 100 per cent mortality at different lower salinities.

Experiments were also performed on the tolerance of the limpets to dry air at different temperatures. Five animals were used in each experiment and they were maintained at different higher temperatures ranging from 32°C to 40°C in a thermostatically controlled hot air oven, for a constant period of 6 hr. Six hours exposure was chosen since the maximum period the animals are likely to be exposed to higher temperatures in the field is only 6 hr because of the semi-diurnal nature of the tides along the coast. After the prescribed period of exposure the animals were brought to sea-water at room temperature and the number of animals recovering was recorded. The results of these experiments are presented in Tables VIII and IX.

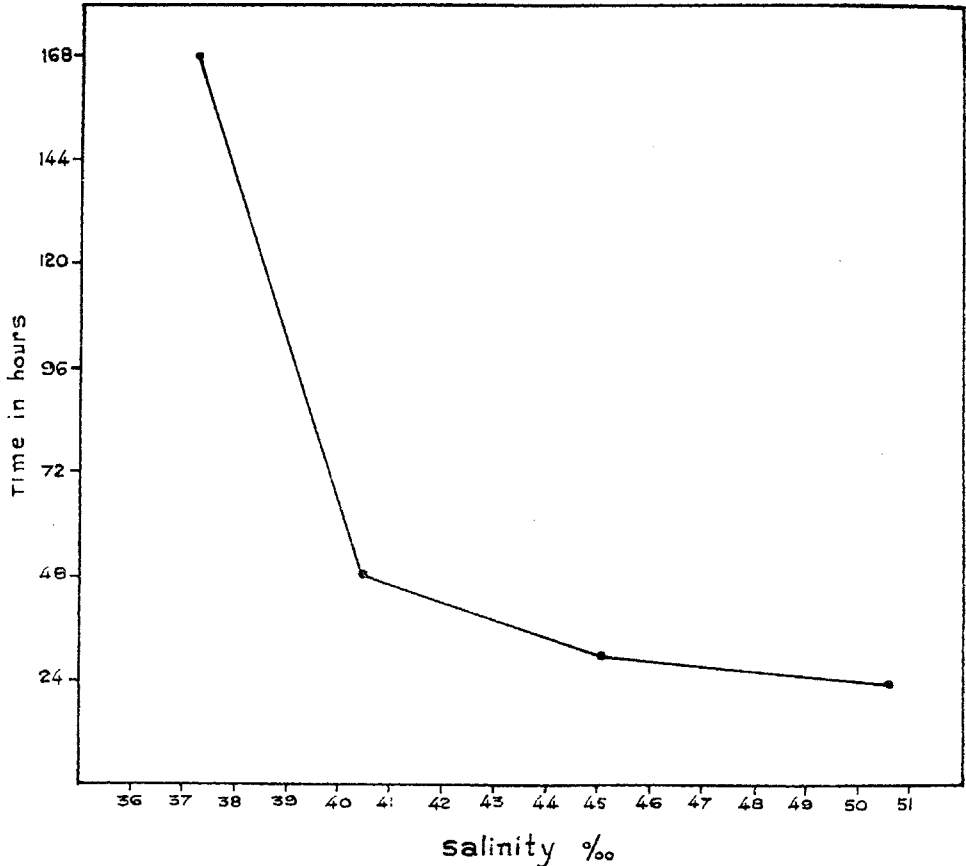


FIG. 3. Time required for 100 per cent mortality at different higher salinities.

It may be seen from these results also that the animals living at the higher levels are more tolerant to higher temperatures in dry air than those living at the lower levels.

To study the rate of water loss in relation to the body weight animals of different weights were selected. The encrustations on the shell were scraped with a scalpel. The initial weight of the animals was determined to the nearest milligram after wiping the animals with a clean cloth. Total weight of the animals was taken into consideration. The animals were first allowed to settle on a glass plate which was then kept in dry air at $28^{\circ} \pm 1^{\circ}\text{C}$ in a hot air oven. During the course of the experiment the weight of the animals was determined at hourly intervals for the first three hours, and later at the end of 6, 12, and 24 hr. The rate of water loss was calculated as the difference between two successive body weights. Experiments were performed on 50 animals. All the animals used in the experiment were sorted into 10 size groups of 50 mg class intervals depending on the body weight and the water loss was expressed in relation to these size groups. Only the average body weight in each size group and the corresponding average water loss at the end of 24 hr were taken into consideration for statistical treatment.

