J. mar. biol. Ass. U.K. (1961) 41, 161–174
Printed in Great Britain

SALINITY TOLERANCE AND PERMEABILITY TO WATER OF THE STARFISH *ASTERIAS RUBENS* L.

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(Text-figs. 1 to 7)

Present-day echinoderms are marine animals and are usually considered to be a stenohaline group, in the sense that they are intolerant of salinities differing greatly from that of normal oceanic sea water. In the Baltic Sea, however, the position is somewhat different. Most groups of echinoderms are to be found in the Kattegat, but the number of species declines eastwards and the asteroids are the only group to extend beyond the Öresund. The farthest penetration is made by *Asterias rubens* which is taken as far east as Rugen Island where the salinity is only 8‰ (Brattström, 1941; Segerstråle, 1949; Schlieper, 1957).

In the British Isles the distribution of echinoderms is fairly well documented. According to Bassindale (1940, 1943), none are to be found in the Bristol Channel above Kilve, where normal salinity conditions obtain. Some post-larval asteroids have, however, been found by Rees (1938) in the Cardiff Roads plankton, where the salinity was 27·1‰. In the Salcombe and Exe estuaries, Allen & Todd (1900) did not report any echinoderms from water of less than 30‰. Percival (1929) and Spooner & Moore (1940) in their Tamar surveys did not record *A. rubens* within the estuary. In north-east England too, echinoderms seem to be absent from the estuaries (Hobson, 1949). Hancock (1955) reported *Asterias* from the River Crouch as far as the western end of Bridgemarsh Island, and the occasional specimen is taken a little higher up the river, where the summer low tide salinity is not reduced. In winter, however, the low tide salinity may fall as low as 18‰, but it is not known whether *Asterias* is also present at these times. Some personal observations made in September 1957, together with the opinion of local fishermen, suggests that in the River Stour *Asterias* is not taken more than 3 miles above Harwich, but full salinity conditions are maintained for a further 8 miles.

The aim of the present study therefore, has been to investigate the salinity tolerance and permeability to water of *A. rubens*. The animals were collected at Whitstable, which is situated at the mouth of the Thames estuary on the north Kent coast. These animals were taken as representative of a population which in nature does not appear to enter water of significantly reduced salinity, their distribution not extending west of the Pollard Spit area (Newell, 1957, personal communication and personal observations) where the lowest recorded salinity for the year 1956/57 was 28·7‰.

SALINITY TOLERANCE

Some preliminary observations were performed in which five animals were kept in each of a series of tanks containing sea water of different dilutions. The dilutions were made with M/400 sodium bicarbonate and the salinities checked daily by chloride titration and adjusted if necessary. No form of temperature control was available for this series of experiments and the variation encountered was from 14 to 17° C. Food was available to the starfish in the form of *Mytilus edulis*. The water was aerated by means of a small pump fitted with stone diffusers and any dead animals were removed as soon as they were noticed. After 1 week, all those in water of 18‰ had died, whereas those at 25‰ were still in good condition and were feeding.

The lower limit of tolerance was narrowed further by using ten animals in 20 l. of medium for each of a number of different salinities. The LD_{50} after 14 days was taken as the criterion of tolerance. A value of between 22 and 24‰ was obtained. Loosanoff (1945) found that he was unable to affect the lower limit of acclimatization of *Asterias forbesi* (Desor) by the protracted dilution of the medium. Similarly, the value obtained here for *A. rubens* was not affected by diluting the medium so as to cause a fall in salinity of 1‰ per day.

WEIGHT REGULATION AND PERMEABILITY TO WATER

The time course of the weight changes of *A. rubens* when placed in diluted sea water has been followed on only two occasions. In 1934, Bethe placed the animal in 75% sea water and found that there was a maximum gain in weight of 10% in from 3 to 6 h, decreasing to the original value in from 6 to 24 h. Similar results were obtained by Maloeuf (1938) who immersed *Asterias* in 50% sea water. The maximum increase in weight occurred after about 7 h, falling back to normal in from 20 to 50 h. As these animals were shown, by measurements of the internal osmotic pressure, to be isotonic with the medium after they had returned to their original weight, it may be supposed that both water and salts had been lost across the body wall, the animal lacking any morphologically differentiated excretory organ. Maloeuf concluded that *A. rubens* is incapable of any osmoregulation; his graphs, however, and those of Bethe, suggest that it is capable of weight regulation.

