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RESPONSES AND ACCLIMATION TO SALINITY IN THE ADULTS OF SOME BALANOMORPH BARNACLES

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CONTENTS

The responses of a number of barnacles to a wide range of salinity have been studied by observation of the activity and measurement of the depression of freezing point of the blood. In active barnacles of the species *Elminius modestus*, *Balanus balanoides*, *B. crenatus*, *B. improvisus*, *B. hameri*, *B. balanus* and *Chthamalus stellatus* the blood concentration conforms with changes in the external salinity. The concentration of the blood tends to remain slightly hyperosmotic to the fluid in the mantle cavity, and to the medium. With sudden changes of external salinity the blood concentration conforms within a few hours if cirral activity is maintained.

When placed in such low salinities that activity is inhibited, *E. modestus*, *B. balanoides*, *B. crenatus*, *B. improvisus*, *B. balanus* and *C. stellatus* close the opercular valves with the result that the blood and mantle cavity fluid are maintained for some time at a level initially considerably hyperosmotic to the medium, but the blood is still only slightly hyperosmotic to the fluid remaining in the mantle cavity. There is no permanent control, and in time the blood concentration approximates to the external level.

E. modestus, *B. balanoides* and *B. improvisus* from low salinity estuarine habitats, and *B. crenatus* after gradual reduction of salinity in the laboratory over a matter of days, exhibit tolerance to lower salinities than do specimens of the same species obtained from, or acclimated to normal salinities. Salinity acclimation is typical of osmoconformers lacking specific organs for effective regulation.

It is concluded that the barnacles here tested are osmoconformers, able to adjust to small changes of environmental salinity by tissue acclimation, but evading too severe salinity changes by withdrawing into the protection of the shell.

The deep sea *B. hameri*, however, does not close up when immersed in dilute sea water, and appears to be relatively stenohaline with limited ability to acclimate to low salinity. The intertidal *E. modestus* and *B. balanoides*, and the low-tidal to sublittoral *B. crenatus*, are tolerant, after experimental or natural acclimation, of salinities down to 14 to 17‰. The estuarine *B. improvisus* can, with gradual acclimation, be induced to be active in a salinity of about 2‰. This species is remarkably tolerant of dilution of the blood, and its distribution into regions of low salinity is evidently due to a wide tissue resistance and not to any ability to regulate.

INTRODUCTION

Although the great majority of cirripedes are sublittoral and strictly marine, some thoracican species have very successfully colonized rocky shores throughout the world. Because of their abundance and often very clear patterns of intertidal zonation, littoral barnacle species lend

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themselves well to experimental ecology, especially in regard to the analysis of the environmental factors causing or influencing distribution. The existence of barnacles in the more rigorous littoral environment implies that these species have special means of mitigating the many stresses presented when the sea recedes. Varying salinity is an important stress, being associated with rainfall and desiccation during emersion on the shore, and a regular feature at all depths in estuarine and shallow land-locked situations.

The literature on the osmotic responses of barnacles is sparse, and has not yet reached any of the reviews of crustacean osmoregulation. Borsuk & Kreps (1929) reported that adult *Balanus crenatus* and *B. balanoides* tolerate a wide range of salinity and that these species are poikilosmotic, but they give no experimental data. Belyaev (1949) determined the depression of freezing point of the blood of specimens of *B. balanoides*, *B. crenatus*, *B. balanus* and *B. improvisus* from regions of low salinities (White and Baltic Seas) and showed that the blood is maintained slightly hyperosmotic to the water in which they were living. Belyaev also found experimentally that *B. balanoides* in very dilute sea water maintained the blood considerably hyperosmotic to the medium. Newman (1967) noted a similar pattern of hyperosmoticity of the blood of *B. glandula* and *B. amphitrite* from California in experimental media of less than about half strength sea water, but found that *B. improvisus*, the most estuarine or brackish-water species, conformed in all dilutions as great as 3% sea water. Bergen (1968) also found that the mantle cavity fluid of *B. glandula* was hyperosmotic to the external dilute medium. Belyaev (1949) noted that the hyperosmoticity of *B. balanoides* was maintained when the barnacles showed no cirral activity, and concluded that an osmoregulatory apparatus existed which was sufficiently effective to maintain a relatively constant blood concentration with the aid of the partial closure of the opercular valves. However, he gave no evidence that the degree of hyperosmoticity of the blood was constant in these conditions. Newman (1967) maintained that *B. amphitrite* and *B. glandula* remained cirrally active in dilute media when the blood was hyperosmotic, and that the effective mechanism was the separation of the mantle cavity from the exterior, the orifice being plugged by the extended prosoma. Again, the constancy of the degree of hyperosmoticity was not demonstrated, nor is the information relating cirral activity with the blood concentration convincing.

In this paper the osmotic responses of a number of balanomorph barnacles are described, and the mechanism by which these species can mitigate the effects of adverse salinities is put forward. The possible effect of salinity in limiting distribution of barnacles is discussed.

The barnacle species studied

The species were selected to cover a wide range of habitats. *Chthamalus stellatus* (Poli) is typically intertidal, and generally forms a distinct 'zone' about the high water mark on moderately exposed shores. The vertical extent of the distribution varies, and can be correlated with the presence or absence of predators and competitors (Connell 1961) and the degree of wave exposure (Moore 1935). *Balanus balanoides* (L.) occurs over much of the intertidal, below the *C. stellatus* zone where it occurs or otherwise from about low high water neap to low tide levels. It is not characteristic of climax sublittoral communities. *B. crenatus* Bruguière is characteristically subtidal, but does extend into the lower littoral particularly in damp microhabitats. *B. improvisus* Darwin will occur in sublittoral situations of full salinity, but is especially characteristic of low intertidal and subtidal estuarine habitats. The immigrant species from Australasia, *Elminius modestus* Darwin, occurs over a wide range of intertidal and subtidal habitats. Its spread in European waters has been closely followed (e.g. Crisp & Southward 1959; Barnes & Barnes 1965), and it is thought that some element of competition for space occurs between *E. modestus* and the indigenous species *B. balanoides*, *B. crenatus* and *B. improvisus* (Crisp 1958; Kühl 1963). It is therefore of interest to relate the fitness to intertidal and estuarine existence of *E. modestus* and its competitors to the outcome of competition between them and the final limits of their distribution. As littoral species have presumably been evolved from marine sublittoral species, the deep water *B. balanus* (L.) and *B. hameri* Ascanius were studied briefly for comparative purposes.

METHODS

Experimental media were prepared from Menai Strait sea water, either diluted with distilled water or concentrated by evaporation at 70 °C. Specimens were immersed in these media in large shallow Pyrex dishes. The volume was maintained by the addition of distilled water when necessary, and the medium was changed daily. The activity of samples was noted as the percentage showing cirral activity, including pumping, normal and fast beating (Crisp & Southward 1961), for a set time of observation. The depression of freezing point of media, mantle cavity fluids and blood were determined by the method and apparatus of Ramsay & Brown (1955). Each determination of mantle cavity fluid or blood was made on a fresh individual, except where these two fluids were compared from the same barnacle. The barnacle was removed from the substratum and the basis, if present, and the basal tissues cleared to reveal the prosoma within the mantle cavity. The prosoma was moved to one side to reveal the subscutal sinus. If the mantle cavity fluid was to be sampled it was collected with a glass capillary from the region between the prosoma and the opercular flaps. Excess mantle cavity fluid was then removed by suction through a glass pipette. Blood was collected by piercing the subscutal sinus with a fine glass capillary and allowing a sample of the blood to be drawn into it. Ample blood could be obtained from a barnacle as small as 4 mm basal diameter. Fluid samples were immediately blown out under liquid paraffin, and small subsamples (about 10^{-3} mm^3) were mounted between liquid paraffin in very fine silica glass capillaries and quickly frozen. The depression of freezing point determination was made as soon as possible, and never more than 1 week afterwards. It was found that as long as the samples were stored permanently frozen in a deep freeze, the melting point was not appreciably affected after 1 week.

RESULTS

The procedure and results of the experiments with *Elminius modestus* are described first. The results for *Balanus balanoides* are presented in the same figures and tables as for *E. modestus* to emphasize the similarity of the responses of these two species. The pattern of response of these species serves as a basis for the description of the responses of the other species.

The blood concentration is given in terms of the depression of freezing point (Δ) which is linearly proportional to salinity, given by the lines of isosmoticity in the figures. The concentration of the medium, or salinity, is expressed in parts per thousand (‰) calculated from depression of freezing point measurements, or the approximate percentage dilution or concentration of normal sea water.

Elminius modestus

Specimens were collected on intertidal stones from under the suspension bridge over the Menai Strait, where the annual salinity range of the water is from 32 to 34‰ (Harvey & Spencer 1962). The rate of cirral beating of these specimens decreased slightly with either an increase or a decrease of the salinity from an optimum of about 30‰ (figure 1). In these specimens there was no activity below 17‰ nor above 53‰; the opercular valves remained closed except for periodic slight gaping to expose the opercular flaps, often with a small opening or pneumostome between them.

