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SALINITY TOLERANCE AND VOLUME REGULATION IN FOUR SPECIES OF POLYCHAETES

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INTRODUCTION

THE present investigation was undertaken to understand the role of osmotic regulation in the distribution of four species of polychaetes, *viz.*, *Glycera embranchiata*, *Onuphis eremita*, *Loimia medusa* and *Clymene insecta* in the brackish-water zones of Adyar, Madras. *O. erimita* along with *G. embranchiata* are restricted to the shore and do not occur in the brackish-water zones: *L. medusa* occurs in the brackish-water zone but confined only to regions of higher salinity equal to the salinity of sea-water. The latter species also occurs in the Madras Harbour predominantly marine in habitat. *C. insecta*, on the contrary, occurs only in the upper purely brackish-water reaches. Volume regulation and their capacities for survival have been used as the criteria for understanding their distribution and their abilities for osmotic regulation.

MATERIAL AND METHODS

All the worms for investigation were collected from the Madras seashore and the brackish-water regions of the Adyar Estuary. Only those worms which were vigorously active were used for experimentation. All worms either mutilated or otherwise showed signs of injury and/or inactivity were discarded. Excepting for change of fresh media given daily, no special care was taken in rearing them in glass troughs in the laboratory. All worms continued to thrive well for weeks together under these conditions. All experiments were made at a room temperature of $28 \cdot 5 \pm 0 \cdot 5°$ C. Animals marked for experimentation or under experimentation were not fed. All dilutions were of sea-water made up to the desired concentration by the addition of distilled water. The volumes were determined by the method of Lowndes (1942). All values given are the averages of six sets of determinations.

RESULTS

Experiment I. Capacity for Survival in Hypotonic Media.—A hundred worms belonging to each of the four species, *viz.*, *G. embranchiata*, *O. ermita* *L. medusa* and *C. insecta*, were exposed to the following three dilutions: 18·62‰, 16·32‰ and 10·54‰. In order to judge their capacities for tolerance of hypotonic media, their rates of mortality were followed at intervals of 24 hours (Table I). The general trend of mortality, which increased with increasing dilutions, was the same irrespective of the species studied. The higher the dilution the greater was the rate of mortality. Further the rate of mortality also increased with the time of exposure. In 24 hours in a medium of 18·62‰, *G. embranchiata* suffered the maximum rate of mortality of 26%. *C. insecta* with only 12% showed the minimum; *L. medusa* and *O. ermita* with respective rates of 24% and 22% ranked in between the two previously mentioned species. At the end of 48 hours all the four species exhibited an increased rate of mortality until 96 hours when 98% of *G. embranchiata*, *O. ermita* and *L. medusa* were dead. *C. insecta* alone reached only 26% of mortality. The rates and trend of mortality were similar in other dilutions of salinities of 16·32‰ and 10·34‰. *C. insecta* alone showed better capacities of survival. They reached as high a per cent. as 98, only in a dilution of salinity of 10·34‰ and that at the end of 96 hours.

TABLE I

Name of species	% Rate of mortality at intervals of 24 hours in dilutions of salinity											
	18·62‰				16·32‰				10·34‰			
	24	48	72	96	24	48	72	96	24	48	72	96
G. embranchiata ..	26	45	82	98	42	56	86	98	98	100	..	..
O. ermita ..	22	41	85	98	36	57	88	98	100	..	..	..
L. medusa ..	24	44	82	98	98	100	..	..	100	..	..	..
C. insecta ..	12	16	18	26	24	32	48	69	55	68	74	98

The variations in the rates of survival exhibited by the different polychaetes could only be due to their capacities for osmotic regulation as reflected by their abilities for volume regulation. In order to test this possibility the following experiments on the effect of hypotonic media on the volume changes were performed on each of the above four species.