Similar experiments to these have been performed on Whitstable animals. In view of the extreme delicacy of the distended starfish, special precautions were taken during the weighing of the animals. These consisted of keeping them for the experimental period in small plastic cages suspended at the corners in the diluted media. When removed for weighing the cage could thus be hung on the balance with the animal in a horizontal position, no matter what attitude it had assumed. The purpose of this was to prevent localized increases in pressure within the coelom and the consequent rupture of the integument. Before weighing, the cages were blotted with filter-paper and care was taken to ensure that no water was trapped under the ambulacral grooves. In experiments where the animals were permitted the free range of the tanks and simply removed and dried with a cloth prior to weighing, frequent and large decreases in weight were observed. This was found invariably to be due to splitting of the integument, generally near the tips of the arms, followed by loss of coelomic fluid. For each experiment five animals of about 30 g were weighed at regular intervals, a further five being kept as controls. Four separate dilutions were employed—95, 85, 70 and 55% sea water. After 50 h in these dilutions the animals were transferred back to normal sea water and their weight changes followed for a further 50 h. During the experiments the control animals did not vary in weight by more than 3%.

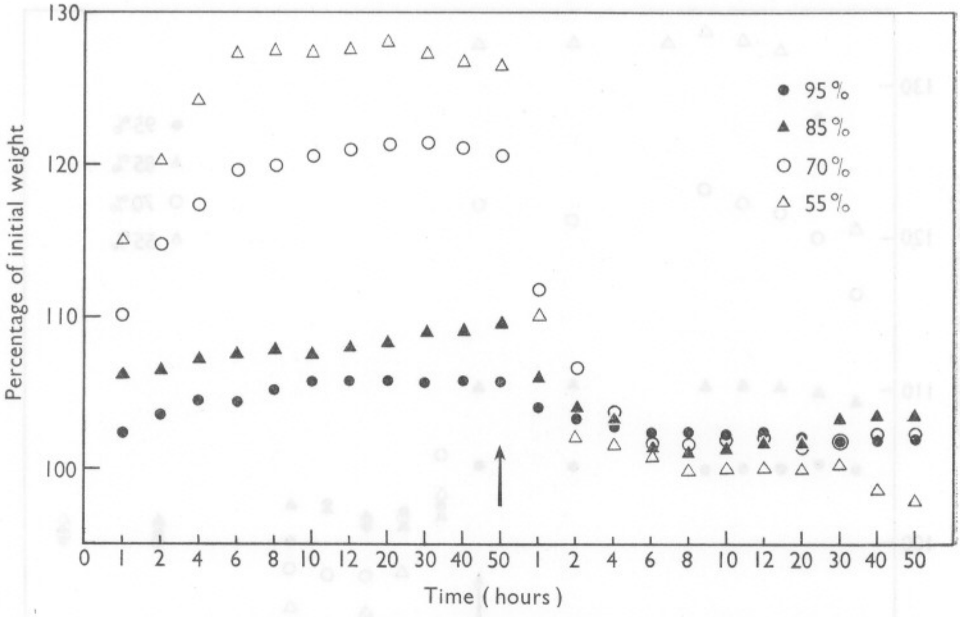

Fig. 1. Weight changes in large animals in diluted media. Arrow indicates replacement in normal sea water.