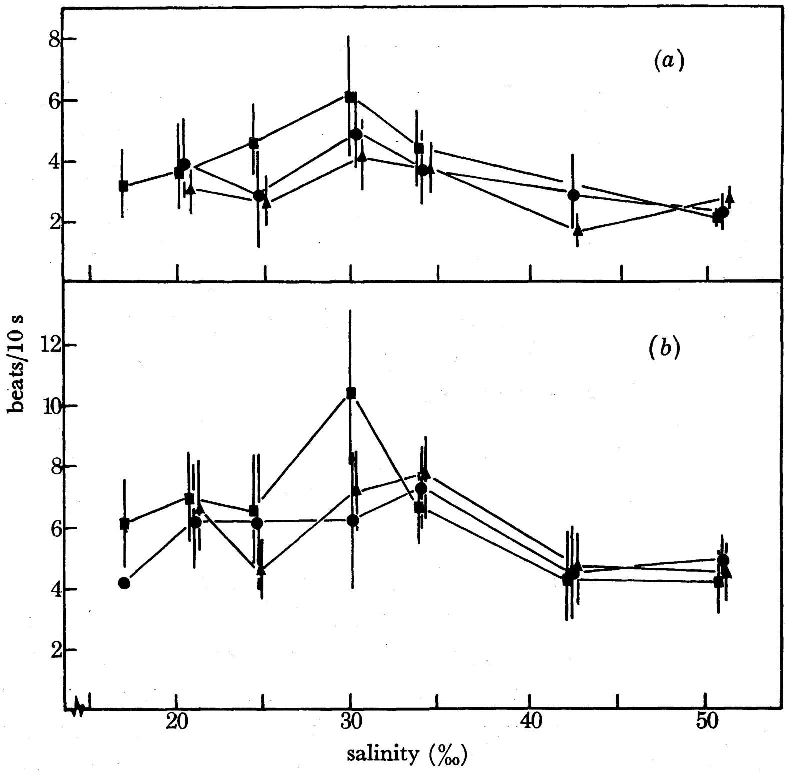

FIGURE 1. The rate of cirral beating of the barnacles (*a*) *B. balanoides* and (*b*) *E. modestus* after continuous submersion in a range of salinities after 1 day (●), 2 days (▲) and 3 days (■). The mean for 10 individuals and the standard deviation is shown for each salinity.

The depression of freezing point of the fluid within the mantle cavity and of the blood of specimens taken sequentially from normal through three dilutions of sea water are given in table 1. The blood was slightly hyperosmotic to the fluid within the mantle cavity, and both the mantle cavity fluid and the blood were considerably hyperosmotic to the external diluted sea water. The depression of freezing point of the blood of specimens after 24 h in media of various concentrations (figure 2) shows that the blood essentially conforms over the range of salinities in which cirral activity occurs; in dilute sea water which inhibited cirral activity the blood was maintained markedly hyperosmotic and at a more or less constant level, and in the concentrated media the blood was somewhat, but not constantly, hyposmotic. A greater degree of hyperosmoticity in dilute media was maintained at lower temperatures (figure 3). The blood of four specimens that were active after 1 day in 50% sea water is compared in table 2 with that of inactive specimens in the same sample and medium. Even with the small number of specimens, the active animals were significantly less hyperosmotic than the inactive ones. In those media where cirral activity was maintained, the blood concentration was never more than slightly hyperosmotic to the medium.

TABLE 1. COMPARISON OF THE DEPRESSION OF FREEZING POINT (Δ) OF THE EXTERNAL MEDIUM, THE MANTLE CAVITY FLUID AND THE BLOOD OF BARNACLES COLLECTED FROM THE MENAI STRAIT

Observations were made sequentially through the dilutions of sea water in which the barnacles were left for the times indicated. Specimens were active in 100 % sea water, otherwise there was no cirral activity. Values are means for five specimens.

		E. modestus		*B. balanoides*	
medium $\Delta(-°\mathrm{C})$	time (h)	mantle cavity fluid $\Delta(-°\mathrm{C})$	blood $\Delta(-°\mathrm{C})$	mantle cavity fluid $\Delta(-°\mathrm{C})$	blood $\Delta(-°\mathrm{C})$
1.83 (100 %)	∞	1.85	1.95	1.83	1.87
1.24 (68 %)	6	1.42	1.47	1.63	1.72
0.97 (53 %)	24	1.18	1.26	1.53	1.57
0.70 (38 %)	24	1.16	1.25	1.13	1.34

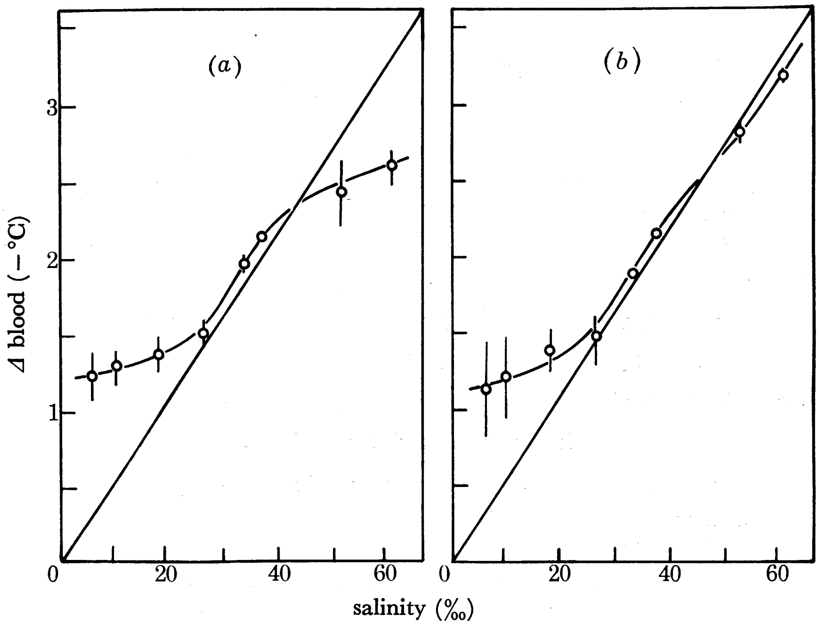

FIGURE 2. The relationship between the blood concentration of Menai Strait specimens of (*a*) *E. modestus* and (*b*) *B. balanoides* and the salinity of the medium. Observations made after 24 h immersion in experimental salinities at 15 °C. Mean and standard deviation given for 5 to 10 freezing point determinations per sample. Diagonal line = isosmoticity.

When immersed directly in media of high or low salinity, the rate of change of the blood concentration was mainly influenced by whether the barnacles displayed cirral activity or not. In media of 75 and 140 % sea water, the barnacles soon regained activity and the blood approximated to a level near isosmotic with the medium within 1 or 2 h (figures 4, 6). After a given time in media of a salinity that inhibited activity, there was a considerable variation in values obtained for blood concentrations. In 25 and 50 % sea-water media (figures 4, 5) the barnacles were initially but briefly active, and the blood concentration fell to a value corresponding to about 80 % of sea water within the first hour. Thereafter the valves were closed except for the occasional formation of the pneumostome between the opercular flaps, and the decrease was correspondingly more gradual. When immersed in 50 % sea-water medium, the blood became nearly isosmotic in 5 days, during which time some of the specimens resumed cirral activity, and none became comatose. In 25 % sea-water medium, the rate of decrease was greater. After 30 h in 25 % sea water, high osmotic pressures were recorded, the blood was opaque and the animals not responsive. It is possible that osmotic disruption of the tissues and the release of organic substances into the blood, either as a result, or cause, of death, may have been responsible. In 200 % sea water (figure 6) the barnacles were closed, and the blood concentration increased more gradually than in 140 % sea water, and near isosmoticity was reached in 1 to 2 days.

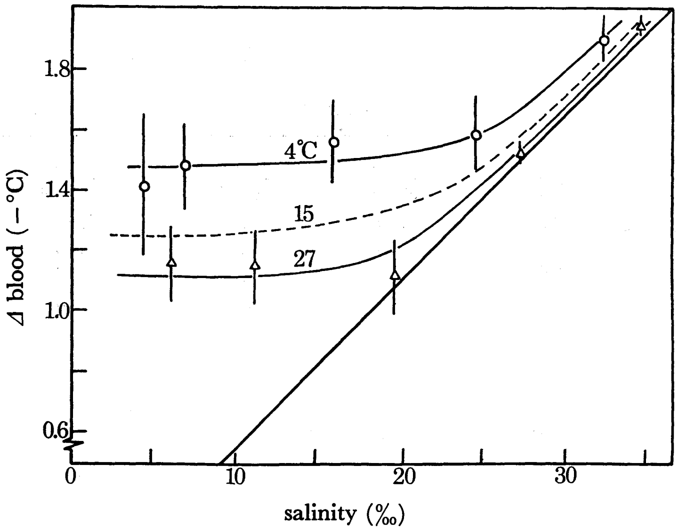

FIGURE 3. The relationship between temperature and the degree of hyperosmoticity of the blood of Menai Strait specimens of *E. modestus* after 24 h in dilute sea-water media. Mean and standard deviation for 5 to 10 individuals per sample. The results at 15 °C (figure 2) are indicated by the broken line. Straight line = isosmoticity.

