Desiccation as a factor in the intertidal zonation of barnacles

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Abstract

Four species of balanomorph barnacles, *Balanus crenatus* Brugière, *B. balanoides* (L.), *Elminius modestus* Darwin and *Chthamalus stellatus* (Poli), were studied to assess the susceptibility of intertidal barnacle species to desiccation. Known sized samples of barnacles were exposed to controlled desiccating conditions and subsequent survival and water loss were determined. It is clear that the ability to live high on the shore is dependent on a reduction of the overall permeability to water loss. Because of greater surface area to volume ratios, small stages are particularly prone to desiccation. In normal intertidal emersion periods, small stages of *B. crenatus* particularly, and also of *B. balanoides* and *E. modestus* which are similar in their desiccation resistance, would be susceptible to desiccation at normal temperatures and low humidities. Large barnacles would be more prone to death from high temperatures when the tide is out. The spat of *C. stellatus*, although surviving much longer than spat of larger dimensions of the other species, must also be prone to prolonged emersion conditions at high shore levels.

Introduction

In the intertidal habitat, marine animals encounter more rapid fluctuations and wider ranges of physical environmental factors than they would below the influence of tides and waves. Intertidal organisms must be able to avoid or tolerate the severe conditions of the emersion environment. Balanomorph barnacles are characteristic rocky shore forms; they cannot move to more favourable habitats when environmental factors become adverse and thus their adaptations for an intertidal existence must include increased tissue tolerance or mechanisms to protect the tissues under all likely conditions. Intertidal barnacles resist the entry of fresh water into the mantle cavity by controlled use of the opercular valves (Foster, 1970). Increased tolerance to low salinity has been observed only in those species inhabiting brackish waters. Intertidal barnacles tolerate more extreme temperatures than do characteristically sublittoral species, but the cumulative effect of continued exposure to high temperatures when the tide is out must occasionally result in some intertidal barnacles living not far below lethal temperature conditions (Foster, 1969). However, before rock surface and barnacle tissue temperatures reach lethal values water may be lost from the organism.

The factors of the environment causing water loss are embodied in the term desiccation, as normally used to describe an obvious hazard to intertidal marine life. Desiccation, unlike temperature, humidity or wind speed, is not a simply measurable factor under natural conditions on the shore; it results from the combined effects of these 3 variables on the rate of water loss from the integument of the organism. Desiccation is commonly held to determine the upper limit of intertidal zonation of animals and plants of marine origin. There are few publications that provide quantitative experimental verification of this view.

For 6 species of South African intertidal gastropods, Brown (1960) found some correlation between the rate of water loss and the intertidal distribution. With pulmonate limpets, Allanson (1958) found that low level *Siphonaria aspersa* had a greater desiccation tolerance (the water loss at death) than the higher shore species *S. capensis* and *S. deflexa*, but lost water at a faster rate which was probably attributable to the greater exposure of the soft parts to the air owing to its lack of a home scar. In the prosobranch limpets *Patella aspersa* and *P. vulgata*, Davies (1969) found some intra and inter-specific differences in the rate of water loss and the tolerance of water loss, both of which were correlated with intertidal distribution. These molluscs are potentially able to change their intertidal positions both in relation to the severity of environmental factors and in relation to their age and size by migrating to different shore levels or to more favourable microhabitats. Crevices present more favourable microclimates than open rock surfaces, and for a number of crevice-inhabiting species Kensler (1967) found a correlation between resistance to desiccation, measured by the time of survival under constant conditions of temperature and humidity, and both the distribution within the crevice and the intertidal position of the occupied crevice.

Barnacles on the open rock surface have survived there since settlement without the advantages of more favourable microhabitats. Adult intertidal barnacles can survive for long periods out of water; Monterosso (1930) noted an occasion when specimens of the high tidal warm-water species *Chthamalus depressus*

survived at least 119 days on a laboratory bench at ordinary temperatures. BARNES et al. (1963) showed that the order of survival of adult *C. stellatus, Balanus balanoides* and *B. crenatus* in dry air at unspecified temperatures was correlated with the intertidal distribution of the species. The adaptations of barnacles for intertidal existence include controlled use of the opercular valves when out of water (BARNES and BARNES, 1957, 1964; CRISP and SOUTHWARD, 1961) which allows intertidal barnacles to use the mantle cavity as a lung for aerobic respiration while minimising water loss from the surface of the mantle cavity and body (BARNES et al., 1963; GRAINGER and NEWELL, 1965; AUGENFELD, 1967). NEWMAN (1967) studied water loss from the intertidal species *Balanus amphitrite* and *B. glandula*, and the low tidal and estuarine *B. improvisus*. Under what are to be assumed constant drying conditions, NEWMAN found that *B. improvisus* lost water at a faster rate than the other 2 species, but the main conclusion was that the problem of desiccation resistance involved dimensional, behavioural and physiological differences, and required more detailed study.

Because barnacle species display obvious zonation patterns on rocky shores, they are suitable organisms for studying the effect of exposure to the air in determining upper limits of zonation. Conversely, from a knowledge of desiccation resistance of the species, some idea of the severity of desiccation conditions on a shore may be inferred. This paper reports some experiments designed to assess susceptibility of the settling, metamorphosing and young spat stages of *B. balanoides* to desiccating combinations of temperature and humidity, and compares the desiccation resistance, based on survival and water loss, of adults of the high tidal *Chthamalus stellatus* (POLI), the 2 characteristically intertidal species *Elminius modestus* DARWIN and *Balanus balanoides* (L.), and the low tidal *Balanus crenatus* BRUGIÈRE.

In the experiments reported here, an attempt was made to standardise some of the variables that contribute to water loss. The need to use moving air in desiccation experiments has been emphasized by RAMSAY (1935) and EDNEY (1957), and the first to put this into practice when studying the desiccation resistance of intertidal animals was KENSLER (1967). Moving air reduces the thickness of the concentration boundary layers of water vapour resulting from the diffusion of water from the animal. The greater the wind speed, the more will the integument of the animal constitute the overriding resistance to water loss. To minimise variations in survival time and water loss due to differences in shape of the barnacle, and also to environmentally induced differences due to the nature of the substratum or to acclimation at varying intertidal levels, the adult barnacles used, except for *Chthamalus stellatus*, were cultivated from the time of settlement at the same place under constant conditions of submersion in the natural conditions of the sea.

Water loss is usually measured gravimetrically. Such a method is not very suitable for barnacles, because of their attachment to portions of the substratum. Weight changes, even if readily measurable, would also include water lost from the shell and from parts of the substratum to which it was attached, neither of which is relevant to the tissue tolerance. The proportionate water lost from the body has been calculated from the depression of freezing point of the body fluids, an approach used by SEGAL and DEHNEL (1962) and DAVIES (1969) to determine the state of desiccation of limpets on the shore.

Materials and methods

Cyprid larvae of *Balanus balanoides* were collected from the plankton. Some were induced to settle on 5 cm × 2 cm Tufnol panels in the laboratory, as reported previously (FOSTER, 1969), and were used in experiments as either settled unmoulted cyprids, or as spat after the moult had been completed and some calcareous constituents of the shell plates had appeared.

Cyprids of *Elminius modestus, Balanus balanoides* and *B. crenatus* were allowed to settle on 10 cm × 5 cm Tufnol panels which were submerged on wooden frames from a moored raft in the Menai Strait. *B. balanoides* settled in early May, and *E. modestus* and *B. crenatus* during the summer and autumn. The panels were allowed to accumulate settled cyprids for a few days, but thereafter they were frequently scrubbed, lightly at first and more vigorously later, to remove any further settlement of barnacles or other fouling organisms. If the initial settlement was too dense, subsequent thinning was necessary to allow for growth without crowding. In some cases initial settlement was sparse, and after some weeks of growth of the first settlers, a subsequent settlement of the same or another species was allowed to occur and grow on the same panels. Some 500 panels were used from May 1967 to May 1969. Except for an occasional specimen of *B. improvisus* and 2 of *B. balanus*, no other barnacle species settled on the panels. In this way, populations of approximately equal sized adult barnacles of the 3 dominant species were cultivated in batches of more or less equal numbers under natural conditions of continuous submergence on a substrate of a standard size and material. Prior to the experiments, the required number of panels with the required sized barnacles were harvested, and scrubbed in sea water to remove mud and unwanted fouling organisms.

Chthamalus stellatus does not settle in the Menai Strait, nor does this species settle readily on artificial surfaces. Thus, the survival experiments performed on the other species could not be replicated exactly on *C. stellatus*, but some idea of its desiccation resistance was obtained with spat settled naturally on high tidal levels at Thurlestone Rocks (Devon, England), and

with adults from high tidal levels at Aberffraw (Anglesey, Wales). Natural settlement in Anglesey was too sparse to provide sufficient numbers and density of young stages.

The desiccation apparatus was that described and figured by KENSLER (1967). Some unavoidable temperature and humidity fluctuations occurred with the opening of the cabinet and desiccators for access, but these were short term. Otherwise, in each desiccator, temperatures and relative humidities (R. H.) were maintained at ± 1 °C and ± 2% R.H., respectively. Temperatures below ambient were obtained by placing the cabinet in a constant temperature cold room.

