PHYSIOLOGICAL STUDIES ON *MARPHYSA GRAVELYI* SOUTHERN.

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A C K N O W L E D G E M E N T S.

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PHYSIOLOGICAL STUDIES ON MARPHYSA GRAVELYI SOUTHERN.

C O N T E N T S.

A P P E N D I C E S.

1. Studies on the Osmotic Properties in Eggs and Larvae of a Brackish water Polychaete, Marphysa gravelyi Southern.

   Proc. Indian Acad. Sci., 34: 199-209, (1951b).

2. Salinity Tolerance and Volume Regulation in four species of Polychaetes.

   Ibid., 56: 363-371, (1962).

3. Activity of Marphysa gravelyi Southern (Polychaeta) under heterosmotic conditions, (WITH S. Krishnaswamy).

   Ibid., 57: 83 - 87, (1963).

4. On the Gross Morphology and Histology of nephridia in four species of Polychaetes.

Ibid., 57: 195-209, (1963a).

5. Volume Regulation in Eggs, Larvae and Adults of a Brackish water Polychaete, Diopatra variabilis (Southern).

Ibid., 57: 275-289, (1963b).

6. Chloride Regulation in Marphysa gravelyi Southern.

Curr. Sci., 32: 463-464, (1963c).

7. On the Distribution of Six Species of Polychaetes in the Adyar Estuary, Madras.

J. Mar. Biol. Ass. India, 5: 97-102, (1963d).

CHAPTER I: I N T R O D U C T I O N.

PHYSIOLOGICAL STUDIES ON MARPHYSA GRAVELYI SOUTHERN.

CHAPTER I: INTRODUCTION.

The growth of our knowledge of osmotic and ionic regulation in invertebrates, can be traced from a series of excellent reviews available on the subject (Gurney,1913; Schlieper,1930,'35; Dakin & Edmonds,1931; Pantin,1931; Dakin,1935; Krogh,1939; Beadle,1943,'57; Panikkar,1950; Prosser *et al*, 1950; Ramsay,1954; Robertson,1957,'60a; Shaw,1960c; Nicol,1960; and Lockwood,1962). It is common knowledge that penetration of brackish waters or regions of lower salinity has been accomplished by several marine animal phyla including the polychaetes. Though predominantly marine, a few like the Nereids have successfully established themselves in regions where changes in salinity and temperature are usual (Zenkevich,1957; Hartman,1960; Smith,1963). Yet others like the Glycerids and the Nephthids, have been reported from fresh water (Wesenberg-Lund,1958; Southern,1931). Such independence over the environment has been made possible by mechanisms for osmotic/ionic regulation or both. Factors like (i) passive tolerance of the tissues to lowered salinities; (ii) volume or weight control; (iii) reduction in permeability; (iv) production of hypo- or isosmotic urine etc., have in varying degrees contributed towards the maintenance

of a relatively constant milieu interieur (Claude Bernard,1885), without which passage from the sea into brackish and fresh water would have been impossible. Study of such species and the factors contributing to their successful survival would aid the elucidation of problems associated with the colonisation of such diverse media as the brackish and fresh waters.

Polychaetes have been observed in various localities involving considerable dilution of the sea water (Florentin,1899; Ferronniere,1901; Annandale,1922; Dehorne,1925). Pearse (1928) examining 18 species of polychaetes found that only two viz., Laonice viridis (Verrill) and Nereis virens Sars, could live and be active 'for periods of two to three weeks in one fourth sea water, but died in weaker solutions'. Nereis virens collected from the brackish waters of the River Narraguagus, were able to withstand 2% dilution of the sea water, whereas the same species from the open sea could tolerate only 7% dilution (Topping & Fuller,1942). Glycera dibranchiata became swollen and turgid even in 50% sea water, the integument bursting in greater dilutions. On the basis of their tolerance, Topping & Fuller (1942) have accounted for the distribution of these polychaetes in the estuary of the River Narraguagas. The distribution of Nereis

diversicolor either at Isefjord, Denmark (Smith,1955b) or at Kames Bay, Millport (Smith,1955a) or in the Tamar Estuary (Smith,1956) has also been governed by salinity tolerance of the animal. Neanthes lighti (=Nereis limnicola), another nereid polychaete, although could be adapted to fresh water in the laboratory, is restricted to the estuarine or lacustrine situations in the Salinas River Estuary (Smith,1953). Based on mortality rates when exposed to hyposmotic media, Krishnamoorthi (1962, Appendix 3.) explained the distribution of Glycera embranchiata, Onuphis eremita, Loimia medusa and Clymene insecta in the Adyar Estuary and followed it up with a similar study to understand the distribution of Diopatra variabilis that occurred in the same estuary (Krishnamoorthi,1963b, Appendix 6.). Although the range of tolerance of a species could be increased by gradual acclimation, the response of an organism to sudden environmental changes, is a prime factor contributing to its successful establishment in diverse situations.

It is common knowledge that the responses to hypo- and hyperosmotic media are different in different polychaetes as seen in Nereis diversicolor and Nereis cultrifera (Beadle,1931,'37; Schlieper,1929a & b,'35) and Nereis virens (Sayles,1935). N. diversicolor being euryhaline, showed better powers of volume regulation than either N. cultrifera

or N. virens. When such studies were extended to four species viz., G. embranchiata, O. eremita, L. medusa and C. insecta, it was seen that both in their capacities for volume regulation and tolerance to hyposmotic media, they showed differences. All of them, without exception, increased in volume. But the extent of increase varied from one species to the other. All but G. embranchiata began to decrease in volume. However, the final volume attained at the end of 4 hrs differed, once again, from species to species. Thus, both the initial increase in volume and the final volume attained was the lowest in C. insecta and the highest in G. embranchiata and O. eremita. L. medusa ranked between the two (Krishnamoorthi,1962). Increase in volume is less in euryhaline than in stenohaline forms (Prosser et al,1950; Jørgensen & Dales,1957). In brackish water animals swelling is followed by volume regulation as reported in a number of polychaetes (Beadle,1931,'37; Ellis,1933,'37,'39; Jurgens,1935; Sayles,1935). Volume regulation, therefore, constitutes a distinct mechanism.

Passive tolerance of dilute media and/or volume regulation, although advantageous by themselves, can but have limited significance for a passage into brackish water regions, unless accompanied by a reduction in permeability. Although direct evidence of reduced permeability in the

forms studied by the author is not available (Krishnamoorthi,1962), the variations in volume control indirectly indicate the extent of permeability. Among the forms studied, in D. variabilis both the initial increase in volume and the final volume attained at the end of 4 hrs were the minimum (Krishnamoorthi,1963b). The others showed varying degrees of volume control depending conceivably upon their permeability. That the rate of chloride exchange and the permeability to water, were lower in the euryhaline N. diversicolor than in either N. pelagica or N. virens (= N. southerni) was demonstrated (Bethe,1934; Jørgensen & Dales,1957). Fretter (1955) also found in N. diversicolor a lower ^{24}Na exchange than in Perinereis clutrifera. From the above evidence it is reasonable to accept that D. variabilis with its pronounced euryhalinity and better volume control would evince reduced permeability to water. But reduced permeability alone is insufficient for life in media of lower salinities. Salt uptake is necessary for the maintenance of a constant milieu interieur. N. diversicolor maintains an internal osmotic pressure higher than that of the outside medium (Schlieper,1929a & b; Beadle,1937) and this Jørgensen & Dales (1957) observed, was due to active uptake of salts from the medium. While Ellis (1937) from his studies on the same species viz., N. diversicolor,

from Plymouth and Roscoff, found no active regulation, Zenkevich (1938a & b) found in forms collected from different localities, an ability to maintain a higher osmotic pressure.

Control over the concentration and quantity of urine is yet another factor contributing to the colonisation of brackish and fresh waters. This has been well demonstrated among Crustaceans (see review of Lockwood,1962) and not reported in polychaetes so far and such evidence as is available is only circumstantial. Taking into account the fact that the more swollen worms (N. diversicolor) and, perhaps, with a higher internal hydrostatic pressure, were able to concentrate their body fluids more rapidly in ~~an~~ isosmotic media than the less swollen ones, Beadle (1937) concluded that there was some circumstantial evidence of the formation of urine hyposmotic to the blood. The relatively greater loss of chloride in N. diversicolor when transferred to hyposmotic solutions as compared with that in P. cultrifera (Ellis,1939) and the greater permeability to 36 Cl in the former species than in the latter, led Jørgensen & Dales (1957) to argue that these factors might contribute to the excretion of blood-isosmotic urine. It is conceivable, therefore, that the kidneys would exhibit structural modifications and size

differences accompanying the production of isosmotic or hyposmotic urine, since the importance of nephridia in volume regulation was demonstrated in the estuarine fan-worm, Sabella pavonina, by Ewer & Ewer (1943). Krishnan (1952) found in comparable species of Nereidae that in Lycastis indica, a fresh water polychaete, the nephridia were larger in size and better vascularised than those of the stenohaline Perinereis nuntia and Nereis chilakaensis. A study of the histology and morphology of nephridia in G. embranchiata, O. eremita, L. medusa and C. insecta, has revealed that while G. embranchiata possessed nephridia of the protonephromixial type with simple solenocytes performing the function of excretion, the rest of the species had nephridia of the mixonephridial type. Furthermore, the extent of the excretory surface available for excretion and the blood supply the nephridia receive, were less in O. eremita than in C. insecta. L. medusa ranked between the two. Such a comparison was not possible in the case of G. embranchiata as the cells forming the nephridium were syncitial in nature. Perhaps the failure of regulation in G. embranchiata is a direct consequence of the kind and structure of the nephridia. It also argues, therefore, that formation of hypo- or isosmotic urine is more to be expected in C. insecta than in either O. eremita or L. medusa (Krishnamoorthi,1963a, Appendix 5.). However, as observed earlier, conclusive

evidence as provided by his classical experiments on Oligochaetes (Ramsay,1949a & b), of the formation of hypo- or isosmotic urine in brackish water polychaetes is not yet available.

Ellis (1937) from his studies of the water and electrolyte exchange in N. diversicolor, came to the conclusion that the weight regulation was not accompanied by osmotic regulation. Beadle (1937) also was of the opinion that since the degree of osmotic regulation was relatively slight, it could not be considered of direct importance for survival in dilute sea water. Isolated muscle preparations of the euryhaline N. diversicolor continued to function in dilute sea water better than those of either Arenicola marina or Perinereis cultrifera (Wells & Leidingham,1940a). This as Beadle (1957) says 'throws doubt on the survival value of its powers of osmotic regulation'. Similar studies on whole anterior ends of Marphysa gravelyi Southern, have shown spontaneous and sustained activity in dilutions ranging from 20% to 50% sea water of salinity 34‰. (Krishnamoorthi & Krishnaswamy, 1963, Appendix 4.). Perhaps the answer to their ability to tolerate hyposmotic media may have to be sought elsewhere and their capacity for ionic regulation, a field little investigated in polychaete physiology, would provide the answer. Cole (1940) found that Amphitrite brunnea

and Glycera dibranchiata had considerable powers of ionic regulation. Arenicola marina has no osmotic control but in dilute sea water the concentration of K had become 118, of Ca 113 and of So_4 90% of the same ions in the external 75% sea water (Robertson,1949). Krishnamoorthi (1963c, Appendix 7) briefly reported on the regulation of chloride in M. gravelyi. It is also possible that maintenance of a constant internal concentration is brought about in either of two ways: adjustment by osmoregulation alone as present in A. marina and P. cultrifera (Duchateau et al,1961) or both by osmo-concentration and intracellular adjustment in which free amino acids are involved, as present in the more euryhaline N. diversicolor (Jeuniaux et al,1961). That glucose may also be regulated has been demonstrated in Amphitrite ornata whose glucose content not only increased with the length of exposure but with increase in temperature (Wilber,1948b; Wilber & MacDonald,1960). Although polychaetes are essentially ammonotelic, some traces of urea have been reported, without comment, in the coelomic fluid of Arenicola (Strunk,1938; Wilber,1948a).

Physiological studies on polychaetes have so far been mainly concerned with investigations on the behaviour of adults. These studies, valuable as they are in their

contribution towards the knowledge of polychaete physiology, can give but a partial picture. It is, therefore, necessary that such studies are extended all through the ontogeny of a species to obtain a complete picture of the regulatory mechanisms of a species as a whole as remarked by Beadle (1957). In this regard the experiments of Bogucki (1954) are very interesting. He cultured N. diversicolor in sea water of 7‰ salinity and subjected various developmental stages to further dilutions. He found the pre-larval and larval stages were most susceptible to salinity changes, while young worms could survive in fresh water for several months. The adults were not so resistant. The eggs of M. gravelyi Southern isolated from their jelly coats, behaved like osmometers increasing in dilute media and decreasing in hyperosmotic media. The larvae of the metatrocophore stage showed slight improvement in the tolerance of dilute media. A progressive increase in the tolerance of [redacted] hyposmotic media was noticed in the next stage viz., the nectochaete stage, while the adults could tolerate salinities over a wide range (Krishnamoorthi,1951b, Appendix 2.). Another Eunicid, Dioptra variabilis, behaved in a manner similar to that exhibited by M. gravelyi. A progressive increase in the tolerance of hyposmotic media was noticed through the egg to metatrochophore to nectochaete to the adult stages (Krishnamoorthi,1963b).

Perhaps, as observed by Beadle (1957), a chemical analysis of the developing regulatory mechanisms would give an insight into the 'embryology' of osmotic and ionic regulation, a field so far little investigated.

From the preceeding account it is, therefore, clear that adaptation of a polychaete species to a brackish water environment, is a series of adjustments. It is also obvious that while some investigators have studied only volume regulation and/or oxygen consumption, yet others have confined their investigations to one or two aspects of the physiology of polychaetes. Since adaptation of a species to regimens of lower salinity, is a sum total of all responses, the need for studying all aspects in a single species requires no emphasis. With this object in view, the following study, hitherto unattempted, was undertaken not only because the brackish waters of Adyar present features peculiar to a tropical country like India (Panikkar & Aiyar,1937; Rao,1951), but they teem with a variety of polychaete fauna. Furthermore, Marphysa gravelyi Southern was chosen because among the polychaetes whose distribution in the Adyar brackish water was studied (Krishnamoorthi,1963d, Appendix 8.), it occurred over a wide range of salinities and penetrated far higher up the estuary.

CHAPTER II: MATERIAL AND METHODS.

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The conventional methods of exposing the animals to the stresses of heterosmotic media, was followed to arrive at the SALINITY TOLERANCE of Marphysa gravelyi Southern. The rate of mortality expressed in terms of percentage mortality, when exposed to different salinities, was taken as the criterion to understand the animal's toleration of media of reduced salinities. (vide Chapter III, Page 15).

The displacement method of weighing aquatic animals using specific gravity bottles (Lowndes,1942), was followed for determining the VOLUME and its REGULATION in Marphysa gravelyi when subjected to the stresses of hyposmotic media. (Details are given in Chapter IV, Page 24).

The OSMOTIC CONCENTRATION of the BODY FLUIDS expressed in terms of %NaCl, was determined by the comparative melting point method of Jones (1941) as modified by Freeman & Rigler (1957) along with certain refinements suggested by Gross (1954 and personal communication) and Sukumaran (1960). The collection of body fluids was aided by the use of capillary pipettes fabricated at the laboratory from glass tubing (Pyrex). Details are given in Chapter V (Page 36).

The STRUCTURE of NEPHRIDIA was followed from histological sections prepared by conventional procedures of microtomy. (vide Chapter VI for details. Page 54).

The body fluid CHLORIDES were determined by the titrimetric method of Sendroy (1937) as modified by Robertson & Webb (1939). The Volhard's method of argentrimetric titration was discarded in favour of this method, since the end point was sharp and clearly detectable. POTASSIUM and SODIUM were estimated on a Zeiss Flame Photo-Meter. The total FREE AMINO ACIDS were determined by the colorimetric method of Harding & MacLean (1916) chosen in favour of Troll & Cannan (1953), on a UNICAM Spectrophotometer (SP 600). Details are given in Chapter VII (Page 71).

All readings given are usually the means of 6 to 10 estimations unless otherwise stated. Experimental media of desired concentrations were prepared by adding required quantities of distilled water to Sea Water of salinity ranging from 32‰ to 34‰. For the estimation of the means, the standard deviation, the error of the mean and for applying the Student's 't' test and the Chi-square tests, Simpson et al (1960) and Snedecor (1961) were followed.

CHAPTER III: SALINITY TOLERANCE.

C O N T E N T S.

CHAPTER III. SALINITY TOLERANCE

1. Introduction: In a survey (Krishnamoorthi,1963d) of the brackish water zones of Adayar, Madras, with special reference to the occurence and extent of penetration of six species of Plychaetes, viz., Onuphis eremita Audouin et Milne Edwards, Glycera embranchiata Ranganathan, Loimia medusa Savignay, Clymene insecta Ehlers, Diopatra variabilis (Southern) and Marphysa gravelyi Southern; it was seen that the last named polychaete showed the farthest penetration occuring in fairly good numbers in waters with salinity ranging from 5‰ to 28‰. Their distribution was explained on the basis of salinity tolerance as reflected by mortality rates, when exposed to heterosmotic media (Krishnamoorthi,1962). The occurance of Nereis virens in the brackish waters of the River Narraguagas was also explained as due to its tolerance to lowered salinity (Topping & Fuller,1942). The importance of salinity tolerance in the occurance of Nereis diversicolor both at Kames Bay, Millport, (Smith,1955a) and at the Tamar Estuary (Smith,1956), although it may have been governed by "competetion and/or predation" at the Isefjord (Smith,1955b), has been stressed. In the Salinas River estuary, California, Neanthes lighti (=Nereis limnicola) is restricted to

the estuarine or lacustrine situations (Smith,1953). It appeared, therefore, that the rates of mortality reflecting tolerance of media of reduced salinities, in Marphysa gravelyi Southern, would help in understanding the wide distribution of this worm in the Adyar estuary. In as much as the salinity range of an animal could often be extended or shifted to one or both directions by gradually changing the salt content of the external medium or by rearing the organisms under different salinity conditions (Sayles,1935; Kinne,1953 a & b, '58; Schlieper,1955), the object of the present investigation being the determination of the responses to sudden changes to salinity, as obtained in nature, was necessarily restricted to the latter aspect.

2. Material and Methods: Marphysa gravelyi used in the experiments were collected from the beds of the Adyar river, where they live burried to a depth of one to two feet in loose, black, muddy soil in knee deep waters. Since the soil is loose, it is not difficult to scoop the mud with bare hands. In fact, shovels, forks and such other metallic appliances were seldom used, since more often they caused considerable damage to the worms while digging them out. The worms were freed from the soil by washing them with water and then

transferred to earthen-pots containing brackish water, and transported to the laboratory. The worms were reared in glass troughs filled with filtered brackish water. The brackish water was filtered to keep the presence of particulate matter to the minimum. Any worm that showed signs of the onset of death or found lethargic was seperated from the collection and never used for experimentation. Similarly worms which were not complete were also discarded. The animals were not fed in the laboratory either during the acclimation period to laboratory conditions or during experimentation; and no experiment was commenced until a day after, a period of time needed for acclimation of the worms to laboratory conditions. Aeration of the waters was not absolutely necessary, but as a precaution the aquaria were aerated. Often the worms had a tendancy to tangle themselves into a bolus which caused unnecessary loss of life. Care was, therefore, taken to untie them as often as it occured. Attempts to keep each worm in a seperate glass tube with a bore big enough to allow for an easy passage in and out of the tube with equal facility, were not fruitful, since they would not readily take to this habitation, although, in nature, they lived in burrows. All dilutions were made up to the desired strengths by the addition of distilled water to sea water

of salinity ranging from 32‰ to 34‰. The sea water was also filtered before it was diluted. The salinities of both the sea water and the dilutions made thereof, were determined by Mohr's method of ~~agrometric~~ argentometric titration, by titrating 10 ml of the sample with Silver Nitrate solution of strength 23.95 gm/l and 5% Potassium Chromate as the indicator (Welsh & Smith,1960). The stock solution of Silver Nitrate was stored in dark bottles and was never used for more than a week once prepared. The Silver Nitrate solution was standardised against Standard Sea Water obtained in ampules from the Hydrographical Laboratories at Copenhagen, Denmark.

3.1. Rate of mortality in a hyposmotic medium of salinity 5‰.

Experimental Procedure: A hyposmotic solution of strength 5‰ was prepared by adding required amounts of distilled water to fresh, filtered sea water and 1500 ml of it was transferred into each of 10 thoroughly cleaned, previously marked (1 to 10), glass, 2 liter beakers (Pyrex). The beakers containing experimental animals were maintained at room temperature of 29.5 ± 0.5°C by immersing them in a large trough containing water. Into each of the beakers, 100 vigourous worms more or less of equal length, selected from a common pool, were transferred.

The number that died at the end of every 24 hrs in each beaker over a period of 96 hrs was recorded. Observations were discontinued after 96 hrs, since trial runs of experiments had shown no significant increase in the mortality rates beyond that period. From the initial number of worms introduced into each beaker and the number that died at the end of 24 hrs, the percentage rate of mortality was a simple calculation of rule of three. The percentage rates of mortality at the end of 48, 72 and 96 hrs was, however, arrived at from the number of survivers at the end of the previous day and the number that died during the corresponding 24 hour period. In other words, attempts to make up as many numbers that died were not resorted to, since reaction of fresh worms would, obviously, be different from those that are already some hours old to the experimental medium and may, therefore, viciate the results if taken into account.