Some small fluctuations in weight were occasionally recorded. These mostly coincided with eversions and retractions of the stomach, similar observations being made by Maloeuf. From the graphs illustrating the time course of their behaviour in diluted media, it will be seen that there is no suggestion of a return to their original weight, and it is concluded that *A. rubens* is incapable of weight or volume regulation. This is contrary to the findings of both Bethe (1934) and Maloeuf (1938), but as neither of them mentions any special precautions taken during the weighing of their animals, and, as even quite delicate handling can cause bursting of the integument, it is not improbable that the more anomalous results of these authors may be due to this cause. This series of experiments was repeated using smaller, and presumably younger, animals of between 2 and 5 g. The time course of their weight changes is illustrated in Fig. 2. In sea water more dilute than about 50 %, the weight changes were less than expected. In both 30 and 20 % sea water a rise of only 20 % in weight was encountered and in tap water many animals actually lost weight. This is contrary to the results of Garrey (1904), who found that *A. vulgaris* became much swollen after 12 h in fresh water ($\Delta = -0{\cdot}02°$ C). It is clear that at these dilutions the integument of the

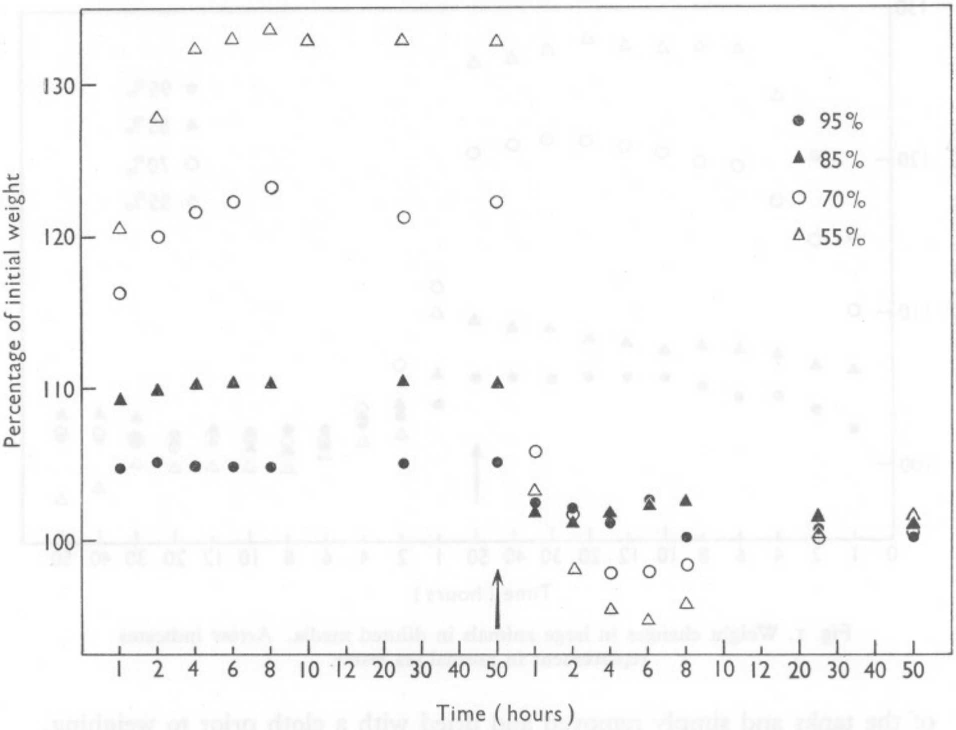

Fig. 2. Weight changes in small animals in diluted media. Arrow indicates replacement in normal sea water.

animal is damaged severely, probably resulting in a rapid loss of salts, thus leading to a reduced water uptake.

In order to compare the results obtained from different animals and different experiments, it is necessary to have some standard by which to measure permeability. The reciprocal of the time taken for a 3 % change in weight to occur, a purely arbitrary figure, was used for the following reasons. Such a value usually fell on the linear portion of the graph, it is a sufficiently small value for the change in the concentration difference between the inside and the outside of the animal to be negligible as also would be the changes in the volume, surface area and thickness of the integument. In Fig. 3 comparison is made between large and small animals for the transfer of water both into, and out of, the animals. It will be seen that the rate of such transfer is nearly twice as great in the smaller animals. However, the total uptake by both large and small animals is very similar at each of the dilutions used and is proportional to the degree of dilution (see Fig. 4).