TABLE 2. COMPARISON OF THE DEPRESSION OF FREEZING POINT OF THE BLOOD OF MENAI STRAIT SPECIMENS OF *ELMINIUS MODESTUS* THAT WERE EITHER REGULARLY ACTIVE OR PERMANENTLY CLOSED AFTER 24 H IN 50 % SEA WATER (Δ OF −0.97 °C)

Most of the barnacles were closed.

activity	no.	mean blood Δ(−°C)	S.D. (°C)
beating	4	1.09	0.05
not beating	10	1.47	0.08

$F = 3.1401$, $t = 2.576$; sig. diff. at 5 % level.

Thus, the apparent hyperosmoticity of the blood in dilute sea water is not maintained at a constant level, and the hyperosmoticity is associated with inactivity and closure of the opercular valves. The resumption of cirral activity by some of the specimens after a period with the valves closed in 50 % sea water, during which time the blood concentration had gradually approached that of the medium, indicated that acclimation to low salinities had occurred.

A direct test for acclimation is to compare the responses of barnacles from a different salinity régime. Specimens of *E. modestus* were collected from a mid-tidal habitat $3\frac{1}{2}$ miles up the Conway

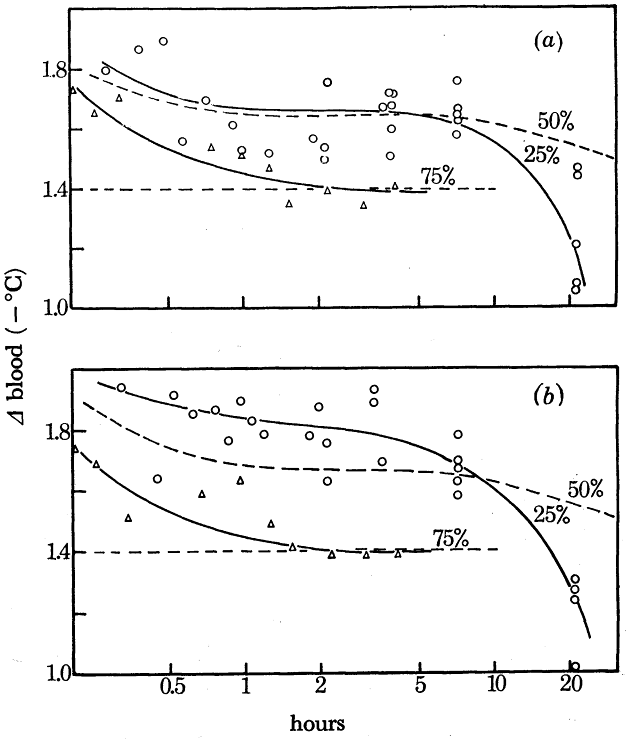

FIGURE 4. The decrease with time of the blood concentration of specimens of (*a*) *E. modestus* and (*b*) *B. balanoides* after immersion straight into 75, 50 and 25 % sea-water media. 75 % (Δ −1.4 °C) ○; 50 % (Δ −0.9 °C) from figure 5; 25 % (Δ −0.45 °C) △. Each point represents the determination for one individual. The curves are fitted by eye. The horizontal dashed line represents isosmoticity in 75 % sea water.

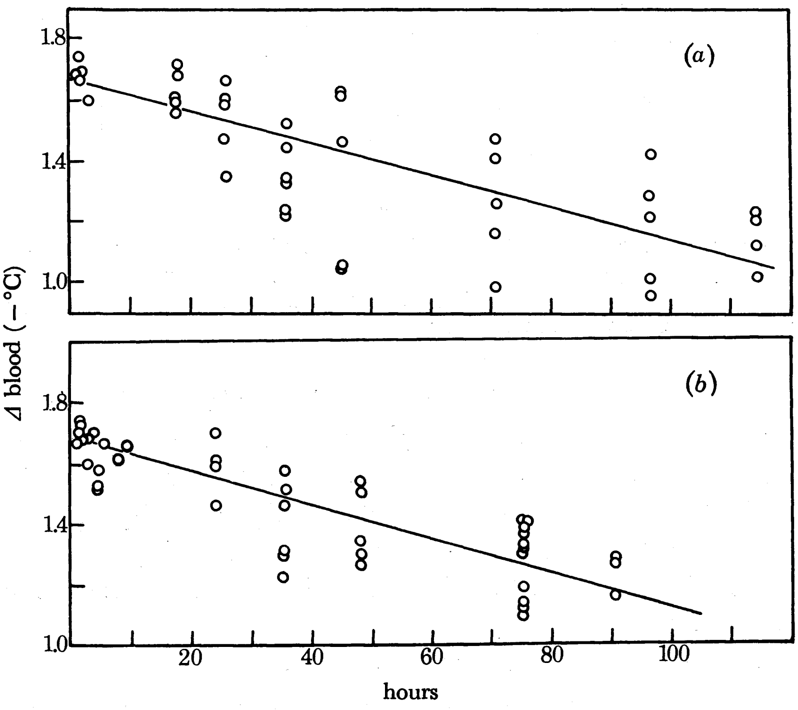

FIGURE 5. The decrease of the blood concentration of Menai Strait specimens of (*a*) *E. modestus* and (*b*) *B. balanoides* after immersion straight into 50 % sea water (Δ −0.9 °C). The points represent individual depression of freezing point determinations, and the line is the linear regression on all points after the first ¼ h. See table 7 for regression data.

Estuary where the general paucity of the intertidal flora and fauna indicated a brackish habitat, and where the surface salinities varied with the tide from about 20‰ at slack high water to near 0‰ at low tide (unpublished student surveys). This location was near the upstream limit of *E. modestus*. The barnacles were active in sea-water dilutions down to about 14‰ (figure 7), compared with 21‰ for the Menai Strait specimens. Even at these lower salinities, the blood remained slightly hyperosmotic in active specimens. Acclimation to low salinity is of obvious advantage in an environment where the salinity only occasionally exceeds 20‰.

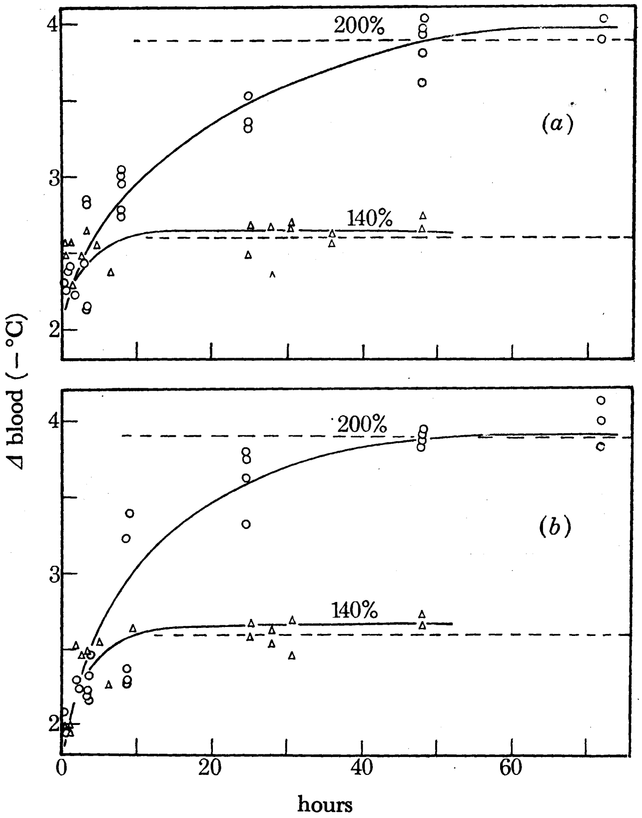

FIGURE 6. The increase of the blood concentration of specimens of (*a*) *E. modestus* and (*b*) *B. balanoides* after immersion straight into 140% (Δ −2.6 °C) and 200% (Δ −3.9 °C) sea-water media. The barnacles were active in 140%, but withdrawn into the shell in 200% sea water. The curves are fitted by eye. The horizontal dashed lines represent isosmoticity in these media.

Conway Estuary specimens were also immersed continually in Menai Strait sea water in the laboratory for 2 and 4 days, and their activity was noted when set up in the same series of dilutions. The results (figure 8) show that there is a rise in the lower salinity limit for activity after a relatively short-term of acclimation in water of higher salinity. Thus the response of *E. modestus* to dilute sea water is variable, and depends on the salinity to which the specimens have been acclimated. This will affect the level at which the barnacles close and the hyperosmoticity of the blood.