The barnacle-bearing panels were vigorously shaken to remove surplus water and then arranged in groups of 10 around the circumference of each desiccator on the gauze tray, but leaning on the desiccator wall so that the barnacled surface was directed towards the fan. The time taken for the surface of the panels to appear dry depended on the quantity of barnacles growing on them, as well as on the temperature and humidity. The amount of moisture retained within the interstices of the shells increased with the size of the animal and has been taken as a property associated with the size of the specimen. The times of survival were measured from such a condition of 'standard wetness', rather than from an appearance of dryness of the surface which would be considerably harder to standardize. Panels were withdrawn from the desiccators at intervals according to the expected time of survival. It became increasingly easier to predict the time of withdrawal to achieve 50% survival without the need for trial or preliminary runs. After withdrawal from the desiccators the panels were immediately reimmersed in trays of running sea water.

In the case of the settled unmoulted cyprids, the completion of metamorphosis after 48 h recovery time was taken as evidence of survival. Successful metamorphosis was judged from the shedding of the cypris carapace and the attachment of the base of the spat to the substratum. The spat and adults were assumed to have survived if the cirri and opercular valves withdrew completely in response to touching the opercular valves with a needle. Unlike specimens subjected to high temperature under water which often appeared moribund, very few specimens exposed to desiccation gave a sluggish response after the recovery time. The numbers dead were converted to percentage kill, and the median lethal times (M.L.T.) obtained from a straight line regression fitted by eye to the probit — log. time plot, as before (FOSTER, 1969).

To determine proportionate water loss, barnacles were removed from the substratum and immediately immersed in liquid paraffin to prevent further water loss. Within the next few minutes blood samples were extracted from the subscutal sinus, mounted between liquid paraffin in silica capillaries and immediately frozen; essentially as described previously (FOSTER,

1970). One barnacle provided blood for only 1 determination. With more severe drying, blood could not be obtained from the sinus without risk of rupture of the body tissues. Contaminated samples were discarded. The depression of freezing point of each sample was determined individually by the apparatus and procedure described by RAMSAY and BROWN (1955). Total water content of barnacles was determined gravimetrically by drying cyprids or isolated prosomas of adult barnacles at 105 °C for 24 h. The basal rostro-carinal diameter was taken as the measure of the size of each barnacle.

Survival of embryos of *Balanus balanoides* within the mantle cavity of adults during desiccation

The developing embryos are retained as ovigerous lamellae within the mantle cavity of the parent, and

Table 1. *Balanus balanoides. Relation between adult survival and viability of egg masses. Parent barnacles, of mean diameter 10.7 mm, were desiccated for the period stated in column I at 0% R.H. and 10 °C, and the numbers surviving and dead after 24 h are given by the totals of columns II and III, respectively. Column IV gives the adult mortality read off the probit regression line*

I Desiccation time (h)	II Adults recovered			III Adults died			IV Adult mortality (%)
	i good hatch	ii poor hatch	iii no hatch	i good hatch	ii poor hatch	iii no hatch	
86	11	0	1	0	0	0	0
97	5	1	0	0	0	0	0
109	4	1	0	0	0	0	0
132	5	0	0	0	1	0	6
146	5	0	0	0	4	1	14
164	5	0	0	0	0	5	27
182	5	1	0	3	0	4	43
192	12	1	0	1	1	8	54
204	3	1	0	0	0	9	65
214	11	1	0	0	0	11	71
228	0	0	0	0	1	11	80
258	0	1	0	0	0	11	92
Totals	66	7	0	4	7	60	

thus experience the same physical stresses as the body of the parent. Table 1 gives the results of a desiccation experiment, carried out in March, 1969 (when the eggs of *Balanus balanoides* were all fully mature and ready to hatch) to assess the survival of the embryos in relation to the survival of the parent barnacles carrying them. The adults were allowed to recover for 24 h in running sea water. Nauplii were not released in this time. The ovigerous lamellae from recovered and dead barnacles were removed and teased apart in finger

bowls of sea water. Hatching of the nauplii occurred within 1h, if it occurred at all. The totals of columns II and III show that there was a strong correlation between, on the one hand, recovery of the adults and the hatching of the nauplii, and on the other hand, death of the adults and failure of the nauplii to hatch. Some lamellae appeared very dry and crumbled readily yet still recovered to yield vigorously swimming nauplii. The embryos were unlikely to have received prolonged protection from water retained in the mantle cavity, as the latter appeared dry well before any noticeable shrinkage of the prosoma or mantle tissue occurred. The nauplii thus received sufficient protection from the thin common membrane of the lamella, their individual egg membranes and external integument, to allow them to survive as long as the parents. Both embryos and parents are, of course, protected by the common parental shell and operculum.

Desiccation tolerance of cyprids of *Balanus balanoides*

The water loss from 0.2 to 0.5 g samples of cyprids in a desiccator at zero humidity and 15 °C is shown in
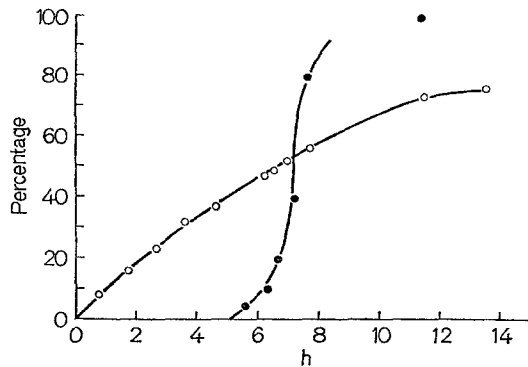

Fig. 1. *Balanus balanoides*. Desiccation of cyprids in still air at zero humidity and 15 °C. o water loss as percentage of the fresh weight; ● percentage mortality

Fig. 1 along with the survival curve determined from the recovery of similar samples after 24 h reimmersion in seawater. Weight losses were determined gravimetrically. At the median lethal time the weight of the cyprids had decreased by 53 %. The water content of fresh cyprids (based on drying 3.7 g of cyprid larvae) was 83 % of the fresh weight. Thus, the cyprids had lost 64 % of the body water at the median lethal time.

The effects of emersion on metamorphosis of settled cyprids of *Balanus balanoides*

After the exploratory phase of settlement activity (Crisp and Meadows, 1963), cyprids irrevocably

cement their antennules to the substratum and undergo metamorphic reorganization culminating in the moult to the juvenile barnacle or spat. The limitation of time spent in pre-settlement behaviour is of increasing importance for settlement at increasing heights on the shore. To ascertain the minimum time required for settlement, small Tufnol panels were "conditioned" for settlement by allowing many cyprids to settle, and then removing them. Such panels accumulated many more exploring and attached cyprids when used again than did unconditioned panels, an expression of the gregarious response to the remnants of previous individuals (Knight-Jones, 1953). By offering these conditioned panels to large

Table 2. *Balanus balanoides*. Numbers of cyprids attached and settled on conditioned Tufnol panels (5 cm × 2 cm) at time intervals after the panels were placed in a rotating dish of cyprids in sea water at 14 °C. The panels were shaken in the sea water of the dish before removal, then rinsed for 2 min in fresh water, when those attached released their adhesion to the panel, leaving behind those permanently settled

Time (min)	Number attached to panel	Number settled on panel	Time (min)	Number attached to panel	Number settled on panel
8	474	0	33	186	13
11	307	0	35	294	54
13	254	0	37	136	14
15	239	0	39	209	30
17	396	0	41	190	70
19	244	2	43	189	16
21	222	2	45	227	25
23	191	4	47	188	25
25	309	2	49	148	27
27	184	3	51	145	10
29	157	10	53	205	16
31	302	21	55	202	63

numbers of cyprids and withdrawing panels at intervals, the number of attached and cemented (settled) individuals was determined. Prolonged rinsing in fresh water caused those that had merely attached to release their adhesion with the substratum and so distinguished them from cemented cyprids which cannot detach. The results are shown in Table 2. Under these conditions, which have been the best for settlement so far attained in the laboratory, settlement began as early as 19 min after initial attachment. Clearly, a cyprid on encountering the shore and all the positive stimuli aiding the settling response, can potentially settle at high shore levels.

After settlement, cyprids of *Balanus balanoides* kept in well oxygenated running sea water moult to the spat in about 20 h at normal temperatures. On a shore with semi-diurnal tides, the settled cyprids of this species will thus be exposed to the atmosphere for

1 or 2 low tides before the moult. In water-saturated atmospheres in desiccators, the Tufnol panels retained a surface film of seawater and most of the cyprids completed metamorphic changes and underwent the moult to the spat, although they took longer to shed the cypris carapace than did specimens kept fully immersed (Fig. 2); indeed, some were not able to shed the carapace completely. Cyprids exposed continuously to drying atmospheres, however, did not moult.