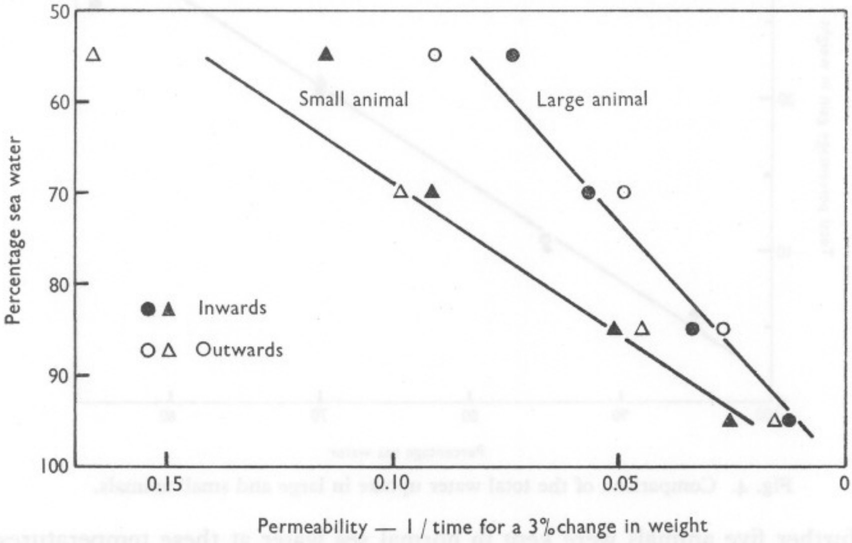

Fig. 3. Comparison of rates of water movement into and out of both large and small animals.

Direct measurement of the surface area of a starfish presents an almost impossible task due to the presence on the aboral surface of numerous dermal branchiae, and on the oral surface, the tube feet. However, some comparison between large and small animals can be made if the starfish arm be considered as a simple geometrical figure, namely a cone. Fig. 5 indicates the relationship between arm length and the (surface area)/volume ratio for some thirty animals. In the weight regulation experiments large animals had an arm length of about 5–6 cm and the small ones 1·5–2 cm. It will be seen therefore, that relative to the body volume, the surface area of the small animals is nearly twice as great as for the large ones. It is suggested, therefore, that this factor alone could account for the increased rate of water transfer found in the smaller animals.

The effect of temperature on the rate of water uptake in large animals was investigated by examining the time course of the weight changes of five animals in a fixed dilution of sea water—70 %—at 0, 5, 10, 15 and 20° C. A further five animals were kept in normal sea water at these temperatures as controls. The same measure of permeability as before was employed, and this value is plotted against the experimental temperature in Fig. 6. Increasing the temperature increases slightly the rate of water movement, the Q_{10} being about 1·5. Furthermore, evidence of weight regulation over the experimental range of temperatures is lacking.

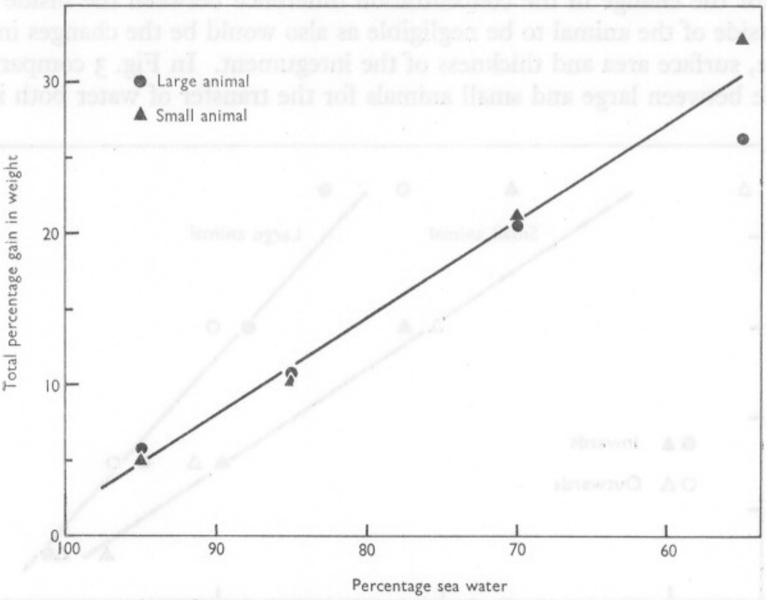

Fig. 4. Comparison of the total water uptake in large and small animals.