Balanus balanoides

This species was collected from the same places and subjected to the same experiments as for *E. modestus*. The results for *B. balanoides* are presented with those for *E. modestus*: the salinities

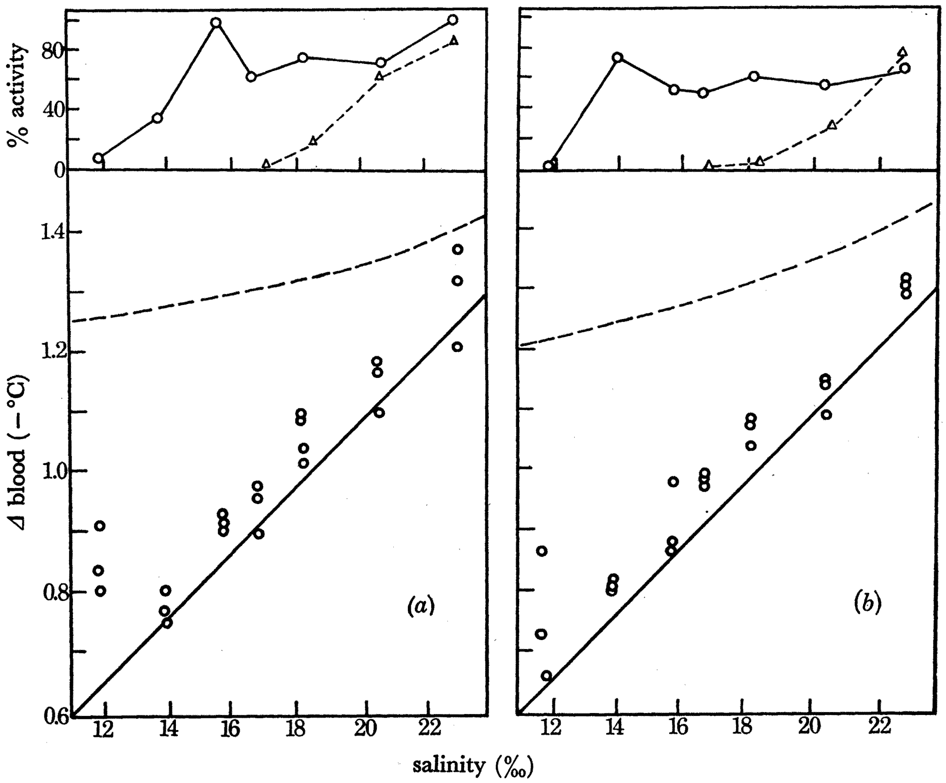

FIGURE 7. The blood concentration and activity of Conway Estuary specimens of (*a*) *E. modestus* and (*b*) *B. balanoides* after 24 h in dilute sea-water media compared with the blood concentration (from figure 2) and activity of Menai Strait specimens shown by the dashed lines. Diagonal straight lines = isosmoticity.

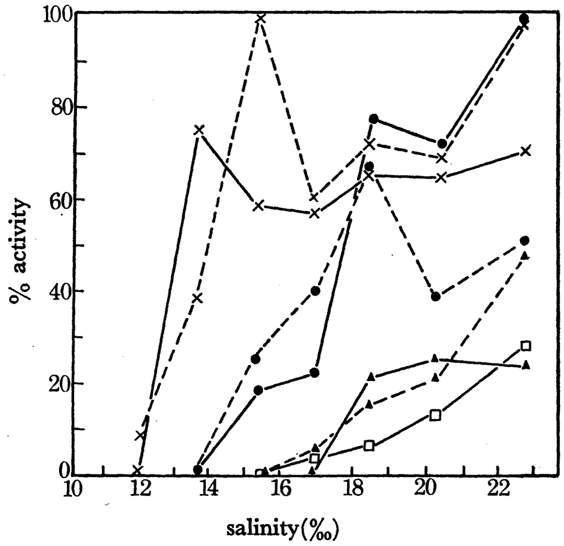

FIGURE 8. The activity with respect to salinity of Conway Estuary specimens of *E. modestus* (broken lines) and *B. balanoides* (solid lines) after no acclimation (×), 2 days acclimation (●), and 4 days acclimation (▲), to normal sea water. The activity (□) of *B. balanoides* from a permanently submerged site (raft) in the Menai Strait is also shown.

inhibiting cirral activity (figure 1), the relationships between the depression of freezing point of the blood and the mantle cavity fluids (tables 1, 3), the depression of freezing point of the blood after 24 h in a wide range of experimental media (figure 2), and the rate of change of the blood concentration in various salinities (figures 4 to 6, table 7), are very similar for the two species. Specimens of *B. balanoides* from the same situation in the Conway Estuary showed a similar degree of acclimation of their activity as did *E. modestus* (figures 7, 8). The activity of *B. balanoides* grown from settlement on permanently submerged panels in the Menai Strait showed a similar response to a dilution series as did the nearby shore specimens (figure 8). As with *E. modestus*, over the range of salinities which permit cirral activity the blood conforms with changes in the external medium, and tends to remain slightly hyperosmotic.

TABLE 3. COMPARISON OF THE DEPRESSION OF FREEZING POINT OF THE MANTLE CAVITY FLUID AND BLOOD OF MENAI STRAIT SPECIMENS OF *BALANUS BALANOIDES* AFTER 36 H IMMERSION IN 12 % SEA WATER (Δ OF -0.34 °C)

	mantle cavity fluid $\Delta(-°\mathrm{C})$	blood $\Delta(-°\mathrm{C})$
specimen 1	1.34	1.33
2	1.25	1.36
3	1.07	1.26
4	0.68	0.63
5	0.79	0.98
mean	1.03	1.11

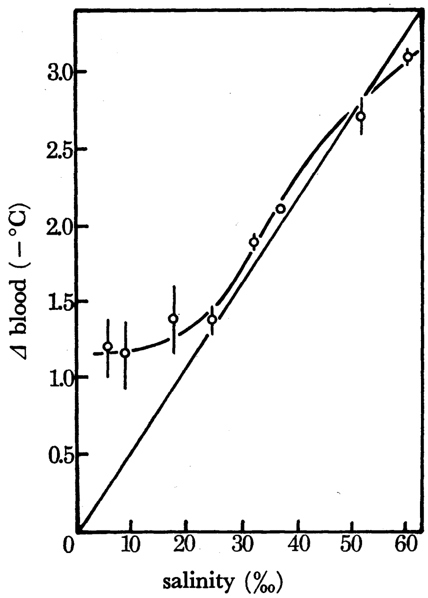

FIGURE 9. The relationship between the blood concentration of Menai Strait specimens of *B. crenatus* and the salinity of the medium. Observations made after 24 h immersion in experimental salinities at 15 °C. Mean and standard deviation given for 5 to 10 freezing point determinations per sample. Diagonal line = isosmoticity.

Balanus crenatus

B. crenatus were collected on mussel shells from a permanently submerged pier pontoon at Menai Bridge. The responses of this species in sea-water media closely parallel those obtained for *B. balanoides* collected from the nearby shore. In dilute sea water, the cirral activity is at first restricted by salinities between 20 and 25‰, but there is gradual acclimation to lower salinities. After 24 h in a wide range of low salinities (figure 9), the hyperosmoticity of the blood indicates an effective separation of the mantle cavity from the exterior in these media. The degree

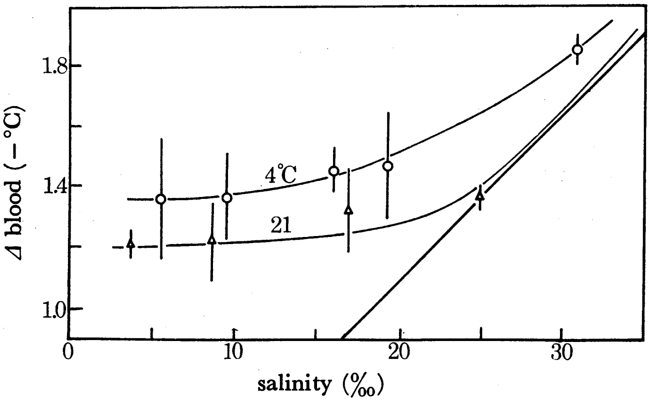

FIGURE 10. The relationship between temperature and the degree of hyperosmoticity of Menai Strait specimens of *B. crenatus* after 24 h in dilute sea-water media. Mean and standard deviation for 5 to 10 individuals per sample. Straight line = isosmoticity.

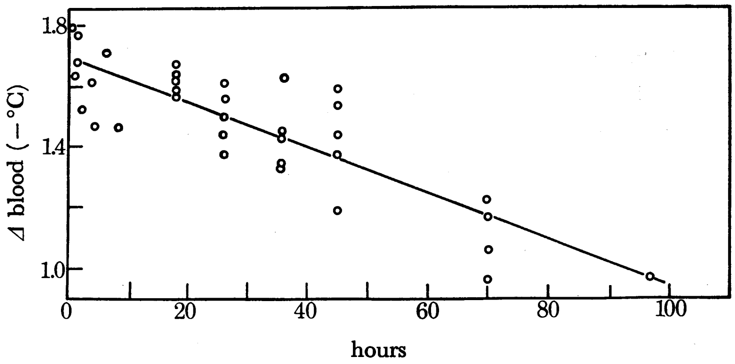

FIGURE 11. The decrease of the blood concentration of Menai Strait specimens of *B. crenatus* after immersion straight into 50% sea water (Δ −0.9 °C). The points represent individual freezing point determinations, and the linear regression is fitted on all points after the first ¼ h. See table 7 for regression data.