To assess the effect of the period of exposure to a drying atmosphere on the rate of metamorphosis,

time of exposure in Fig. 4. If all metamorphic processes had stopped during emersion and recommenced at the normal rate on reimmersion then the delay
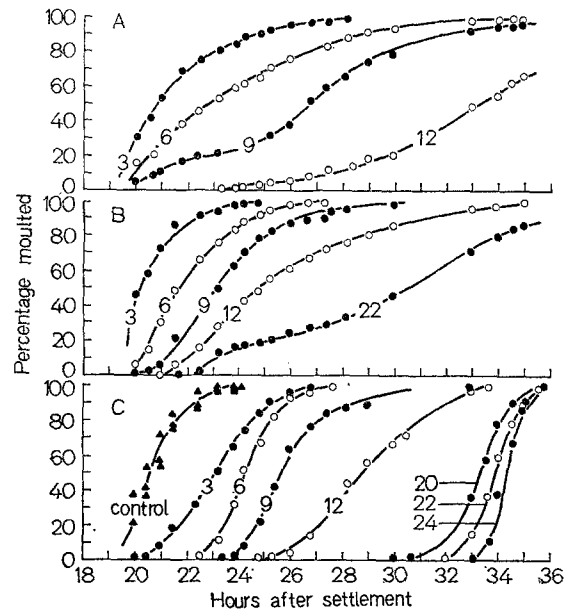

Fig. 3. *Balanus balanoides*. Moulting of cyprids which, immediately after settlement, were exposed in an atmosphere of 75% R.H. at 21 °C (A), 13 °C (B) and 4 °C (C) for the duration, in h, as indicated. Specimens were reimmersed in running sea water at 13 °C. 200 to 500 specimens were used in each sample. The time axis includes the time of emersion. Controls, continuously immersed, in C (▲)

Fig. 2. *Balanus balanoides*. The time from attachment to moulting at 15 °C of cyprids immersed in still sea water (●), or exposed to an atmosphere of 100% R. H. (o). No moulting occurred in 75% R.H. or lower humidities

3 batches of experimental panels with recently settled cyprids were exposed to atmospheres of 75% R.H. in desiccators at 21°, 13° and 4 °C. At 21° and 13 °C the panels dried off in about 3 h, but they took slightly longer at 4 °C. Panels were withdrawn at appropriate intervals of time and reimmersed in running seawater at 13 °C. The progress of the cyprids was compared with ones that had been settled on corresponding control panels that had been continuously immersed in seawater at the same temperature as the experimental panels. The numbers that had moulted were counted at frequent intervals up to 36 h after the start of the experiment. The cumulative percentage moults are shown in Fig. 3. It is apparent that after the surface had dried, the time required to complete moulting was increased roughly in proportion to the time spent out of water. The delay in the time taken for 50% of each sample to moult is plotted against the
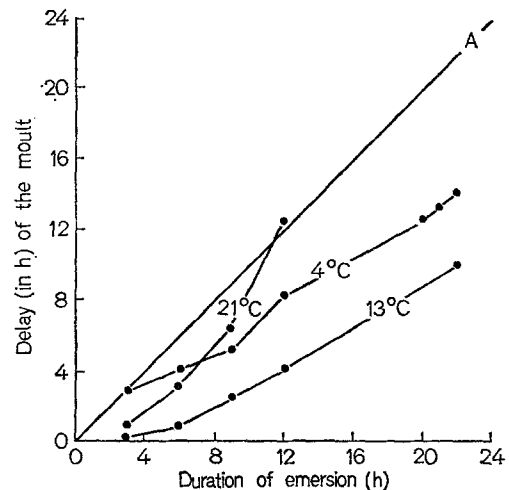

Fig. 4. *Balanus balanoides*. Delay in time for 50% of settled cyprids to moult after periods of exposure to 75% R.H. and the temperatures indicated, as in Fig. 3, compared with the time immersed in sea water at the same temperature. The line OA represents a delay equal to the time of emersion. The abcissa represents no effect of emersion on the time to moulting

would be equivalent to the time of emersion and be represented by the line OA. It is clear that the delay is normally less than the time spent out of water; hence, either some premoult changes took place during emersion, or such changes proceeded faster than normal after reimmersion. However, prolonged emersion at high temperatures, as after 9 h at 21 °C and 75% R.H., resulted in a delay at least equivalent to the time of emersion. These more extreme drying conditions evidently further delayed metamorphic processes or prevented any compensatory acceleration from taking place. After 12 h at 21 °C and 75% R.H. 42% of the cyprids failed to complete metamorphosis within 48 h reimmersion, and after 24 h at 4 °C and 75% R.H., 20% failed to recover. The desiccation effects are first externally apparent as lateral indentations of the anterior carapace. At some stage subsequent to this the cyprid died and literally shrivelled, although still remaining firmly cemented to the panel. Thus, settled cyprids can proceed with premoult metamorphic processes out of water, but if drying conditions are too severe such changes are retarded and stopped, at first temporarily, then permanently with ensuing death.

Survival of settled cyprids and spat of *Balanus balanoides* under desiccating conditions

The median lethal times (M.L.T.) determined from the regression lines fitted to probit — log. time transformations of mortality data of settled cyprids and spat of *Balanus balanoides* under stated conditions of temperature and humidity are plotted in Fig. 5 against temperature for the 4 humidities used. Also included are the time-temperature-survival curves (broken lines) determined for these stages when continuously submerged (FOSTER, 1969). As would be expected, at lower temperatures a quicker kill was obtained at lower humidities. At higher temperatures, the convergence of the curves for each humidity towards the time-temperature-survival curve when immersed indicates the increasing importance of high temperature as a cause of death. A comparison of the corresponding curves for cyprids and spat (Fig. 5) reveals that the spat were more tolerant of high temperatures in agreement with their higher upper lethal temperatures measured under conditions of submersion (FOSTER, 1969), and that they also survive longer at low temperatures where desiccation is the main cause of death. The difference between the actual survival times of spat and cyprids was greater at high humidities. Table 3 gives the survival times at a temperature of 25 °C, which is often attained by intertidal rock surfaces in the summer. At higher humidities the difference is substantial and could be critical for survival from one period of immersion to the next.

The relationship between temperature, humidity, and time of survival can be illustrated in a variant of

Fig. 5 as contours of survival time against temperature and relative humidity. The appropriate diagram for the spat is shown in Fig. 6a. The interpretation of such diagrams is aided by describing the relative drying

Table 3. *Balanus balanoides*. Time for 50% mortality of settled cyprids and spat when exposed to various humidities at 25 °C (from Fig. 4)

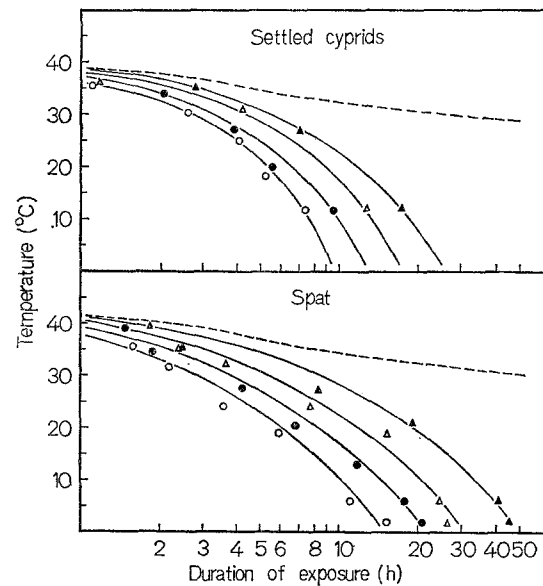| Humidity (% R.H.)	Median lethal time (h) Cyprids	Spat
0	4.1	4.3
25	5.0	5.8
50	6.6	8.3
75	8.6	13.2

Fig. 5. *Balanus balanoides*. Median lethal time (h) of settled cyprids plotted on a logarithmic scale in relation to temperature at 0% R.H. (o), 25% R.H. (●), 50% R.H. (△) and 75% R.H. (▲). The dashed curves are the time-temperature survival curves for settled cyprids and spat when continuously immersed in sea water

power, or 'desiccation potential' (KENSLER, 1967), of various combinations of the temperature and humidity in terms of the saturation deficit which is the amount by which the water vapour present falls short of the saturation value at that temperature. We may write

$$\text{saturation deficit} = p_s - p_o = p_s (1 - \text{R.H.})$$

where p_o is the actual water vapour pressure, and p_s is the saturated water vapour pressure which varies

with the temperature. Contours of equivalent desiccating conditions (all other factors being taken as equal) are shown as broken lines in Fig. 6. The desiccation potential refers to the main body of air in the desiccators, not necessarily to the conditions at the surface of the animals where evaporation occurs. The rate of evaporation from the integument will depend on the humidity gradient established from water diffusing from the animal, and the temperature difference between the surface and the air, although moving air will tend to minimise these gradients.

correspondence between survival contours and desiccation potential as humidity falls suggests that dehydration becomes the main cause of death.

Survival of adult *Balanus balanoides*, *B. crenatus* and *Elminius modestus* under desiccating conditions

Adult barnacles of known size, grown under continuous submersion from a raft, were subjected to various temperature and humidity combinations for known periods of time. The resulting regression lines
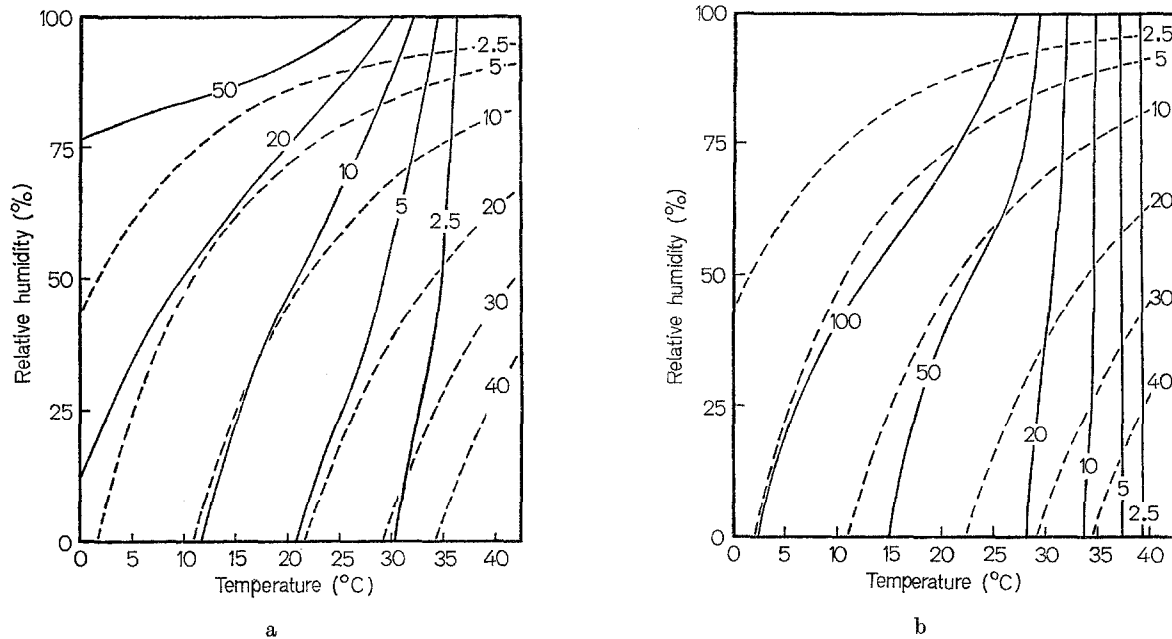