TABLE VII
Tolerance of Cellana radiata to desiccation

Level of collection	Time required for mortalities (hr)	
	50%	100%
High level	24-27	30
Low level	18-21	27

TABLE VIII
Tolerance of the limpets collected at high level, to dry air, at different temperatures, after exposure for six hours

Temp. °C	Period of exposure	Numbers in experiment	Numbers recovered	Remarks
32	6	5	5	Recovered within 5 min.
34	6	5	5	
36	6	5	5	Recovered within 15 min.
38	6	5	4	
		5	5	Recovered after one hour.
40	6	5	2	Recovered after 6 hr, two
		5	1	reactive after treatment, but did not recover.

TABLE IX
Tolerance of the limpets collected at the low level, to dry air, at different temperatures, after exposure for six hours

Temp. °C	Exposure period	Numbers in experiment	Numbers recovered	Remarks
32	6	5	5	All recovered within 5 min
34	6	5	4	
		5	5	Recovered after one hour.
36	6	5	3	
		5	3	Recovered after one hour, two reactive but did not recover.
38	6	5	2	
		5	3	Recovered after six hour.
40	6	5	-	
		5	-	One reactive after treatment, but did not recover.

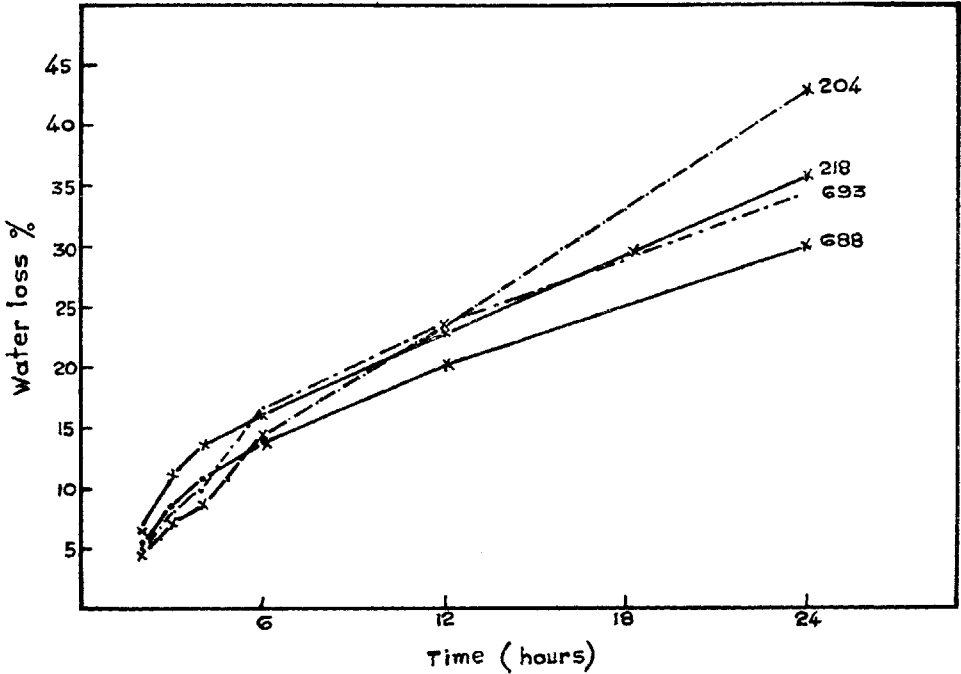


FIG. 4. The relationship between water-loss and time for limpets of different body weights collected at the high and low-water zones. (Solid Line, high level; Broken Line, low level).