Results: A perusal of the table and the figure (Table I, Fig.1.) clearly shows that the rate of mortality in this salinity (5‰) was as high as 13.5% at the end of 24 hrs. Further, there is a progressive increase in the rate of mortality, 19.4% of them dying at the end of 48 hrs; 32.5% at the end of 72 hrs; and reaching the highest rate of mortality, 68.5%, at the

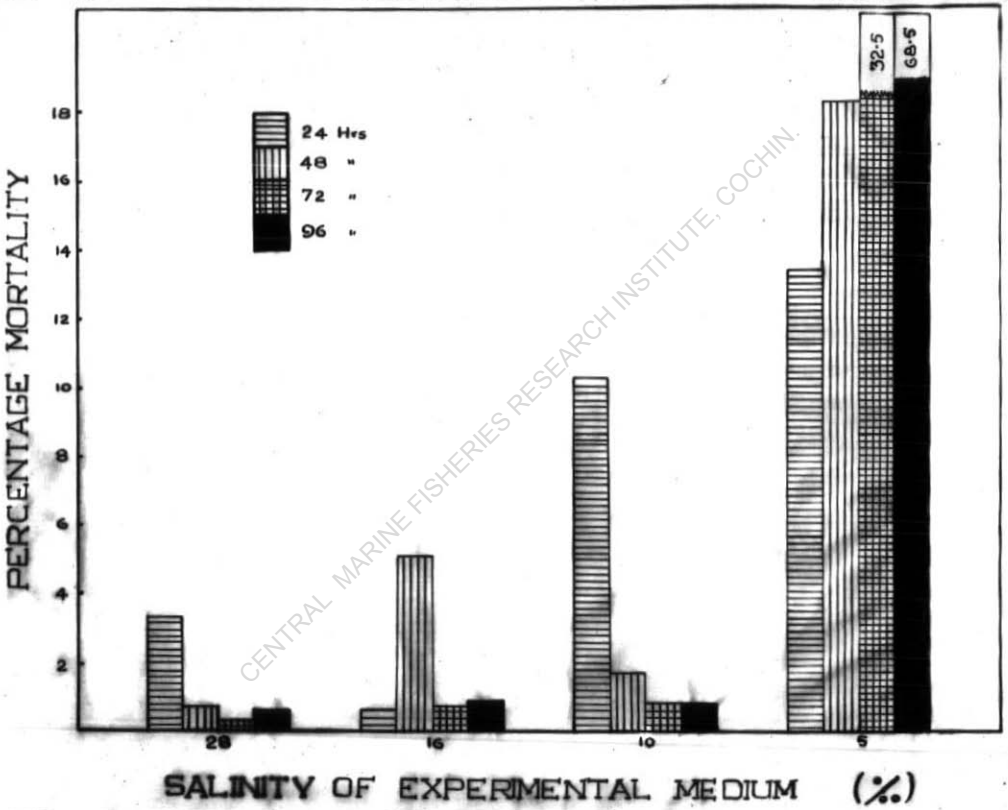


Fig.1. Histogram showing percentage rates of mortality after every 24 hrs over a period of 96 hrs in media of salinities, 5‰, 10‰, 16‰ and 28‰. Temp.: 29.5 ± 0.5°C.

T A B L E. I.

Time in Hours.	% Mortality after daily periods in beakers												
	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e.
24	16	12	8	17	10	11	18	20	13	10	13.5	4.41	±1.39
48	14	13	25	28	12	26	23	14	16	23	19.4	6.16	±1.95
72	41	32	31	21	42	21	32	42	42	21	32.5	9.44	±2.99
96	71	62	82	71	53	61	81	73	60	71	68.5	9.36	±2.96

Table.I. Percentage rate of mortality after every 24 hrs over a period of 96 hrs in a medium of salinity 5‰. Temp.: 29.5± 0.5°C.

end of 96 hrs. A salinity of 5‰ could perhaps be the lowest limit that M. gravelyi would tolerate.

3.2 Rate of mortality in a hyposmotic medium of salinity 10‰.

An experiment similar in procedure and design to one detailed above was performed but with a hyposmotic medium of strength 10‰.

Results: Unlike in the previous salinity of 5‰, the tolerance of M. gravelyi to this salinity (10‰) was different as reflected by the rates of mortality at the end of 24, 48, 72 and 96 hrs. The highest rate of mortality at 10.3% was evident at the end of the first day (24 hrs.) and then there was a fall to 1.7% at the end of 48 hrs (2 days). During both the 3rd and the 4th days i.e., at the end of 72 and 96 hrs, the percentage of mortality was only 0.8% (Table II, Fig.1.). These results indicate that a minimum period of 24 hrs is required to get acclimated to this salinity and further, that the highest rate of mortality is observed during this period of acclimation.

3.3. Rate of mortality in a medium of salinity 16‰.

Results: At this salinity, 16‰, the highest rate (5.1%) of mortality was observed at the end of the 2nd day (48 hrs), (Table III, Fig.1.). The mortality rates between

T A B L E. II.

Time in Hours.	% Mortality after daily periods in beakers												
	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e.
24	6	10	8	12	15	10	10	8	11	13	10.3	0.91	±0.29
48	2	2	1	1	2	1	1	2	2	3	1.7	0.66	±0.21
72	1	2	1	1	nil	nil	1	1	nil	1	0.8	0.63	±0.20
96	nil	nil	1	2	2	1	1	nil	nil	1	0.8	0.82	±0.26

Table. II. Percentage rate of mortality after every 24 hrs over a period of 96 hrs in a medium of salinity 10‰. Temp.: 29.5 ± 0.5 °C.

T A B L E. III.

Time in Hours.	% Mortality after daily periods in beakers												
	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e.
24	2	1	nil	nil	2	nil	1	nil	nil	nil	0.6	0.84	±0.26
48	5	6	6	8	2	6	8	5	3	2	5.1	1.50	±0.47
72	1	1	nil	nil	1	1	nil	1	nil	2	0.7	0.68	±0.21
96	2	nil	1	1	2	nil	1	2	nil	nil	0.9	0.73	±0.84

Table. III. Percentage rate of mortality after every 24 hrs over a period of 96 hrs in a medium ofsalinity 16‰. Temp.: 29.5 ± 0.5°C.

1st and 3rd, 3rd and 4th days were not significant (<0.1%). The highest rate of mortality (5.1%) noted at the end of the 2nd day at this salinity (16‰) is low, compared to the figures obtained in 10‰ (1st day) and 5‰ (any day). This observed variation, at best, could be due to differences between individuals.

3.4. Rate of mortality in hyperosmotic medium of salinity 28‰.

Results: The salinity in the Adyar estuary reaches upto 28‰. The rate of mortality at this salinity was the highest (3.3%) at the end of 24 hrs (c.f. 10‰). During the following three days, however, the mortality rates were very low, ranging between 0.2 to 0.7 per cent. At the end of the 4th day it was only 0.6% (vide Table IV, Fig.1). It appears, that M.gravelyi is capable of surviving even as high a salinity as 28‰, and, perhaps, this marks the upper limit of salinity tolerance of this animal.

4. Remarks: The importance of the above studies to account for the wide distribution of Marphysa gravelyi in the Adyar estuary, would gain significance only when it is viewed against a background of similar studies conducted on other species of polychaetes viz., Onuphis eremita, Glycera embranchiata, Loimia medusa and Clymene insecta (Krishnamoorthi,1962) and Diopatra varaiabilis

T A B L E IV.

Time in Hours.	% Mortality after daily periods in beakers.												
	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e.
24	5	2	2	4	3	6	2	2	3	4	3.30	0.45	±0.14
48	1	1	nil	2	1	nil	nil	1	1	nil	0.70	0.67	±0.21
72	nil	nil	nil	1	nil	nil	1	nil	1	nil	0.30	0.48	±0.15
96	1	1	1	nil	nil	1	nil	nil	1	1	0.60	0.27	±0.09

Table. IV. Percentage rate of mortality after every 24 hrs over a period of 96 hrs in a medium of salinity 28‰. Temp.: 29.5 ± 0.5 C.

T A B L E V.

Concentration of the medium (‰)	% Rate of mortality of species of polychaetes at the end 24 hrs in heterosmotic media.																							
	G. embranchiata				O. eremita				L. medusa				C. insecta				D. variabilis				M. gravelyi			
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
28																					3.3	0.7	0.2	0.6
25																	0	0	0	0				
18	26	45	82	98	22	41	85	98	24	44	92	98	12	16	18	26								
17																	10	10	15	20				
16	42	56	86	98	36	57	88	98	98	100	-	-	24	32	48	69					0.6	5.1	0.7	0.8
10	98	100	-	-	100	-	-	-	100	-	-	-	55	68	74	98					10.3	1.7	0.8	0.8
8																	23	32	48	67				
5																					13.5	19.4	32.5	68.5

Table V. Rate of mortality in heterosmotic media over daily periods.

(Krishnamoorthi,1963b), coinhabitants with M. gravelyi in the Adyar estuary. Summarising, it will be seen (Table V) that M. gravelyi suffered a rate of mortality as high as 68.5% only in the lowest salinity of 5‰ and that after an exposure extending over a period of 96 hrs (4 days). Whereas D. variabilis registered an almost similar mortality rate (67%) in a lower salinity (8‰) at the end of 96 hrs, the others namely C. insecta could not tolerate a salinity of even 10‰, the rate of mortality reaching as high a percentage as 69% even in a salinity of 16‰, at the end of 96 hrs. This (16‰) seems to be the lowest salinity limit of the other three polychaetes, G. embrachiata, O. eremita and L. medusa. The last named three species showed considerable rates of mortality even in a salinity of 18‰. Although the rates of mortality of C. insecta were not as high as those of G. embranchiata, O. eremita and L. medusa in this salinity (18‰), the rates are significantly high and, perhaps, would be considerable if the experiment had continued beyond a period of 96 hrs since a steady increase in mortality from 24 to 96 hrs is seen. In a medium with 25‰, there were no deaths for D. variabilis. In the absence of similar investigation with an experimental medium of 28‰, it is difficult to say whether D. variabilis compares well in its salinity tolerance with M. gravelyi which tolerated

fairly well this salinity. However, in nature, it occurs almost in equal proportions in regions where M. gravelyi occurs. (Krishnamoorthi,1963d). Considering this, it could probably, be said that D. variabilis shares one feature in common with M. gravelyi i.e., tolerance of higher salinities, but not as low a salinity as 5‰, the lowest tolerance limit of M. gravelyi. In the light of these findings, the wide distribution of M. gravelyi to regions ranging over as wide salinities as from 5‰ to 28‰, becomes intelligible. On the basis of tolerance of reduced salinities, Topping & Fuller (1942) traced the distribution of N. virens in the River Naraguagas estuary. Out of 18 species of Polychaetes, Pearse (1929) found only Laonice viridis and Nereis virens capable of withstanding one fourth sea water for periods of two to three weeks, but died in weaker in solutions. Endurance of reduced salinities alone would be of little advantage, unless accompanied by equally developed abilities for volume control, since polychaetes are known to increase in volume when transferred to hyposmotic media (Beadle,1931, '37; Ellis,1933, '37, '39; Jurgens,1935; Sayles,1935; Jørgensen & Dales, 1957; Krishnamoorthi,1962, '63b). In order to understand the regulatory ability of M. gravelyi over volume when subjected to stresses of hyposmotic media, the following series of experiments were planned and performed.

CHAPTER IV: VOLUME REGULATION.

C O N T E N T S.

CHAPTER IV. VOLUME REGULATION.

1. Introduction: It was seen in the previous section that Marphysa gravelyi Southern tolerated lowered salinities over a wider range - 5‰ to 28‰, than any other polychaete studied from the same region viz., Glycera embranchiata, Onuphis eremita, Loimia medusa, Clymene insecta (Krishnamoorthi,1962) and Diopatra variabilis (Krishnamoorthi,1963b). The inequal distribution of this worm in Adyar estuary may be explained as due to its ability to tolerate low salinity as indicated by mortality rates. Further, it was suggested that the tolerance of dilute media alone would be insufficient unless matched with equal abilities for regulation of volume. It is common knowledge that when polychaetes are transferred to dilute media they increase in volume. It has been demonstrated that the rate of increase in volume is pronounced in stenohaline forms than in euryhaline forms; and that in euryhaline species swelling is followed by volume regulation. Even among the members of the same genus, varying capacities for volume regulation have been reported. Thus Nereis diversicolor showed better abilities over volume control than either Nereis pelagica (Schlieper,1929a & b) or Nereis cultrifera (Beadle,1931,'37). The volume regulation was less developed in Nereis virens (= N. southereni)

than in N. diversicolor (Sayles,1935) and that N. virens could be placed intermediate between N. pelagica and N. diversicolor in their capacities for volume regulation has been shown (Jørgensen & Dales,1957). A suggestion that the differences in volume regulation may also be a consequence of the geographical location of the animal and could be racial in character was offered by Ellis (1935) when he found that the same species of N. diversicolor collected from Roscoff waters showed a volume regulation different from those that were collected from Bangor, although it has now been disproved by Smith (1955c). Nevertheless, the fact that volume regulation could constitute a distinct mechanism enabling brackish water forms withstand changes, however big or moderate, in the salinity of the medium, is inescapble. In the light of the above observations, a study of the capacities for volume regulation in M. gravelyi appeared the next most obvious course.

2. Material and Methods: Volume changes were followed by the displacement method of weighing aquatic animals using specific gravity bottles as described by Lowndes (1942). The method is as follows: The volume of the specific gravity bottle is first determined by filling it with a given sample of sea water or dilutions

of sea water and the contents being poured into excess of Silver Nitrate solution, from which a definite quantity of silver halide is obtained. This will give the weight of the silver halide present in the quantity of sea water contained in the specific gravity bottle. If now the specific gravity bottle is filled with the same sample of sea water together with the worm and the water contained in the bottle is carefully poured out into excess of Silver Nitrate solution of the same concentration as used previously, a smaller weight of silver halide will be obtained, since the worm would have occupied some space within the bottle. Therefore, the amount of silver haldie so obtained would faithfully record any change in the volume of the animal. From these two weights of silver halide thus obtained, the calculation of volume is a simple procedure adopting the formulae governing the two (Lowndes,1942). An ordinary specific gravity bottle of 25 ml was found quite suitable. All precautions in the collection of the silver halide by filtration were taken and generally drying in a hot-air ovefn at 200° C overnight was found adequate. Whatman Filter Paper No.2 only was used and the weight of the filter paper was also taken into consideration to arrive at the absolute weight of the silver halide

so collected. The error due to adhesion of water to the animal was eliminated by rinsing the animal with isotonic solution every time the animal was introduced into or removed from the bottle. The isotonic solution was prepared according to the recipe given by Lowndes (1942). The method was found quite suitable, since the animal could be easily introduced into the narrow neck of the specific gravity bottle, without disturbing much the animal while transferring it from the experimental medium into the specific gravity bottle.

3.1. Volume Regulation over a period of 24 hrs in a hyposmotic medium of strength 6‰.

Experimental Procedure: The initial volume of 10 worms of more or less equal size, selected from a common pool, was determined. Another batch of 50 worms similar in size to that of the former batch, also selected from the common pool, was transferred into 1500 ml of hyposmotic medium (salinity 6‰), contained in clean, 2 liter, glass beakers (Pyrex) previously marked 1 to 10. Undue variation in temperature was prevented by keeping beakers in large troughs of water. The experiments were conducted at 27.5 ± 0.5°C. The dilute media was prepared as usual from sea water of salinity ranging from 32‰ to 34‰. It was filtered. At the end of every hour a worm from each of the beakers, was selected and its volume determined. The experiment was continued

over a period of 24 hrs; and since preliminary experiments had shown that there was no appreciable change in volume beyond this period, further observations were discontinued and the experiment terminated.

Results: The volume in all the worms reached the maximum percentage increase (40.49%) at the end of 1st hour (Table VI, Fig.2). Thereafter there was a steady fall reaching the lowest volume viz., 4.29% at the end of the 4th hour. Beyond the 4th hour, intermittant increase and decrease in the volume were noticed. But the increase was never as high as that observed during either the 1st or the 2nd or the 3rd hours when the percentage increases were 40.49%, 31.90% and 12.27% respectively. The initial increase in volume is perhaps, due to absorption of water from the medium against an osmotic gradiant and the subsequent fall a consequence of loss of salts as reported in a number of polychaetes viz., N. diversicolor, N. pelagica, N. virens, Perinereis cultrifera (Schlieper,1929a & b; Beadle,1931, '37; Sayles,1935; Jørgensen & Dales,1957); in N. limnicola (=Neathes lighti) (Smith,1956,'59); in Onuphis eremita, Loimia medusa and Clymene insecta (Krishnamoorthi,1962) and in Diopatra variabilis (Krishnamoorthi,1963b). The oscillations in the volume after the 4th hour, are, perhaps, individual

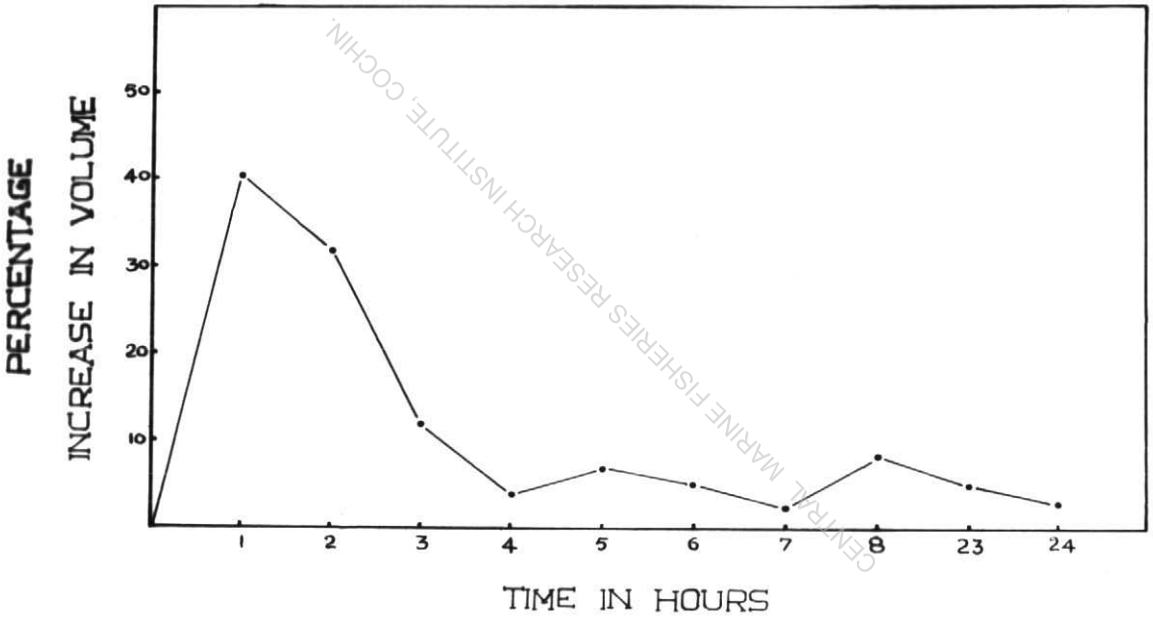


Fig.2. Indicating changes in volume after every hour of exposure to a hyposmotic medium of 6‰ over a period of 24 hrs. Temp.: 29.5 ± 0.5°C. Each point is the mean of 10 estimations.

TABLE VI.

Time in Hours.	Final volume in cc after hourly periods in beakers.													Increase in Volume	
	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e.	Actual	%
Initial.	1.6	1.8	1.5	1.7	1.6	1.6	1.8	1.5	1.6	1.6	1.63	0.336	±0.120	-	-
1	2.3	2.4	2.1	2.3	2.3	2.4	2.2	2.3	2.3	2.3	2.29	0.260	±0.090	0.66	40.49
2	2.2	2.0	2.1	2.2	2.2	2.2	2.1	2.1	2.2	2.2	2.15	0.022	±0.007	0.52	31.90
3	1.8	1.9	1.8	1.8	1.7	1.8	1.8	1.8	1.9	2.0	1.83	0.080	±0.030	0.20	12.27
4	1.7	1.7	1.6	1.7	1.8	1.7	1.7	1.7	1.8	1.6	1.70	0.020	±0.007	0.07	4.29
5	1.8	1.6	1.8	1.8	1.7	1.7	1.7	1.7	1.8	1.8	1.74	0.070	±0.020	0.11	6.74
6	1.7	1.6	1.5	1.9	1.7	1.7	1.7	1.8	1.8	1.7	1.71	0.340	±0.110	0.08	4.91
7	1.6	1.6	1.5	1.6	1.4	1.4	1.6	1.6	1.8	1.6	1.67	0.450	±0.150	0.04	2.45
8	1.9	1.9	1.8	1.9	2.0	1.8	1.9	1.9	1.8	1.8	1.77	0.400	±0.130	0.14	8.59
23	1.8	1.7	1.6	1.7	1.6	1.8	1.8	1.7	1.6	1.8	1.71	0.090	±0.030	0.08	4.91
24	1.7	1.7	1.6	1.5	1.7	1.7	1.8	1.7	1.7	1.7	1.68	0.080	±0.030	0.05	3.07

Table. VI. Volume changes after every hour of exposure over a period of 24 hrs to a hyposmotic medium of salinity 6‰. Temp.: 29.5 ± 0.5 C.

efforts of the worm to keep down the increase in volume. Also it is seen that the final volume attained at the end of the 4th hour and maintained till the 24th hour, is always higher than the original volume. The final volume ranged between 2.45% to 8.59%.

3.2. Volume Regulation over a period of 24 hrs in a hyposmotic medium of strength 8‰.

An experiment similar in approach and plan, was performed, but with a hyposmotic saline of strength 8‰.

Results: It may be seen that in this salinity also as in the previous hyposmotic medium, all the worms swelled to the maximum of 36.13% at the end of the 1st hour and subsequently decreased in volume till the end of the 4th hour, the percentage increase in volume noted at the end of the 4th hour being 2.58% (Table VII, Fig.3). It steadily increased to 9.03% till the end of the 7th hour, only to fall to 2.58% at the end of the 24th hour. In other words, the final volume reached at the end of the 4th hour upto the 24th hour, must be due to similar factors as that prescribed for the behaviour of the worms in the previous experiment. However, one observation is irresistable, i.e., the final volume attained at the end of the 4th hour and

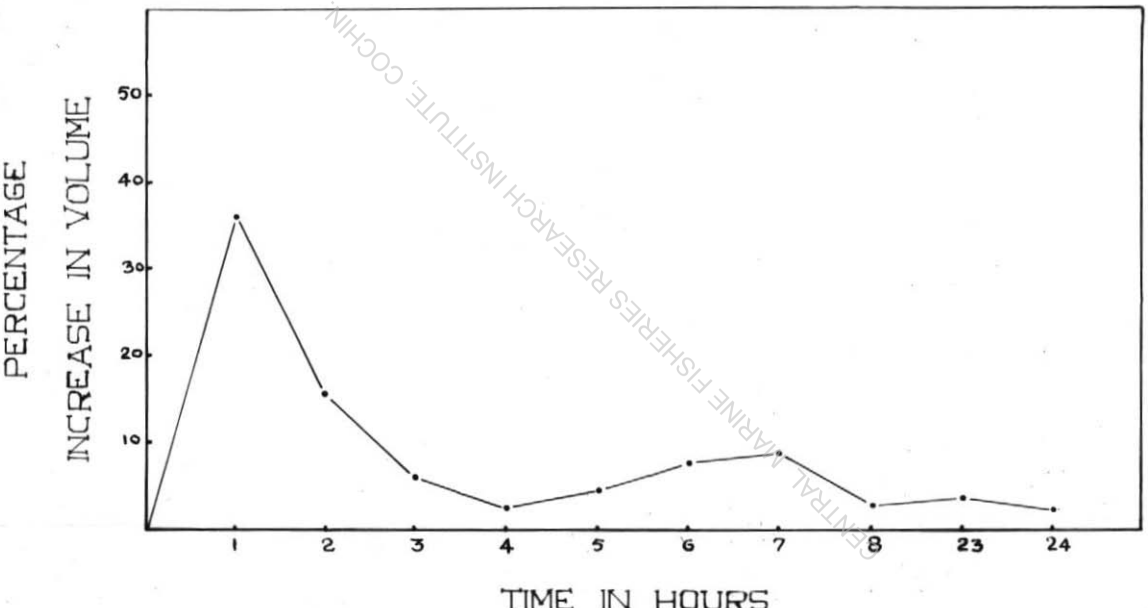


Fig.3. Indicating changes in volume after every hour of exposure to a hyposmotic medium of 8‰ over a period of 24 hrs. Temp.: 29.5 ± 0.5°C. Each point is the mean of 10 estimations.