It is not inconceivable that some seasonal influence may affect the permeability to water. For this reason the rate of water uptake in 70 % sea water was measured at monthly intervals during the year 1956/57. The permeability values so obtained are also plotted on Fig. 6 against the environmental temperature at which they were performed. As they fall within the limits of variation set by the effects of temperature, it is suggested that in so far as permeability to water is concerned, any innate seasonal difference is negligible. In none of these experiments was there any tendency for weight regulation to occur.

So far, the account has been concerned solely with the permeability of the animal as a whole, no attempt being made to assess the relative importance of the contributions made by the two major surfaces of the body, namely, the integument and the tube feet, in the transfer of water. Therefore, several attempts were made to examine the rate of water uptake with the ambulacral grooves sealed with such substances as dental cement and various plastic compounds. Unfortunately, any substance giving a good seal also resulted in the splitting of the integument at its junction with the sealing compound, so no reliable measurements were possible. Some estimate of the relative permeability of the two surfaces was, however, obtained by measuring the loss in weight due to evaporation when either the aboral or the oral surface was exposed to the atmosphere. For this purpose a bed of dry gravel was used with twenty animals exposed in each of the positions mentioned. After 3 h five animals from each group were removed, weighed and the chloride concentration in both the perivisceral and water vascular cavities estimated by the method of Milton & Waters (1949). A further five animals from each group were replaced in normal sea water and weighed at intervals. After 18 h they had fully recovered. Similar estimations were made after a 6 h period

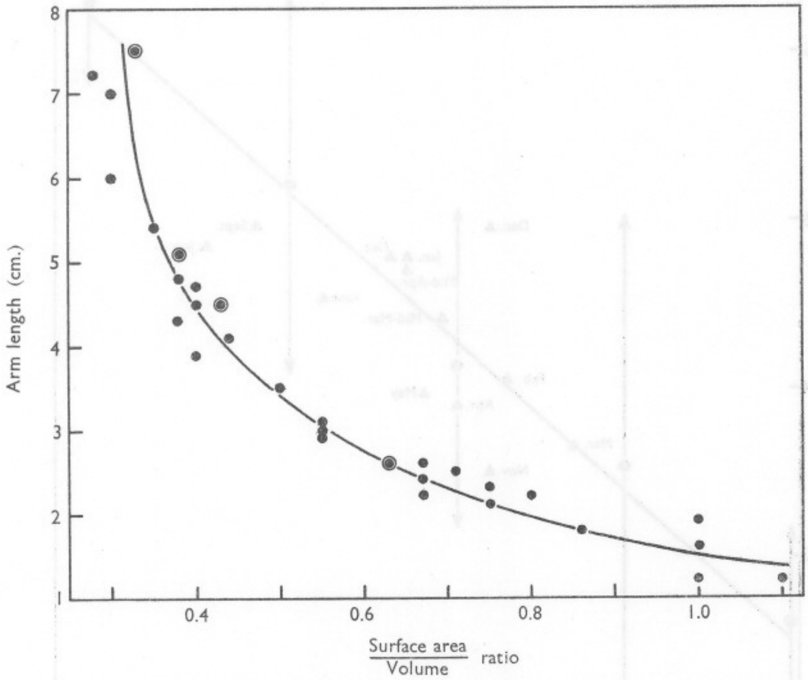

Fig. 5. Relationship between arm length and the (surface area)/volume ratio for a range of animals.

of exposure, but on this occasion there was no recovery in sea water. Table 1 shows the values obtained and it suggests that the amount of water lost was independent of the surface exposed, furthermore, the chloride concentration was increased by a similar amount in both coelomic cavities. Bearing in mind