Fig. 6. *Balanus balanoides*. Contours of median lethal time in h (solid lines) in relation to temperature and humidity for spat (a) and 5 mm basal diameter *B. balanoides* (b). The dashed lines represent contours of desiccation potential in mm Hg aqueous vapour pressure deficiency

At low humidities the desiccation potential curves closely parallel the contours of survival times. Hence, below the points of inflection of the survival curves, the M.L.T. is roughly proportional to the reciprocal of desiccation potential. At high humidities and at high temperatures the survival curves depart from the desiccation potential curves, becoming nearly parallel to the humidity axis: this implies that temperature was the main determinant of survival time in such environments. In water saturated atmospheres there should be no water loss and the survival contours read off along the 100% R.H. axis agree with the values obtained from time-temperature-survival curves under continuous submersion. At lower humidities, loss of water and its consequent lethal effects cause the survival contours to diverge from the upper lethal temperature values for continuous submersion. The

fitted to the probit of mortality on the logarithm of time of exposure, gave M.L.T.s which, when plotted against temperature for different size groups and humidities, suggested that all the curves were of the same type and resembled those shown in Fig. 5. The curves were then arranged as contour diagrams similar to those shown in Fig. 6. As an example, the diagram for individuals of *Balanus balanoides* of 5 mm rostro-carinal basal diameter is shown in Fig. 6b for comparison with survival of the spat. The contour diagrams in the plane XOY for each size group were then arranged along a third axis (OZ) representing the basal rostro-carinal diameter taken as a characteristic linear dimension. The 3 dimensional diagrams relating M.L.T. to size, temperature and humidity are illustrated in Fig. 7 for *B. balanoides*, *B. crenatus* and *Elminius modestus*. The form of the contours represent-

ing interaction of the humidity and temperature of the air in causing death of each size group lies on the appropriate XOY plane. Immediately obvious, by following the changes along the Z axis, is the advantage of increased size in delaying death under otherwise equivalent desiccating conditions. The most favourable region for survival in the space enclosed by the axes is that of highest humidity, lowest temperature and

greatest size. Since lethal temperatures are not size dependent in adult barnacles, the larger sized barnacles may be killed directly by high temperatures, whereas the smaller sizes under identical conditions may die earlier as a result, primarily, of desiccation.

Comparison of the diagram for *Elminius modestus* with that of either *Balanus balanoides* or *B. crenatus* (Fig. 7) reveals the advantage of higher temperature
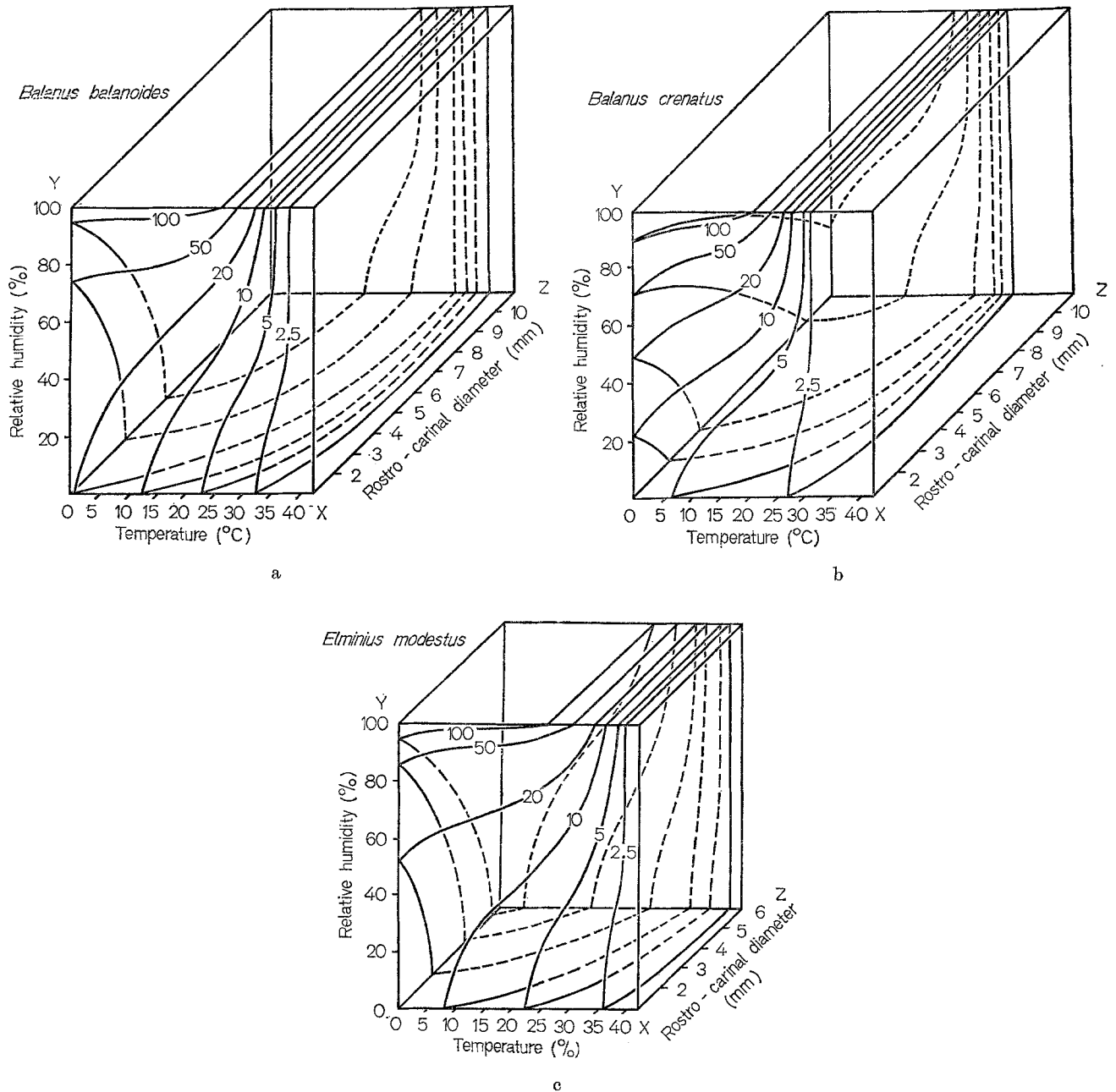

Fig. 7. Diagrams of 50% survival contours of (a) *Balanus balanoides*, (b) *B. crenatus* and (c) *Elminius modestus* when exposed to atmospheric temperature (OX) and humidity (OY) in relation to the basal rostrocarinal diameter (OZ) of the barnacle

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tolerance in shifting further to the right the regions of survival contours which diverge from the desiccation potential contours.

In Fig. 8, the M.L.T.s are plotted against the temperature which caused 50% mortality in various size groups at a desiccation potential selected to cover the whole temperature range, namely 5 mm Hg aqueous vapour pressure deficiency, all other experimental

conditions being assumed constant. The lethal effects of desiccation alone are represented by the vertical parts of the curves where the M.L.T. is affected by temperature only through its influence on the rate of evaporation and not by any harmful effect on the tissues. At the highest temperatures endured, the curves are asymptotic to the time-temperature-survival curves, and temperature is clearly the lethal
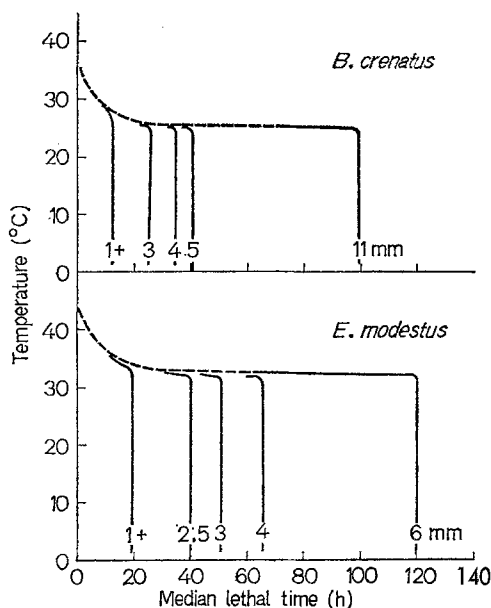

Fig. 8. *Balanus crenatus* and *Elminius modestus*. Median lethal time of various sized specimens (basal rostro-carinal diameter in mm) in relation to temperature at a standard desiccation potential of 5 mm Hg aqueous vapour pressure deficiency. The broken lines are the time-temperature survival curves under continuous submersion