T A B L E VII.

Time in Hours.	Final Volume in cc after hourly periods in beakers										Increase in Volume				
	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e	Actual	%
Initial.	1.5	1.6	1.7	1.5	1.5	1.5	1.6	1.6	1.5	1.5	1.55	0.020	±0.016	-	-
1	2.1	2.2	2.0	2.1	2.1	2.0	2.2	2.1	2.1	2.2	2.11	0.230	±0.017	0.56	36.13
2	1.8	1.9	1.7	1.8	1.8	1.8	1.7	1.9	1.8	1.7	1.79	0.230	±0.017	0.24	15.81
3	1.7	1.7	1.5	1.6	1.6	1.6	1.7	1.7	1.7	1.6	1.64	0.220	±0.015	0.09	5.81
4	1.6	1.6	1.7	1.7	1.5	1.5	1.6	1.6	1.5	1.6	1.59	0.230	±0.017	0.04	2.58
5	1.6	1.5	1.6	1.7	1.6	1.7	1.7	1.5	1.6	1.7	1.62	0.250	±0.017	0.07	4.52
6	1.7	1.7	1.7	1.6	1.6	1.7	1.7	1.7	1.6	1.7	1.67	0.150	±0.007	0.12	7.81
7	1.8	1.6	1.8	1.7	1.6	1.7	1.6	1.8	1.7	1.6	1.69	0.270	±0.085	0.14	9.03
8	1.6	1.6	1.5	1.6	1.6	1.6	1.5	1.5	1.8	1.7	1.60	0.094	±0.030	0.05	3.23
23	1.6	1.6	1.5	1.5	1.6	1.6	1.7	1.7	1.8	1.6	1.61	0.090	±0.028	0.06	3.87
24	1.6	1.5	1.6	1.6	1.5	1.5	1.7	1.6	1.7	1.6	1.59	0.075	±0.024	0.04	2.58

Table. VII. Volume changes after every hour of exposure over a period of 24 hrs to hyposmotic medium of salinity 8‰. Temp.: 29.5 ± 0.5°C.

maintained at that volume till the end of the 24th hour is not only higher than the original volume, similar to the behaviour of the worms in the previous salinity, but more or less of the same magnitude as that attained in the previous hyposmotic medium.

3.3. Volume Regulation over a period of 24 hours in a hyposmotic medium of strength 13‰.

Another experiment identical in procedure to the previous experiments, was performed with but one departure. The experimental medium had a slightly higher concentration, viz., 13‰.

Results: In this medium also the responses of the worm were not disimilar from those observed in the two previous media (Table VIII, Fig.4). They attained the maximum volume of 25.60% at the end of the 1st hour; and as in the previous two salinities, the volume subsequently decreased reaching the minimum of 1.19% at the end of the 4th hour. Thereafter, till the end of the 24th hour, the volume fluctuated between 1.19% to 6.55%, the latter percentage increase registered at the end of the 6th hour. The same factors are, perhaps, applicable to explain the responses of the worms observed in the present experiment too. However, while the final volume fluctuated in the present medium between 1.19% and 5.36%, similar ranges were higher in the two previous media.

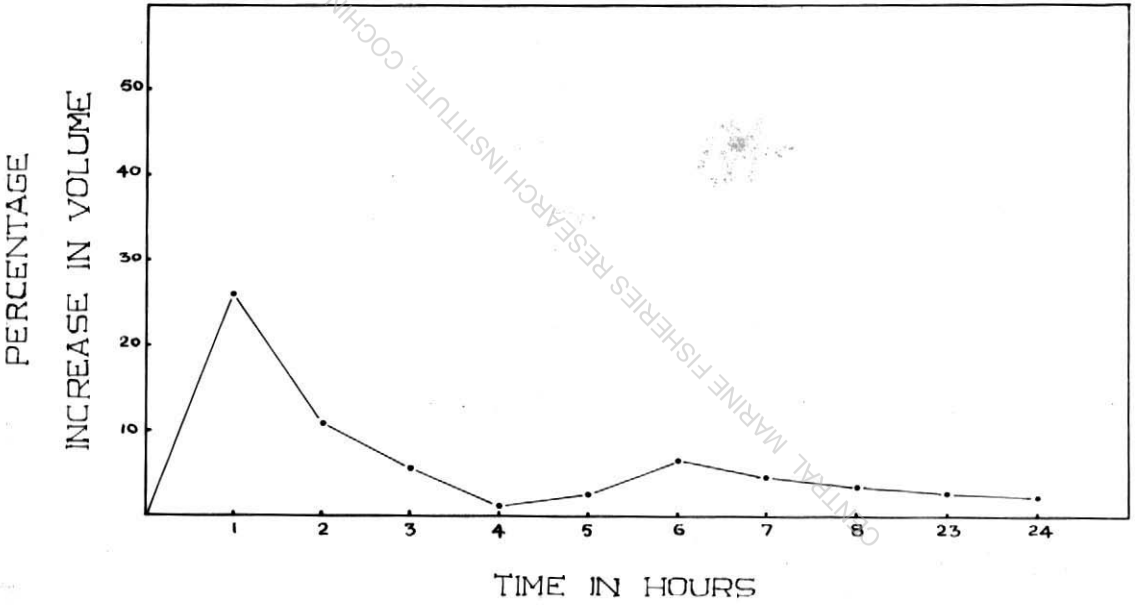


Fig.4. Indicating changes in volume after every hour of exposure to a hyposmotic medium of 13‰ over a period of 24 hrs. Temp.: 29.5 ± 0.5°C. Each point is the mean of 10 estimations.

T A B L E VIII.

Time in Hours.	Final Volume in cc after hourly periods in beakers.												Increase in Volume		
	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e.	Actual	%
Initial.	1.7	1.7	1.6	1.8	1.6	1.7	1.6	1.6	1.7	1.8	1.68	0.078	±0.025	-	-
1	2.1	2.1	2.0	2.2	2.2	2.1	2.1	2.0	2.1	2.2	2.11	0.075	±0.024	0.43	25.60
2	1.9	1.8	1.8	1.9	1.9	1.8	1.9	2.0	1.8	1.8	1.86	0.070	±0.020	0.18	10.71
3	1.8	1.7	1.8	1.9	1.7	1.7	1.8	1.8	1.7	1.8	1.77	0.068	±0.021	0.09	5.36
4	1.7	1.7	1.6	1.6	1.7	1.7	1.8	1.7	1.8	1.7	1.70	0.066	±0.020	0.02	1.19
5	1.7	1.6	1.6	1.6	1.7	1.7	1.6	1.6	1.6	1.6	1.73	0.370	±0.120	0.05	2.98
6	1.8	1.7	1.7	1.8	1.8	1.7	1.9	1.7	1.9	1.9	1.79	0.088	±0.027	0.11	6.55
7	1.7	1.7	1.6	1.6	1.7	1.7	1.8	1.7	1.8	1.8	1.76	0.090	±0.028	0.08	4.76
8	1.6	1.5	1.6	1.9	1.8	1.8	1.7	1.6	1.9	1.8	1.74	0.417	±0.130	0.06	3.57
23	1.7	1.7	1.8	1.8	1.7	1.8	1.8	1.7	1.7	1.7	1.73	0.050	±0.015	0.05	2.98
24	1.8	1.8	1.6	1.6	1.6	1.8	1.8	1.8	1.7	1.7	1.72	0.090	±0.028	0.04	2.38

Table. VIII. Volume changes after every hour of exposure over a period of 24 hrs, to a hyposmotic medium of salinity 13‰. Temp.:29.5 ± 0.5°C.

4. Remarks: In all the experiments the behaviour of Marphysa gravelyi was consistantly similar. It attained the maximum volume at the end of the 1st hour and subsequently decreased to the lowest value observed at the end of the 4th hour. Thereafter, it was characterised by interimittant rises and falls till the end of the experiment i.e., 24 hrs. M. gravelyi, thus, resembles Nereis diversicolor and Nereis pelagica (Schlieper,1929a & b); N. diversicolor and Perinereis cultrifera (Beadle,1931, '37); Nereis virens (Sayles,1935); N. virens, N. pelagica and N. diversicolor (Jørgensen & Dales,1957) and Nereis limnicola (Smith,1959) in its response to hyposmotic media. However, even in this similarity of pattern, there is yet a difference apparent. The magnitude of increase in the volume at the end of the 1st hour as well as the final volume attained and maintained from the 4th to 24th hour were different in different hyposmotic media. While the maximum increase in volume in hyposmotic media of strength 6‰ was 40.49%, the respective increases in volume in media of salinities 8‰ and 13‰ were 36.13% and 25.60%. Similarly, the final volumes reached in sea water diluted to have a concentration of 6‰, 8‰ and 13‰ were 4.29%, 2.58% and 1.19% respectively. In other words, both the initial increase in volume and the final volume attained is,

perhaps, a function of the dilution of the experimental media. Once again, the responses of M. gravelyi to the stresses of hyposmotic media, are, thus, not far different from those observed in N. diversicolor, N. virens, N. pelagica, Perinereis cultrifera and N. lighti (Schlieper,1929a & b; Beadle,1931, '37; Sayles,1935; Joregensen & Dales,1957; Smith,1959). But the magnitude of change in volume under identical stresses of an hyposmotic or hyperosmotic media, was more in N. virens than in N. diversicolor (Schlieper,1929a & b). P. cultrifera swelled considerably compared with N. diversicolor (Beadle,1931), while N. virens ranked between N. pelagica and N. diversicolor (Jørgensen & Dales,1957). The extent of volume change in M. gravelyi, therefore, would be significant only when compared with similar parameters obtained from the responses of other polychaetes viz., G. embranchiata, O. eremita, L. medusa and C. insecta (Krishnamoorthi,1962) and D. variabilis (Krishnamoorthi, 1963b) that coexist with M. gravelyi in the Adyar estuary. It has already been shown that volume control in C. insecta was better developed than in either G. embranchiata or L. medusa or O. eremita, since the magnitudes of increase in volume and the final volume attained at the end of the experiment were the lowest compared with those of L. medusa and O. eremita (Krishnamoorthi,1962). Between C. insecta

and D. variabilis, the latter exhibited better capacities for volume regulation (Krishnamoorthi,1963b). Comparing the increase in volume of D. variabilis with that of M. gravelyi in hyposmotic media of salinities 8‰ and 13‰, it would be seen that, whereas the percentage increase in volume at the end of the 1st hour in the respective dilutions were 50% and 36% for D. variabilis, similar figures for M. gravelyi were only 36% and 25%. Also the final volumes attained were lower in M. gravelyi than in D. variabilis. In M. gravelyi, therefore, the mechanism of volume regulation is far superior to that obtained in other forms. Observing lesser degree of swelling in N. limnicola obtained from Lake Merced than in those from the estuary Walker Creek, Smith (1959) suggested better volume control in the former population.

The increase in volume in M. gravelyi is, probably, due to absorption of water against an osmotic gradiant, in agreement with the observations of earlier workers in the field of physiology of Nereids. But the differences in the magnitude of increase could only be a reflection of the extent of permeability. Among the polychaetes studied (Krishnamoorthi,1962, '63b), M. gravelyi shows the lowest rate of increase in volume and, therefore, the rate of permeability in this worm is, perhaps, the lowest. Jørgensen & Dales (1957) following the volume

changes in three species of Nereids viz., N. virens, N. pelagica and N. diversicolor, suggested and later proved with experiments involving use of radio active isotope, Cl^{36}; that the lower values of increase in volume in N. diversicolor could only be due to it being less permeable than Perinereis cultrifera. In the light of recent evidences let in (Smith,1963) that quicker rate of loss of salts may also be advantageous, a reduction in permeability coupled with the above factor may considerably increase the chances of establishment of a species faster to a changing environment. Although similar studies in M. gravelyi must await future investigation, the observed rate of increase in volume being comparatively lower, offers itself an explanation that in M. gravelyi the rate of permeability is the lowest.

Factors like tolerance of salinity over a wider range; greater capacities for volume regulation and reduction in the permeability, admirable and meaningful as they are, for a successful penetration into and establishment of a polychaete such as M. gravelyi in brackish waters, fail to answer the question of maintenance of a constant internal concentration, a factor equally profitable and necessary to meet the hazards of a changing environment. If it is assumed that the initial increase in volume in M. gravelyi is due to

absorption of water against an osmotic gradiant and the subsequent decrease a consequence of loss of salts, such an assumption presupposes profound changes in the body fluids, any imbalance of which would be disastrous. It appeared, therefore, that the next stage in the course of the present investigations, should be directed to the study of body fluids and its consequential changes as reflected by the depressions in the freezing point, under stresses of heterosmotic media.

CHAPTER V: REGULATION OF BODY FLUID CONCENTRATION.

C O N T E N T S.

CHAPTER V: REGULATION OF BODY FLUID CONCENTRATION.

1. Introduction: Among the invertebrate phyla, no other group shows peak of osmotic independence in all the forms known to science, better than the Crustaceans. Here, one comes across the conformers, the blood being isosmotic with the medium over the entire salinity range tolerated. There are the regulators, some being hyperosmotic in the lower dilutions but becoming isosmotic in more concentrated solutions. Yet others being hyperosmotic in dilute and hyposmotic in higher concentrations (see Lockwood, 1962, for an exhaustive treatment). Although polychaetes have not achieved an osmotic independence comparable with that of the Crustaceans, yet there are the conformers represented by Arenicola mariana, Nereis palarica and Perinereis cultrifera which show very little swelling and adjust their osmoconcentration to suit the strength of the medium in which they are bathed (Schlieper,1929a & b). Forms like Nereis diversicolor, however, regulate their body fluid concentration such that it is hyperosmotic in dilute media and isosmotic in higher concentrations (Schlieper,1929a & b; Beadle,1937; Smith,1955c). Further, in N. diversicolor, a regulator, the mechanism for volume control is better developed than either in

Nereis pelagica or Nereis virens or Perinereis cultrifera (Schlieper,1929a & b; Beadle,1931, '37; Sayles,1935; Jørgensen & Dales,1957). It was seen in the two previous sections, that Marphysa gravelyi Southern not only exhibited tolerance of lower salinities over a wider range but its capacities for volume regulation were far superior to any other polychaete studied (Krishnamoorthi, 1962, '63b). These features of Marphysa gravelyi prompted the following investigations to understand its abilities for osmoconcentration under stresses of hyposmotic media.

2. Material & Methods: The method followed was basically similar to the comparative melting point method of Jones (1941) as modified by Freeman & Rigler (1957). In principle it requires comparison of melting time of known concentrations of NaCl solutions with those of unknown solutions such as body fluids of animals. In the present method refinements like the use of polaroids and a binocular microscope to observe with as suggested by Gross (1954 and personal communication), facilitated timing the disappearance of the last crystal. A series of capillary tubes of sizes usually ranging from 6 to 8 cm in length, and with an uniform bore of 0.1 mm, were selected, thoroughly cleaned with Teepol (B.D.H.), washed in distilled water and dried in a hot-air oven overnight

at 200°C. This procedure besides freeing the tubes of any contamination, helped in rapid introduction of either the NaCl samples or body fluids with the aid of capillary action alone. More often the latter failed, perhaps, due to some contamination in the bore. Such tubes were discarded and never used till a free flow was ensured by repeated washings. NaCl solutions of known concentrations ranging from 0.1% to 1.0% at 0.1% intervals and from 1% to 5% at 0.5% intervals were carefully prepared and since the melting time of these concentrations formed the basis for comparison with the unknown, particular attention was paid in their preparation. These prepared solutions were introduced with the help of a glass pipette with a fine drawn-out nozzle also prepared at the laboratory from glass tubing (Pyrex), such that each tube contained about 6 to 10 columns of 0.2 mm in size and each column was sandwiched between columns of paraffin oil ranging in size from 0.5 to 1 cm. The ends of the capillary tubes were sealed with sealing wax. Depending upon the availability of dry-ice (Solid Co_2), they were either transferred to a deepfreeze for later examination or immediately utilised for estimation of the melting time. For determining the melting time, they were quick frozen in dry-ice and transferred into a glass

trough of size 10 x 4 x 4 c.cms containing 94 % alcohol previously cooled in a refrigirator. To ensure constancy of temperature of the alcohol at 2°C at which the melting time was noted, the entire trough was encased in another transparent polythene box with dry-ice packed between the glass trough and the outer box on all sides except at the bottom and the top. A stirrer gently rotating, kept up a constant circulation of the alcohol also aimed at the maintenance of a constant temperature. Addition of suitable quantities of dry-ice encasing the glass trough helped in bringing down the temperature of the alcohol to the required level i.e., at 2°C, whenever it showed signs of shooting up. The entire process of melting was observed under a binocular microscope (Leitz) with a built-in illumination, at a constant magnification and with the help of a pair of polaroids. One piece of polaroid was placed in between the trough and the source of illumination and the other was placed over the trough but below the objectives. The plane of the second polaroid, since in practise this was easier than the adjustment of the first polariod, was rotated in relation to the plane of the first polariod, [redacted] until the frozen crystals being birefringent, appeared with a brilliant pinkish glow against a dark background. With the help of a stop-watch, the time taken from the moment the

frozen column started melting untill the disappearance of the last crystal, was noted. The data so obtained formed the basis for finding out the concentration of the unknown in terms of %NaCl. A procedure similar as above was followed to find the melting time of samples of frozen body fluids and from it, its concentration. Since the melting times are comparable only when equal amounts of samples are taken, it is essential that the column of body fluid introduced is equal in length to that of known concentrations of NaCl i.e., 0.2 mm. It is also equally important that the capillary tubes are quickly transferred to the trough from the freezing mixture. The entire estimation was carried out in a cold room at a temperature of 16°C. The body fluid was drawn from the worms by means of a glass pipette with a fine nozzle, made at the laboratory from glass tubing (Pyrex). Normally the body fluids flowed into the nozzle by mere capillary action. At times when this failed, moving the nozzle back and forth was sufficient to drive the body fluid in. Often blockage of the nozzle resulted and as often as this happened, the pipette was withdrawn, thoroughly cleaned in distilled water and dried before a second insertion.

3.1 Body Fluid Concentration under stresses of heterosmotic media.

Experimental Procedure: Experimental media of differing concentrations ranging from 9.54‰ to 28.62‰ (30% to 90% of sea water) were prepared by adding required amounts of distilled water to sea water to bring it ato the desired concentration, filtered; and transfered to beakers kept and maintained at room temperature, i.e., 28 ±0.5°C. Into each of the beakers a single entire, active worm selected from a pool of worms equal in size, was introduced and exposed over a period of 24 hrs. At the end of 24 hrs, the body fluid was drawn and its osmotic concentration determined in terms of %NaCl.

Results: The lowest concentration of 0.930 %NaCl was obtained in a worm exposed to the stresses of medium of 12.72‰ and the highest at a value of 0.962 %NaCl in a medium of strength 28.62‰ (Table IX, Fig.5). If it is assumed that the animal is isosmotic with a medium of strength 45% (Fig.5), the two previous concentrations indicate the two extremes of the range of tolerance of the animal. The range of body fluid concentration from 0.930 %NaCl to 0.962 %NaCl is, thus, small when compared to the range of concentration of the experimental media - 9.54‰ to 28.62‰. In other words, the animal appears a perfect regulator, maintaining its body fluid

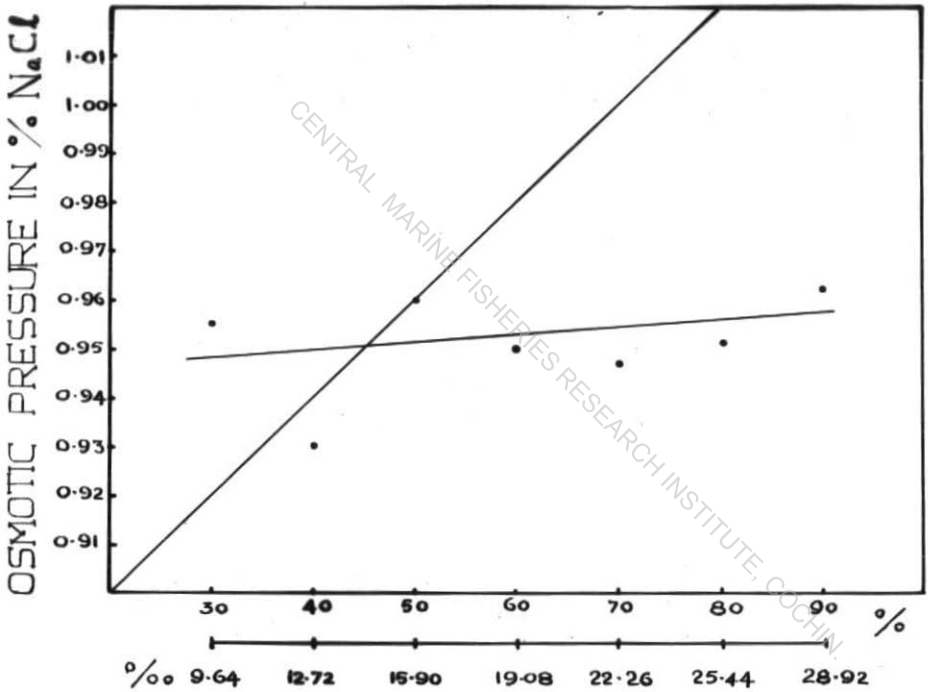


Fig.5. Showing the osmotic pressure of body fluids, in terms of %NaCl, after an exposure of 24 hrs to heterosmotic media. Temp.: 28.0 ± 0.5°C. Each point is the mean of 5 estimations. The diagonal line is the line of isosmocity.

TABLE IX

Experimental Medium %	Melting Time in Seconds in Sample								Osmotic Pressure as % NaCl	Depression in Freezing Point (Δ/0.6 = %NaCl)
	1	2	3	4	5	Mean	s.d.	s.e.		
9.54	57	58	57	57	58	57.4	0.30	±0.13	0.955	0.5730
12.72	57.5	59	59	58	58	58.3	0.90	±0.40	0.930	0.5580
15.90	57	55	56	55	55	55.6	1.10	±0.49	0.960	0.5760
19.08	59	56	57	56	58	57.2	3.40	±1.52	0.950	0.5700
22.26	57	58	58	59	57	57.8	1.40	±0.63	0.946	0.5676
25.44	58	57	57	58	58	57.6	0.60	±0.27	0.951	0.5886
28.62	54	58	56	58	57	56.6	5.60	±0.25	0.962	0.5772

Table. IX. To indicate osmotic pressure of body fluids when exposed to heterosmotic media. Temp.: 28.0 ± 0.5°C. The body fluid concentration is expressed in terms of both %Nacl and in depression in the freezing point.

concentration within a very low range over a wide range of experimental media. That this is so, is well borne out by Fig.5. M. gravelyi, therefore, hyper-regulates in the lower dilutions and hyporegulates in the higher concentrations.