Experiment II. Volume Regulation in Hypotonic Media in G. embranchiata.—Out of a lot of worms collected from the natural habitat and acclimatised to laboratory conditions, six vigorous worms of similar sizes were selected and exposed to three dilutions of salinities of 8·62‰, 13·70‰ and 20·72‰. They were exposed for a period of 8 hours and their volumes measured at intervals of 1 hour (Fig. 1). All the worms increased in volume, in the respective salinities, by 55%, 40% and 28% at the end of the 1st hour and continued to increase thereafter. By the end of 4 hours they reached in the respective salinities, the maximum per cent. increase in volume registered at 60%, 45·6% and 32·7%. These final volumes reached at the end of 4 hours were maintained even at the end of 24 hours when further observations were discontinued. It may be seen that the per cent. increase in volume increased with increasing dilutions and that the final volumes reached were also proportionate to the dilutions to which the worms were exposed (Table II).

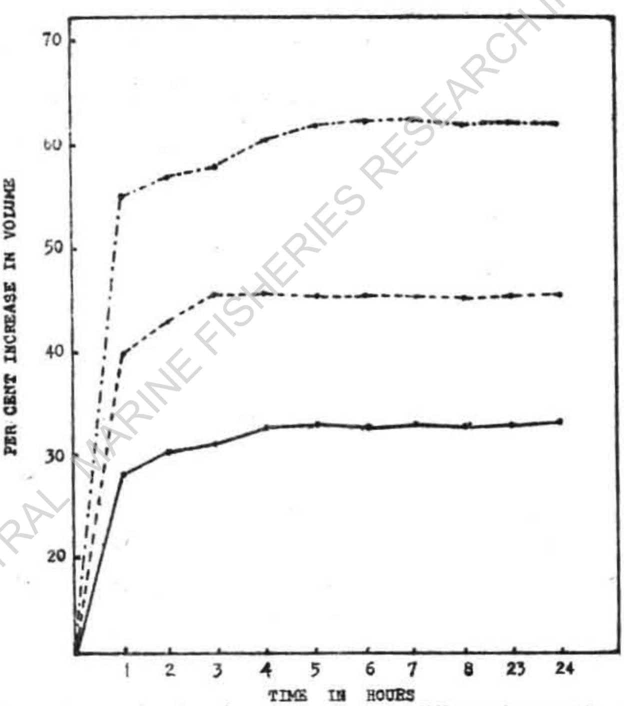

FIG. 1. Volume changes in *G. embranchiata* in three different hypotonic media during different intervals. (·—·—·—· 8·62‰; 13·70‰ ; ·—— · 20·72‰.) - - - -

Experiment III. Volume Regulation in Hypotonic Media in O. eremita.—A batch of six worms of equal sizes were exposed to hypotonic media of similar salinities as used in the previous experiment. The changes in volumes at intervals of 1 hour over a period of 8 hours were followed. In all the experimental media the worms in 1 hour reached the maximum volumes of 60%, 44% and 29·40% in the respective dilutions of 8·62‰, 13·70‰ and 20·72‰. At the end of 1 hour the volumes decreased reaching in the respective dilutions, the final volumes of 18·1%, 17·4% and 12·6% at the end of 3 hours (Table II).

TABLE II

Name of species	% Increase of volume after 1 hour in dilutions of salinities			% Final volume after 4 hours in dilutions of salinities		
	8·62‰	13·70‰	20·72‰	8·62‰	13·70‰	20·72‰
O. ermita ..	60·0	44·7	29·4	18·1	17·4	12·6
L. medusa ..	58·6	42·2	26·8	17·6	15·6	10·3
C. insecta ..	50·8	35·8	21·1	10·4	8·2	5·2
G. embranchiata	55·0	40·0	28·0	60·0	45·5	32·5

These final volumes were maintained during the rest of the period and even at the end of 24 hours (Fig. 2). The initial increase in volume during the first hour must be due to the inrush of water against an osmotic gradient and the subsequent decrease must be due to loss of salts as has been observed in a number of polychaetes by Schlieper (1930), Beadle (1937) and Krishnamoorthi and Krishnaswamy (1962).