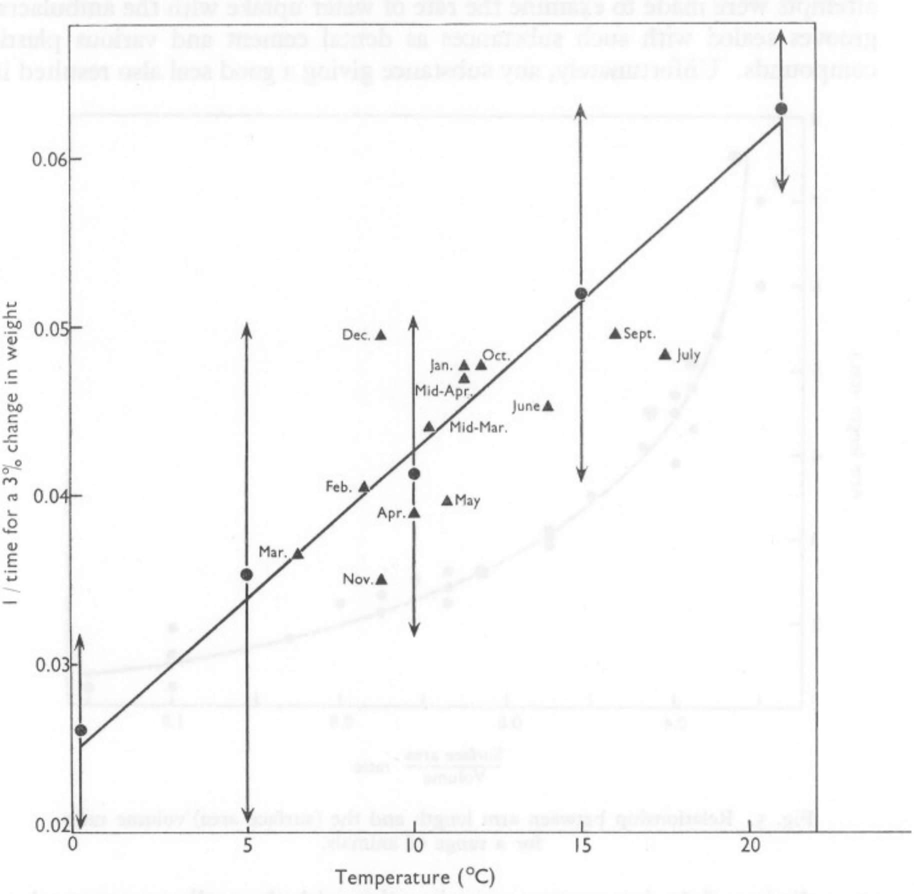

Fig. 6. Effect of temperature and season on the rate of water transfer. Arrows indicate the standard deviation due to the effects of temperature.

TABLE 1. RATES OF WATER LOSS AND CHLORIDE CONCENTRATION IN BODY FLUIDS OF EXPOSED ANIMALS

	Aboral surface uppermost			Oral surface uppermost		
		Increase in Cl⁻ conc. in fluids (%)			Increase in Cl⁻ conc. in fluids (%)	
Time of exposure	Loss in weight (%)	PVC	Amb.	Loss in weight (%)	PVC	Amb.
After 3 h	13	19·4	23·5	11	22·0	20·6
After 6 h	20	26·0	28·4	21	28·4	26·6

the relative volumes of these two cavities (Smith, 1946), it would be necessary for water to pass into the ampullae from the perivisceral cavity in order to permit such a large quantity to be lost via the ambulacral surface.

In order to examine the permeability of the various structures present in the starfish, isolated preparations of the tube feet, tube foot ampulla systems, stomach and gut caeca were made. The tube feet were ligatured in the retracted condition, for otherwise bursting was often encountered when they were placed in diluted media, which for this series of experiments was 70% sea water at 15° C. As the weight changes were very rapid compared with those in the intact animal, weighings were made at 20 min intervals for 1 h before returning the preparation to normal sea water for a similar period. The osmotically inactive material, in the form of adherent ossicles in the case of the tube-foot ampulla systems, was removed after the experiment and the weight thus allowed for when calculating the percentage changes in weight. The results of this experiment, shown in Fig. 7, indicate that the tube feet, ampullae, stomach and gut caeca are all extremely permeable to water and therefore a movement of water from one cavity to another as suggested above is quite feasible.