The relationship between the water-loss and time for animals of different body weights collected at the high and low-water zones is shown in Fig. 4. It is clear from the Figure that the rate of water loss is high in the first 6 hr and more or less constant after this period. The high initial rate of water loss may be due to the evaporation of superficial and shell water.

The desiccation experiments have also shown that the total water loss increased with an increase in body weight. The increase in the total water loss was found to have an exponential relationship with increasing body weight. As shown in Fig. 5, when the relative log water loss/24 hr is plotted against the relative body weight, it shows a positive linear relation and the regression of relative log water loss/24 hours on the relative body weight is of the form;

$$Y = aW^b \dots \dots \dots (1)$$

where Y is the water loss/24 hours and W is the weight in mg.

Using the method of least squares the regression value in the above equation (1) was calculated independently for the limpets collected at the high and low-water zones. The results showed that in the high water limpets the water loss is proportional to the power 0.776 of the body weight and in the low water limpets to the power 0.825 of the body weight. The regression equations at the two levels are ;

- High level : $\text{Log. } Y = 0.776. \text{ Log. } W + 0.138.$
- Low level : $\text{Log. } Y = 0.825. \text{ Log. } W + 0.050.$

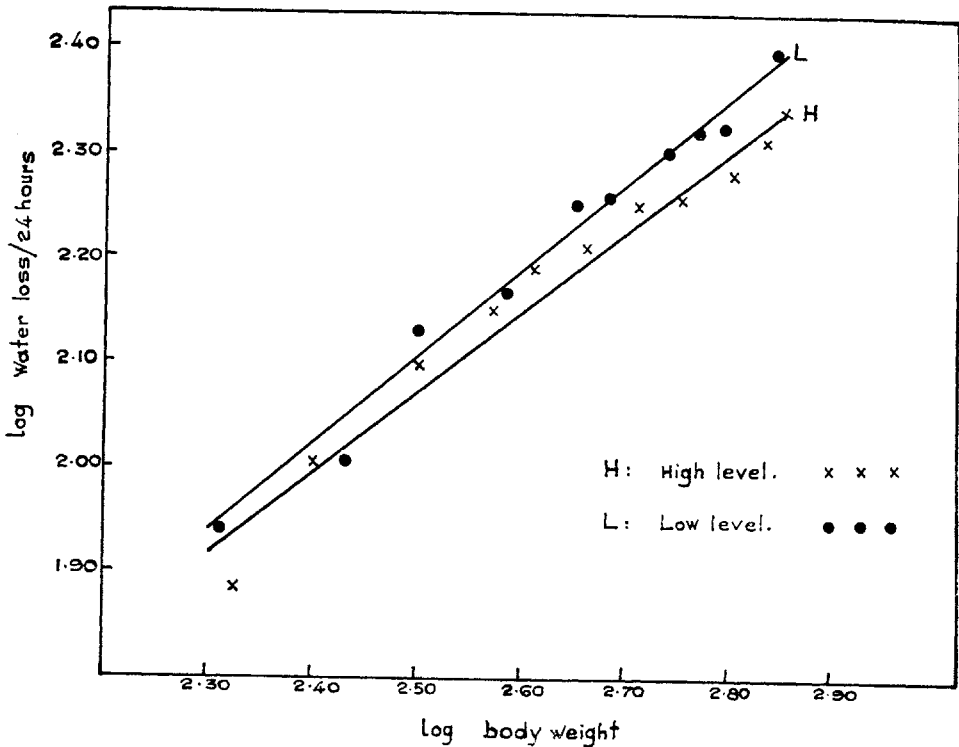


FIG. 5. Relationship between water-loss and body weight in the limpets living at the high and low-water zones.

As shown in Fig. 5 when the log water loss/24 hr is plotted against the log body weight, it shows that the regression line for the low water habitat lies above that of the high-water habitat.

The weight specific water loss i.e., water, loss per unit body weight/24 hr shows a decline with an increase in body weight (Fig. 6), a linear negative regression which is of the form :

$$\frac{Y}{W} = a W^{(b-1)} \dots \quad (2)$$

The regression values calculated independently for the weight specific water loss in the limpets living at the high and low-water zones are negative. The results showed that in the high-water limpets the total water loss is proportional to the power—0.224 of the body weight and in the low water limpets to the power—0.175 of the body weight.