Experiment IV. Volume Regulation in Hypotonic Media in L. medusa.—An experiment similar in features and procedure was repeated with *L. medusa* as the experimental material. Figure 3 represents diagrammatically the results of the experiment. This species also increased in volume reaching the maximum volumes of 58·6%, 42·2% and 26·8% in hypotonic media of 8·62‰, 13·70‰ and 20·72‰ at the end of one hour and later decreased in the respective media to the final volumes of 17·6%, 15·6% and 10·3% at the end of 3 hours (Table II). The final volumes reached at the end of 3 hours were maintained till the end of 8 hours and even at the end of 24 hours (Fig. 3). The initial increase and subsequent decrease in volume must be due to similar factors reported for *O. ermita*.

Experiment V. Volume Regulation in Hypotonic Media in C. insecta.—A similar experiment as described earlier was repeated to understand the osmotic behaviour of *C. insecta* when exposed to hypotonic media. The

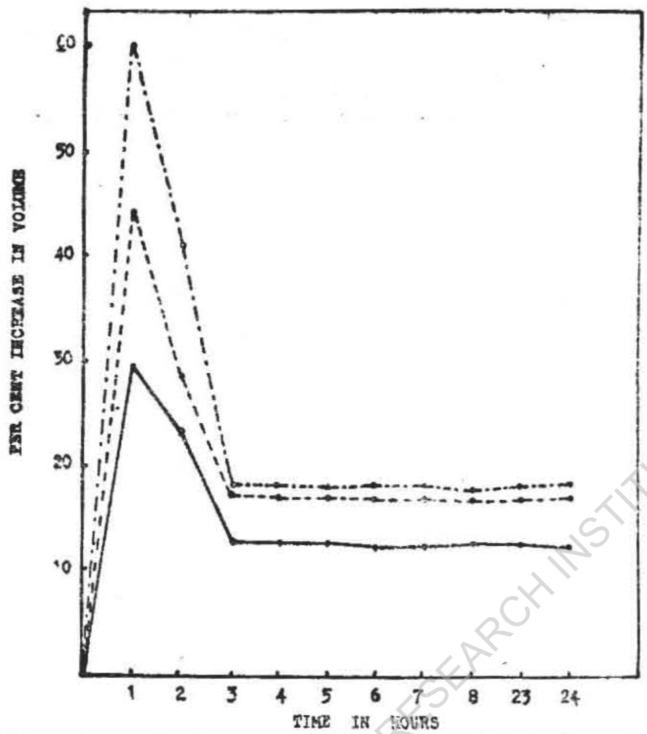

FIG. 2. Volume changes in *O. erimita* in three different hypotonic media during different intervals. (·—·—·—· 8·62‰; 17·30‰; ·——· 20·72‰.) ←---

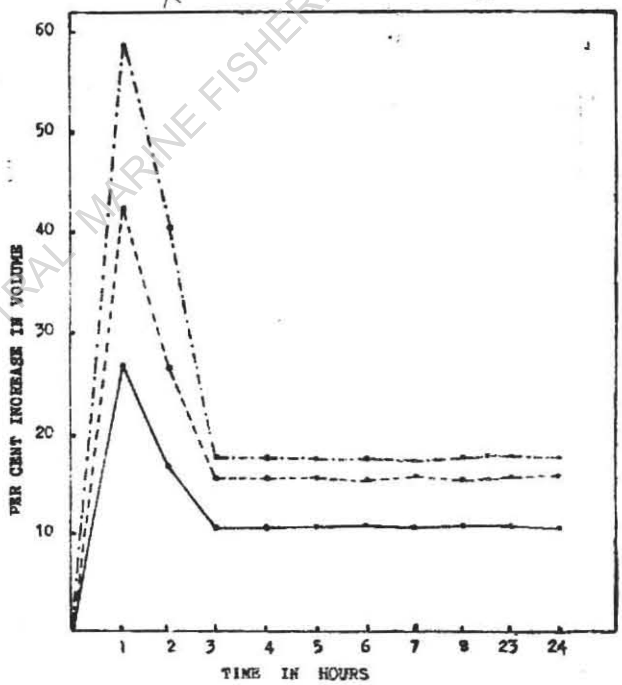

FIG. 3. Volume changes in *L. medusa* in three different hypotonic media during different intervals. (·—·—·—· 8·62‰; 13·70‰; ·——· 20·72‰.) ←---→