3.2. Body Fluid Concentration at hourly intervals in hyposmotic medium of strength 10.68‰.

Experimental Procedure: An experimental medium of strength 10.68‰ was prepared from filtered sea water and 1500 ml of it was transferred into a clean Pyrex glass beaker of 2 liter capacity. The beaker with the experimental medium was placed in another larger trough containing tap water such that the experimental medium contained in the beaker was in level with that of the tap water surrounding the beaker. This helped the fluctuations in the temperature of the experimental medium at 28.5°C within a narrow range of ±0.5°C. A batch of 50 complete vigourous worms equal in size, was slected from the aquarium, and transferred into a beaker containing medium of salinity 10.68‰. At the end of every hour over a period of 31 hours, body fluid was drawn from a worm and its osmotic concentration determined.

Results: A steady fall from 1.05 %NaCl registered at the end of the 1st hour, to 0.88 %NaCl at the end of

the 4th hour was noticed (Table X, Fig.6). Beyond the fifth hour, it progressively increased to reach the highest concentration of 1.06 %NaCl, only to fall during the next hour to 1.00 %NaCl. During the 26th hour to the 31st hour, it fluctuated hourly between 1.00 %NaCl and 1.05 %NaCl a figure similar to that registered at the end of the 1st hour. Although the character of the graph is not a mirror image of that obtained for N. diversicolor (Beadle,1937), the steady fall in the concentration and subsequent increase must be due to loss of salts and/or removal of fluid by the excretory organs. It is also possible that at this salinity the loss of fluid either through the body surface or via excretory organs becomes effective after period of 4 to 5 hours.

3.3. Body Fluid Concentration at hourly intervals in a hyposmotic medium of 13.52‰.

Experimental Procedure: An experiment similar in all respects to the previous one, was performed but with an experimental medium of 13.52‰. Worms chosen from the same pool formed the experimental material.

Results: The results are summarised in Table XI and represented in Fig.7. From the character of the graph,

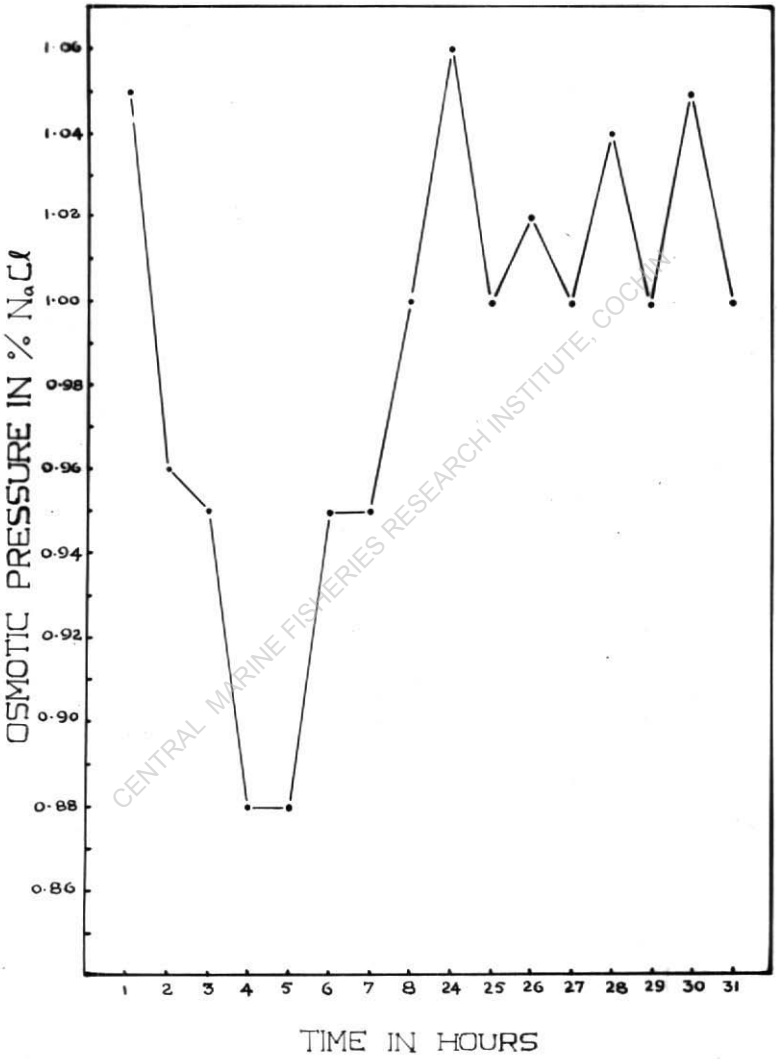


Fig.6. Showing the changes in the osmotic pressure, in terms of %NaCl, at hourly intervals overa period of 31 hrs in a hyposmotic medium of 10.68‰. Temp.: 28.5 ± 0.5°C. Each point is the mean of 10 estimations.

TABLE X.

Time in Hours.	Melting time in Seconds in sample after exposure over hourly periods.										Mean	s.d.	s.e.	Osmotic Pressure in % NaCl	Depression in Freezing Point (Δ=0.6 x %NaCl)
	1	2	3	4	5	6	7	8	9	10					
1	51	50	51	49	51	51	50	51	51	50	50.5	1.35	±0.42	1.05	0.630
2	59	57	59	56	57	56	59	58	56	56	56.3	1.70	±0.51	0.96	0.576
3	59	58	59	57	59	59	60	60	59	59	58.9	0.88	±0.28	0.94	0.564
4	62	64	60	62	62	61	62	60	64	62	61.9	1.37	±0.43	0.88	0.528
5	60	63	62	60	60	60	61	62	61	60	60.9	1.09	±0.34	0.88	0.528
6	57	59	59	57	57	57	59	57	59	57	57.8	1.03	±0.33	0.95	0.570
7	58	56	58	58	60	60	58	58	58	56	58.0	1.33	±0.42	0.95	0.570
8	52	50	50	52	52	54	50	51	50	51	51.2	1.31	±0.41	1.00	0.600
24	49	49	51	50	48	48	49	46	49	48	48.7	1.33	±0.42	1.06	0.636
25	53	50	53	54	53	52	50	53	53	53	52.0	1.41	±0.45	1.00	0.600
26	52	50	48	50	52	52	52	50	52	50	50.8	1.40	±0.44	1.02	0.600
27	51	50	48	48	50	51	51	50	50	50	49.9	1.10	±0.34	1.00	0.600
28	51	48	49	50	51	51	50	50	51	50	50.1	1.00	±0.32	1.04	0.624
29	49	49	48	50	51	49	48	49	50	49	49.2	0.92	±0.29	1.00	0.600
30	52	50	49	50	50	52	52	51	50	51	50.7	1.06	±3.35	1.05	0.630
31	52	52	52	50	54	50	49	50	52	52	51.3	1.49	±0.47	1.00	0.600

Table. X. Showing changes in the osmotic pressure in terms of %NaCl at hourly intervals over a period of 31hrs in a medium of salinity 10.68‰. Temp.:28.5 ± 0.5°C.

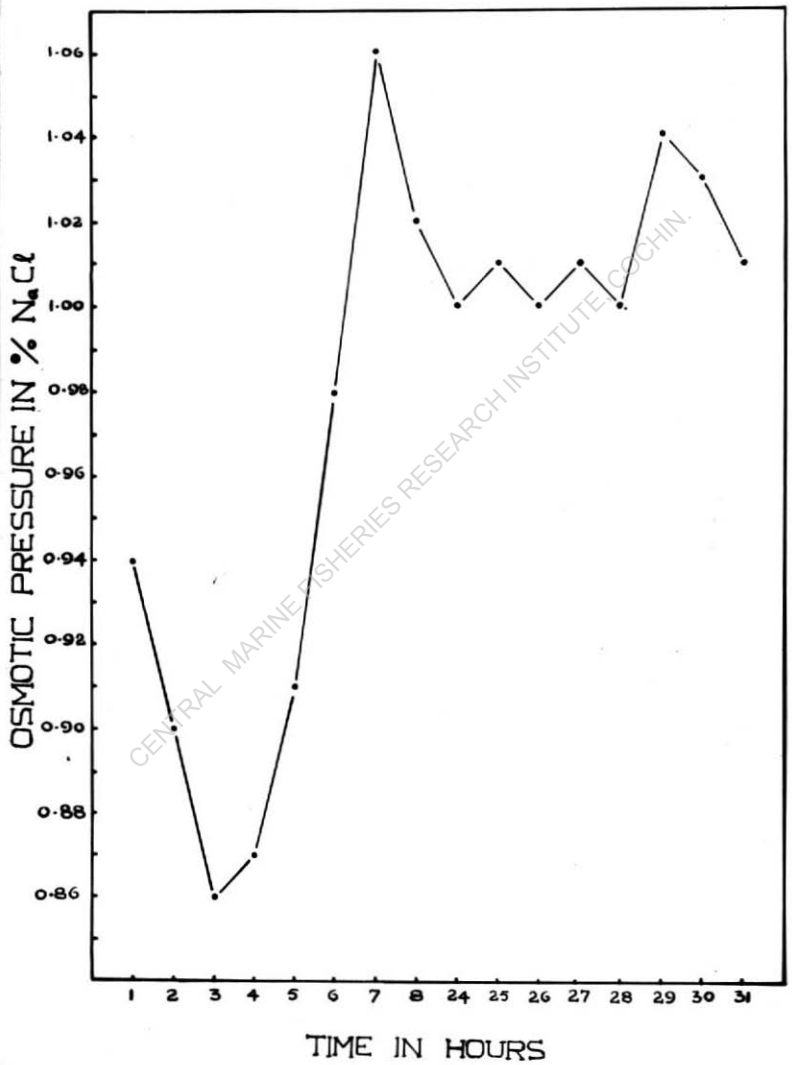


Fig.7. Showing the changes in the osmotic pressure, in terms of %NaCl, at hourly intervals over a period of 31 hrs in a hyposmotic medium of 13.52‰. Temp.: 28.5 ± 0.5°C. Each point is the mean of 10 estimations.

T A B L E XI.

Time in Hours.	Melting time in Seconds in sample after exposure over hourly periods.													Osmotic Pressure in ‰NaCl.	Depression in Freezing Point (Δ=0.6 x ‰NaCl)
	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e.		
1	57	57	55	59	57	59	57	57	55	57	57.0	1.33	±0.42	0.94	0.564
2	59	60	60	58	58	59	55	58	59	59	58.5	1.43	±0.45	0.90	0.540
3	59	59	60	58	58	59	58	59	59	59	58.8	0.69	±0.22	0.86	0.516
4	60	60	61	59	60	61	61	60	60	60	61.2	1.23	±0.38	0.87	0.522
5	59	59	58	60	56	56	58	58	59	59	58.2	1.32	±0.42	0.91	0.546
6	55	55	53	53	54	55	56	56	54	55	54.6	1.05	±0.33	0.98	0.588
7	51	51	50	48	50	51	50	51	50	51	50.3	0.95	±0.30	1.06	0.636
8	53	51	50	53	53	51	51	53	51	50	51.6	1.27	±0.40	1.02	0.612
24	53	53	52	53	52	50	51	53	52	51	52.0	1.41	±0.43	1.00	0.600
25	52	50	50	52	51	52	50	51	52	50	51.0	0.94	±0.27	1.01	0.606
26	53	52	53	50	54	53	55	50	51	50	52.1	1.79	±0.57	1.00	0.600
27	52	51	50	53	52	50	50	50	52	51	51.1	1.01	±0.35	1.01	0.606
28	52	53	52	53	52	50	52	50	51	50	51.5	1.18	±0.34	1.00	0.600
29	49	50	49	51	47	48	49	50	49	51	49.3	1.25	±0.40	1.04	0.624
30	50	50	50	51	48	51	48	50	50	49	49.9	1.12	±0.36	1.03	0.618
31	52	51	52	50	50	53	50	53	50	52	51.3	1.25	±0.40	1.01	0.606

Table. XI. Showing changes in osmotic pressure in terms of ‰NaCl, at hourly intervals over a period of 31 hrs in a medium of salinity 13.52‰. Temp.:28.5 ± 0.5°C.

it is clear that the adjustment of the body fluid concentration followed the same pattern as in the previous experiment. However, at this salinity (13.52‰) the steady fall in the body fluid concentration from 0.940 %NaCl to 0.860 %NaCl continued upto a period of 3 hrs only. Thereafter, it progressively increased reaching the maximum concentration of 1.060 %NaCl at the end of the 7th hour, only to fall to 1.0 %NaCl at the end of the 24th hour. During the 24th to 31st hours it fluctuated between 1.0 %NaCl to 1.04 %NaCl. The governing factors are, perhaps, the same as that visualised to explain the course of osmoconcentration of body fluids observed in the previous experiment. Further, the variations between the two must be due to concentration differences of the experimental media; and, perhaps, a function of the differences in stresses imposed.

4. Remarks: Nereis diversicolor is the only Nereid polychaete on which fairly exhaustive studies on the regulation of osmconcentration of body fluid exist. At Kiel its body fluid concentration (Table XII), represented as gm/l chlorides, over its regulatory phase, ranges from 7.10 to 18.20 gm/l or from Δ 0.48 to 1.90 in terms of the depression in the freezing

point (Schlieper,1929a & b). At Millport, Tvarminne, Isefjord and the Upper Tamar Estuary, it ranges from △0.33 to 1.90 (Smith,1955c). Beadle (1937) reports a range of 0.84 to 1.67 for worms obtained from the River Blythe estuary. In other words, the freezing point depressions reflecting the osmotic concentrations

TABLE XII.

Population From	Range of Body Fluid Concentration gm/l	△	Name of Polychaete.	Reference
Millport.	4.80-19.15	0.48-1.90*	N.diversicolor.	Smith,1955c
Tvarminne.	3.35- 9.50	0.33-0.94*	"	"
Isefjord.	3.59- 8.94	0.36-0.89*	"	"
Upper Tamar Estuary	3.38- 9.40	0.33-0.93*	"	"
Kiel.	7.10-18.20*	0.70-1.80	"	Schlieper, 1929a & b.
Lake Merced.	4.40-10.20	0.44-1.01*	N.lighti.	Smith,1959.
Walker Creek.	4.00-10.00	0.40-0.99*	"	" (From Fig.12)
Kiel.	-	0.76-0.77	Arenicola marina	Schlieper, 1929a & b.
Helgoland.	-	1.70	"	"
Adyar Estuary.	9.64-16.46	0.56-0.59	Marphysa gravelyi.	Present study.

* Calculated Figures from references cited.

of the body fluid of N. diversicolor ranges from 0.33 to 1.90 in worms studied over its entire range of distribution. In Arenicola mariana at Kiel when △ of the external medium was 0.76-0.77, the body fluid concentration was that of the external medium (Schlieper,1929a & b). At Helgoland it was 1.70 in conformity with the concentation of the △ external medium. While the body fluid concentration of N. limnicola from Lake Merced (Fresh water) ranged from 0.44-1.01, it was 0.40-0.99 in those obtained from Walker Creek estuary. The range of 0.56 to 0.59 for Marphysa gravelyi was not only narrow but very low compared with the above forms (Table XII). Thus in M. gravelyi we come accross the lowest concentration of body fluid when compared with the euryhaline N. diversicolor or, more or less stenohaline A. marina. Such a situation could only be a consequence of better regulatory powers for osmoconcentration. The fresh water Gammarid, Gammarus fasciatus, regulated at the lowest level, whereas the marine species Marinogammarus finmarchicus and Gammarus oceanicus, regulated its blood at the highest level, with Gammarus tigrinus, a brackish water species, ranking between the two (Beadle & Cragg,1940a & b; Werntz,1963). A reduction in the body fluid concentration is,

perhaps, a general feature common to organisms attempting colonisation of regimens of diverse habitats of reduced salinities (Beadle,1943,'57); and according to Potts (1954c) and Croghan (1961), the chief means of easing the strain on osmoregulatory mechanisms.

Making parallel determinations of weight and body fluid concentration in N. diversicolor at equal intervals over a period of 24 hrs under stresses of hyposmotic media, Beadle (1937) concluded that the concentration curve followed 'a logarithmic course towards a value above that of the external medium'. Similar determinations made in M. graveli also (compare Fig.4 with Fig.7), indicate a trend identical with that obtained in N. diversicolor. However, in M. gravelyi restoration of the body fluid concentration does not begin until after the 3rd or 4th hour, by which time volume regulation is completed, since the highest increase in volume, irrespective of the strength of the experimental medium, is reached at the end of the 1st hour (vide infra). Independent of the final volume attained at the end of the 4th hour and maintained at that level over a period of 24 hrs, the final concentration fluctuates between 1.00 %NaCl and 1.06 %NaCl. The regulation of body

fluid concentration and the control of volume in M. gravelyi are, perhaps, two factors which are not interdependent in agreement with that concluded by Beadle (1937). It follows, therefore, that the fall in volume, as in N. diversicolor (Beadle,1937), should be due to forces other than mere diffusion or osmotic pressure; and, perhaps, nephridia act as channels for vacating the excess of fluid in the coelomic cavity. This is probably brought about by either the hydrostatic pressure or the tonus of the body wall muscles. Or it may also be due to the size of the nephridia that M. gravelyi possesses. There is evidence that they (nephridia) are larger in size (vide Chapter VI). There is also evidence that the tissues are quite active over a wide range (20% to 70% sea water) of salinities (Krishnamoorthi & Krishnaswamy, 1963). Preliminary results have shown (unpublished data) that the hydrostatic pressure increases with increasing concentration of the external media.

Invertebrates have achieved osmotic independence through one of three familiar ways viz., either it is brought about by conforming its internal concentration on a par with that of the external; or being hyperosmotic in the lower dilutions but becoming isosmotic

in higher concentrations; or hyperregulating in dilute media but being hyposmotic in concentrated media. All these forms find representatives among Crustacea, the so called higher invertebrates. Among polychaetes, however, only the first and the second are known. Arenicola marina and N. pelagica are conformers (Schlieper,1929a & b). N. diversicolor be it from Kiel (Schlieper 1929a & b) or the River Blythe estuary (Beadle,1937) or Millport, Tvarminne, Isefjord, Upper Tamar Estuary (Smith,1955c), hyperregulates in lower dilutions but becomes isosmotic in higher concentrations, exemplefying the second type of osmotic independence. Nereis limnicola is yet another Nereid belonging to this type. (Smith,1959). The present investigations show yet another type as represented by M. gravelyi which is hyperosmotic in media of reduced salinities and hyposmotic in concentrated media, a pattern which recalls that obtained in most grapsoid and ocypode crabs (Gross,1957; Green et al,1959) and some prawns like Palaemonetes varians, Leander serratus and Crangon crangon (Panikkar,1941a; Parry,1954,'55; Broekema,1942). The underlying mechanism or mechanisms leading to regulation must await future investigation. There is, however, circumstantial evidence that hyposmotic

urine is being formed, since M. gravelyi possesses nephridia bigger in size (Chapter VI) than those found in polychaetes obtained from similar situations (Krishnamoorthi, 1963b) and as reported in N. diversicolor (Jurgens,1935) and in Lycastis indica (Krishnan, 1952), if it is assumed that bigger sized nephridia could be associated with the production of hypo- or isosmotic urine. Attempts to collect urine following either the method suggested by Bhal (1945b;'47) or under paraffin oil, did not yield desirable results. The maintenance of an internal aqueous and saline steady state, inspite of a constant inward diffusion of water, is due not only to the elimination of isosmotic/hyposmotic urine by the invertebrate nephron, but also due to the varying quantities of urine production as demonstrated in Palaemonetes varians (Parry,1955); in Gammarids (Werntz,1963) and in a host of other crustaceans (see Lockwood,1962, for complete literature). An attempt to study the rate of urine production in N. gravelyi by the inulin clearance method of Roe et al (1949) as followed by Potts (1954) for the estimation of blood volume and rate of urine production in Anodonta cygnea, a fresh water lemellibranch, yielded erratic results. Perhaps, the inulin clearance method successful for crustaceans and molluscans, is not

suitable for soft-bodied polychaetes. Similarly whether there is extrarenal elimination of chlorides characteristic of teleosts and crustaceans hyporegulating needs future investigation, since the presence of well developed branchiae all along the major length of the worm (Aiyar,1933) point to this possibility. The present investigations, however, have brought to light the occurrence among polychaetes a third type of osmotic independence i.e., hyperregulation in dilute media and hyporegulation in concentrated media.

A review of all the literature on polychaete physiology with special reference to osmoregulation, reveals that no other group has been more experimented upon as the Nereids. This is, perhaps, due to the fact that several members of the Nereidae viz., N. diversicolor, N. virens (N. southerni), N. pelagica, N. limnicola (Neanthes lighti), Nereis (Neanthes) succina and Perenereis cultrifera are found to penetrate more or less deeply into brackish water and a few are found even in fresh waters (Johnson,1903; Hartman,1938; Smith,1953). The nereids, therefore, have constituted an excellent group of animals for the study of mechanisms of osmoregulation as pointed out by Jórgensen & Dales (1957). The present investigation has brought to light the fact that, it is by no means confined to

Nereidae alone. For, the Eunicidae of the Adyar estuary show adjustments matching with those of Nereidae in their capacities for osmoregulation. Onuphis eremita, an Eunicid, with no regulatory ability over volume control and a limited range of tolerance of reduced salinities, represents, perhaps, one end of the scale of osmotic independence (Krishnamoorthi,1962). Another Eunicid, Diopatra variabilis, shows osmotic independence a shade better, with greater capacities for volume control and tolerance of reduced salinities over a wider range compared to O. eremita (Krishnamoorthi,1963b). Perhaps, the peak of independence is attained in Marphysa gravelyi, also an eunicid. Another such group may be the Glyceerids, for Glycera spp. have been reported from brackish water situations in her survey of the brackish and fresh water polychaetes (Wesenberg-Lund,1958) and also from the Chilka Lake by Southern (1931). Nephtys spp., may be yet another group (Southern,1931). It is reasonable, therefore, attention is drawn to groups other than the Nereidae, if only to obtain a fuller knowledge of osmoregulation in polychaetes. If the results obtained in the present investigation, revealing that it (osmoregulation) is much better

developed in Marphysa gravelyi and far superior to that obtained in Nereis diversicolor, are of any significance, it is certainly a pointer that such studies are extended to other groups as well.

CHAPTER VI: STRUCTURE OF NEPHRIDIA.

C O N T E N T S.