TABLE 4. COMPARISON OF THE DEPRESSION OF FREEZING POINT OF THE BLOOD OF SPECIMENS OF *BALANUS CRENATUS* THAT WERE ACTIVE IN THE MEDIA INDICATED

Those in 57 and 45% sea water were active after gradual acclimation to low salinities.

	blood	
medium Δ(−°C)	mean Δ(−°C)	S.D. (°C)
1.78 (100%)	1.86	0.05
1.35 (75%)	1.37	0.05
1.03 (57%)	1.06	0.01
0.82 (45%)	0.86	0.02

of hyperosmoticity in dilute media is dependent on the temperature (figure 10) and time (figure 11). In 50% sea water the blood of *B. crenatus* collected from a similar salinity régime as *B. balanoides* and *E. modestus* attains near isosmoticity at a faster rate than these latter two species (figure 11 and table 7), but nevertheless some control over the entry of water into the mantle cavity is apparent. Gradual reduction of the salinity over a period of a week and observation of activity and survival, indicated that the ultimate dilution tolerated is between 12 and 14‰. With low salinity acclimation the blood remained only slightly hyperosmotic to the medium (table 4). In the same sample, *E. modestus* survived the course of dilution to 15‰, but coma resulted at 14‰.

Balanus improvisus

Specimens of *B. improvisus* were collected from a low tidal situation at Cross Parks Wood on the Tamar Estuary. At this locality the salinity can fluctuate between 24 and 1‰ (Percival 1929; Milne 1938). At the time of collection, low tide, the salinity was 1.5‰. On return to the laboratory, the barnacles were divided into three groups; the first sample was immersed straight into Plymouth Laboratory circulation sea water for 5 days; the second was placed into 50% sea water and, over the following 6 days, the water was changed regularly and the salinity gradually reduced to about 3‰, each change in salinity being made only when most of the barnacles had been observed to be active; the third sample was used directly in the following procedure to which the other two samples were subjected after acclimation. The sample was subdivided and placed in a series of dilute sea water, and the activity was observed over a period of half an hour when each specimen that showed any sustained rhythmic cirral activity was noted. The results are shown in figure 12. For the specimens tested directly after collection, activity ceased at salinities less than 7‰. Those acclimated to normal sea water were less active in corresponding dilutions. Those acclimated to 3‰ were active down to 2.5‰, but

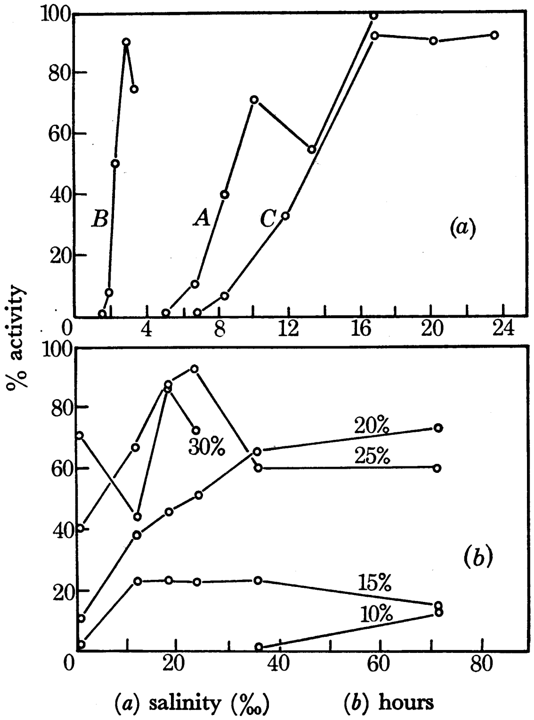

FIGURE 12. The activity of *B. improvisus* in dilute sea-water media. (*a*) The effect of acclimation to high and low salinities on the activity shown when reimmersed in a dilution series: *A*, no acclimation; *B*, 6 days acclimation to 8% sea water; *C*, 5 days acclimation to normal sea water. (*b*) To show acclimation as resumption of activity in dilute media after immersion straight into the dilutions indicated.

only a few were active in 2.1‰ and none at all in 1.7‰ or lower. In this last group, the salinity was further lowered to about 1.1‰ (3% of sea water), at which many specimens were active but with rather weak pumping movements of the cirri only.

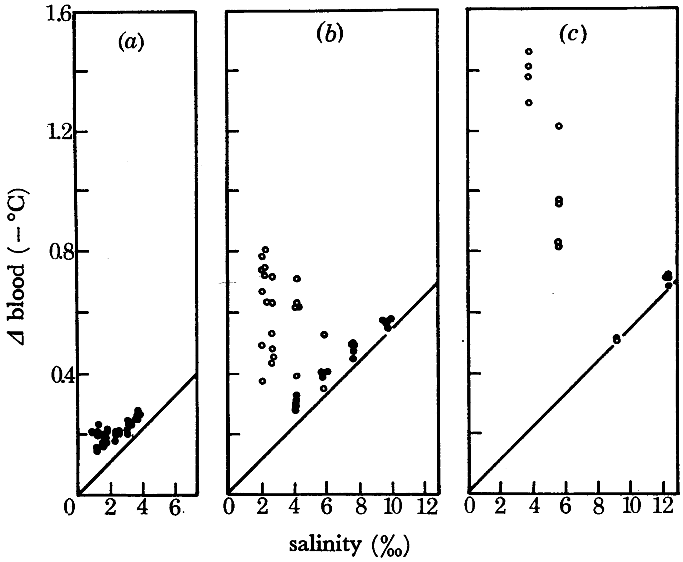

FIGURE 13. The depression of freezing point of the blood of specimens of *B. improvisus* after 1 to 2 days immersion in dilute sea-water media: (*a*) after acclimation to 8% sea water; (*b*) with no acclimation; (*c*) after acclimation to normal sea water. Solid symbols denote that the specimen was seen to sustain rhythmic cirral activity, and open symbols denote that no cirral activity was noted. Straight line = isosmoticity.

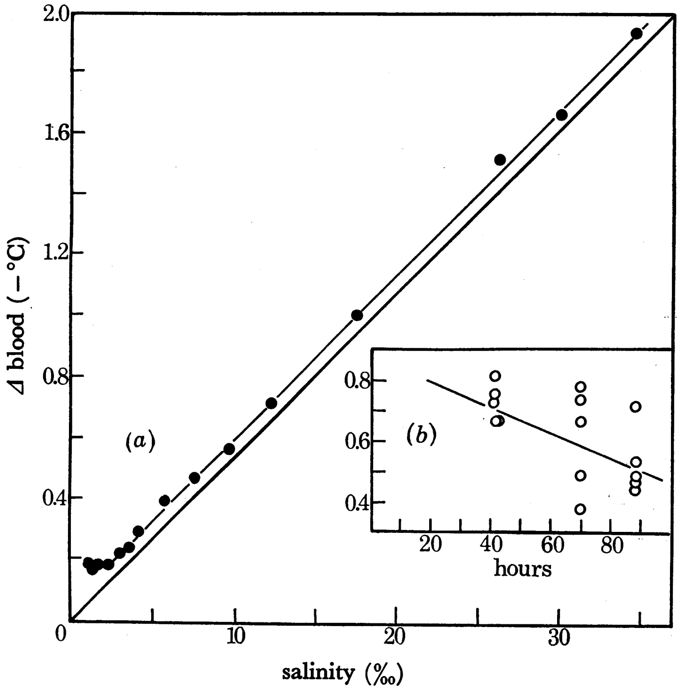

FIGURE 14. The relationship between the blood concentration of *B. improvisus* and the salinity of the medium: (*a*) active specimens only, each point representing the mean of five or six depression of freezing point determinations; (*b*) the trend of the decrease of the blood concentration of unacclimated specimens after being placed directly in 5% sea water (Δ −0.09 °C). Diagonal line = isosmoticity.

The depression of freezing point of the blood of specimens designated as active, if they showed any sustained cirral activity prior to sampling the blood, or inactive, if no activity had been noticed, is shown in figure 13. The blood of the active specimens was slightly hyperosmotic to the medium, whereas that of inactive specimens was variable but mostly considerably hyperosmotic. The acclimation salinity affects the salinity below which the specimens close the opercular valves. In very dilute media the opercular apparatus maintains the blood concentration at a high level, but with less severe dilution there is a corresponding lower degree of hyperosmoticity. The decrease in the blood concentration of specimens immersed directly into 5 % sea water (1.7 ‰) is shown inset in figure 14. There was a gradual fall in the concentration, but after 5 days these specimens were not active.

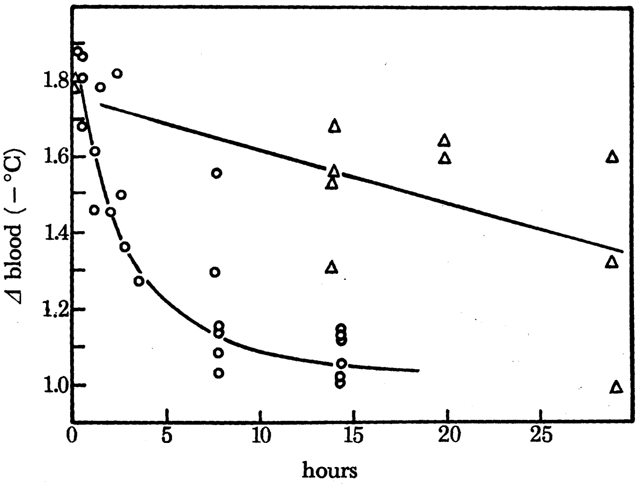

FIGURE 15. The decrease of the blood concentration of *B. balanus* (△) and *B. hameri* (○) after immersion straight into 50 % sea water (Δ − 0.95 °C). The points represent individual depression of freezing point determinations. See table 7.