Fig. 10. Survival of barnacles with respect to size and temperature after 6 h at zero humidity, taken to represent conditions on the shore. The curves separate regions of greater than 50% mortality above, from regions of less than 50% mortality below the curves for each species
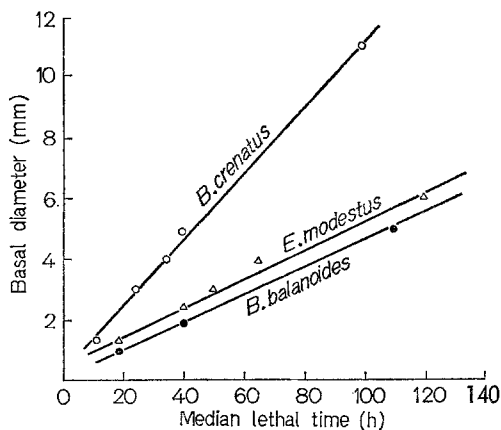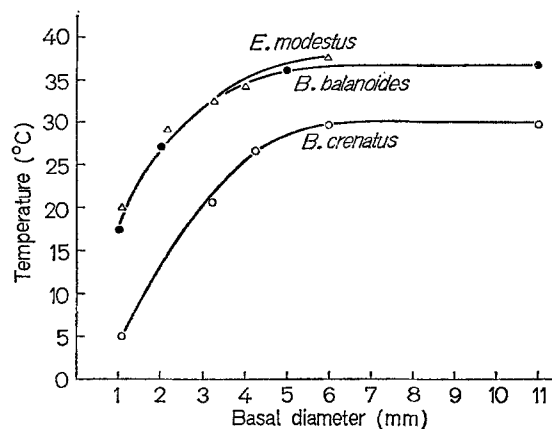

Fig. 9. Relation between the median lethal time and the size of the barnacle for a standard desiccation potential of 5 mm Hg aqueous vapour pressure deficiency. Under these conditions 11 mm *Balanus balanoides* survived for at least 200 h

factor. Fig. 8 has the advantage of displaying clearly the time of survival under the stress of desiccation alone. In Fig. 9, these times are plotted against size for the 3 species studied. Fig. 9 provides empirical evidence that survival is linearly proportional to a characteristic linear dimension of the animal.

Balanus crenatus is more susceptible to desiccation than either of the other species. When individuals of equal size are compared, the proximity of the lines for *B. balanoides* and *Elminius modestus* in Fig. 9 indicates the similarity of these two species in their desiccation resistance. If any significance could be attached to the difference between the lines, then *B. balanoides* appears to have survived slightly longer than *E. modestus* under conditions of desiccation only. At the upper extremes of temperature *E. modestus* survived better by virtue of its greater tolerance of high temperatures.

To relate the survival times of individuals of various sizes to drying conditions in the intertidal zone, the temperatures causing 50% mortality at zero humidity after 6 h exposure are compared in Fig. 10. Other times could be selected to correspond to periods of emersion at various tidal levels. The horizontal parts of the curves in Fig. 10 are asymptotes to the 6 h

upper-lethal-temperature. The smaller sizes, being more quickly susceptible to drying out, became lethally dehydrated at temperatures well below the upper limit in less than 6 h. It is apparent from this graph, with the time interval chosen to correspond to the period of emersion at mean sea level, and given that the conditions of the experiments approximate those on the shore, that young settled stages of these barnacles are in danger of desiccation at temperatures commonly experienced, and more so if the drying conditions (for example — with greater wind speed) were more severe than those applied in the experiments.

Survival of *Chthamalus stellatus* under desiccating conditions

The probit mortality — log. time plots for spat at 18 °C and adults at 19° and 28 °C, all at zero humidity, are shown in Fig. 11. The M.L.T.s were 48, 165 and 270 h, respectively. The rock substrata on which *Chthamalus stellatus* had settled differed in nature from the Tufnol panels used for the other species. However, as the pieces of rock were shaken dry before putting into the desiccators and appeared quite dry after 4 h, it seems reasonable to attribute much of the enhanced survival time, when compared with those for the other 3 species, to characteristics of the animals. In contrast to the other species, and to expectation, the survival time of the adults at 28 °C was not shorter than at 19 °C, but longer. The M.L.T.s of spat and adults at 18° and 19 °C, respectively, are compared with those obtained for *Elminius modestus*, *Balanus crenatus* and *B. balanoides* in Table 4. Obviously *C. stellatus* is well adapted to withstand desiccation, the spat survived as long or longer than individuals of *B. crenatus* well over 1,000 times greater in volume, or individuals of *E. modestus* over 200 times larger.

Desiccation stress and water loss

To be able to compare the degree of desiccation of different barnacles under different desiccating conditions for varying periods of time, it is necessary to relate proportionate water loss to the applied desiccation conditions which can be defined in terms of the duration of the drying power of the atmosphere and the permeability and size of the barnacle. If we write

P   = permeability, defined to include all the properties of the barnacle that allow water loss, and measured as mass flow/unit area/unit water vapour pressure difference/unit time;

p_s = saturated water vapour pressure, which varies with temperature;

(1 — R.H.) = the humidity deficit;

l   = a characteristic linear dimension of the barnacle; and
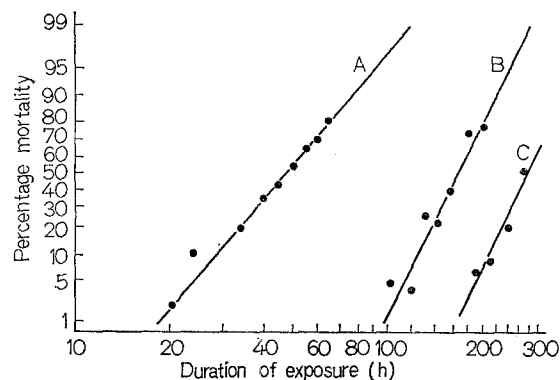
t   = the time of exposure;

Fig. 11. *Chthamalus stellatus*. Probit — log. time plot of mortality at 0% R.H.: A: spat at 18 °C; B: 6 mm diameter specimens at 19 °C, C: 6 mm diameter specimens at 28 °C. 40 to 100 spat, and 30 to 70 adults used for each mortality point

Table 4. *Comparison of the estimates of median lethal times of barnacles desiccated at 0% R.H. The volume (v) is a straight line function of the cube of the length (l): Balanus balanoides* $v = 0.07\ l^3$; *Balanus crenatus* $v = 0.06\ l^3$ *and Elminius modestus* $v = 0.05\ l^3$. *The spat of Chthamalus stellatus are assumed to be similar in shape to those of E. modestus*

Species	Spat at 18 °C		Adults at 19 °C		
	Volume (mm³)	M.L.T. (h)	Diameter (mm)	Volume (mm³)	M.L.T. (h)
Chthamalus stellatus	0.05	48	6	11	165
Elminius modestus	0.05	7	6	11	46
Balanus balanoides	0.07	6	5	9	45
Balanus balanoides			11	93	92
Balanus crenatus	0.06	3.5	6	13	17
Balanus crenatus			11	80	40

M.L.T. = median lethal time.

then the amount of water lost (ΔW) after a certain time of exposure will be proportional to the product of the permeability, saturation deficit, surface area and time:

$$\Delta W = K_1\ P\ p_s\ (1 - R.H.)\ l^2\ t \qquad (1)$$

which has units of mass only (the constant of proportionality being dimensionless). Note that p_s (1 — R.H.) is the "desiccation potential", the difference between the vapour pressure of the evaporating surface and that of the atmosphere. In the case of a desiccating barnacle, p_s refers to the wet tissue of the animal within the shell. The vapour pressure of sea

water is related to that of pure water by the equation (HARVEY, 1955):

$$p_{seawater} = p_s \left(1 - 0.00097 \, Cl^o/_{oo}\right) .$$

In this work the blood concentrations rarely exceeded a depression of freezing point of $-5.0 \, ^\circ C$, corresponding to a chlorinity of $47^o/_{oo}$ (calculated from equations in HARVEY, 1955 and NICOL, 1960). At this concentration there is about 5% drop in the saturated water vapour pressure of the saline solution compared with pure water. Therefore, p_s is only slightly affected by increasing concentration of solutes in the blood and may be regarded as approximately constant.

The initial water content (W) of a barnacle is proportional to the cube of a characteristic linear dimension: i.e.

$$W = K_2 \, l^3 \tag{2}$$

where the constant of proportionality has units of mass/volume. Therefore, the water loss per unit of water content at the outset, the proportionate water loss (X), after t hours exposure is given from Eqs. (1) and (2):

$$X = \frac{\Delta W}{W} = \frac{K_1}{K_2} \, P \, p_s \, (1 - R.H.) \, l^{-1} \, t . \tag{3}$$

It has already been shown experimentally that the duration of desiccation stress for 50% mortality of barnacles is proportional to a linear dimension of the barnacle. The expression $p_s \, (1 - R.H.) \, l^{-1} \, t$, which we will refer to as Z, the desiccation stress, contains readily measurable factors. The permeability is given by [from Eq. (3)]:

$$P = \frac{K_2}{K_1} \frac{X}{Z} . \tag{4}$$

The constants K_1 and K_2 are shape factors and, assuming that the barnacles used are similar in shape because they were freely growing on flat substrata, then $\frac{K_2}{K_1}$ will be similar for each species. The plot of X on Z, the ratio of proportionate water loss to desiccation stress, should thus give lines of slope proportional to the permeability of the species.