CHAPTER VI: STRUCTURE OF NEPHRIDIA.

1. Introduction: Although considerable work on the anatomy and histology of the polychaete nephron exists (Goodrich,1945), much of the earlier studies were hardly orientated towards forming a 'better basis for physiological work' as pointed out by Jones (1957). It was not until Jurgens (1935) let in morphological evidence that Nereis diversicolor possessed a nephridium bigger and more convoluted than those possessed by species allied to it; and the importance of nephridia in the osmoregulation of Sabella pavonina was demonstrated (Ewer & Ewer,1943), that its functional implications were brought to the fore-front. The greater loss of chlorides in N. diversicolor (Ellis,1939) and subsequently confirmed by Jørgensen & Dales (1957) as compared to that obtained in Nereis pelagica and Nereis virens, led these authors believe a rapid excretion of isosmotic/hyposmotic urine resulting in a rapid regulation of volume. Examining nephridia in a number of nereids, Krishnan (1952) found that they were larger in Lycastis indica than either in Nereis chilkaensis or Perinereis nuntia. Krishnamoorthi (1963b) extending similar studies to polychaetes other than Nereidae, related the greater penetration of

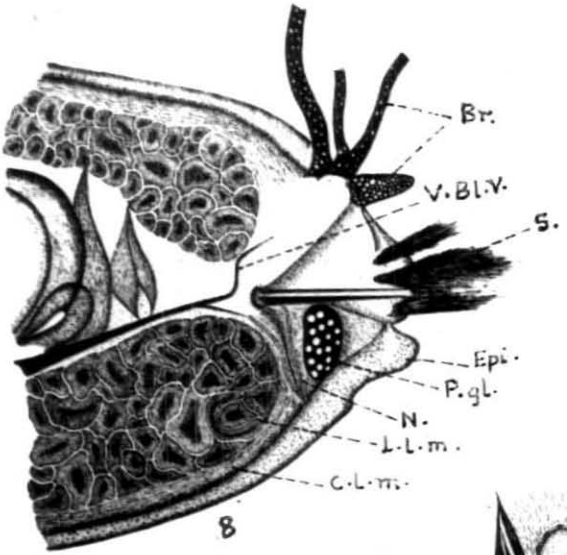
Clymene insecta as being due to the possession of bigger nephridia implying greater efficiency of the kidney. A bigger nephron, therefore, is associated with the production of iso- or hyposmotic urine as observed in a number of annelids (Grobben,1881) and in crustaceans (Schwabe,1933; Panikkar,1941a; Hynes, 1954). In the previous section it was presumed that Marphysa gravelyi Southern may be producing hyposmotic urine. It, therefore, appeared that a study of the structure of the nephridia in M. gravelyi should be helpful.

2. Material and Methods: From a collection of worms collected from Adyar, a few, equal in size with those that formed the experimental animals, were selected and narcotised with Chloral Hydrate or Menthol to ensure an extended condition. Not infrequently gradual addition of 30% alcohol was also found useful in killing the worm in an extended condition. A number of fixatives like Duboscq Bouin, Zenker's Fluid and Susa were tried and discarded in favour of Bouin's Fluid which yielded the best results. The worms were starved a day or two before they were fixed, this being necessary to remove from the gut, fine particles of mud and sand which impede obtaining good sections. The material fixed in Bouin was thoroughly

cleaned until it had only traces of Bouin in it. dehydrated in alcohol series; cleared in cedar-wood oil and embeded in Tissue-Mat (58° C M.P.). Serial sections at eight micra were cut, mounted on Gold-Seal slides and stained with Haematoxylin either Heidenhain's or Ehrilich's, after taking them through the conventional alcohol series. They were counter-stained with Borax Carmine or Orange G. All diagrams were made with the aid of a Camera Lucida. An ordinary ocular micrometer was used for measurements that are recorded here, under constant magnification of 5 x 8.

3. Structure of Nephridia in Marphysa gravelyi:

(a) Previous Work: Nephridia in Eunicidae were first studied by Ehlers (1864) in Eunice harasii, E. rubrocinta, E. limosa, E. siciliensis, Onuphis tubicola and Marphysa sanguinea. However, it was not until 1900, that Goodrich, describing the nephridia of Eunicidae in detail, pointed out the homology of the large trumpet shaped nephrostome with the coelomostome of primitive forms and explained the composite nature of the mixonephridia, combined as they are, to form simple nephridia and coelomoducts. Since then, Fage (1906) has added to our knowledge of the nephridia of Eunicidae by giving details of the form and structure



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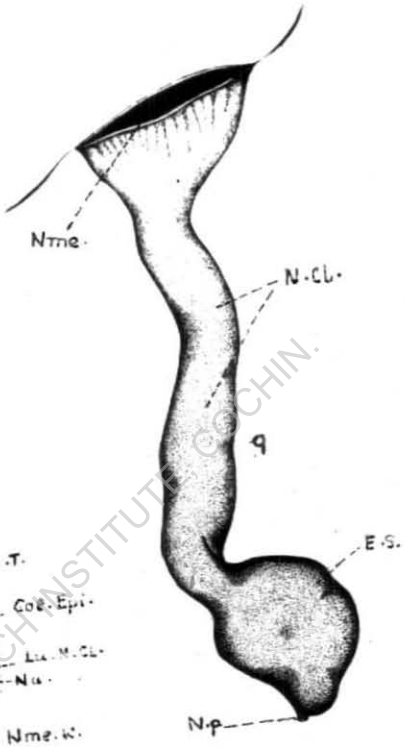

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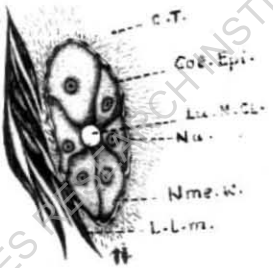

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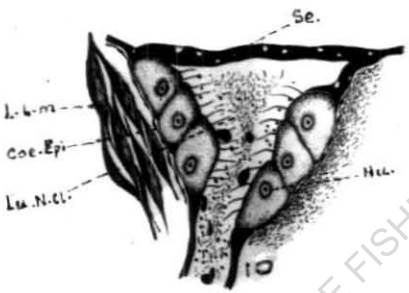

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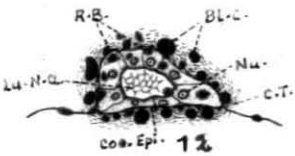

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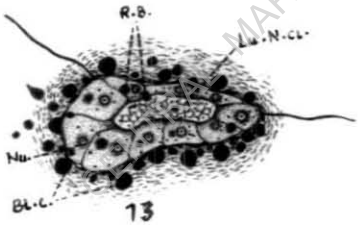

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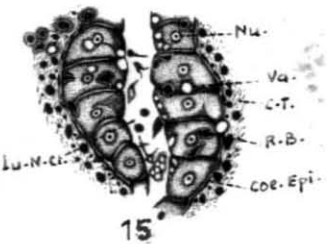

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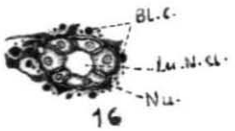

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EXPLANATION TO THE FIGURES

Fig.

8. Diagrammatic cross-section of the worm, Marphysa gravelyi Southern, indicating the relation of nephridium to other structures in the body cavity. X 56.

9. Diagrammatic representation of a single whole nephridium of Marphysa gravelyi. X 400.

10. Longitudinal section of the nephrostome. X 450.

11. Cross-section of the nephrostome. X 450.

12. Cross-section through the nephridial canal. X 450.

13. Cross-section through the middle part of the nephridial canal. X 450.

14. Longitudianl section of the nephridial canal. X 450.

15. Longitudinal section of the end-sac. X 450.

16. Cross-section of the end-sac. X 450.

ABBREVIATIONS USED.

Bl.c: Blind ending capillaries; Br.: Branchiae; Coel.Epi.: Coelomic Epithelium; C.l.m.: Circular layer of muscles; C.T.: Connective Tissue; Con.: Concrements; Ci.: Cilia; E.s.: End sac. Ep.: Epidermis; L.l.m.: Longitudinal layer of muscles; Lu.Nme.: Lumen of the nephrostome; Lu.N.Cl.: Lumen of the nephridial canal; Lu.E.: Lumen of the End sac; N.: Nephridium; Nme.: Nephrostome; Nme.W.: Nephrostomial wall; N.Cl.: Nephridial canal; N.P.: Nephridiopore; Nu.: Nucleus; P.gl.: Parapodial gland; R.B.: Refringent bodies; S.: Setae; Se.: Septum; V.Bl.V.: Ventral blood vessel; Va.: Vacuoles.

in some more genera. He figured the coelomostome or Hyalinoecia tubicola and Lumbriconeries impatians as simple and smooth. But Goodrich (1945) observed deep groves on the ciliated inner surface. Aiyar (1933) while confirming the mixed nature of the nephridmium in his study on the anatomy of M. gravelyi, has failed to describe the histology of the nephridium.

(b) Structure: In Marphysa gravelyi, as in all Eunicidae, a pair of NEPHRIDIA are found in each segment except in a few of the anterior and posterior segments. As can be made out from sections (Fig.8), the nephridium (Fig.9) is located between the lateral border of the ventral longitudinal muscle on the one side and the vental border of the pigment gland on the other. It consists of a wide trumpet shaped nephrostome opening into the coelome along the outer edge of the ventral longitudinal muscle and a long narrow almost straight nephridial canal which runs outwards piercing the circular layer of muscles and the epidermis to open by the nephridiopore situated near the ventral edge of the neuropodium. While the funnel has been observed in Marphysa sanguinea (Cosmovice,1880), there has been no mention of the external opening. Just before opening to the exterior the nephridial canal dilates,

perhaps, comparable to the end-sac of earthworms (Bhal,1947) or of Carcinus maenas and Potamobius fluviatilis (Picken,1936).

The wide, oval mouth of the trumpet shaped NEPHROSTOME, measuring 80 µ, gradually narrows down to 18 µ before it imperceptbly fuses with the nephridial canal. The wall of the nephrostome (Figs. 10 & 11) is made up of a single layer of 3 to 4 similar cubical and well defined cells 10 µ in length. The deeply stained nucleus is invariably at the proximal end of the cells. Only the inner walls of the cells bear numerous cilia measuring about 20 µ in length. The cilia are packed together and are directed towards the nephridial canal. The lumen of the nephrostome is full of concrements, probably, of an excretory nature. The nephrostome externally is lined by the coelomic epithelium and surrounding all around except in the vicinity of the opening of the nephrostome into the coelomic cavity, is the loose connective tissue which is much vacuolated. The cytoplasm of the cells are free of any inclusions. The waste matter present in the coelomic cavity appears to be collected by the nephrostome and pumped out by means of cilia through the nephridial canal as observed in a number of polychaetes by Cunningham (1887).

Immediately following the nephrostome is the NEPRIDIAL CANAL which measures about 120 μ in length and is uniformly 18 μ in width. The lumen of the canal which contains concrements throughout its length is bounded on all sides, forming the walls of the canal, by a single layer of similar cubical cells measuring about 6 μ in length. The cell boundries are well demarkated. The cytoplasm is densely granular. Among the granules large, bright refringent bodies could be distinguished by their taking a deep eosin stain and by their smooth reflecting surfaces. Such bodies are found outside the cells and also in the coelomic cavity (Figs.12, 13 & 14). The presence of such bodies within the cells and outside the cells and the uniform granulation of the cytoplasm suggests that this part of the nephridial canal is, perhaps, concerned with filtration. Racovitza (1895) saw, after injecting sepia-black ink into the coelomic cavity of Leiocephalus leiphygas, masses of black granules deposited in the cells of the nephridial canal and concluded that the particles may have been ingested by the cells of the walls of the nephridial canal. Schneider (1899) confirmed this observation in Arenicola marina, Travisia forbesi, Pectinaria hyperboraea, Terebellides stromii, Polymnia nebulosa

and P. nidensis as well as a number of oligochaetes. Willem & Minne (1900) have also corroborated the same for some more polychaetes. Ingestion of particles by the cells from the coelomic fluid, has been regarded by them as the process of filtration in excretion. In the present form the presence of granules and refringent bodies in the cells, probably, indicate that they have been ingested from the coelomic cavity and, hence, it may be concluded that the cells of the nephridial canal take part in the process of filtration. Spherical and unstained vacant spaces similar to those found in the cells of the nephridial canal in Pectinaria belgica and Terebella conchilega (Willem & Minne,1900; Maziarski,1905) and regarded as water vacuoles concerned in the elimination of water from the coelomic cavity, are also apparent in the cells forming the nephridial canal in M.gravelyi. It is likely that the vacant spaces in the cells of the walls of the nephridial canal in the present form, are vacoules concerned with the function of water excretion. Since such vacuoles are present in all the cells of the nephridial canal, it appears that all the cells are concerned with water elimination. Thus no cells are set apart for water elimination.

From longitudinal and transverse sections (Figs.15 & 16), the walls of the END-SAC could be seen to be made up of a single layer of cells, 5 to 6 in number, cubical and 8 μ in length. As the cells are thick the lumen of this enlarged part of the nephridial canal is as narrow as elsewhere. The cytoplasm is granular, especially at the proximal end of the cells close to the lumen. Refringent bodies and water vacuoles such as those seen in the cells of the nephridial canal are very scarce suggesting that filtration may not be a functional feature of this sac. Coelomic epithelium and connective tissue are, however, present surrounding the end sac, as in the case of the nephridial canal and the nephrostome. In its morphological features and in the absence of cellular concrements, it resembles the end-sac of the earthworms like Pontoscolex corethrurus, Thamnodrilus crassus (Bhal,1942a & b) and Pheretima posthuma (Bhal,1919). This sack in these earthworms aids in the reabsorption of water or of water and other dissolved substances that pass down the nephridial canal (Bhal,1947). The end-sac of Carcinus maenas and Potamobius fluviatilis have also been ascribed similar function (Picken,1936). It is, therefore, probable that the end-sac in the present form aids in reabsorption of the water and dissolved solutes.

(c) Blood Supply: The nephridium, as in all Eunicids, in M. gravelyi is supplied with blood by a branch of the ventral blood vessel, resembling in many respects that obtained in Eunice sp. (Goodrich,1900). The main branch of the ventral vessel supplies the parapodia as well as the branchia. But before it proceeds to the parapodia and the branchia proper it gives off a subsidiary branch to the nephridium which breaks into capillaries on the nephridial body and is brought back to the general circulation of the blood from the epidermis (Fig.8). Several of the the capillaries end blind in the form of dilations within the nephridia as in Marphysa sanguinea (Fuchs,1907). Such blind ending capillaries, known as ampullae, are of common occurrence in oligochaetes (Stephensen,1930) and have also been observed in Arenicola sp. (Benham,1891) and Lanice conchilega (Meyer,1888). Although Ewer (1941) noted them in Travesia forbesii, he concluded that their possible function must remain a matter of conjecture. However, considering their intimate 'juxtaposition' with the nephridial body (Krishnan,1952), the number of such ampullae could be a conveniant measure of not only the metabolic activity but the nature of activity of the nephridium. With this object in view, serial

sections obtained from a number of polychaetes were examined and the number of blind ending capillaries noted (Table XIII) similar to previous studies,

TABLE XIII.

S. No.	Number of Blind Ending Capillaries in Specimen						
	1	2	3	4	5	6	M e a n.
1	70	72	72	70	72	72	71.33
2	66	74	70	68	72	76	71.00
3	73	70	68	68	74	72	70.83
4	70	70	71	75	69	72	71.17
5	72	72	70	71	70	72	71.17
6	68	74	75	72	72	70	71.87
						Mean:	71.23
						S.d.	2.22
						S.e.	±0.37

(Krishnamoorthi,1963a). The number of blind ending capillaries in M. gravelyi ranged from 66 to 75 with a mean of 71.23 ± 0.37.

(d) Excretory Surface relative to the Size of the worm:

A study of the structure of the nephridium in M. gravelyi, has revealed that excepting the nephrostome, the entire nephridium takes part in the process of excretion.

TABLE XIV.

S. No.	Length of the worm in mm.	No. of Segments.	Length of each cell of the middle part of the nephridial canal in μ.	Length of each cell of the end-sac in μ.	No. of cells in the middle part.	No. of cells in the end-sac.	Length of the middle part in μ (c x e).	Length of the end-sac in μ (d x f).	Total length of the middle part in μ (g x b x 2).	Total length of the end-sac in μ (h x b x 2).	Sum total of the Excretory surface in μ (i + j).	Ratio of the Excretory surface to the length of the worm.
	a	b	c	d	e	f	g	h	i	j	k	l
1	195	411	6	8	13	5	78	40	64116	32880	96996	0.497
2	179	253	6	8	18	5	108	40	54648	20240	74888	0.418
3	250	397	6	8	13	6	78	48	61932	38112	100044	0.401
4	229	475	6	8	13	5	78	40	75100	38000	113100	0.494
5	249	470	6	8	15	6	90	48	84600	45120	129720	0.521
6	209	431	6	8	15	5	90	40	77580	34480	112060	0.536
	219										104301	0.478 - Mean. 0.156 - S.d. ±0.061 - S.e.

Table XIV. Showing the ratio of the excretory surface to the length of the worm.

The nephrostome, perhaps, aids in the collection of the nitrogenous waste matter to be extruded out by the nephridiopore through the nephridial canal. The length of the canal implying the number of cells may, therefore, be taken as an index of the excretory capacity of the nephridium of any animal. Although the length, breadth and the height of the nephridial body may give a comparative picture (Krishnan,1952), it is not strictly accurate to take into account those sections of the nephridium which do not participate in the different renal processes of excretion. In order to arrive at some value likely to be constant and comparable for different genera of polychaetes, the ratio between the length of the nephridial canal to the length of the worm was determined in M. gravelyi and it can be seen that (Table XIV) M. gravelyi had a mean ratio of 1 : 0.478 ± 0.061 and ranged from 1 : 0.401 to 1 : 0.536 in the six worms studied.

4. Remarks: In Marphysa gravelyi Southern, the nephridium is simple in form and structure, similar to that obtained either in Onuphis eremita (Krishnamoorthi, 1963a) or Diopatra variabilis (unpublished data) both belonging to the Eunicidae. In all the three species the nephridium belonging to the mixonephridial type

(Goodrich,1945), consists of a wide funnel, the nephrostome and a long, uncoiled narrow canal, the nephridial canal, which opens out by the nephridiopore at the base of the neuropodium. Its topography in relation to other organ systems, is much the same in all the three forms. While the nephrostome situated closely along the septum dividing two consequetive segments, opens into that segment previous to the one that contains the nephridial body; the nephridiopore gains its exit in the same segment as that holds the nephridial body. The disposition of the connective tissue and the coelomic epithelium around the nephridia in M. gravelyi is much similar to that observed in O. eremita and D. variabilis. However, the nephridia in M. gravelyi differ from those of the others in the possession of an end-sac and resembles in this respect the nephridia of Oligochaetes (Bhal,1942,'45) and many crustaceans (Picken,1936; Panikkar,1941a). Although the presence of refringent bodies and vacuoles in the cells of the nephridial canal point fairly to its function as the site of filtration, in the absence of any such concrements and the cytoplasm remaining uniformly granular in the cells forming the end-sac, it is difficult to attribute precise a function to the end-sac in the renal processes of

excretion. Bhal (1942,'45) assigns a function of reabsorption to the end-sac in the Oligochaetes he studied. Whether a similar function could be attributed to the end-sac in M. gravelyi, a study of the nephridial physiology alone could reveal. But taking into consideration the structural similarities, it may be possible to assign a similar function, namely, reabsorption to the end-sac in M. gravelyi Southern. Since it is seen that M. gravelyi is a hyporegulator (vide infra) the importance of reabsorption of salts and water that might otherwise be lost, needs no further emphasis and, perhaps, the possession of an end-sac is an adaptational response.

Based on circumstantial evidence, Beadle (1937) presumed that hyposmotic urine was being formed in N. diversicolor. Examining the nephridia of N. diversicolor and P. cultrifera, Jurgens (1935) associated the formation of hyposmotic urine with the long, coiled nephridial canal obtained in N. diversicolor and which provided ample epithelium for reabsorption, whereas it was a simple sac in P. clutrifera. With the positive demonstration of the function of nephridia in the osmoregulation of Sabella pavonina (Ewer & Ewer, 1943), its importance gained additional strength.

Krishnan (1952) without providing experimental evidence, related the size of the nephridia in Lycastis indica, Nereis chilkaensis and Perinereis nuntia to the habitat. Krishnamoorthi (1962,'63a) provided both experimental and structural evidences to account for the penetration and distribution of a number of polychaetes in the Adyar estuary. In M. gravelyi also there is circumstantial evidence that hyposmotic urine is being formed (Chapter IV). If association of a bigger nephridium with the production of hyposmotic urine is accepted and Jørgensen & Dales (1957) suspect that it might well be, it is seen (Table XV) that M. gravelyi Southern possesses a nephridium which has a ratio between the excretory surface and the length of the worm, considerably higher than that obtained either in O. eremita, L. medusa and C. insecta (Krishnamoorthi,1963a) or D. variabilis (unpublished data). While the ratio in M. gravelyi was 1 : 0.478, the respective ratios in O. eremita, L. medusa, C. insecta and D. variabilis were viz., 1 : 0.247; 1 : 0.220; 1 : 0.346; and 1 : 0.357. Now, applying the 'student's t' test for the significance of the difference of two sample means, the 't' value between the mean ratios of

TABLE XV.

S.No.	Ratio of the excretory surface to the length of the worm in				
	O. eremita*	L. medusa*	C. insecta*	D. variabilis#	M. gravelyi
1	0.270	0.224	0.355	0.355	0.497
2	0.236	0.213	0.335	0.368	0.418
3	0.254	0.227	0.342	0.330	0.401
4	0.233	0.210	0.348	0.354	0.494
5	0.240	0.224	0.352	0.377	0.521
6	0.246	0.224	0.342	0.359	0.536
Mean:	0.247	0.220	0.346	0.357	0.478
S.d.:	0.011	0.007	0.023	0.050	0.156
S.e.:	±0.004	±0.003	±0.001	±0.020	±0.061

* taken from Krishnamoorthi, 1963a. #unpublished data.

M. gravelyi and D. variabilis was 0.653 and the corresponding probability, read from Table III of Fisher & Yates (1948) for 10 degrees of freedom was at the 50% level. Similar values of 't' between M. gravelyi and C. insecta; between M. gravelyi and O. eremita; and M. gravelyi and L. medusa respectively were 0.765, 1.384 and 1.568 with the corresponding probabilities for 10 degrees of freedom at 60%, 80% and 90%. The conclusion is, therefore, irresistible that

M. gravelyi Southern owes its better powers of osmoconcentration and volume regulation to the bigger nephridia that it possesses. An examination of Table XV also reveals that the ratios of *C. insecta* and *D. variabilis* are not significantly different. In the Adyar estuary, *C. insecta* and *D. variabilis* occur together with *M. gravelyi* at Station D (Krishnamoorthi, 1963d). The range of distribution of these two species viewed in this light is, therefore, self explanatory, as well as the obvious advantage of possessing a bigger nephridia which alone could account for the wide distribution of *M. gravelyi* Southern in the Adyar estuary and the osmotic independence it has gained thereof.