B. improvisus clearly tolerates considerable dilution of its blood, and over the greater part of the salinity range the blood conforms with the medium (figure 14). At salinities lower than 3 ‰, however, there was a tendency for the blood to be maintained more or less constantly hyperosmotic in active specimens, and as these specimens showed rather sluggish cirral movements, some mechanism other than the opercular apparatus is responsible.

Unfortunately, the blood of specimens collected straight from their collection locality was not sampled, but the degree of hyperosmoticity even after 40 h in 5 % sea water (figure 14*b*) indicates that the blood concentration corresponded to an external medium of not less than 14 ‰ (Δ = − 0.8 °C). At the time of collection at low spring tide, the salinity was 1.5 ‰, and it would appear therefore that the barnacles had spent some time of the falling tide inactive, namely after the salinity had fallen below 14 ‰.

Balanus hameri and *Balanus balanus*

B. hameri was collected on the mussel *Modiolus modiolus* dredged from off Langness Point, Isle of Man; and *B. balanus* from the same habitat and also on scallops dredged southwest of the Isle of Man. These regions would experience permanently normal salinities. When placed directly into 50 % sea water, *B. balanus* very soon closed up, thereafter occasionally relaxing the valves. The decrease in the blood concentration (figure 15) indicates that some of the specimens maintained as effective a control over water entering the mantle cavity after 24 h as *B. balanoides*. One specimen resumed activity after 27 h, and its blood had become nearly isosmotic with the medium.

TABLE 5. THE DEPRESSION OF FREEZING POINT OF THE BLOOD OF SPECIMENS OF *BALANUS HAMERI* THAT WERE ACTIVE AFTER 24 H IN THE DILUTIONS OF SEA WATER INDICATED

medium $\Delta(-°C)$	blood $\Delta(-°C)$		
1.78	1.80	1.85	1.78
1.44	1.48†	1.41	1.47
1.43	1.45	1.42	1.40
1.26	1.26	1.27	

† Mantle cavity fluid of this specimen $\Delta = -1.44$ °C.

TABLE 6. COMPARISON OF THE DEPRESSION OF FREEZING POINT OF THE BLOOD OF SPECIMENS OF *BALANUS HAMERI* AFTER 8 H IN 50 % SEA WATER (Δ OF -0.89 °C) THAT WERE EITHER SHOWING CIRRAL AND VALVE MOVEMENTS OR HAD THE OPERCULAR VALVES RESTRICTED BY A WIRE HARNESS

activity	no.	mean blood $\Delta(-°C)$	S.D. (°C)
beating	6	1.20	0.04
restricted	4	1.68	0.02

$F = 1.9353$, $t = 5.677$; sig. diff. at 5% level.

TABLE 7. COMPARISON OF THE RATE OF DECREASE (b = REGRESSION COEFFICIENT) OF THE BLOOD CONCENTRATION OF BARNACLE SPECIES WHEN PLACED IN 50 % SEA WATER

Over the first ¼ h the concentration fell rapidly concurrent with initial activity, and these values were not used in the regression. a = regression constant. In *B. hameri* the regression was applied over eight hours only (see figure 15).

species	no.	b (°C/h)	a	half life (i.e. a rise from -1.7 to -1.3 °C) in hours
B. hameri	18	0.0770	1.77	5
B. balanus	9	0.0136	1.75	29
B. crenatus	33	0.0074	1.69	54
B. balanoides	51	0.0056	1.68	71
E. modestus	36	0.0052	1.65	77

The blood of specimens of *B. hameri* active in dilute sea-water media was isosmotic with the media (table 5). When placed in 50% sea water, the blood concentration dropped quickly, approaching that of the external medium within 10 h (figure 15 and table 7). Coma ensued and death occurred within a day. In table 6 the blood concentration of four specimens of *B. hameri* that had been prevented from opening the opercular valves by a wire harness, and then placed in 50% sea water is compared with that of five specimens not so restricted. The blood of these specimens was significantly more hyperosmotic, equivalent to that of *B. balanus*, *B. crenatus*, *B. balanoides* and *E. modestus* after an equal time in similar conditions. When placed directly into dilute sea-water media, activity ceased at salinities of 22 to 24‰. Even with gradual reduction of the salinity of the medium, no specimens were active in salinities of less than 22‰.

B. hameri clearly differs from the other species studied in its inability to use the opercular apparatus to close itself off from adverse salinities, and the erratic valve and cirral behaviour leads to a relatively rapid exchange of the medium into the mantle cavity. With the onset of coma in activity-limiting salinities, the opercular valves are held in an extruded position often with the cirri also protruded. It also appears that *B. hameri* is not able to acclimate to reduced salinity.

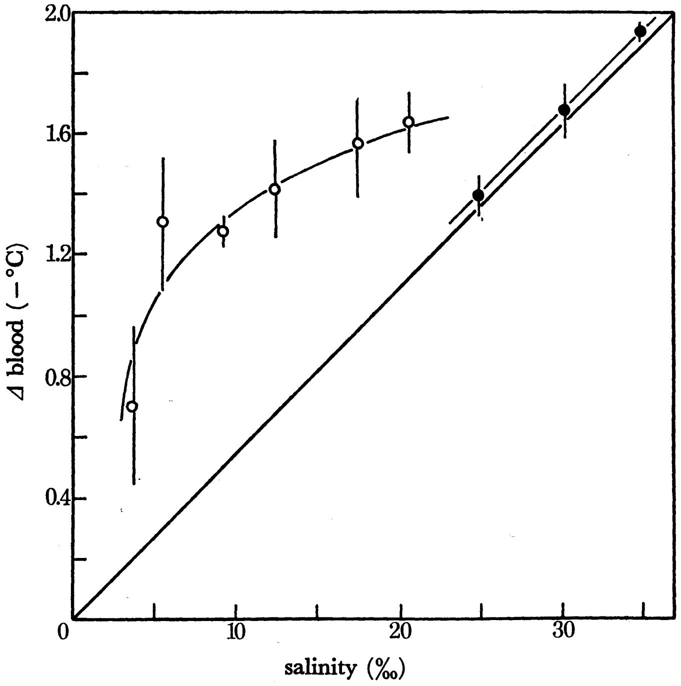

FIGURE 16. The relationship between the blood concentration of *C. stellatus* and the salinity of the medium, 24 h after immersion. Solid symbols represent cirrally active specimens; open symbols represent specimens in which no cirral activity was seen. The mean and standard deviation for five or six determinations is given for each medium. Diagonal line = isosmoticity.

Chthamalus stellatus

Specimens were collected from Thurlestone Rocks, Devon, and when placed directly into dilute sea-water media showed cirral activity in 30 and 25‰, but not in 20‰ or lower. In dilute media where there was no cirral activity, the opercular flaps were visible between the valves, often with a wide gap leading into the mantle cavity. The blood concentrations (figure 16) show that the active specimens are in almost complete osmotic conformity, whereas the inactive ones in dilute media retain a blood concentration that is markedly hyperosmotic. However, in the latter, the blood is not maintained constantly hyperosmotic over the whole range of dilution to the same extent as in *B. balanoides*, *B. crenatus* and *E. modestus*, probably indicative of a less effective separation of the mantle cavity from the medium.

THE RESPONSE TO SALINITY

The beating of the cirri and the movements of the prosoma of barnacles produce a flow of medium in and out of the mantle cavity, fulfilling respiratory and excretory needs (Crisp & Southward 1961). When normal beating, fast beating or respiratory pumping of the cirri occurs, at least in all the species of barnacles examined in this paper, the concentration of the blood is held at a level slightly above that of the medium, and conforms with changes in the external medium. On transfer from one salinity to another in which cirral activity is maintained, the adjustment of the blood concentration occurs within a few hours, and the change is roughly exponential.

The slightly higher osmoticity maintained by the blood over the external medium and the fluid within the mantle cavity is apparent in the results given by Belyaev (1949) for *B. balanoides*. A similar relation has also been established for the isopod *Limnoria* by Eltringham (1964). Many, if not all, crustaceans possess blood proteins which may bind ions to establish an osmotic gradient across the integument (Robertson 1953). Active ionic regulation could also result in an osmotic gradient without osmotic regulation. The degree of ionic regulation may vary with the physiological condition, e.g. the stage of the moulting cycle (Robertson 1960), which would result in a range of freezing point values for a heterogeneous sample of animals. However, nothing is known about the ionic or organic composition of the body fluid of cirripedes, nor about the nature of the excretory products. Whatever the mechanism by which the blood is maintained hyperosmotic, the blood concentration passively parallels any change in the concentration of the surrounding medium, and the active barnacles must be regarded as osmoconformers.