If the depression of freezing point of a solution before any evaporation has occurred is Δ_1, which is proportional to the concentration of solutes, then

$$\Delta_1 = K' \frac{s}{s + W} \tag{5}$$

where s = weight of the solutes, and W = initial water content. After the evaporation of a quantity of water (ΔW), and assuming no withdrawal of osmotically active solutes from the system, then the new value of freezing point depression will be

$$\Delta_2 = K'' \frac{s}{s + W - \Delta W} . \tag{6}$$

If Δ is linearly proportional to the concentration, as it is by definition in the case of an ideal solution, and

is approximated by low concentrations of a pure solution of sodium chloride (SCATCHARD and PRENTISS, 1933), then K′ and K″ will be the same, and by combining Eqs. (5) and (6)

$$W = (s + W) \left(1 - \frac{\Delta_1}{\Delta_2}\right) . \tag{7}$$

Therefore, the proportionate water loss X (as a proportion of the weight of the original solution) from a barnacle whose blood has a depression of freezing point of $-1.9 \, ^\circ C$ in sea water of salinity $34^o/_{oo}$ (FOSTER, 1970), is given by

$$X = 1 - \frac{1.9}{\Delta} . \tag{8}$$

When nearly all the water has been lost by evaporation, Δ is very large. Calculation of water loss is facilitated by the approximate linear relationship between water loss and the reciprocal of Δ.

The above relation of Δ determinations of barnacle blood to water loss assumes that barnacle blood evaporates like an ideal salt solution with no ionic interaction at high concentrations, i.e. that $K' = K''$. Also, the calculations relate to the blood only, or strictly to the blood of the subscutal sinus where the blood was sampled. However, since the thermodynamic activity of water would probably equalise throughout the tissues, it is reasonable to assume that the proportionate water loss applies to the body water as a whole. It is then possible to express the water loss per unit body weight from the proportion of water present in the undesiccated animal. For example, for *Balanus balanoides*, the prosomas of which contain 80.0% water, a Δ of $-3.0 \, ^\circ C$ (36.7% water loss from the blood) will represent 29.6% weight loss from the prosoma.

Increase in blood concentration of desiccating adult barnacles

It was noted that excess water in the mantle cavity evaporated before any noticeable decrease in the distension of the subscutal sinus or shrivelling of the prosoma. Body movements continued in barnacles with dry mantle cavities when under liquid paraffin. Prolonged drying was accompanied by shrivelling of the basal and mantle tissues, and retraction of the previously distended sinus. Even when the sinus cuticle had partially collapsed, sufficient blood could usually be obtained by exploring the sinus with the capillary tip. With more prolonged drying, the prosomas shrivelled. Examples of the increase in the blood concentration under constant drying conditions are shown in Fig. 12 together with mortality results on independent samples in the same experiments.

In the experiments with *Balanus crenatus*, *B. balanoides* and *Elminius modestus*, but not with *Chthamalus stellatus* which will be considered later, the rate of increase of Δ, which depended on the rate
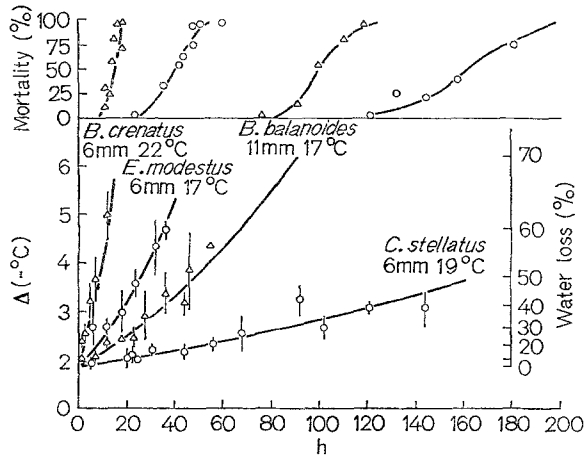
Fig. 12. Below: increase in Δ of the blood of given sized barnacles at the temperatures shown and all at zero humidity. 5 to 10 individuals contribute to each mean, and the standard deviation is given. Δ is related to water loss on the right hand scale. Above: mortality curves of barnacle samples in the same experiment
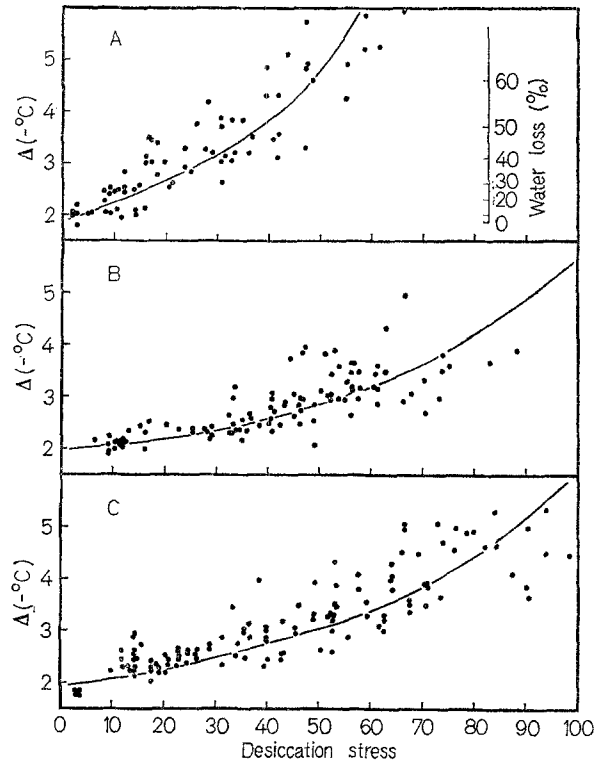

Fig. 13. Increase in Δ of the blood of the barnacles (A) *Balanus crenatus*; (B) *B. balanoides*; and (C) *Elminius modestus* in relation to the time spent at a certain desiccation potential per the size of the barnacle (the desiccation stress, Z). The curves are transformed regressions of the reciprocal of Δ on Z

of water loss from the sinus, was influenced by the saturation deficit of the atmosphere and also the size of the barnacle. To take into account the temperature, humidity and the size of each specimen, blood concentrations were plotted against "stress of desiccation", Z, as defined for Eq. (4) above, excluding those experiments in which the time of exposure was equal to or greater than the median lethal time resulting from temperature effects alone (FOSTER, 1969). Similar straight line functions were obtained when the reciprocal of Δ was plotted against desiccation stress. The pooled data are shown in Fig. 13. The curves applied to the points are the transformed regressions of 1/Δ on the desiccation stress, since the reciprocal of Δ gave the best linear fit in agreement with the theoretical relationship between Δ and increasing water loss. The details of the regressions are given in Table 5. Note

that the regression lines all intersect the ordinate (no stress) at values less than the expected value of 1/1.9, perhaps indicating that water was initially lost at a greater rate. Significance tests on the regression coefficients reveal that there is more than a 10% chance (P > 0.10) that the slopes of the curves in Fig. 13 for *B. balanoides* and *E. modestus* could be the

Table 5. *A comparison of the increase in Δ of the blood of different species of barnacles under desiccating conditions. Duration of exposure, saturation deficit and size of the barnacle are measured by the desiccation stress (Z) as defined in the text. The reciprocal of Δ was regressed by the method of least squares on a linear axis of the desiccation stress Z. The regressions are applied to the range of Δ values shown in Fig. 5 for the first 3 species; the equation for C. stellatus applies only at a temperature of 19 °C*

Species	Experimental temperatures	No. of specimens	Regression equation	Variance (s^2)
Balanus crenatus	16, 20.5, 22 °C	72	$1/\Delta = 0.470 - 0.00523\ Z$	0.00257
Balanus balanoides	17, 24 °C	104	$1/\Delta = 0.515 - 0.00337\ Z$	0.00191
Elminius modestus	3, 10, 17, 24 °C	126	$1/\Delta = 0.477 - 0.00311\ Z$	0.00191
Chthamalus stellatus	19 °C	60	$1/\Delta = 0.515 - 0.00051\ Z$	0.00184

Table 6. *Chthamalus stellatus*. *The increase in* Δ *of the blood of specimens of mean basal diameter of 6 mm at zero humidity in 2 experiments at 19° and 28 °C. The reciprocal of* Δ *was regressed by the method of least squares on a linear axis of time (t) in h (See Fig. 14)*

Temperature (°C)	No of specimens	Regression equation	Variance (s^2)	M.L.T. (h)
19	60	$1/\Delta = 0.494 - 0.00107\ t$	0.00412	170
28	70	$1/\Delta = 0.488 - 0.00075\ t$	0.00166	250

M.L.T. = median lethal time.

same, but the difference between the slopes of the curves for *B. crenatus* and either *B. balanoides* or *E. modestus* is highly significant (P > 0.001). Plotting the curves in Fig. 13 as proportionate water loss [by Eq. (8)] against the stress of desiccation as in Eq. (4), straight lines through the origin (no water loss with no stress) were obtained. Assuming the shape factors K_1 and K_2 to be similar for all species, the slopes of these lines are proportional to the permeability of the species, and are given in Table 8.
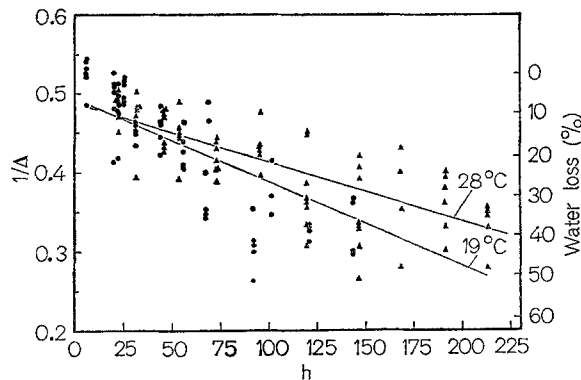

Fig. 14. *Chthamalus stellatus*. Increase in Δ (plotted as the reciprocal) of the blood of 6 mm sized individuals at zero humidity and at 19 °C (●) and 28 °C (▲)

Chthamalus stellatus, in contrast to the other species, lost water at about the same rate at 19° and 28 °C under otherwise equivalent conditions. The results of the 2 experiments are shown in Fig. 14. There is no significant difference (P > 0.10) between the regression of $1/\Delta$ on time, the details of which are given in Table 6. In other words, the temperature did not affect the rate of water loss. From Eq. 4 if the water loss X is constant, then at a higher stress 'Z' corresponding to higher temperatures, the permeability was lower. As it is unlikely that the integument could be made more impermeable at higher temperatures, it is concluded that *C. stellatus* reduced the permeability,

which has been taken in its broadest sense, by closing the opercular valves more tightly and not allowing any water loss via a pneumostome.