It is clear from the desiccation experiments that the rate of water loss, when compared at the same body weight is different in the limpets living at the high and low-water zones and that the former lose water at a lesser rate than the latter.

The desiccation experiments have also indicated that there are differences in the tolerance of the animals living at the high and low-water zones to loss of body water. In a sample of low level limpets 50 per cent succumbed to a loss of water of

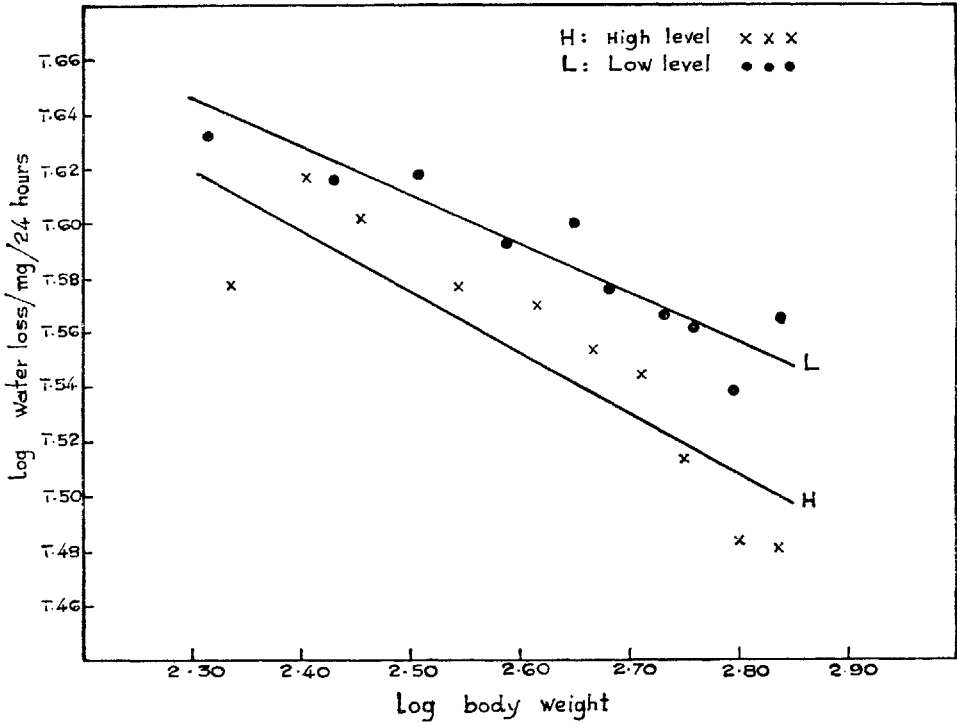


FIG. 6 : Relationship between weight specific water-loss and body weight in the limpets living at the high and low-water zones.

25–30 per cent of their body weight, while 50 per cent of the sample of the high level limpets were able to withstand a loss of water of 30–35 per cent of their body weight.

DISCUSSION

The results of the experiments have shown that *Cellana radiata* tolerates for short periods higher and lower temperatures and salinities above and below the ambient conditions. No marked differences were observed in the tolerance of the animals collected at the high and low-water zones to lower and higher temperatures and salinities. The lethal temperature of the animals was found to be 42°C. Salinities below 20‰ and above 37‰ appear to be critical to the animals.

The desiccation experiments showed that the animals living at higher levels are more tolerant to dry air, and also to higher temperatures in dry air than those living at the lower levels. The experiments also showed that the rate at which water is lost is related to the weight and the vertical position of the limpets. In both the high and low-water limpets the total water loss in the smaller animals is less than that of the larger ones, but the weight specific water loss is more. When compared at the same body weight the low-water limpets are found to lose water at a greater rate than those at higher levels.