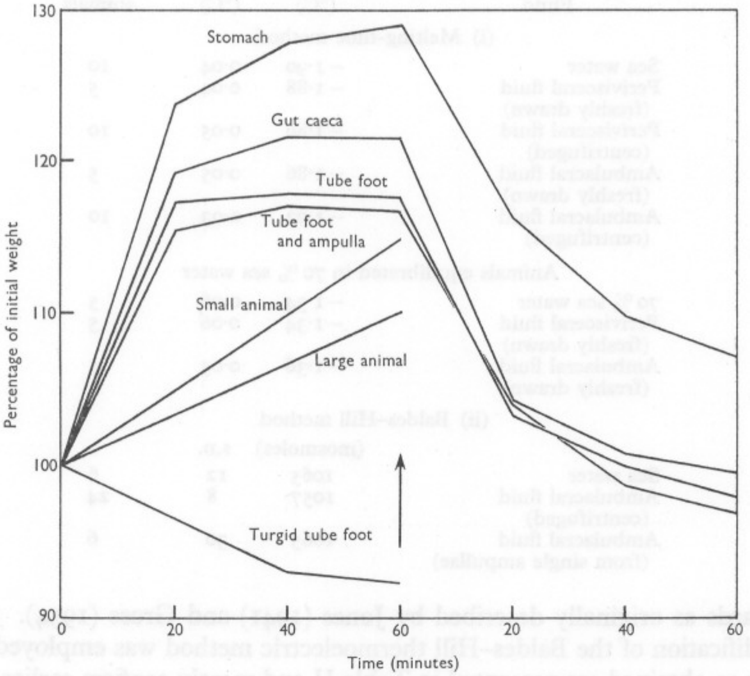

Fig. 7. Weight changes in isolated preparations from *Asterias rubens* in 70% sea water. Arrow indicates replacement in normal sea water.

Finally, osmotic pressure measurements were made on the perivisceral and ambulacral fluids of animals living under environmental conditions and upon those which had reached weight equilibrium in diluted media. Both freshly drawn and centrifuged samples were employed, but no difference was to be found between them. The method used was that of Freeman & Rigler (1957) based upon the melting times of the samples and a number of standards as originally described by Jones (1941) and Gross (1954). Later, a modification of the Baldes–Hill thermoelectric method was employed. The values so obtained are presented in Table II and merely confirm earlier work. Both the perivisceral and ambulacral fluids of *A. rubens* are isotonic with the environment and the animal is incapable of any osmoregulation in either coelomic cavity should the environment become diluted.

TABLE 2. OSMOTIC PRESSURE OF THE BODY FLUIDS UNDER ENVIRONMENTAL AND EXPERIMENTAL CONDITIONS

Fluid	Value (°C)	S.D. (°C)	No. of animals
(i) Melting-time method			
Sea water	−1·90	0·04	10
Perivisceral fluid (freshly drawn)	−1·88	0·04	5
Perivisceral fluid (centrifuged)	−1·86	0·05	10
Ambulacral fluid (freshly drawn)	−1·86	0·05	5
Ambulacral fluid (centrifuged)	−1·92	0·03	10
Animals equilibrated in 70 % sea water			
70 % sea water	−1·34	0·06	5
Perivisceral fluid (freshly drawn)	−1·34	0·06	5
Ambulacral fluid (freshly drawn)	−1·36	0·05	5
(ii) Baldes–Hill method			
	(mosmoles)	S.D.	
Sea water	1065	12	6
Ambulacral fluid (centrifuged)	1057	8	24
Ambulacral fluid (from single ampullae)	1065	30	6