In *Chthamalus stellatus*, therefore, the relation of Δ of the blood to desiccation stress cannot be expressed through Eqs. (4) and (8) for all ranges of temperature and humidity. However, for comparison with the other species, the reciprocals of Δ were regressed on desiccation stress at 19 °C at which the permeability was greater, and the details are included in Table 5. There is a highly significant difference (P > 0.001) between the coefficients for *C. stellatus* and those of *Balanus crenatus*, *B. balanoides* and *Elminius modestus*. Clearly *C. stellatus* lost water at a relatively slow rate.

Thus, in equivalent desiccating conditions, equivalent sized barnacles of *Balanus balanoides* and *Elminius modestus* lost water at about the same rate, both faster than *Chthamalus stellatus*, and all slower than *B. crenatus*. These differences in rates of water loss reflect difference in permeability (Table 8).

Tolerance of water loss in adult barnacles

The range of Δ values observed for blood samples from desiccating barnacles are as shown in Figs. 13 and 14, and mostly fell in the range from near isosmoticity with sea water to just below −5 °C (i.e. to about 63 % water loss). The mortality in samples of *Elminius modestus* and *Balanus crenatus* occurred during the period when the blood first became unobtainable from a few specimens, and progressed until blood was entirely unobtainable. In *B. balanoides*, the samples did not show any mortality until after the blood became unobtainable. The concentration of the blood was estimated by extrapolating the change in Δ to the median lethal time (M.L.T.) determined for barnacles in the same desiccating conditions. For *E. modestus*, the estimated proportionate water loss from the blood is plotted against the temperature of the experiment in Fig. 15. At temperatures below 30 °C, the water loss from the blood at death ranged from 59 to 70 % with little apparent trend. Above 30 °C the water loss at death was less and approached low levels towards the

upper lethal temperature of 45 °C (FOSTER, 1969). At temperatures near the upper lethal temperature, death occurred through the harmful effect of high temperature, water loss occurring between the time the barnacles were placed in the desiccator and the time they succumbed. The mean water loss at death for 11 experiments at temperatures less than 30 °C was 65% (Δ of −5.15 °C), which is near the maximum measured concentrations for this species as shown in Fig. 13.

No experiments at temperatures less than 16 °C were carried out with *Balanus balanoides* and *B. crenatus*, but the estimated water loss at death at
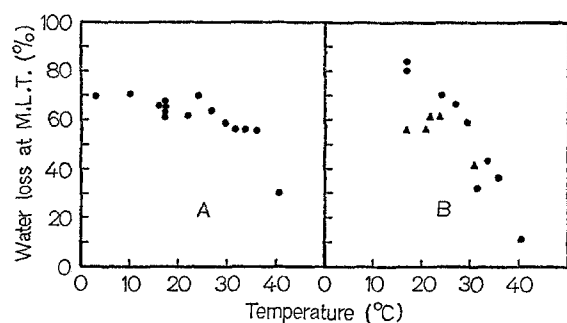
The water content of the prosomas of barnacles that had been in sea water for at least 12 h previously are given in Table 7 as the means of repeated determinations. There is no significant difference between the means of the different species. Taking all the determinations together, a common water content of 81.3 ± 0.2% may be applied to the prosomas of these species. Most gravimetric studies of desiccation express water loss per weight of the body. To aid comparison with other animals, the tolerances of the barnacles are given as weight losses per the fresh prosoma weight at the median lethal time at 17 °C in the last column of Table 8.

Fig. 15. Calculated proportion of water lost at the median lethal time, based on the increase in Δ, and plotted against the temperature of each experiment. (A) *Elminius modestus* (●); (B) *Balanus balanoides* (●) and *B. crenatus* (▲)

Table 8. *Summary of the water relations of desiccating barnacles. The proportionate permeability was derived as in Eq. (7), and represents the ratio of the proportionate water loss (X) to the desiccation stress (Z) as defined in the text*

Species	Proportionate permeability	% water lost from the blood at M.L.T.	% weight loss from the body at M.L.T.
Balanus crenatus	0.0122	60	49
Elminius modestus	0.0070	65	53
Balanus balanoides	0.0069	80	64
Chthamalus stellatus[a]	0.0012	49	40

[a] Data for *C. stellatus* for experiment at 19 °C only.

Table 7. *Water content of the prosomas of barnacles, determined in early June 1969. Each sample contained 20 to 50 prosomas*

Species	No. of samples	Mean water content (%)	Standard deviation of the mean
Balanus crenatus	6	81.68	0.84
Balanus balanoides	8	80.85	1.53
Elminius modestus	5	81.52	0.89
Chthamalus stellatus	9	81.37	2.67

higher temperatures are shown in Fig. 15b. The effect of high temperatures in producing death at relatively low levels of water loss is apparent. At 17 °C, *B. balanoides* lost about 80% of the water from the blood by the median lethal time, and at 16 °C half the *B. crenatus* died of desiccation with about 60% water loss.

At 19° and 28 °C, the blood of *Chthamalus stellatus* had lost 49 and 46% of its water, respectively, at the median lethal times of 170 and 257 h.

Discussion

With positive chemical and physical stimuli, cyprids of *Balanus balanoides* can settle within ½ h of initially exploring a surface, and thus may fix themselves at high shore levels that experience relatively brief periods of immersion. With a 20 h interval between settlement and the moult into the spat, cyprids settled between tide marks must experience 1 or 2 periods of exposure to the air. The increase in temperature tolerance of 3 C° from the free-swimming cyprid to the settled cyprid stage (FOSTER, 1969) will be of only slight value in offsetting the harmful effects of high temperature régimes in the more hazardous intertidal environment. The small settling stages of any intertidal organism must be very susceptible to desiccation. The cyprid carapace may provide some diffusional resistance to water loss, and unless the effects of emersion become too severe, premoulting stages of metamorphosis can proceed to some extent during the intervals that the animal is out of water. Evidently, either the change in shape or integumental structure on metamorphosis, or both,

confer a slightly increased protection from water loss. It follows that any reduction in the time taken from attachment to the moult and completion of meta-morphosis will favour survival on the shore, while the subsequent increase in size and degree of calcification of the valves and shell plates to produce close-fitting structures will retard further the loss of water from the integument and increase the chances of survival.

For *Balanus balanoides*, *B. crenatus* and *Elminius modestus*, it is clear that over a wide range of combinations of atmospheric temperature and humidity, all other factors being taken as equal, there is a pattern of survival times that can be associated with the known lethal effects of temperature alone or with the drying power of non-saturated atmospheres at non-lethal temperatures. If high temperature does not kill the barnacle in a given time, then death will result from desiccation when the lethal effects of water loss are felt. The advantage of increased size is obvious: the larger the barnacle, the greater the volume to surface area ratio and the longer it will take to lose an equivalent proportion of water. As the rate of evaporation is reduced, for example with a decrease in the saturation deficiency of the air, the survival time is increased but with increased chances of high temperatures being the cause of death. Rapid metamorphosis and growth is therefore of advantage for survival between tides.

Comparing barnacles of equivalent size, *Balanus crenatus* is particularly susceptible to desiccation, much more so than equivalent sized and shaped *B. balanoides* and *Elminius modestus* which themselves are nearly equitolerant. From the evidence of the experiments, *B. balanoides* should survive slightly better than *E. modestus*, at least in the summer when the adults were tested. *Chthamalus stellatus* is the most resistant species to desiccation. The linear relationship established for *B. balanoides*, *E. modestus* and *B. crenatus* between a characteristic dimension and the survival time under desiccating conditions suggests that the proportion of water lost at death is the same for all sizes. Since the survival time of parent *B. balanoides* and of the embryos contained in the ovigerous lamellae are equal, there is no reason to suspect that there is any marked alteration of tolerance to desiccation with ontogeny. After all, adults and embryos are exposed to similar environments within the mantle cavity before the nauplii are released.