initial increase in volume at the end of 1 hour in the respective dilutions of 8·62‰, 13·70‰ and 20·72‰, however, were 50·8%, 35·8% and 21·1% and the final volumes 10·4%, 8·2% and 5·2% (Table II, Fig. 4).

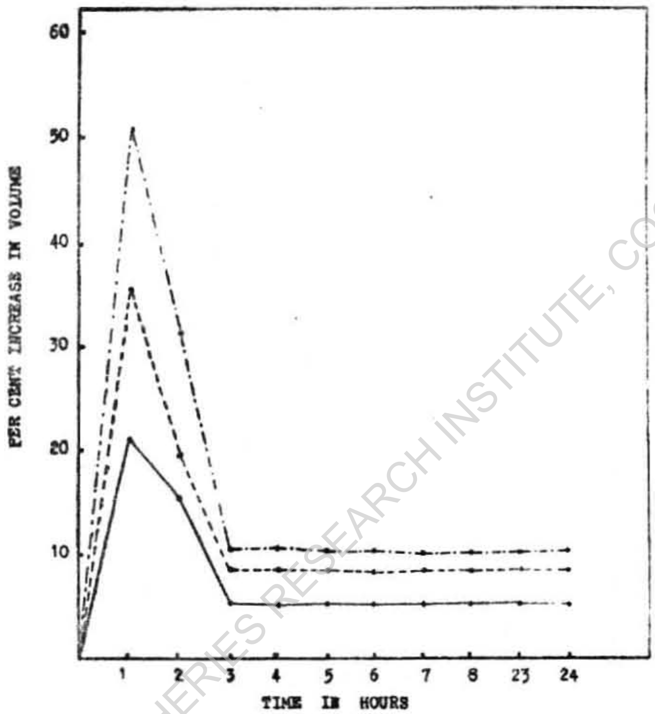

FIG. 4. Volume changes in *C. insecta* in three different hypotonic media during different intervals. (·—·—·—· 8·62‰; 13·70‰; ·——· 20·72‰.) ·- - - ·

It may thus be seen that except *G. embranchiata* the rest of the worms, *viz.*, *O. eremita*, *L. medusa* and *C. insecta*, showed similar behaviour when subjected to stresses of hypotonic media. All of them increased in volume at the end of the first hour and subsequently decreased reaching a final and steady volume at the end of 3 hours which was maintained even at the end of 24 hours. The initial increase and the subsequent decrease in volume must be due to inrush of water against an osmotic gradient to begin with and subsequent loss of salts. *G. embranchiata* alone did not exhibit this behaviour. Indeed it also increased in volume at the end of 1 hour. But it continued to increase reaching the maximum at the end of 3 hours. This was maintained even at the end of 24 hours. Among the former three species in an apparently similar behaviour a difference yet could be noticed. The initial increase in volume at the end of the 1st hour and subsequent decrease to a final volume at the end of 3 hours were different in the different species. While, no matter what the dilution was, both the initial increase in volume and the final volume attained were the highest in *O. eremita*; in *C. insecta* it was the lowest; and *L. medusa* ranked in between the two. The initial increase in volume at the end of 1 hour in *G. embranchiata* was as high as that of *O. eremita*.