CHAPTER VII: IONIC REGULATION.

C O N T E N T S.

CHAPTER VII: IONIC REGULATION.

1. Introduction. There seems to be little doubt that osmotic regulation of body fluids in invertebrates is secondary to ionic regulation (Pantin,1931) as demonstrated by a series of studies on crustaceans (Robertson, 1939,'49,'53,'57,'60a; Webb,1940; Parry,1954; Shaw,1955a &b, '60c; Bryan,1960a,b & c). Although considerable work on osmotic regulation in polychaetes exists, paucity of similar studies on ionic regulation stands out by contrast. Since isolated muscle preparations of Nereis diversicolor exhibited sustained activity in media as dilute as the least saline water (Wells & Ledingham,1940), its powers of osmotic regulation appear to be of doubtful survival value as rightly pointed out by Beadle (1957). Analysing the inorganic constituents of the body fluids of Arenicola sp., Amphitrite sp., and Aphrodite sp., Bethe & Burger (1931) and Bialaszewicz (1933) indicated an accumulation of potassium in the worms without explaining the inherent implications. Cole (1940) examined a few more forms and found that the purely marine polychaetes, Amphitrite brunnea and Glycera dibranchiata, had considerable powers of ionic regulation. When Arenicola marina which has its body fluid isosmotic with the external medium of strength of 44% sea water (Schlieper,1929a),

were exposed to dilutions, equilibrium with the surrounding medium was soon established except in the case of potassium and sulphate (Robertson,1949). He presumed that it may be a consequence of the selective activity of the nephromixia and the control of the absorption of these ions by the body wall. A study, therefore, of ionic regulation could throw more light on understanding the osmotic independence gained by an animal over media of unequal and changing osmotic stresses. It is also possible that other compensatory mechanism for osmotic adjustment, may come into play as demonstrated in Sialis lutaria (Beadle & Shaw,1950); larvae of Aedes aegypti (Wigglesworth,1938); in Phascolosma gouldii and Amphitrite ornata (Wilber,1948; Wilber & MacDonald,1950) and in Perinereis cultrifera and N. diversicolor (Jeuniaux et al,1961; Duchateau-Bosson & Florkin,1961). Evidences from the previous sections and the fact that anterior bits of Marphysa gravelyi maintained a sustained activity over a wide range of dilute media (Krishnamoorthi & Krishnaswamy,1963), emphasised the need for a study of ionic regulation as well as the regulation of organic solutes in the body fluid. That the chlorides are regulated by M. gravelyi has already been briefly reported (Krishnamoorthi,1963c).

2. Material and Methods: The experimental procedure was similar to that detailed earlier. The body fluids were drawn with the help of pipettes fabricated from glass tubing (Pyrex) at the laboratory. The chlorides were estimated by the method of Sendroy (1937) as modified by Robertson & Webb (1939). In principle, the insoluable silver iodate reacts with the chlorides present to form insoluable silver chloride and soluable iodate. From this iodine is liberated and titrated with Sodium Thiosulphate. The Volhard's method of titration was discarded in favour of this method since the end point being sharp, could be easily detected. A sample of 0.1 ml of body fluid made up to 1 ml by the addition of glass-distilled water, was transferred into a 100 ml standard flask and was further made up to 60ml. Into this solution 15 ml of Phosphoric acid of 23% strength was drawn from a burette. To the solution enough silver iodate was added such that an excess of silver iodate was always present when shaken well. In practise 20 to 30 mg of silver iodate was found quite suitable to bring about the desired reaction. When froth was present, a drop or two of caprylic alcohol (Octonal) as suggested by Robertson & Webb (1939), controlled its formation. Since polychaete body fluids contain negligible quantities of protein in their body

fluids (Robertson & Webb,1939), the need for adding caprylic alcohol arose only rarely. After shaking the solution thoroughly for about 10 minutes, it was made upto 100 ml and filtered with Whatman Filter Paper No.2. To 10 ml of filtered solution a few drops of 1% Potassium Iodide solution, to liberate the iodine, was added and titrated with 0.5N Sodium Thiosulphate, with a few drops of 1% starch solution as the indicator. From the titre value, the amount of chlorides present was calculated using the formula: Cl = a x N x 29.67, where 'a' is the titre value and 'N' the normality of the Sodium Thiosulphate solution used. The chloride values so obtained are in mg per ml. Blanks were run. The Sodium Thiosulphate solution was standardised as suggested by (Hawk et al,1954). All reagents used were of Analar grade.

Sodium and Potassium were determined on a Zeiss Flame Photo-meter. Since preliminary estimations had shown that a minimum dilution of body fluids to 200 times was necessary to read it on the scale of the Flame Photo-meter all determinations were made on samples (0.05 ml) diluted to 200 times. Standard graphs prepared from estimations on solutions of Sodium and Potassium of known concentrations and similarly diluted to 200 times, formed the basis to arrive at the concentrations of Sodium and Potassium in the unknown samples of body fluids. Blanks were run.

Total Free Amino Acids were determined by the colorimetric method of Harding & MacLean (1916) in preference to the more elaborate method of Troll & Cannan (1953), since it had the additional advantage of being both quick and less cumbersome. Speaking of this method, Block & Weiss (1956) comment (p.29) "this excellent method has been largely forgotten". The method is as follows: to 0.1 ml of body fluid made upto 1 ml by adding glass-distilled water, 1 ml of 10% aqueous Pyridine and 1 ml of 2% aqueous Ninhydrine are added. The Pyrex test tube containing this solution is plugged with cotton and placed in boiling water bath for 20 minutes, when the solution develops an amber colour which stays stable for about 12 hrs. Then it is cooled under running water. The colour intensity which is dependent upon the amount of free amino acids present, is read off on a UNICAM (S,P.600) Spectrophotometer at 565 μ. Standard graphs were prepared with Leucine as suggested by Green & Stahmann (1955) and Cowgill & Pardee (1957). Blanks were run.

3.1 Regulation of Chlorides: Results: The results of a series of experiments to understand the extent of regulation of chlorides when M. gravelyi is exposed to experimental media of varying concentrations are summarised in Table XVI and represented in Fig.17. The mean chloride

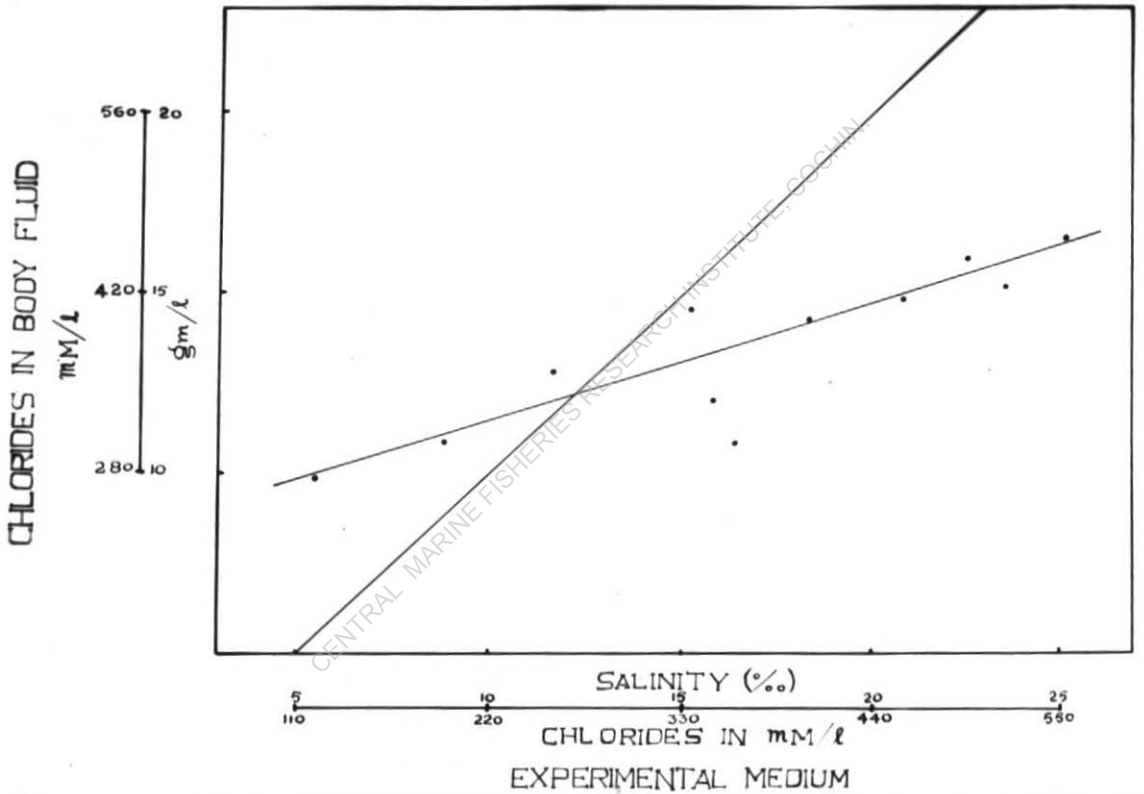

Fig.17. To indicate the relation between the body fluid Chlorides (gm/l) and the external media of varying salinities. Temp.: 27.5 ± 0.5°C. Each point is the mean of 3 to 12 estimations. The line running diagonal is the line of isosmocity.

TABLE XVI

Experimental Medium.		Chloride Values of Body Fluid after 24 hours of exposure to Experimental Medium																	No. of Estimations.
‰	Chlorides in mM/l	Titre values in CC.												Chloride values in					
		1	2	3	4	5	6	7	8	9	10	11	12	Mean	mM/l	gm/l	s.d.	s.e.	
5.68	137*	0.60	0.65	0.60										0.65	272	9.64	0.011	±0.005	3
8.52	197*	0.70	0.70	0.75										0.72	301	10.68	0.029	±0.016	3
10.60	256*	0.85	0.85	0.80	0.90	0.85	0.90	0.85	0.90					0.86	358	12.76	0.011	±0.004	8
14.60	333*	0.95	0.90	1.00	1.00	0.95	0.95	1.00	1.00					0.97	406	14.39	0.014	±0.005	8
15.30	348*	0.75	0.75	0.75	0.80	0.80	0.80	0.75	0.85	0.80	0.85	0.85	0.80	0.80	335	11.87	0.014	±0.004	12
16.20	358*	0.70	0.70	0.70	0.65	0.75	0.75	0.80	0.80					0.73	305	10.83	0.017	±0.006	8
18.40	399*	0.95	0.95	0.95	0.95	0.95								0.95	397	14.09	-	-	5
19.50	456*	1.10	1.05	1.15	1.10	0.90	0.90	0.90	0.90					1.00	418	14.84	0.035	±0.012	8
21.60	493*	1.10	1.05	1.05	1.05	1.05	1.00	1.05	1.05	1.05	1.00	1.05	1.00	1.04	435	15.42	0.009	±0.003	12
23.00	517*	0.95	1.00	1.00	0.95	0.95	0.90	0.95	0.90					0.95	397	14.09	0.012	±0.003	8
29.52	554*	1.15	1.10	1.15	1.15	1.15	1.15	1.20	1.15	1.05	1.00	1.00	1.05	1.11	464	16.46	0.021	±0.006	12

Table XVI. Body fluid chlorides in gm/l and mM/l, after exposure over a period of 24 hours to heterosmotic media. Temp.: 27.5 + 0.5°C.
*Calculated from Barnes, H J. exp. Biol. 31: 582-588, (1954).

values ranged from 272 mM/l (9.64 gm/l) to 464 mM/l (16.46 gm/l) in animals exposed to media of strengths ranging from 5.68‰ to 25.92‰ whose chloride values were respectively 137 mM/l and 554 mM/l. The body fluid chlorides are maintained at higher levels of 272 mM/l; 301 mM/l and 358 mM/l in the respective external concentrations of 137 mM/l; 197 mM/l and 256 mM/l (Fig.7). Although it is still at a high level of 406 mM/l, in a medium of 333 mM/l, it represents rather a large deviation from expected value as could be seen clearly from the Fig.17. In the rest of the dilutions from 15.30‰ (348 mM/l) to 25.93‰ (554 mM/l), the body fluid chloride values ranged from 335 mM/l to 464 mM/l respectively. The very low body fluid chloride value of 305 mM/l in a medium of 16.20‰ (368 mM/l) similarly is a large departure. These two departures may have been due to the physiological condition of the worms, although they were aparently well looking when chosen for experimentation, for Chi-square tests indicate a probability <1% and, therefore, could not be mistakes due to experimental procedure. Excepting for these two departures, that the chlorides are being maintained at a steady level irrespective of the external concentration is also clearly aparent from the results presented. In other words, in hyposmotic media the chlorides of the body fluid are held high, while in hyperosmotic media they are low.

3.2 Regulation of Sodium: Results: The Sodium content of body fluids of worms exposed to 5 different experimental media viz., 9.60‰, 11.04‰, 16.10‰, 25.76‰ and 27.36‰, ranged from 90 mM/l (16.10‰) to 285 mM/l (27.36‰), as is evident from Tables XVII and XX, and Fig.18. Sodium content of the external media ranged from 142.5 mM/l to 362.5 mM/l (Tables XVIII & XX, Fig.18). The two low values of 90 mM/l and 135 mM/l obtained from body fluids of worms subjected to the stresses of external media of 16.10‰ and 11.04‰, may be attributed to the physiological condition of the animal prior to its exposure to experimental media (probability <1%). However, that there is an increase in the sodium content with the increase in the concentration of the external medium is evident. Also, except in the lowest dilution when the sodium content of both the body fluids and the external medium are more or less similar, the sodium content of the body fluids in the rest of the experimental media are less than that of the external medium. This further supports the view that there is a regulation of Na ions.

3.3 Regulation of Potassium: Results: The potassium content in the body fluids of worms subjected to similar dilutions as in the previous experiment ranged from 18 mM/l (16.10‰) to 36 mM/l (27.36‰). The low value of 18 mM/l

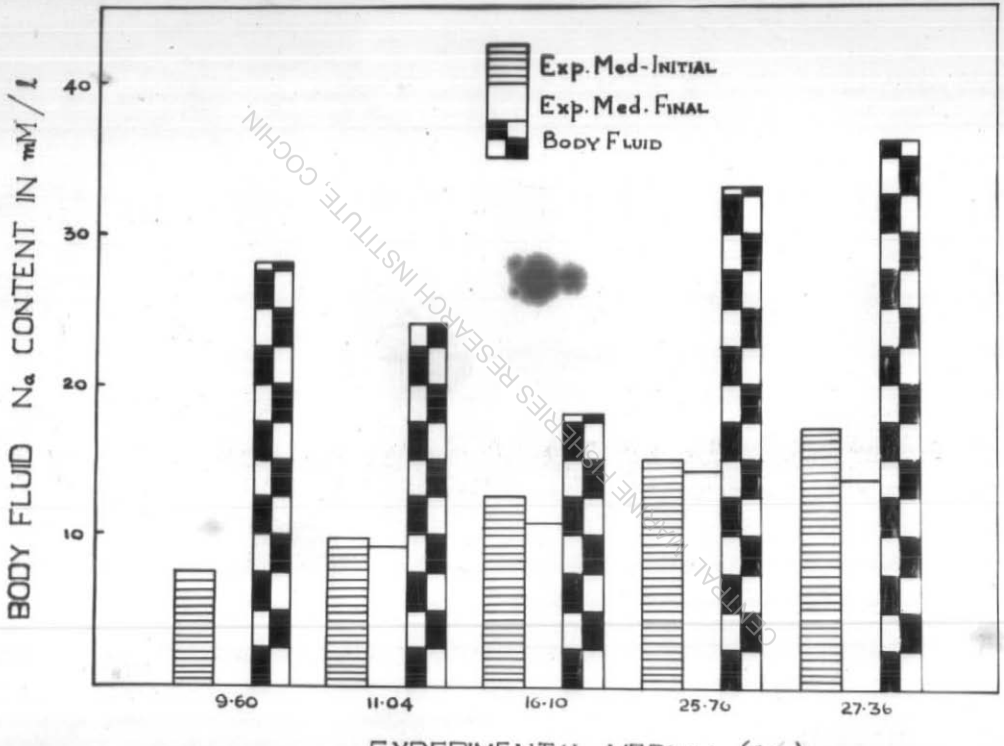

Fig.18. Histogram to indicate the Sodium levels (mM/l) of the body fluids in five differing salinities. Temp.: 28.0 ± 0.5°C. Exp. Med.: Experimental medium.

Experimental Medium.		BODY FLUID - Flame Photo Meter Reading after 24 hours exposure to Experimental Medium																				
		Na Content of Sample											K Content of Sample									
%	‰	1	2	3	4	5	6	7	8	9	Mean	s.d.	S.e	mM/l	1	2	3	4	5	6	7	8	9	Mean	S.d.	S.e.	mM/l
25	9.60	50	55	42	49	47	49	44			48.00	4.30	±1.62	150	100	140	93	116	86	64	67			95.07	26.83	±10.12	28
30	11.04	46	45	44							45.00	1.00	±0.58	138	90	80								85.00	7.07	± 5.00	24
50	16.10	37	30	32	33	33	32	31	35	34	33.33	2.65	±0.88	90*	65	62	60	61	66	66	61	69	69	64.33	3.46	± 1.15	18*
70	25.76	47	43	50	52	67					51.80	9.38	±4.20	163	87	102	101	130	142					112.40	22.73	±10.00	33
80	27.36	71	81	86	74	98	84				82.33	9.60	±3.92	285	83	145	133	98	123	144				120.83	25.34	±11.31	36

Table XVII. To indicate the Na and K content (mM/l) of body fluids which subjected to media of differing osmotic stress over a period of 24 hours. Tem: 28.0 ±0.5° C. *The two values are a departure from the expected values. But the probablity (Chi-square tests) being <1% they may, perhaps, be attributed to the physiological condition of the animals prior to experimentation. Dilution: 1/200

TABLE XVIII

Strength.		Experimental Medium - Flame Photo meter Reading /mM/l.																							
		Initial Na Content of Sample														Final Na Content of Sample									
%	‰	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e.	mM/l	1	2	3	4	5	6	7	8	9	Mean	s.d.	s.e.	mM/l
25	9.60	35	41	41	38	39	52	44	62	58	65	47.40	10.98	±3.44	142.5	-	-	-	-	-	-	-	-	-	-	-	-	-
30	11.04	125	68	65	67	60	50	56				70.14	25.03	±9.45	240.0	76	59	65	58	69	61	67	61	71	65.22	6.06	±2.02	215.0
50	16.10	55	68	66	82	82	72	70	70			70.64	8.73	±3.09	242.5	78	85	78	46	58	74	72			70.14	12.50	±5.10	237.5
70	25.76	146	110	108	86	110	96	102				108.29	17.47	±7.10	390.0	95	92	100	125	92					100.18	13.90	±6.22	360.0
80	27.36	123	100	83	85	100	90	97	96	94		96.33	11.25	±3.75	342.5	100	98	98	105	111					100.67	6.31	±2.58	362.5

Table XVIII. To indicate the Na content (mM/l) of the experimental media of differing salinities both before and after the experiment.
Temp: 28.0 ±0.5°C. Dilution: 1/200.

may be due to reasons offered for the low values of sodium in the previous experiment, since the same body fluid formed the basis for its estimation. As in the previous experiment, increase in the potassium content of the body fluids with increasing concentrations of the external media, is similar to that observed for sodium (Tables XVII, XIX & XX, Fig.19). However, unlike sodium, the potassium content of the body fluids was always higher than those of the experimental media (Table XX, Fig.19).

3.4 Regulation of Total free Amino Acids: Results:

The total free amino acid content of the body fluid of worms exposed to experimental media of salinity varying from 6.7‰ to 29.3‰, ranged from 10 µg/ml to 32 µg/ml obtained in the respective dilutions of 6.7‰ and 21.6‰. A progressive increase in the amino acid content could be seen (Table XXI, Fig.20), until a dilution of 21.6‰ is reached. In dilutions beyond 21.6‰, a fall was noticed and, perhaps, the mechanism or mechanisms responsible for the increase in the lower dilutions, breaks down and hence, a down-ward trend. In other words, the increase in the amino acid content of the body fluids upto an experimental medium of 21.6‰, is a function of the osmotic stresses of the media imposed.

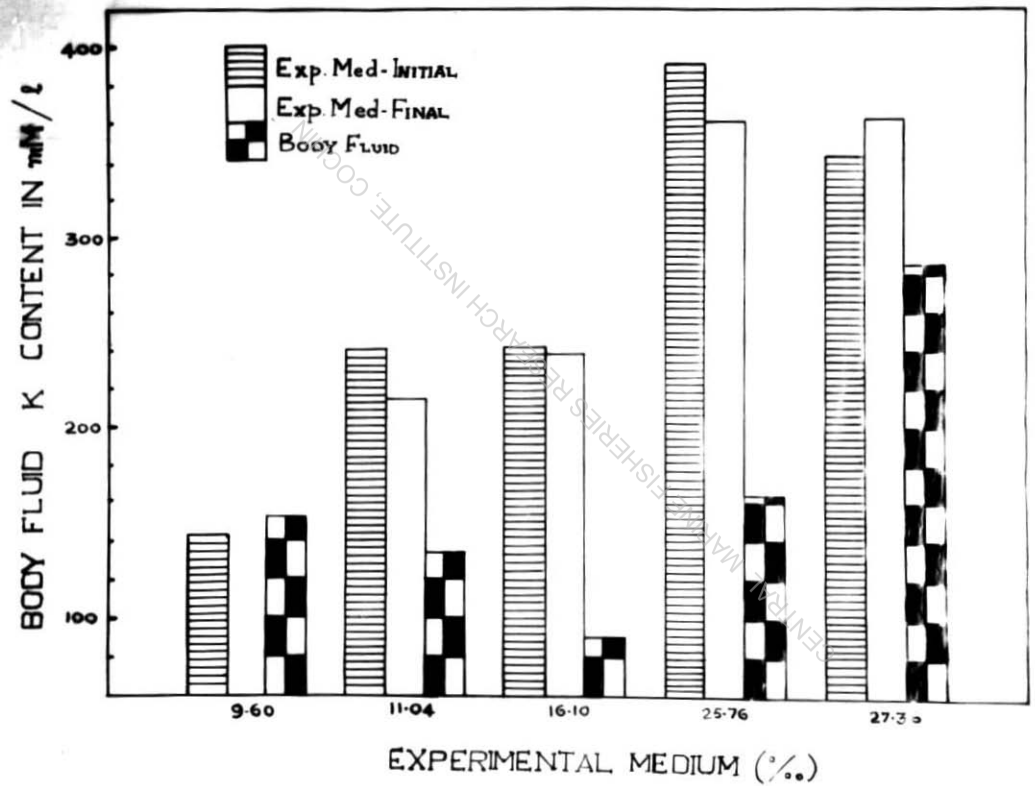
Exp. Med-Initial
Exp. Med-Final
Body Fluid
BODY FLUID K CONTENT IN mM/l
400
300
200
100
9·60
11·04
16·10
25·76
27·3
EXPERIMENTAL MEDIUM (‰)

Fig.19. Histogram to indicate the Potassium levels (mM/l) of the body fluids in five differing salinities. Temp.: 28.0 ± 0.5°C. Exp. Med.: Experimental medium.