DISCUSSION

A. forbesi (Desor) has a lower limit of acclimatization of about 17‰ (Loosanoff, 1945) and can withstand temperatures of up to 33·5° C (Huntsman & Sparks, 1924). Lee (1941) also notes that *A. forbesi* cannot endure a salinity below 18‰ for very long. *A. vulgaris* (Verrill) is by comparison less hardy, and hence is physiologically at least more like our own *A. rubens*. G. Smith (1940) found that this latter American species could live for a short while in water of salinity 14‰, but this experiment was criticized by Loosanoff as being of too short a duration for a true value for the lower limit of acclimatization to be obtained. In a more detailed study Topping & Fuller (1942) arrived at a figure of about 22‰ and backed this with field observations in the Narraguagus estuary (Maine, U.S.A.) where they found that its distribution ceased when the low tide salinity fell below 27·4‰. Furthermore, it seems unable to withstand temperatures in excess of about 25° C, similar to the maximum for *A. rubens*. If physiological differences such as these can exist in separate species of *Asterias*, then it seems reasonable to suppose that such differences as have been found between the North Sea and Baltic Sea populations of *A. rubens* might be justifiable grounds for postulating that they belong to different physiological races. The value for the lower limit of salinity tolerance obtained here for the North Sea *A. rubens* of around 23‰ must be viewed with some caution. No experiments were of a sufficient duration to test whether the animals could mature and breed at this dilution. Sagara & Ino (1954) showed that the bipinnaria of the Japanese starfish *A. amurensis* (Lutken) could tolerate a salinity of 13‰. However, even if the tolerance of the larva of *A. rubens* is greater than the adult, the value already obtained for the adult will still be a factor limiting its distribution. Even so, there is a considerable gap between a value of 23‰ and the 8‰ at which the Baltic Sea population can live and reproduce, although the 'spawning intensity' of animals in this locality is much reduced and asteroid larvae seem very sensitive to reduced salinity (Thorson, 1946). In addition to differences in salinity tolerance, the gross chemical composition, size of ova at maturity, breeding cycle and reaction times for the 'righting process', have also been shown to differ (Bock & Schlieper, 1953; Kowalski, 1955; Schlieper, 1957).

It is now stated in certain textbooks (e.g. Prosser *et al.* 1950) upon the authority of Bethe (1934) and Maloeuf (1938), that *A. rubens* is capable of regulating its weight in dilute media. In the present investigation nearly 300 animals have been examined and in no instance was there the slightest tendency for them to regulate their weight, even after as long as 50 h in diluted media. Furthermore, this situation is uninfluenced by temperature, season and size.

It was not possible to obtain a direct estimate of the contributions made by the oral and the aboral surfaces in the uptake of water when the animal is in a diluted medium. Rates of evaporation, however, suggest that there is very little difference between the two. When stranded, *A. rubens* is nearly always found with the oral surface against the substratum. In many instances it was discovered that the stomach was everted and penetrated some little distance down between the gravel. At the lower levels of the beach, where the animal is most frequently to be found, the water table is usually only a few centimetres below the surface and it is feasible that water evaporated from the surface of the animal can be replaced osmotically from this source.

Eversion of the stomach seems primarily to be due to oxygen lack, but may thus also be effective in preventing desiccation. Analysis of the body fluids of a few stranded animals indicated that they were only slightly more concentrated than the sea water. Animals stranded for any length of time on dry gravel seem invariably to be desiccated. Meyer (1935) found that each surface contributed equally to the total oxygen uptake. Experiments involving the weight changes of isolated preparations in diluted sea water confirmed that the bounding membranes of the animal are virtually freely permeable to water. Measurements of osmotic pressure under environmental and experimental conditions indicated that the animal is incapable of any osmotic regulation, and this evidence taken in conjunction with the permeability studies suggests that the two main coelomic cavities of the starfish behave, osmotically, as one, and that differences induced in one are reflected in the other.

This work was carried out during the tenure of a Research Assistantship at Sir John Cass College, London. My thanks are due to Professor J. E. Smith, F.R.S., for his supervision of the thesis of which this paper forms a part, and for the use of his Department's Marine Laboratory at Whitstable. I am also grateful to the Director and Staff of the Plymouth Marine Biological Laboratory for their hospitality whilst I occupied the London and British Association Tables there. Part of the travelling expenses were defrayed by a grant from the Central Research Fund of London University.

SUMMARY

Evidence is presented to suggest that the salinity tolerance of the adult North Sea *Asterias rubens* is around 23‰, whereas that of the Baltic Sea animals is 8‰. This, together with other evidence outlined, is thought to be sufficient reason to justify the use of the term 'physiological race' where these two populations are concerned.

Adult *A. rubens* from the North Sea is incapable of any degree of weight regulation in diluted media within the ecological temperature range, size or state of the breeding cycle.

Similarly, it is incapable of any osmotic regulation and it is suggested that the two main coelomic cavities constitute an osmotic unity.

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