Broekhuysen (1940) while studying the resistance of six species of prosobranchs in False Bay, South Africa, to desiccation, high temperatures and salinities above and below that of normal sea-water observed that their resistance is correlated with their position on the shore, and found that the high-level species are more tolerant than those at lower levels. In the present investigation the results show no marked differences in the tolerance of the animals collected at the high and low-water zones, to higher and lower temperatures and salinities. Marked differences, however, were observed in the tolerance of the animals collected at the high and low-water zones to desiccation and also to higher temperatures in dry air. The animals living at the high level were found to be more resistant than those living at low level. Evans (1948) while studying the lethal temperatures of some common British littoral molluscs found no marked differences in the lethal temperature of *Patella vulgata* collected at the high and low-water zones. He, however, points out that the animals are temporarily tolerant to higher temperatures, the possibility of which is very rare in the natural environment and that pure temperature effects are not of much importance in determining the upper limits of distribution of the animals. The results of the present study also suggest that variations in temperature or salinity alone may not be important in determining the upper limits of distribution of *Cellana radiata*. Southward (1958) while studying the temperatures tolerances of some intertidal animals in relation to the environmental temperature and geographical distribution found that the evidence for casual relation between temperature and distribution must be sought in non-lethal terms such as debilitating effects or indirectly through competition between species, or in combination with other factors. Newell (1964) summarizes that the heat-light-desiccation complex whose intensity will vary with the times of exposure directly or indirectly determines the upper limit of the animals in the intertidal region.

A comparison of the lethal temperatures of *Cellana radiata* and *Patella* spp. shows that the temperatures at which the spontaneous movement and irritability of the animals are lost were more in the former than in the latter (Table X). Southward (1958) while studying the temperature tolerance of some intertidal animals inhabiting the coasts around Britain found higher tolerance to high temperature by species of southern distribution and to low temperatures by species of northern distribution. The higher resistance of *Cellana radiata* is probably due to its tropical distribution, where it experiences uniformly higher temperatures in the ambient conditions unlike the temperature forms. Further, it can also be seen from the Table X that the range of temperature which *Cellana radiata* can tolerate after their spontaneous movement ceases is less (3° - 4° C) when compared with *Patella* spp. (10° - 12° C). Mayer (1914) points out that the tropical animals often live at temperatures very near to their thermal death points unlike the arctic forms which live in water as much as 13° C to 16° C below their death points.

Evans (1948) while studying the thermal resistance of British limpets observed that the thermal death points of the animals vary according to the time of the year. He found that the animals collected during the very cold months are less tolerant than those collected during the summer months. In the present investigation the results show no evidence of seasonal adaptation to temperature. This is probably

due to the absence of well-marked seasonal fluctuations in temperature in a tropical location like Waltair.

TABLE X

Comparison of the lethal temperatures of British limpets and Cellana radiata and the percentage of exposure to air experienced by these species

Species	Exposure limits (%)	Temp (°C) at which spontaneous movement ceases	Temp (°C) at which irritability lost (Heat coma)	Temperature (°C) of thermal death	Author
<i>Patella vulgata</i>	0-100	31-32	37	42.8	Evans (1948)
<i>Patella depressa</i>	0-92	31-32	37-38	43.3	Evans (1948)
<i>Patella athletica</i>	0-42	30-32	37	41.7	Evans (1948)
<i>Cellana radiata</i>	30-80	37-38	39-40	41-42	Present study

The results of the experiments carried out on the rate of water-loss in the limpets living at the high and low-water zones show that when compared at the same body weight the high-water limpets lose water at a lesser rate than those living at the lower levels. Davies (1965, 1969) while studying the desiccation effects in *Patella vulgata* made a similar observation in the animals collected at the high and low water zones. He points out that it is advantageous to the animals living in adverse environmental conditions at higher levels to withstand greater water-loss and lose water more slowly than those at lower levels and attributes these differences to the variations in the shape of the shell of the limpets living at the high and low-water zones. He states that the reduced rate of water-loss in the high level limpets is due to the steeper angle of their shell which appears to be true in *Cellana radiata* also.

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