If no attention is paid to the behaviour of the barnacles, a superficial consideration of the blood concentration after a given time in sea-water solutions sufficiently dilute to inhibit cirral activity, might be held to indicate some level of hyperosmoregulation. Thus, Belyaev (1949) sampled the blood of *B. balanoides* after 3 days in a range of dilute media, and concluded that the hyperosmoticity was due to an effective osmoregulatory apparatus aided by the partial closure of the opercular valves to reduce the inflow of water. This fails to answer the question of how the excreted water is voided from the mantle cavity. An active barnacle with the valves wide apart has a definite inflow and outflow from the mantle cavity (Crisp & Southward 1961), but currents have not been demonstrated associated with the pneumostome of the 'testing' barnacle.

Newman (1967) determined the depression of freezing point of the blood of the intertidal species *B. glandula* and *B. amphitrite* after periods varying from 18 h to 3 days in a range of experimental media. He first established that 'the mantle cavity fluid is essentially isosmotic to the blood', and then noted that below 25 % sea-water feeding activity ceases, but 'both species continue to feed below 50 % sea water during which time the mantle cavity fluid is maintained hypertonic to the medium and isotonic to the blood'. Newman concluded that these species 'regulate below 50 % sea water', and postulated that the plugging of the opercular orifice by the prosoma 'allows the animals to feed while affecting a mechanical separation between the mantle cavity and the external milieu'. Obviously Newman is using the term osmoregulation to mean osmotic evasion, but the theory implies that either only the integument of the mantle cavity is at all permeable and that of the cirri, mouthparts and the exposed regions of the prosoma are impermeable, or that the barnacle is in fact effectively regulating the osmotic pressure of the blood and the excreted water is being voided by organs on the mouthparts or cirri. The maxillary organs are very likely excretory in function (Bruntz 1902; Nilsson-Cantell 1921), but it has not been demonstrated that they are effective osmotic pumps. During fast beating of the cirri the prosoma is held in an elevated position (Crisp & Southward 1961), but this behaviour is usually of short duration and a response to food stimuli. In none of the species of the present study was this raised position of the prosoma and active cirral beating noted as being especially characteristic in dilute sea-water media, even though it was particularly looked for to see whether this anatomical arrangement for osmotic evasion was used and effective in European species. In the absence of food stimuli, it is rather the pattern of normal beating and respiratory pumping that is commonly displayed in dilute media, and in these activities there is a regular and rapid exchange of medium between the mantle cavity and the exterior. In all specimens that were observed to show any activity in which the cirri were extruded, the concentration of the blood conformed with the medium. Therefore, either the European species here studied are very different in their physiology and behaviour from the Californian *B. glandula* and *B. amphitrite* when exposed to osmotic stress, or the latter's activity and blood concentration relationships were incorrectly interpreted. Bergen (1968) also noted the hyperosmoticity of the mantle cavity fluid of *B. glandula* in 50 % sea water and lower, and attributed this only to closure of the opercular valves.

In adverse salinities barnacles respond by withdrawing the cirri and prosoma, and close the opercular valves. This is an intrinsic response of any barnacle to any adverse stimulus. It is well known that characteristically intertidal barnacles remain closed for the duration of the adverse conditions associated with emersion, whereas more characteristically sublittoral ones do not (Barnes & Barnes 1957). The opercular valves are, however, relaxed from time to time and a small pneumostome appears between the opercular flaps. Grainger & Newell (1965) have shown that the pneumostome allows gaseous diffusion to occur during emersion, facilitating aerial respiration. The pneumostome allows the intertidal species *C. stellatus*, *B. balanoides* and *E. modestus* to respire during emersion and yet expose the mantle cavity minimally to the atmosphere. The more continuously submerged species, *B. crenatus*, *B. improvisus*, *B. balanus* and *B. hameri*, however, do not form a pneumostome during emersion, but continue to make cirral and opercular movements that can only result in enhanced water loss. Now, in sea water dilute enough to inhibit activity and cause valve closure, the barnacles *E. modestus*, *B. balanoides*, *B. crenatus*, *B. improvisus* do occasionally relax the opercular valves slightly, and a pneumostome-like opening appears between the opercular flaps. Barnes, Finlayson & Piatigorsky (1963) have shown that in water of low salinity anaerobic conditions are set up within *B. balanoides*, *B. crenatus* and *C. stellatus*, with the accumulation of some lactic acid which is possibly excreted at intervals. It is suggestive that the infrequent appearance of the opening is probably a response to an oxygen lack. Whatever the causes of its formation, an occasional communication afforded by the pneumostome will allow salt exchange between the mantle cavity and the external medium. In the absence of any cirral or body movements to aid convection, the salt exchange will be by the slow process of molecular diffusion through the pneumostome. Crisp & Southward (1961) have applied the name 'testing' to this level of behaviour, and it is conceivable that the animal is able to test the nature of the environment, and if the salinity remains too low the response will be to close the valves again.

B. hameri does not keep its opercular valves closed when in media of low salinity, but continues cirral movements until succumbing to coma as a prelude to death. The valves are then fully extended, often with the cirri and prosoma protruding. *C. stellatus* also does not close the opercular valves to form a distinct pneumostome when in dilute sea water, but nevertheless restricts its cirral activity. Diffusion then occurs through a relatively wider opening which is permanently present, resulting in a poorer protection in dilute sea water.

There is a wide range in the degree to which the blood remains hyperosmotic in those specimens that are inactive in dilute sea-water media. The variation could reflect individual differences in the duration and frequency of pneumostome formation. In dilute sea water, the blood solute concentration approaches that of the external medium and, depending on the tolerance of the tissues and the salinity of the medium, may result in the death of the animals. In *E. modestus*, *B. balanoides*, *B. crenatus* and *B. balanus* the concentration of the blood decreases more or less linearly with time, whereas in active specimens in more saline media, and in *B. hameri*, the rate of change is more nearly exponential. Obviously, the closed valves slow down the rate of entry of solutes into or out of the mantle cavity. For *E. modestus* and *B. crenatus* at least, the rate of change of the blood concentration is greater at higher temperatures, which accords with the positive temperature coefficient of passive diffusion, but is contrary to what would be expected of an effective regulating mechanism which would probably be more efficient at higher temperatures, judging from the results on active regulators such as *Crangon crangon* (Broekema 1941) and *Penaeus* spp. (Williams 1960).

Even without direct communication between the exterior and the mantle cavity, it is probable that some of the integument underlying the opercular valves and exposed between the valves or between the valves and the parietal plates of the shell will be exposed to the medium. The shell itself may not be entirely impermeable. No crustacean integument has been shown to be completely impermeable, and it is possible therefore that some exchange of water or solutes occurs across the general integument. The rate of change of the concentration of the blood will, therefore, depend on a number of factors: initially on the activity of the animals, and if there is some control over the behaviour in adverse salinities then it will be proportional to the size and frequency of the pneumostome, the area and permeability of any exposed integument, the porosity of the shell, and on the temperature and the concentration gradient.

Clearly, the hyperosmoticity of the blood of barnacles in dilute sea water is a result of a partial separation of the mantle cavity from the exterior. If an osmoregulatory apparatus is contributing to the maintenance of the hyperosmotic blood, dilute urine would be excreted into the mantle cavity of the withdrawn barnacle, and then would need to be forced out against the concentration gradient between the opercular valves as there is no other outlet to the exterior. The enclosure of the animal by a hard calcareous shell therefore prevents the animal combining osmotic evasion with an active regulatory mechanism. In any case, closing the valves is not of permanent advantage; the blood salts are ultimately lost and in the meantime the barnacle cannot perform such vital activities as feeding and respiration.

It is of interest that in a variety of molluscs the shell is similarly used temporarily to separate the animal from osmotic stress. In *Mytilus edulis* (Cronklin & Krogh 1938), *Scrobicularia plana* (Freeman & Rigler 1957), *Littorina* spp. (Avens & Sleigh 1965), *Acmaea limulata* (Segal & Dehnel 1962), *Patella vulgata* (Arnold 1957) and *Siphonaria pectinata* (McAlister & Fisher 1968) the response to osmotic stress is to withdraw into the shell, or between the shell and rock substratum in the limpet forms, and 'wait out' the duration of the stress. The intertidal barnacles *E. modestus*, *B. balanoides* and *C. stellatus*, which have membranous bases, as well as the calcareous-based sublittoral species *B. crenatus*, *B. improvisus* and *B. balanus* behave similarly but with varying effectiveness.