The low tidal *Balanus crenatus*, which was killed soonest, lost water at the fastest rate. *B. balanoides* and *Elminius modestus* lost water at similar rates. The high tidal *Chthamalus stellatus*, which survived for the longest time, appears to be relatively impermeable. Any mechanism to restrict water loss will prolong the time taken to reach a lethal level of dehydration. Alternatively, the greater the proportionate water loss tolerated, the longer will barnacles survive if the rates of water loss are the same. However, there is no correlation between the estimated degree of water loss

endured up to the point where death results from desiccation at normal temperatures and the respective intertidal levels occupied by each species. *C. stellatus*, the species most tolerant of desiccating conditions, died at blood concentrations no higher than those leading to death in *B. crenatus*, the most susceptible species. If individuals of *E. modestus* are desiccated up to the median lethal time, the blood reaches a depression of freezing point of about −5.1 °C, and then it is not possible to take samples. This indicates that death occurred at this concentration, and was probably due to the osmotic effects of the solutes at this concentration. If desiccation at first induces coma rather than causing immediate death, the rate of water loss may rapidly increase through the relaxed opercular valves, and there will be less chance of sampling blood more concentrated than that causing coma. *B. balanoides* died at higher blood concentrations than did *E. modestus*, and since mortality occurred in samples of *B. balanoides* after blood had become unobtainable from the subscutal sinus, it would seem that the effects of high blood concentrations that were sufficient to kill *E. modestus* were not immediately lethal in *B. balanoides*. There is some suggestion in Fig. 9 that *B. balanoides* may survive slightly longer than *E. modestus* in similar desiccating conditions at non-lethal temperatures. However, no such enhanced tolerance is reflected in the respective intertidal distributions of these species. On the contrary, the spread of *E. modestus* along the European coast to more southern shores than where the indigenous *B. balanoides* is found, and the slightly higher upper limit which it does extend to in the intertidal zones, indicates that *E. modestus* survives better under warmer and, presumably, dryer environments.

Desiccation resembles freezing in the sense that water is withdrawn from the tissues. CRISP and RITZ (1967) and RITZ (1968) have determined the subzero temperatures that caused 50% mortality after 18 h exposure as −4.3° in the summer to −6.0 °C in the winter for *Elminius modestus*, −4.8 °C with no seasonal trend for *Balanus crenatus*, and −6.0 °C in summer to −16.0 °C in winter for *B. balanoides*. The marked increased winter cold tolerance of *B. balanoides* apart, these temperatures approximate to the freezing point of the blood at death under desiccating conditions, suggesting that in both conditions death may be influenced by excessive osmotic pressure of body fluids. The increased cold tolerance of *B. balanoides* might be explained by the formation of protective substances to lower the freezing point of tissue fluids as in certain insects (see CRISP and RITZ, 1967). However, in the present study, no seasonal variation in the osmoticity of the blood of undesiccated *B. balanoides*, or any of the other species, has been detected, showing clearly that the increase in tolerance in *B. balanoides* cannot be due to an increase in the concentration of solutes in the blood. This does not rule out the possible secretion of

protective agents in tissues particularly vulnerable to cold temperature damage, or of substances that promote supercooling in such tissues.

SEGAL and DEHNEL (1962) maintain that "extra visceral water" in the limpet *Acmaea limatula* functions "both in an osmotic and temperature buffering capacity". In the intertidal barnacle *Tetraclita squamosa japonica*, SUZUKI and MORI (1963) claim that high level specimens have a greater quantity of "extra visceral water" and "shell water" than the low level specimens, although the bodies of specimens from both levels had the same water content. SUZUKI and MORI (1963) ascribe evaporative cooling and desiccation protection to the surplus extra visceral water. Since the mantle space of barnacles during desiccation experiments was without surplus water, a result of its expulsion soon after emersion, it is clear that it is the water which is lost subsequently from the body tissues that is ultimately lethal. Surplus water will be essentially sea water, and its evaporation will lead to concentration of salt around the animal; it may, however, prolong slightly the time taken to reach overall lethal salt concentrations. Evaporative cooling and water conservation are conflicting interests; but surplus water may be advantageous with short-term high temperatures during emersion.

Longer survival times under similar desiccating conditions in the barnacle species studied are apparently entirely due to mechanisms which restrict water loss. Even from *a priori* reasoning, the unpredictable severity and duration of desiccation factors at high tidal levels would make adaptations to increase the impermeability and slow down the rate of water loss more advantageous than an increase in tolerance to water loss by itself. The fused parietal plates of high tidal adult chthamalid barnacles is an obvious feature restricting water loss. The opercular plates, however, must necessarily open for the animal to perform vital activities. Most barnacles expel surplus water from the mantle cavity on emersion, and these initial periods of activity may lead to a high rate of water loss. Sublittoral barnacles like *Balanus crenatus* continue with erratic opercular plate and cirral movements when maintained out of sea water. Water loss then occurs through the loosely held opercular plates, and at some point while the body fluid concentration is increasing death ensues. When barnacles that are adapted to survive in the intertidal habitat are emersed, they too initially expel surplus water, and then the opercular plates are withdrawn and fitted closely together. This controlled behaviour must restrict gaseous diffusion between the mantle cavity and the air. The plates, however, are relaxed occasionally and slightly, a pneumostome is formed between the opercular flaps underlying the plates, and small-scale cirral activity takes place in the mantle space (BARNES and BARNES, 1957; CRISP and SOUTHWARD, 1961; GRAINGER and NEWELL, 1965). Inter-specific differences in the rate of

water loss in intertidal barnacles, although perhaps partially due to differing shell porosities, may be influenced more by differences in the size of the pneumostome and the frequency of its formation.

It is well known that intertidal barnacles respire aerobically when exposed to the air (GRAINGER and NEWELL, 1965; AUGENFELD, 1967). BARNES et al. (1963) have also shown that barnacles can respire anaerobically when placed in nitrogen or in low salinities in which activity is inhibited and the opercular plates closed. In *Chthamalus depressus* from high shore levels of the Mediterranean, BARNES and BARNES (1964) claim that, with prolonged exposure to desiccation stress, the mantle cavity is occluded completely by the opercular plates and anaerobic respiration occurs. BARNES and BARNES (1964) maintain that it is the toxic metabolic waste products that eventually kill the animal, and that dehydration is not the immediate problem. *C. stellatus* probably shares this attribute of decreasing the overall permeability with higher desiccation stress, judging from the greater resistance at 28 °C than at 19 °C. The relatively low water loss that had occurred at death may not have been near the tolerance levels that this species can actually endure.

The conditions causing mortality in the cyprids and young adult stages of *Balanus balanoides, Elminius modestus* and especially *B. crenatus* are quite conceivably to be met naturally and regularly during periods of intertidal emersion in dry weather. The colonisation of higher shore levels by these species must, therefore, depend on the coincidence of settlement with a sustained period of humid conditions of emersion.

The high-tidal distribution of *Chthamalus stellatus*, in contrast to the other species, suggests that, at times, particularly at neap tides, the species may have to endure long periods of emersion which are unpredictable because of variable wave action, atmospheric pressure and precipitation. Apart from a greater tolerance of high temperatures, the spat of *C. stellatus* have a much greater resistance to desiccation than equivalent stages of the other species. This might be explained on a behavioural basis, but a closer investigation of the permeability characteristics of the settling and spat stages of chthamalids is called for, which may yield clues as to how such species as *C. stellatus* colonise the extreme reaches of the tides and waves, and survive under conditions which are often more typical of terrestrial than marine environments. However, in the experiment that killed the spat in 2 days, the conditions were not as severe as might sometimes be obtained at high levels on the shore over a period of neap tides. Therefore, even in this species the weather during emersion at, and immediately after, settlement must play a significant part in successful colonisation of the highest zones. The affinity of *C. stellatus* for very wave exposed shores may, in part, result from the greater chance of successful settlement

where wave splash and resulting high humidity is of frequent occurrence.

The prosomas of the 4 barnacle species studied have essentially the same proportion of water; and the cyprids of *Balanus balanoides* have the same water content as the prosomas of the adults, and died at similar water losses. Within the shell, the adult intertidal barnacle seems to be relatively unmodified in its osmotic tolerances; the shell and its associated structures would thus appear to afford the main protection from the osmotic effects of low salinity (FOSTER, 1970) and desiccation.

Summary

1. Survival and water loss of the balanomorph barnacles *Balanus balanoides* (L.), *B. crenatus* BRU-GIÈRE and *Elminius modestus* DARWIN, when exposed to atmospheres of known temperature and humidity, were determined with respect to the size of the barnacle.

2. At high temperatures survival can be related to known lethal effects of temperature alone; at lower temperatures survival was inversely proportional to the saturation deficit of the air, and the survival time was directly proportional to a linear dimension of the individual barnacle.

3. The water loss was followed by measuring the increase in the depression of freezing point of the blood.

4. Size for size, *B. crenatus* was the most susceptible to desiccation and lost water at a faster rate than either *B. balanoides* or *E. modestus* which survived for equivalent times and lost water at about the same rate.

5. By relating the increase in the concentration of the blood to the median lethal time of survival, the water loss at death was estimated. There was no correlation between the tolerance of water loss and the survival time or the intertidal distribution patterns of the species.

6. Experiments with spat and adults of *Chthamalus stellatus* (POLI) showed that this species, size for size, loses water at a much slower rate and survives for much longer periods under the same conditions as the other species. Furthermore, survival time appears not to be proportional to desiccation stress.

7. The desiccation resistance, in terms of survival time or the rate of water loss, correlates with the intertidal height to which each species extends.

8. The first stage nauplii of *B. balanoides*, retained as ovigerous lamellae in the mantle cavity of the adult, survived as long under desiccating conditions as the parents.

9. The cyprids of *B. balanoides* settle irrevocably in about 20 min, and undergo the moult into the adult form about 20 h later unless delayed by prolonged emersion. The cyprids tolerate about the same propor-

tionate water loss as the adults, but survive for slightly less time than the metamorphosed and calcified spat.

10. A comparison of the desiccation resistance with natural periods of intertidal emersion suggests that the small sizes of *B. crenatus*, particularly, and also of *B. balanoides* and *E. modestus*, are susceptible to desiccation at normal temperatures and low humidities. The spat of *C. stellatus*, although markedly more resistant than the spat of the other species, must also be prone to extreme conditions at high tidal levels.

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