Remarks

All the worms used in the present study without exception exhibited increase in volume reaching a maximum during the first hour of their introduction to experimental dilute media and, excepting *G. embranchiata*, decreased subsequently reaching a final steady volume at the end of 3 hours. This agrees with the observations of Schlieper (1930); Beadle (1937); and Topping and Fuller (1942) made on a number of polychaetes. However, both the maximum volume reached at the end of the first hour and the final volume attained at the end of 3 hours, irrespective of the media used, varied in the different species. This variation can probably be correlated with the habitats these worms have been taken from. Whereas *G. embranchiata* and *O. eremita* were taken from the shore and *L. medusa* from shoreward regions of the Adyar brackish-water zones; *C. insecta* was taken from the upper reaches purely brackish-water in character. *O. eremita* showed the highest initial increase and the lowest subsequent decrease. *L. medusa* ranked in between *O. eremita* and *C. insecta*. In this latter species, namely *C. insecta*, both the initial increase and the final volume attained were the lowest. It is known that increase in volume is pronounced in poikilosmotic than in homoiosmotic animals (Prosser *et al.*, 1950; Jørgensen and Dales, 1957). Therefore the occurrence of *C. insecta* in the upper reaches of the brackish-water zones could only be due to greater powers of volume regulation being an euryhaline form. The stenohaline forms *O. eremita* and *L. medusa* with lesser abilities for volume regulation have, therefore, very limited distribution restricted only to the marine dominant regions. *G. embranchiata* alone among the forms studied exhibited a behaviour quite different from the others. It also increased in volume initially. But it continued to increase reaching the maximum volume by the end of 4 hours which was maintained even at the end of 24 hours. Although taken from the shore along with *O. eremita*, the responses of this worm to osmotic stresses were thus different from those exhibited by either *O. eremita* or *L. medusa* both taken from marine-dominated regions. This may perhaps be attributed to the structure of the nephridia which, in this worm, is different from those of either *O. eremita* or *L. medusa* or *C. insecta* (the anatomy and histology of nephridia of these polychaetes are being published elsewhere). *G. embranchiata* possesses nephridia of the protonephromixial type with simple solenocytes performing the excretory functions. The others possess excretory organs of the mixonephridial type.

Perhaps the type of excretory organs present in *G. embranchiata* are inefficient to meet the demands of baling out copious water that is absorbed against an osmotic gradient. Among the other polychaetes studied in *C. insecta* the proportion of the size of the nephridia to the size of the segment was greater as also the degree of blood supply (Krishnamoorthi, 1951, unpublished). The importance of the role played by the nephridia in osmotic regulation has been stressed in *Sabella pavonina* by Ewer and Ewer (1943) and in some Nereidae by Krishnan (1952).

If rates of mortality could be taken a measure of their capacities for survival in different anisotonic media, a comparison of mortality rates with volume regulation would be of interest. It was evident that of all the worms studied only *C. insecta* showed better capacities for acclimation to dilute media by suitable volume regulation. While the per cent. mortality of this polychaete in a salinity of 18·62‰ was only 26% at the end of 96 hours, 98% of *G. embranchiata, O. eremita* and *L. medusa* died in that dilution at the end of that period. In lesser dilutions the rates of mortality even at the end of 24 hours were higher in the latter three species than in *C. insecta*. The rates of survival among other factors, responsible for the distribution of a species in space, could only be explained in the light of their capacities for volume regulation, the greater the regulation the lesser the rates of mortality and farther the extent of penetration into a brackish-water region. It was small wonder, therefore, that *G. embranchiata, L. medusa* and *O. eremita* which exhibited less regulation showed the highest mortality and lesser penetration; and *C. insecta* the lowest rate of mortality, better powers of regulation and greater penetration in the brackish-water zones of Adyar, Madras. Similar observations on *Nereis virens* have been made by Sayles (1935).

SUMMARY

1. Salinity tolerance and volume regulation in four species of polychaetes, *viz.*, *G. embranchiata, O. eremita, L. medusa* and *C. insecta* have been studied. *C. insecta* showed lower mortality rates and greater powers of osmoregulation than those of the other three species of polychaetes.

2. The distribution of these polychaetes in the brackish-water zones of Adyar, Madras, have been explained in the light of their capacities for tolerance and volume regulation. The probable role of nephridia in the unusual behaviour of *G. embranchiata* has been shown.

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