TABLE XIX

Strength.		Experimental Medium - Flame Photo-meter Reading /mM/l.																					
		Initial K Content of Sample														Final K Content of Sample									
‰	‰	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e.	mM/l	1	2	3	4	5	6	7	8	Mean	s.d.	s.e.	mM/l
25	9.60	29	37	36	26	32	30	22	21	25		29.83	5.46	±1.64	7.50	-	-	-	-	-	-	-	-	-	-	-	-
30	11.04	48	31	39	32	36	34	45	36	38		37.66	5.24	±1.58	9.75	48	37	40	35	31	34	34		36.71	5.70	±2.20	9.25
50	16.10	48	43	53	44	47	47	52				47.29	3.97	±2.08	12.50	36	49	39	39	47	48	36	38	41.38	5.98	±2.11	10.75
70	25.76	43	44	54	80	59						56.00	15.00	±6.70	15.00	67	60	50	38	53	50	51		52.43	9.90	±3.78	14.25
80	27.36	98	76	56	52	51	54	55	53	59		61.33	15.60	±5.20	17.50	45	57	58	43	42	50	66		51.57	9.04	±3.42	13.75

Table XIX. To indicate the K Content (mM/l) of the experimental media of differing salinities both before and after the experiment.
Temp: 28.0 ±0.5° C. Dilution: 1/200.

TABLE XX

Flame Photo-meter Reading /mM/1.											
EXPERIMENTAL MEDIUM.								BODY FLUID.			
Strength.	Initial Na Content		Final Na Content		Initial K Content		Final K Content		Final Na Content		Final K Content	
‰	‰	Mean	mM/1	Mean	mM/1	Mean	mM/1	Mean	mM/1	Mean	mM/1	Mean	mM/1
25	9.60	47.40	142.5	-	-	29.83	7.50	-	-	48.00	150.0	95.07	28.0
30	11.04	70.14	240.0	65.22	215.0	37.66	9.75	36.71	9.25	45.00	135.0	85.00	24.0
50	16.10	70.64	242.5	70.14	237.5	47.29	12.50	41.38	10.75	33.33	90.0*	64.33	18.0*
70	25.76	108.29	390.0	100.18	360.0	56.00	15.00	52.43	14.25	51.80	163.0	112.40	33.0
80.	27.36	96.33	342.5	100.67	362.5	61.33	17.00	51.57	13.75	82.33	285.0	120.83	36.0

Table XX. Consolidated statement to show the relation between the Na and K Contents (mM/1) of the body fluids and experimental media of differing salinities. Temp: 28.0 ±0.5°C *For explanation of the low values, see caption to Table XVII. and also the text. Dilution: 1/200.

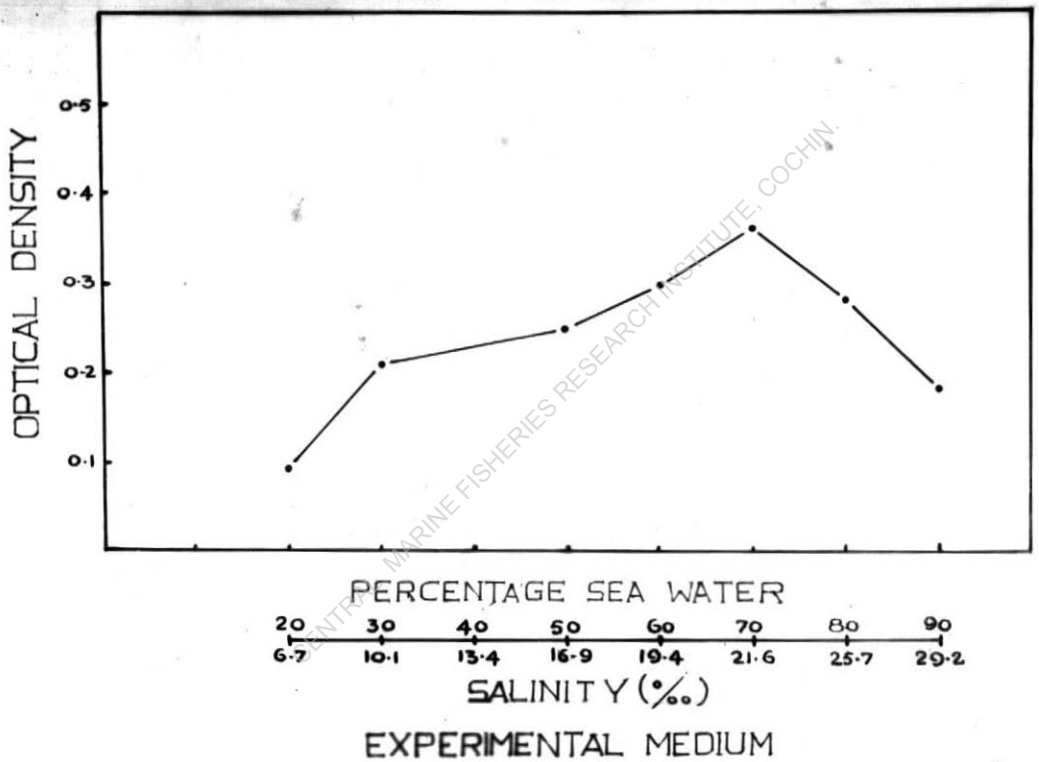

Fig.20. Showing the relation between the Total Free Amino Acid levels (µg/cc) in the body fluids and the concentration of the experimental media. Temp.: 29.5 ± 0.5°C. Each point is the mean of 5 to 10 estimations.

TABLE XXI

Experimental Medium		Optical Density at 565 /u in Sample													Total Free amino acids in /ug/cc.
%	%.	1	2	3	4	5	6	7	8	9	10	Mean	s.d.	s.e.	
20	6.7	0.065	0.050	0.116	0.095	0.091	0.080					0.083	0.073	±0.030	10
30	10.1	0.117	0.174	0.170	0.216	0.216						0.206	0.163	±0.094	18
50	15.9	0.245	0.400	0.270	0.200	0.335	0.227	0.295	0.150	0.220	0.180	0.252	0.238	±0.070	25
60	19.4	0.328	0.297	0.310	0.270	0.297	0.310					0.302	0.061	±0.024	26
70	21.6	0.390	0.355	0.305	0.315	0.285	0.325	0.365	0.400	0.440	0.450	0.363	0.181	±0.057	32
80	25.7	0.310	0.212	0.293	0.265	0.360						0.288	0.173	±0.077	27
90	29.2	0.115	0.187	0.210	0.230	0.127	0.195					0.177	0.146	±0.056	15

Table XXI. To show the total free amino acids levels (/ug/cc) in the body fluids of worms exposed to media of differing osmotic stresses. Temp. 29.5 ± 0.5°C Dilution: 1/1000

4. Remarks: The only polychaete in which chloride regulation has been extensively studied is Nereis diversicolor collected from Millport, Tvarminne, Isefjord and the Upper Tamer Estuary (Smith,1955c). Schlieper (1929a) while giving the depression in the freezing point of N. diversicolor from Kiel, has failed to give the chloride content of its body fluid over its range of tolerance of the external medium. However, it could be calculated. The chloride content of N. diversicolor, thus, ranges during its regulatory phase, from 4.80 gm/l to 19.15 gm/l at Millport; 3.35 to 9.50 gm/l at Tvarminne; 3.59 to 8.94 gm/l at Isefjord; 3.38 to 9.40 gm/l at the Upper Tamar Estuary and from 7.10 to 18.20 gm/l at Kiel. In other words the overall range of chloride content in the body fluid of N. diversicolor varies from 3.35 to 19.15 gm/l over its entire geographical distribution. Nereis limnicola another nereid polychaete, also has a chloride content ranging from 4.0 to 10.20 gm/l (Smith,1959, from Fig.12). Since N. diversicolor is a hyperregulator, a comparison of chloride values obtained in N. diversicolor with that obtained in M. gravelyi, being a hyporegulator and an Eunicid, is strictly not tenable. However, the chloride values obtained in M. gravelyi viz., 9.64 to 16.46 gm/l, are rather high and are close to chloride

values obtained for N. diversicolor from Kiel (Schlieper, 1929). Since in N. diversicolor, regulation is restricted over the lower range of dilutions, the lower chloride values are as they should be and understandable. Perhaps the high chloride values obtained in M. gravelyi is a necessary consequence of it being a hyporegulator.

Smith (1955c) without making parallel determinations of the depression in the freezing point and the corresponding chloride content of the body fluid during its (N. diversicolor) regulatory phase, suspected that the chloride concentration may not parallel the osmotic pressure of the coelomic fluid, especially at low salinities. Comparing the osmotic pressure of the body fluids obtained in M. gravelyi (Chapter IV) with the chloride values, the conclusion that they parallel each other is irresistable.

The need for studies on ionic regulation in animals attempting to invade regions other than they are normally accustomed to, largely remained in the background, although its impàrtance was realised. An attempt in this direction was made very early by Schlieper (1929) in Arenicola marina and later by Bethe & Berger (1931) and Bialaszewicz (1933) in Arenicola sp., Amphitrite sp,, and Aphrodite sp., and was closely folled by Cole (1940) in some more genera of

polychaetes. However, they hardly forged much, apart from providing a catalogue of the inorganic constituents of the body fluids of the animals. It is to Webb (1940) who in a thought provoking paper on Carcinus maenas, goes the credit of giving an impetus to this aspect. In a series of papers Robertson (1949,'53,'57,'60a) brought to light not only the existence of ionic regulation among lower invertebrates but also interspecific differences. The results presented here, strictly are not comparable with those obtained by Robertson (1949,'53), for the methods followed differ with each other. While Robertson (1949,'53) estimated the inorganic constitutents of the body fluids both before and after dialysis, in the present investigation chemical analyses of the body fluids obtained as such after exposure to experimental media, were made without dialysis. However, it was seen that all the ions studied viz., Chlorides, Sodium and Potassium, increased with increasing concentration of the external medium. The accumulation of potassium needs no further explaining, since it is of common occurrence (Robertson,1949,'53; Parry,1953,'54). Whether the increase of sodium and chloride is also a case of accumalation, perhaps, dialysing experiments as suggested by Robertson (1949) may provide the answer. However, that in M. gravelyi, regulation is extended to all ions should be admitted, if the ratios are

considered (Table XXII). Although the Cl values

TABLE XXII.

Expt., Medium. ‰	Final Concentration (mM/l) in Body Fluid.						
	Values.			Ratios.			
	Cl.	Na.	K.	Na:Cl.	K:Cl.	K:Na.	(Na+Cl):(K+Cl)
9.60	301 (197)	150 (142.5)	28 (7.50)	1:2	1:11	1:5	1:1.4
11.04	358 (256)	135 (215)	24 (9.75)	1:3	1:15	1:5	1:1.3
16.10	305 (358)	90 (237.5)	18 (12.5)	1:3	1:17	1:5	1:1.2
25.76	464 (554)	163 (360)	33 (15)	1:3	1:14	1:5	1:1.2

(Figures in brackets are values of respective ions present in the experimental media).

ranged from 301 mM/l to 464 mM/l and the Na values ranged from 90 mM/l to 163 mM/l in the four experimental media chosen, yet when the ratios of Cl:Na is considered, it will be seen that it is maintained at 1:3. Similarly the ratios between K:Cl is maintained at 1:11 to 1:17 and the ratio between K:Na is remarkably consistent at 1:5. Even when they are considered together, it is seen that the ratio

between (Na+Cl):(K+Cl) is maintained at a fairly constant figure. It is, therefore, probable that such a situation is a consequence of regulation and, perhaps, due to accumulation of these ions in the body fluids. In the light of these results, the observation of Robertson (1949, '53) that only in decapod crustaceans an accumulation of sodium and potassium could be possible, cannot be supported. An accumulation of sodium is, perhaps, not possible in Arenicola marina (studied by Robertson, 1949), which is a purely marine species and which shows toleration of reduced salinities over a relatively narrower range. Arenicola marina is, therefore, certainly not a good choice to generalise that regulation of sodium, potassium and chloride is restricted to only members belonging to Crustacea and Cephalopoda, and that polychaetes lack the ability for regulation of these ions, for in M. gravleyi regulation extends to all the three ions studied. The mechanism or mechanisms responsible for this regulation is obscure. Perhaps, the excretory organs play an important role. Until more refined techniques for the collection of urine in polychaetes are developed, the question of the part played by nephridia in the conservation of these salts must remain a matter of speculation. Further, it is possible that sodium/chloride in M. gravleyi is being taken up by well developed branchiae situated along the whole length of the worm.

Since chlorides are the major ions in the body fluids of animals (Robertson,1953), it may be assumed that it contributes to a major part of the osmotic pressure. It was seen earlier, that the chloride values parallel, fairly well, the depression in the freezing point of the body fluid in M. gravelyi. However, even this ion does not make up all the osmotic pressure needed to keep it on a par with the osmotic pressure of the external medium, especially when exposed to media of higher concentrations. Perhaps, it does, in the lower dilutions (Fig.17). Recent work indicates that, the organic constituents like the amino acids (Bricteux-Gregoire et al,1961; Duchateau-Bosson et al,1961; Duchateau-Bosson & Florkin,1961) and the glycogen (Wilber,1948; Wilber & MacDonald,1950) also help adjustment of the osmotic pressure. The results presented here on the regulation of the total free amino acids in M. gravelyi, support such a view since a progressive increase of amino acid content with increasing concentration upto a point, is a clear proof of such a possibility. Jeuniaux et al (1961) and Duchateau-Bosson & Florkin (1961) have shown that such an adjustment although present in Arenicola marina and Perinereis cultrifera, is not so well developed as in the more euryhaline Nereis diversicolor which osmoregulates better than the two previously mentioned

polychaetes. In other words, in M. gravelyi Southern, there is not only osmoregulation of the body fluid but also intracellular adjustment, realised at least partly by marked change of concentration of intracellular free amino acids. Preliminary results, by chromatographic separation of the amino acids of the body fluids (unpublished data) have shown that M. gravelyi may be regulating glycine, similar to that reported in Arenicola marina (Duchateau-Bosson et al,1961) and Perinereis cultrifera and Nereis diversicolor (Jeuniaux et al, 1961).

CHAPTER VIII: C O N C L U S I O N S.

CHAPTER VIII: CONCLUSIONS.

Marphysa gravelyi Southern, an Eunicid Polychaete, as revealed in the present investigations, presents features which are both common and unique in polychaete physiology. Like the other polychaetes known from the Adyar estuary viz., *Glycera embranchiata*, *Onuphis gremita*, *Loimia medusa*, *Clymene insecta* (Krishnamoorthi,1962) and *Diopatra variabilis* (Krishnamoorthi,1963b), *M. gravelyi* also tolerates media of lowered salinities. But unlike the others *M. gravelyi* withstood a salinity range as wide as 5‰ to 28‰, which is exactly the range over which it is distributed in the Adyar estuary (Krishnamoorthi,1963d). Matching with its capacities for tolerance of reduced saline media, are its abilities for volume regulation. This feature, it shares with most nereids viz., *Nereis diversicolor*, *Perenereis cultrifera*, *Nereis virens*, *Nereis pelagica* and *Nereis limnicola* (Schlieper,1929a & b; Beadle, 1931,'37; Sayles,1935; Jøregensen & Dales,1957; Smith,1959); and with all the other polychaetes, except *G. embranchiata*, studied from the Adyar estuary (Krishnamoorthi,1962,'63b). Furthermore, like *N. diversicolor*, *M. gravelyi* exhibits the most developed ability for regulation of volume. If regulation of volume is an indication of permeability, *M. gravelyi* shows the

greatest reduction like N. diversicolor (Fretter,1955; Jørgensen & Dales,1957). M. gravelyi possesses both volume control and regulation of body fluid concentration and shares in these features with those reported in N. diversicolor; and as in N. diversicolor, they are not interdependent (Beadle,1937). However, M. gravelyi differs from both N. diversicolor (Schlieper,1929a & b; Beadle,1937; Smith,1955c) and Arenicola marina and N. pelagica (Schlieper,1929a & b), in the manner of its regulation of the body fluid concentration. While N.diversicolor is a hyperregulator; both A. marina and N.pelagica are conformers; M. gravelyi is unique in possessing a mechanism for hyporegulation commonly met with in the crustaceans and teleosteans. The present investigations have, thus, brought to light a third type of osmoregulation among polychaetes. The mechanism or mechanisms responsible for hyporegulation in M. gravelyi is yet obscure. As in N. diversicolor (Beadle,1937), there is circumstantial evidence that hyposmotic urine is being produced in M.gravelyi. If bigger nephridia could be associated with the nature and quantity of urine produced as suggested by Jurgens (1935) for N. diversicolor and implied in Lycastis indica (Krishnan, 1952), but amply demonstrated in Palaemonetes varians (Panikkar,1941a; Parry,1955) and in Gammarids (Beadle & Cragg,1940; Werntz,1963), then production of hyposmotic urine is certainly a possibility in M. gravelyi for the

nephridia are not only bigger in size than those of other polychaetes that co-exist with M. gravelyi in the Adyar estuary, but also better vascularised. Further, histological studies of the nephridia indicate that reabsorption of salts and water is provided for. As in N. diversicolor (Jeuniaux et al,1961) there is not only osmoregulation, but intracellular adjustment in which amino acids are involved. There is regulation extended to chlorides, potassium and sodium as well. The mechanism for this ionic regulation, just as the mechanism for osmoconcentration, is yet obscure. M. gravelyi is unique in yet another feature. Although it is not viviparous like Nereis lighti (= N. limnicola) (Smith,1950), M. gravelyi lays eggs in well protected jelly-coats which act like barriers, both for the eggs and the larvae that develop inside the jelly-coat, against a fluctuating environment. The eggs and the larvae denuded from the jelly-coat, showed limited abilities of survival against stresses of hyposmotic media (Krishnamoorthi,1951b). Thus, Marphysa gravelyi Southern with a wider range of salinity tolerance; better volume regulation; possessing bigger nephridia; a mechanism for hyporegulation; regulation extended not only to the three ions viz., Cl, K, Na but to the regulation of free amino acids; and providing protection for the developing eggs

and larvae, is far better conditioned for a greater penetration into and successful establishment in the brackish water regions of the Adyar estuary, Madras, than anyother polychaete occuring there.

CHAPTER IX: S U M M A R Y.

CHAPTER IX: S U M M A R Y.

1. M. gravelyi Southern exposed to hyposmotic media showed considerable toleration of reduced salinities ranging from 5‰ to 28‰. While the rate of mortality was only 3.3% in a salinity of 28‰, it was as high as 68.5% in 5‰ at the end of 96 hrs. Compared with the rates of mortality of other polychaetes viz., Glycera embranchiata, Onuphis eremita, Loimia medusa, Clymene insecta and Diopatra variabilis all taken from the Adyar estuary, and similarly subjected to stresses of hyposmotic media, the rates of mortality in Marphysa gravelyi were considerably low emphasising the wide range of toleration of reduced salinities in this worm.

2. Like all other polychaetes, M. gravelyi also swells when exposed to hyposmotic media. But the extent of increase was considerably low. In this worm, as in other polychaetes, the initial increase and the final volume attained, are a function of the external medium. Both the final volume and the initial increase being very low in M. gravelyi, reduction in permeability is, perhaps, the maximum in this worm.

3. Following volume regulation, there is also regulation of the body fluids in M. gravelyi. However, as in

Nereis diversicolor, both the factors are not inter-dependent. Unlike N. diversicolor, a hyperegulator, and Nereis pelagica and Perimereis cultrifera, both conformers; M. gravelyi is a hyporegulator. The mechanism or mechanisms are still obscure. But there is circumstantial evidence that hyposmotic urine is being formed, just as in N. diversicolor. The range of the osmotic pressure of the body fluids in M. gravelyi is very low (0.56 to 0.59 %NaCl) and, perhaps, this is the chief means of easing the strain on osmoregulatory mechanisms.

4. M. gravelyi possesses nephridia of the mixo-nephridial type as obtained in most Eunicids. However, a structure comparable to the end sac of the nephridium in earthworms and crustaceans, is met with in the nephridium of M. gravelyi. Histological preparations support the view that it may be taking part in reabsorption, while the nephridial canal takes part in the filtration. Not only the ratio between the excretory surface and the length of the worm, but also the magnitude of vascularisation of the nephridium are higher than that obtained in other polychaetes that co-exist with M. gravelyi in the Adyar estuary.

5. Regulation in M. gravelyi is extended to all ions viz., Chlorides, Sodium and Potassium. The ratios between Na:Cl; K:Cl; K:Na and (Na+Cl):(K+Cl) are remarkably held constant. In addition to osmoconcentration of the body fluids, M. gravelyi also resorts to intracellular regulation in which amino acids, partly at least, are involved.

CHAPTER X: R E F E R E N C E S.

CHAPTER X: REFERENCES.

Aiyar, R.G. 1933 On the Anatomy of Marphysa gravelyi Southern.
Rec. Indian Mus., 35: 287-323.

Annandale, N. 1922 The marine element in the Fauna of the Ganges.
Bijdr. Dierk. Amsterdam, (Feest-num, M.Weber): 143-154.

Bhal, K.N. 1919 New type of nephridia found in the genus Pheretima.
Quart. J. micr. Sci., 64:67-

——— 1942a Structure, development and physiology of the nephridia of Oligochaeta, Part.I. Nephridia of the Octochaetinae.
Ibid., 83:423-

——— 1942b Idem., Part II. Multiple funnels of the nephridia.
Ibid., 83:450-

——— 1945b Idem., Part VI. The physiology of excretion and the significance of the enteronephric type of nephridial system.
Ibid., 85:343-

——— 1947 Excretion in Oligochaeta.
Biol. Rev., 22:109-147.

Barcroft, J. 1934 "Features in the Architecture of Physiological Function". Cambridge, The University Press, pp.ix + 368.

Beadle, L.C. 1931 The effect of salinity changes on the water content and respiration of marine invertebrates.

J. exp. Biol., 8:211-227.

——— 1932 Scientific results of the Cambridge Expedition to the East African Lakes, 1930-1, -4. The waters of some East African Lakes in relation to their fauna and flora.

J. Linn. Soc.(Zool.)., 38:157-211.

——— 1934 Osmotic regulation in Gunda ulvae.