ACCLIMATION TO SALINITY

Salinity acclimation may obscure any attempt to measure the absolute salinity tolerance of a species unless it is first allowed to adapt. Belyaev (1949) showed that the activity of *B. balanoides* from the White Sea was adjusted to lower salinities than was that of specimens of the same species from the much higher salinities of the Barents Sea. Savvateev (1952) found that the time taken for loss of irritability of specimens, supposedly of *B. balanoides*, when placed in a range of salinities was related to the salinity of the habitat: those from an environment of 5‰ retained irritability in salinities from 7 to 51‰, those from 18‰ in 11 to 51‰, and those from 34‰ in 17 to 51‰. Specimens from a habitat with a salinity of 34‰ were tolerant of 9‰ after 25 days acclimation to 5‰. In the present study, *E. modestus* and *B. balanoides* from an estuarine location were initially much more tolerant of low salinities than specimens of the same species from a habitat experiencing normal salinity. But the activity of the estuarine specimens after 4 days of acclimation in normal sea water showed a decrease in the salinity range tolerated. *B. improvisus* from an estuarine location showed adjustment of its activity after acclimation to high and low salinities. Acclimation towards low salinities must, it seems, proceed gradually. Whereas the salinity can be reduced gradually by say 2‰ per day and activity still be maintained, if the barnacles are placed directly into the dilute sea water the response is to close up. In time, though, activity may be resumed, and it is assumed that in this interval the gradualness of the fall in tonicity is of advantage in preventing drastic osmotic damage. Limitation of rapid changes of the blood concentration, in this case by appropriate control of an anatomical mechanism, allows time for adjustment of the intracellular concentration and perhaps of the biochemical organization. The activity exhibited with respect to low salinities is modifiable within the limits imposed by the genotype. It is indeed typical of osmoconformers lacking specific organs for effective regulation to show such non-genetic adaptation in which the tissues as a whole can acclimate to a gradual change (Kinne 1964). The ultimate salinity tolerance is probably set by the lower limit of solute concentration in the body fluids which allows the protoplasmic organization to perform its vital metabolic functions.

The lower salinity limit measured for a particular species will depend on the criteria of the experiment and on the acclimation state of the specimens. Although there has been no attempt here to determine ultimate salinity tolerance, casual observations of activity of the various species suggest that there is considerable variation in the degree of blood dilution that is tolerated. The deep-water barnacle *B. hameri* showed no salinity acclimation, no defensive response to adverse salinities, and succumbed at salinities lower than about 24‰. This salinity is about that which initally inhibited activity in specimens of *E. modestus*, *B. balanoides* and *B. crenatus* acclimated to normal salinity, although these three species are capable of activity after acclimation in salinities down to about 14‰. *B. improvisus* is especially tolerant of blood dilution, maintaining activity in salinities down to 1 or 2‰.

DISTRIBUTION IN RELATION TO SALINITY

As barnacles appear in general to be osmoconformers, the distribution patterns with respect to salinity will give some idea of the tolerances as affecting survival and growth, assuming that salinity is the limiting factor. In estuaries other factors such as nature of the substratum, ionic composition of the water, and barriers to larval dispersal may be just as important. Furthermore, since barnacles can resist even fresh water for short periods, the salinity limits to distribution in an estuary will depend on the cumulative time of immersion during which feeding and other vital activities will enable survival and growth. The description of the fluctuating salinity régime of an estuarine location would require a prohibitive amount of physical observation, and predictions would have to take into account the variation in freshwater run-off. The distribution of a species with respect to a more stable salinity gradient such as that of the Baltic

Sea is easier to interpret. *B. improvisus* is limited in the Baltic by salinities of 3 to 5‰ (Segerstrale 1957; Barnes & Barnes 1961), where it mingles with the freshwater component of the fauna; small specimens are figured on the exoskeleton of a water beetle by Tarasov & Zevina (1957). Darwin (1854) noted the extreme euryhalinity of this species in a probably not perfectly controlled experiment; after 'placing them in perfectly fresh water they continued for many hours expanding and retracting their cirri with perfect regularity and vigour'. Although *B. improvisus* can maintain activity in salinities of 1 to 2‰, it does not mean that the species can survive to grow in such low salinities. The ultimate salinity may well be 3‰, and at this and higher salinities, the species conforms. The marine mussel *Mytilus edulis* also closes its valves as a response to osmotic stress, and shows acclimation to low salinities (Cronklin & Krogh 1938). It penetrates into the Baltic Sea to a salinity limit of 4.5 to 5‰, and is an osmotic conformer. The apparent regulation in very low salinities in *B. improvisus* may be due to an increased amount of organic constituents in the blood, resulting perhaps from their release from the tissues as a result of low salinity acclimation. If, however, it is due to effective ionic regulation, then the response is reminiscent of the regulation in the freshwater mussel *Anodonta cygnea* (Duval 1925). But *B. improvisus* has not yet been recorded from a permanently freshwater habitat, and this is suggestive of a lack of an active and effective osmotic regulation in very low salinities; the hyperosmoticity is probably merely a result of organic changes in the tissues.

The effect of salinity on *B. pallidus stutsburi* in Lagos Harbour has been investigated by Sandison (1966). This estuarine species, like *B. improvisus*, appears to be tolerant of very low salinities, but Sandison showed (experimentally) that 20‰ was the highest salinity for normal life processes. The osmotic relationships of *B. pallidus* have not been investigated, but its stenohalinity is in contrast to the euryhalinity of *B. improvisus*.

As with all sessile organisms, limiting factors may often operate on the more delicate and unprotected larval stages. After settlement, metamophosis and the calcification of the shell plates, the young barnacle would be able to survive periodic low salinities by evasion. The larval tolerance will thus be relevant in relating distribution to salinity. The evasive response of the adult barnacle to low salinity will protect the eggs and embryos retained in the mantle cavity. Crisp & Costlow (1963) have shown that the egg masses of three North American barnacles can withstand wide ranges of salinity for long periods, 20 to 50‰ permitting normal development, and found a similar tolerance in the first and second-stage nauplii. Barnes (1953) found that the first-stage nauplii of *B. balanoides* and *B. crenatus* tolerated a similar range; a salinity of 18‰ permitted full swimming activity, and considerable recovery occurred after 2 days at 15‰. Bhatnagar & Crisp (1965) investigated fully the time–temperature–salinity relationships for survival of the first-stage nauplii of *B. balanoides*, *E. modestus* and *C. stellatus*, and showed that at normal temperatures salinities below 12‰ or above 50‰ caused complete immobility. Although the salinity values defined as tolerable depend on the experimental criteria, the values quoted above for the larvae agree with the salinity ranges to which the adults are potentially able to acclimate.

Barnes & Barnes (1962) have described the distribution of *B. balanoides* in the Baltic Sea; this species penetrates to the southern end of the Kattegat where salinities of 10 to 15‰ are experienced. This agrees with the limiting salinity for activity of the Conway Estuary specimens which were collected from near the upstream limit of distribution, and with the tolerance of the nauplii.

E. modestus extends further into estuaries than *B. balanoides* (see Lewis 1964), and although specimens of both species collected from the same habitat show similar responses to osmotic stress because of acclimation, the former species must be potentially, or genetically, more euryhaline. This is apparent in the tolerances of the nauplii (Bhatnagar & Crisp 1965). *E. modestus* has not yet spread into the Baltic, and if temperature or some other factor does not prevent it spreading into more northern latitudes, it will be interesting to mark its limit in the Baltic Sea. *B. crenatus* occurs in estuarine locations, and it extends into the Tamar almost as far as *E. modestus*, further than *B. balanoides*, but not as far as *B. improvisus*. It is confined to low tidal situations, and would experience generally lower salinities than mid-tide barnacles at the same station. *B. crenatus* has about the same degree of tissue tolerance to low salinities as *E. modestus*, and their responses to osmotic stresses are similar.

The distribution of barnacles with respect to salinity is thus a consequence of the degree of euryhalinity of the tissues, and the pattern of evasive behaviour. None of the species studied here possess the advantage of being able to regulate and thereby maintain life processes in otherwise adverse salinities. The results of competition between *E. modestus*, *B. balanoides* and *B. crenatus* may therefore depend on factors other than the salinity experienced. Where *E. modestus* is becoming dominant it is probably because of greater fecundity, prolonged breeding and settling seasons, enabling prior colonization of vacant sites on the substrate. On the other hand, the extreme tolerance of *B. improvisus* should enable it to remain dominant in those regions from which the other species are excluded by prolonged periods of low salinity.

C. stellatus, the highest intertidal barnacle in British waters, does not behave efficiently in low salinities, and communication between the mantle cavity and the exterior is only partially restricted. Not surprisingly, *C. stellatus* is not characteristically an estuarine barnacle. Lowered salinity on wave-exposed shores will be primarily associated with rainfall during emersion, and from the osmotic responses of *C. stellatus* it would seem that during the short periods that rainfall is a factor, it would be unlikely to affect the vertical distribution of this or the other species that close the opercular valves in adverse conditions.

In hypersaline media also, *E. modestus*, *B. balanoides* and *B. crenatus* are essentially osmotic conformers, remaining active in concentrations up to 50 to 55‰. Above these concentrations, the immediate response is to close up, thereby retarding the exchange between the mantle cavity and the outside medium. But the control in hypersaline conditions is not as effective as in dilute media. Thus, in 200% sea water, equilibrium occurs within 2 days. High salinities in the environment, apart from certain hypersaline lagoons in tropical areas, will always be associated with emersion in the intertidal zone. On emersion the barnacles will encounter quite different problems of water conservation and heat tolerance, as well as the increase of salt concentrations of the body and tissue fluids. Not surprisingly the response of barnacles under hypersaline conditions is not entirely appropriate to conditions of submersion.

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