J. exp. Biol., 11:382-396.

——— 1937 Adaptation to changes of salinity in the polychaetes: I. Control of body volume and of body fluid concentration in Nereis diversicolor.

Ibid., 14:56-70.

——— 1943a Osmotic regulation and the faunas of inland waters.

Biol. Rev., 18:172-183.

——— 1943b An ecological survey of some Inland Saline Waters of Algeria.

J. Linn. Soc. (Zool.)., 41:218-242.

——— 1957 Comparative Physiology: Osmotic and Ionic Regulation in Aquatic Animals.

Anna. Rev. Physiol., 19:329-358.

——— 1958 The Classification of Brackish and Inland Saline Waters.

Nature, London, 182:27-28.

Beadle, L.C. 1959 Osmotic and Ionic regulation in relation to the classification of brackish and inland saline waters.

Estratto Dall'Archivio Di Oceanografia E Limnologia, Vol.XI, Supplemento, pp, 143-151.

_____ & Cragg, J.B. 1940a Studies on adaptation to salinity in Gammarus spp.I. Regulation of blood and tissues and the problem of adaptation to fresh water.

J. exp. Biol., 17:153-163.

_____, & Cragg, J.B. 1940b Osmotic equilibrium in Fresh water animals.

Nature, London, 146:588.

_____, & Shaw, J. 1950 The retention of salt and the regulation of the non-protein nitrogen fraction in the blood of the aquatic larva, Sialis lutaria.

J. exp. Biol., 27:96-109.

Benham, W.B. 1891 Nephridium of Lumbricus and its blood supply with remarks on the Nephridia of Chaetopods.

Quart. J. micr. Sci., London, 32:293-

Bethe, A. 1934 Die Salz- und Wasser-Permaeabilitat der Korperberflachen verschiendener Seetheiere in ihrem gegenseitigen Verhaltnis.

Pflugers Arch. ges. Physiol., 234:629-644.

_____, & Berger, E. 1931 Variationen im Mineralbestand verschiedener Blutarten.

Ibid., 227:571-584.

Bialaszewicz, K. 1933 Contribution a l'etude de la composition minerale des liquides nourricieres chez les animaux marins.

Arch. int. Physiol., 36:41-53.

Block, R.J. & Weiss, K.W. 1956 "Amino Acid Handbook": Methods and Results of Protein Analysis" Charles C.Thomas, Springfield, Illinos, U.S.A., pp.xiii+386.

*Bogucki, M. 1954 Adaptacja Nereis diversicolor (O.F.Muller) do rozcienzonej wody morskiej i wody slodkiej.

Polsk. Arch. Hydrobiol., 2(XV):237-251.

*Botazzi, F. 1897 La pression osmotique du sang des animaux marins.

Arch. ital. biol., 28:61-72.

Bryan, W.G. 1960a Sodium regulation in the crayfish, Astacus fluviatilis. I. The Normal Animal.

J. exp. Biol., 37:83-99.

——— 1960b Idem., II. Experiments with sodium depleted animals.

Ibid., 37:100-112.

——— 1960c Idem., III. Experiments with NaCl loaded animals.

Ibid., 37:113-128.

——— 1961 The accumulation of radioactive Caesium in Crabs.

J. Mar. biol. Ass., U.K.,41:551-575.

——— 1963a The accumalation of radioactive Caesium by marine invertebrates.

Ibid., 43:519-539.

Bryan, W.G. 1963b The accumalation of ^{137}Cs by brackish water invertebrates and its relation to the regulation of potassium and sodium.

Ibid., 43:541-565.

_____, & Ward, E. 1962 Potassium metabolism and the accumalation of ^{137}Cs by Decapod Crustacea.

Ibid., 42:199-241.

Bricteux-Gregoire, S. Jeuniaux, Ch., & Florkin, M. 1961 Role de la variation de la composante aminoacide intracellulaire dans l'euryhalinite de Leander serratus F., et de Leander squilla L.

Arch. int. Physiol., 69:744-745.

Broekema, M.M.M. 1941 Seasonal movements and the osomotic behaviour of the shrimp, Crangon crangon L.

Arch. neerl. Zool., 6:1-100.

Broekhuysen, G.J. 1936 On development, growth and distribution of Carcinus maenas (L).

Ibid., 2:257-399.

Cole, H.W. 1940 The composition of fluids and sera of some marine animals and of the sea water in which they live.

J. gen. Physiol., 23:575-584.

Cosmovice, L.C. 1880 Glandes, genitales et organes segmentaires des Annelides Polychaetes.

Arch. Zool. exp. gen. 8: 232-372.

Claude Bernard. 1885 Quoted by Brarcroft, J., in 'Features in the Architecture of Physiological Function'.

Cowgill, R.W., & Pardee, A.B. 1957 "Experiments in Biochemical Research Techniques", John Wiley & Sons., New York; Chapman & Hall, Ltd., London, pp.ix+189.

Croghan, P.C. 1961 Competetion and mechanisms of Osmotic adaptation.

Smp. Soc. exp. Biol., 15:156-167

Cunningham, J.T. 1887 Some points on the anatomy of Polychaeta.

Quart. J. micr. Sci., 28:239-277.

Dakin, W.J. 1935 The aquatic animal and its environment.

Proc. Linn. Soc. N.S.W., 60:8-32.

_____, & Edmonds, E. 1931 Regulation of salt contents of blood of aquatic animals and the problem of permeability of the bounding membranes of aquatic invertebrates.

Aust. J. exp. Biol. med. Sci., 8:170-187.

Dehorne, A. 1925 Observations sur la biologie, de Nereis diversicolor.

C.R. Acad. Sci., Paris, 180:1441-1443.

Duchateau-Bosson, Gh. & Florkin, M. 1961 Change in intracellular concentration of free amino acids as a factor of eurybalinity in the cray fish, Astacus astacus.

Comp. Biochem. Physiol., 3:245-250.

Duchateau-Bosson, Gh., Jeuniaux, C., & Florkin, M. 1961 Role de la variation de la composante aminoacide intracellulaire dans l'euryhalinite d'Arenicola marina L.

Arch. int. Physiol., 69:30-35.

Duval, M. 1935 Recherches physico-chimiques et physiologiques sur le milieu interieur des animaux aquatiques Modifications sous l'inflence dum milieu exterieus.

Ann. Inst. oceanogr., Paris, 2:232-407.

Ellis, W.G. 1933 Calcium and the resistance of Nereis to brackish water.

Nature, London, 132:748.

——— 1937 The water and electrolyte exchange of Nereis diversicolor (Muller).

J. exp. Biol., 14:340-350.

——— 1939 Comparative measurements of water and electrolyte exchange in a stenohaline and in a euryhaline polychaete.

Ibid., 16: 483-486.

Ewer, D.W. 1941 The blood system of Sabella and Spirographis.

Quart. J. micr. Sci., 82:587-

———, & Ewer, R.F. 1943 Osmotic regulation in Sabella pavonina.

Nature, London, 152:598.

Ehlres, E. 1864-68. "Die Borstenwurmer nach systematischen und anatomischen Untershuchungen dargestellt", Leipzig, Wilhelm Englemann, pp.xxx + 748.

Fage, L. 1906 Recherches sur les organes segmentaires des Annelides Polychaetes.

Sci. Nat. Zool. Paris, 3:261-410.

Ferronniere, G. 1901 Etudes biologique sur les Zones supralittorales de la Loire-inferieure.

Bull. Soc. Sci. nat. Ouest, 11: 1-45.

Fisher, R.A., & Yates, F. 1948 "Statistical Tables for Biological and Medical Research", 3rd Edition, Oliver & Boyd, Edinburgh.

Florentin, R. 1899 Etudes sur la faune des mares salees de Lorraine.

Ann. dess. Sci. Natur.(8), 10:209-346

Freeman, R.F.H., & Rigler, F.H. 1957 Reponses of Scrobicularia plana (Da Costa) to osmotic pressure changes.

J. Mar. biol. Ass., U.K., 36:553-567

Fretter, V. 1955 Uptake of radioactive sodium (^{24}Na) by Nereis diversicolor Mueller and Perinereis cultrifera (Grube).

Jour. Mar. Biol. Ass. U.K., 34:151-160.

Fuchs, K. 1906 Die topographie des Blutgefas systems der Chaetopodon.

Jena. Z. Naturz., 42:374-484.

Garrey, W.E. 1905 The Osmotic Pressure of Sea Water and the Blood of Marine Animals.

Biol. Bull., Woods Hole, 8:257-270

Goodrich, E.S. 1900 On the Nephridia of Polychaetes.

Quart. J. micr. Sci., 43:699-

Goodrich, E.S. 1945 The study of Nephridia and genital duct since 1895.

Ibid., 86:113-392.

Green, M., & Stahmann, M.R. 1955 Preperation and enzymatic hydrolysis of poly-γ-ethyglutamyl bovine plasma albumin.

J. biol. Chem., 213:259-269.

Green, J.W., Harsch, M., Barr, L., & Prosser, C.L. 1959 The regulation of water and salt by the fiddler crabs, Uca pugnax and Uca pugilator.

Biol. Bull., Woods Hole, 112: 34-42.

*Grobben, C. 1881 Die Antennendruse der Crustacean.

Arb. Zool. Inst. Univ. Wien., 3:

Gross, W.J. 1954 Osmotic responses in the sipunculid Dendrostomium zostericolum.

J. exp. Biol., 31:402-423.

——— 1955 Aspects of osmotic regulation in crabs showing the terrestrial habit.

Amer. Nat., 89:205-222.

——— 1957 An analysis of response to osmotic stress in selected decapod Crustacea.

Biol. Bull., Woods Hole, 112: 43-62.

——— 1957 A behavioural mechanism for osmotic regulation in a semi-terrestrial crab.

Ibid., 113:268-274.

Gross, W. J. 1958 Potassium and Sodium regulation in an intertidal crab.

Ibid., 114:334-347.

_____ 1959 The effect of osmotic stress on the ionic exchange of a shore crab.

Ibid., 116:248-257.

_____ 1961 Osmotic tolerance and regulation in crabs from a hypersaline lagoon.

Ibid., 121:290-301.

_____, & Holland, P.V. 1960 Water and ionic regulation in a terrestrial hermit crab.

Physiol. Zool., 33:21-28.

_____, & Marshall, L.A. 1960 The influence of salinity on the Mg and water fluxes of a crab.

Biol. Bull., Woods Hole, 119: 440-453.

Gurney, R. 1913 The origin and condition of existence of the fauna of fresh water.

Trans. Norfolk Norw. Nat. Soc., 9:461-485.

Harding, V.J., & MacLean, R.M. 1916 A colorimetric method for the estimation of amino-acid a-nitrogen. II. Application to the hydrolysis of proteins by pancreatic enzymes.

J. biol. Chem., 24:503-517.

Hartman, O. 1938 Brackish and fresh-water Nereidae from the northeast Pacific, with the description of a new species from Central California.

Univ. Calif. Publ. Zool., 43:79-82.

Hartman, O. 1960 An account of the nereid worm Neanthes diversicolor, comb. n. in the Caspian Sea and its more extensive distribution.

Zool. Zhurnal., 39:35-39.

Hawk, P.B., Oser, B.L., & Summerson, W.H. 1954 "Practical Physiological Chemistry", J. & A. Churchill & Co., London, pp.xvi + 1439.

Hukuda, K. 1932 Change of weight of marine animals in diluted media.

J. exp. Biol., 9:61-68.

Hynes, H.B.N. 1954 The ecology of Gammarus duebeni Lilleborg and its occurrence in fresh water in western Britain.

J. Anim. Ecol., 23:38-84.

Jeuniaux, Ch., Duchateau-Bosson, Ghislaine & Florkin, M. 1961 Variation de la composante amino-acide des tissues et euryhalinite chez Perinereis cultrifera Gr. et Nereis diversicolor (O.F.Muller).

J. Biochem., Tokyo, 49:527-531.

_____, Duchateau-Bosson, Ghislaine & Florkin, M. 1961 Free amino acids in the intracellular osmoregulation of euryhaline marine worms.

Biochem. J., 79:24-25.

Johnson, H.P. 1903 Fresh-water nereids from the Pacific coast and Hawaii with remarks on fresh-water Polychaetes in general.

Mark Anniversary Volume, Art. 10: 205-223.

Jones, L.L. 1941 Osmotic regulation in several crabs of the Pacific Coast of North America.

J. cell. comp. Physiol., 18:79-92.

Jones, M.L. 1957 On the morphology of the nephridium of Nereis vexillosa Grube.

Biol. Bull., Woods Hole, 113:407-413.

Jørgensen, C.B., & Dales, R.P. 1957 The regulation of volume and osmotic regulation in some nereid polychaetes.

Physiol. comp., 4:357-374.

Jurgens, O. 1935 Die Wechselbeziehungen von Blutkreislauf, Atmung und Osmoregulation bie Polychaeten (Nereis diversicolor O.F. Muller).

Zool. Jb. allgem. Zool., 55: 1 - 46.

Kinne, O. 1952 Zur Biologie und Physiologie von Gammarus duebeni Lillj. V. Untersuchungen uber Blutkonzentration, Herzfrequenz und Atmung.

Kieler Meeresforsch., 9:134-15

——— 1954 Experementelle Unterschungen uber den Einfluss des salzgehaltes auf die Hitzresisten von Brackwassertieren.

Zool. Anz., 152:10-16.

——— 1953a Zur Biologie und Physiologie von Gammarus duebeni Lillj.

Z. wiss. Zool., 157:427-491.

——— 1953b Zur Biologie und Physiologie von Gammarus duebeni Lillj. I Uber die Hautungsfrequenz, ihre Abhangigkeit von Temperatur und Salzgehalt, sowie uber ihre Verhalten beiisoliert gehaltenen und amputierten Versuchstieren.

Zool. Jb. allgem. Zool., 64: 183-206.

Kinne, O. 1956a Uber Temperatur und Salzgehalt und ihre physiologisch-biologische Bedeutung.

Biol. Zbl., 75:314-327.

——— 1956b Uber den Einfluss des Salzgehaltes und der Temperatur auf Wachstum, Form und vermehrung bei dem Hydroidpolypen Cordylophora caspia (Pallas), Thecata, Clavidae.

Zool. Jb. allgem. Zool., 66: 565-638.

——— 1957 A programatic study of comparative biology of marine and brackish water animals.

Anne. Biol., 33:87-92.

——— 1958a Adaptation to salinity variations: Some factors and Problems., in Physiological Adaptation, Ed., C.L. Prosser, pp.92-106 (Washington D.C.: Physiological American Society).

——— 1958b Uber die Teaktion erbgleichen coelenteraten gewebes aur verschiedene Salzgehalts und Temperaturbedingungen.

Zool. Jb. allgem. Zool., 67:407-486.

Krishnan, G. 1952 On the nephridia of Nereidae in relation to habitat.

Proc. nat. Inst. Sci. India, 18: 241-255.

Krishnamoorthi, B. 1951a "Studies on the Nephridia of Polychaetes". Thesis submitted for the M.Sc., Degree of the University of Madras.

——— 1951b Studies on the Osmotic properties of Eggs, and Larvae of a brackish water polychaete, Marphysa gravelyi Southern.

Proc. Ind. Acad. Sci., 34:199-209.

Krishnamoorthi, B.	1962	Salinity Tolerance and Volume Regulation in four species of Polychaetes. Ibid., 56:363-371.
———	1963a	Gross morphology and histology of nephridia in four species of polychaetes. Ibid., 57:195-209.
———	1963b	Volume regulation in eggs, larvae and adults of a brackish water polychaete, Dionatra variabilis (Southern). Ibid., 57:275-289.
———	1963c	Chloride regulation in Marphysa gravelyi Southern. Curr. Sci., 32:463-464.
———	1963d	On the distribution of six species of polychaetes in the Adyar Estuary, Madras. J. Mar. Biol. Assoc. India, 5: 97-102.
———, & Daniel, A.	1951	A rare case of commensal in a polychaete, Amphinome anatifera n. sp. J. Madras Univ., 28:34-40.
———, & Krishnaswamy, S.	1963	Activity of Marphysa gravelyi Southern (Polychaeta) under heterosmotic conditions. Proc. Ind. Acad. Sci., 57:83-87.
Krogh, A.	1938	The active absorption of ions in some fresh water animals. Z. vergl. Physiol., 25:335-350.

Krogh, A. 1939 "Osmotic Regulation in Aquatic Animals", Cambridge University Press, Cambridge, pp.1-242.

Kromhout, G.A. 1943 A comparison of the protonephridia of fresh water, brackish water and marine specimens of Gyrâtrix hermâphroditicus.

J. Morph., 72:167-177.

Lockwood, A.P.M. 1962 The Osmoregulation in Crustacea.

Biol. Rev., 37:257-308.

Lowndes, A.G. 1942 The displacement method of weighing aquatic animals.

J. Mar. biol. Ass. U.K., 25:555-572.

Margaria, R. 1931 The osmotic changes in some marine animals.

Proc. roy. Soc. (B), 107:606-

Martens, P. von, 1858 On the occurrence of Marine Animal forms in fresh water.

Ann. Nat. Hist. London, 1, 3rd Ser.,:

*Maziarski, S. 1905 Sur la structure des nephridis des vers de terre.

C. R. Soc. Biol., Paris, 53:

Meyer, E. 1888 Studies uber des Korperbaue der Anneliden.

Zool. Stat. Neapel, Mitt., 8:462-662.

Nagel, H. 1924 Die Aufgaben der Exkretionsorgane und der Kiemen bei der Osmoregulation von Carcinus maenas.

Z. vergel. Physiol., 21:468-491.

Nicol, J.A.C. 1960 "The Biology of Marine Animals", Sir Isaac Pitman & Sons Ltd., London, pp.ix+707.

Panikkar, N.K. 1940 Influence of temperature on osmotic behaviour of some crustacea and its bearing upon problems of animal distribution.

Nature, London, 146:366-367.

——— 1941a Osmoregulation in some Palaemonid prawns.

J. Mar. biol. Ass. U.K., 25:317-359.

——— 1941b Osmotic behaviour of the fairy shrimp, Chirocephalus diaphanus.

J. exp. Biol., 18:110-114.

——— 1948 Osmotic regulation in animals and man - Sir Subramania Ayyar Lectures, University of Madras, 1943-44, given in Jan., 1944.

J. Madras Univ., 17:1-19.

——— 1950 Physiological aspects of adaptation to estuarine conditions.

Proc. Indo-Pacific Fish. Counc. Australia, Sect. 3:168-175.

———, & Aiyar, R.G. 1937 The brackish water fauna of Madras.

Proc. Ind. Acad. Sci., 6:288-357.

Pantin, C.F.A. 1931 The origin of the composition of the body fluids of animals.

Biol. Rev., 6:459-482.

Parry, G. 1953 Osmotic and Ionic regulation in the isopod crustacean, Ligia Oceanica.

J. exp. Biol., 30:567-574.

——— 1954 Ionic regulation in the palemonid prawn, Palaemon (=Leander) serratus.

J. exp. Biol., 31:601-613.

Parry, G. 1955 Urine production by antennary glands of Palaemonetes varians Leach.

Ibid., 32:408- 22.

——— 1957 Osmoregulation in some fresh water prawns.

Ibid., 34:417-423.

Pearse, A.S. 1927 The migration of animals from the Ocean to fresh water and land habitats.

Amer. Nat., 61:466-476.

——— 1928 On the ability of certain marine invertebrates to live in diluted sea water.

Biol. Bull., Woods Hole, 54:405-409.

——— 1934 Freezing points of blood of certain littoral and estuarine animals.

Carnegie Inst. of Washington, Papers from Tortugas Laboratory, 28:93-102.

Picken, L.E.R. 1936 The mechanism of urine formation in invertebrates. I. The excretory mechanism in certain Arthropods.

J. exp. Biol., 13:309-328.

*Pieh, S. 1936 Uber die Beziehungen zwischen Atmung, Osmoregulation und Hydration der Gewebe bei euryhalinen Meeresevetebraten.

Zool. Jb. allem. Zool., 56:129-160.

*Pora, E.A., & Rosca, D.I. 1952 Efectual suprasalurii Lacurilor Dela Eforie Asupra Rezistentei La Salinitati Variable a species Nereis diversicolor.

Studii si Cercetari Stiinitifice, 3:209-213.

Potts, W. T. W. 1952 Measurement of osmotic pressure in single cells.

Nature, London, 169:834.

——— 1954 The energetics of osmotic regulation in brackish - and fresh water animals.

J. exp. Biol., 31:618-630.

——— 1954a The inorganic composition of the blood of Mytilus edulis and Anodonta cygnea.

Ibid., 31:367-385.

——— 1954b The rate of urine production of Anodonta cygnea.

Ibid., 31:614-617.

——— 1959 The sodium fluxes in the muscle fibres of a marine and fresh water lamellibranch.

Potts, W.T.W., & Parry, G. 1964 "Osmotic and Ionic Regulation in Animals", Pergamon Press, Oxford, London, New York, Paris, pp.xiii + 428.

Bishop, D.W., Brown, F.A, John, T.L. & Wulff, V.J. Philadelphia, pp.[illegible]

Racovitza, E.G. 1895 Sur la role des amibocytes chez les Annelides polychaetes.

C. R. Acad. Sci., Paris, 120:464-467

Ramsay, J.A. 1949a The osmotic relations of the earth worm.

J. exp. Biol., 26:46-56.

——— 1949b The site of formation of hypotonic urine in the nephridium of Lumbricus.

Ibid., 26:65-75.

Ramsay, J.A. 1954 Movements of water and electrolytes in invertebrates. In Active Transport and Secretion.

Symp. Soc. exp. Biol., 8:1-15.

_____ 1957 "Physiological Approach to the Lower Animals", The Cambridge University Press, Cambridge, pp.vii + 148.

_____, Falcon, S.W.H.W., & Machin, E.E. 1951 An intigrating flame photometer for small quantities.

J. sci. Instrum., 28:

_____, Brown, R.H.J., & Falcon, S.W.H.W. 1953 Simultaneous determination of sodium and potassium in small volumes of fluid by flame photometry.

J. exp. Biol., 30:1-

_____, & Brown, R.H.J. 1955 Simplified apparatus and procedure for freezing point determination upon small volumes of fluid.

J. sci. Instrum., 32:372-375.

_____, Brown, R.H.J., & Croghan, P.C. 1955 Electrometric titration of chloride in small volumes.

J. exp. Biol., 32:822-829.

Rao, K.V. 1951 Observations on the probable effects of salinity on the spawning, development and setting of the Indian backwater Oyster, Ostrea madrasensis Preston.

Proc. Ind. Acad. Sci., 33:231-256.

Remane, A., & Schlieper, C. 1958 "Die Biologie des Brackwassers", Die Binnengewasser, 22: pp.348.

Robertson, J.D. 1939 The inorganic composition of the body fluids of three marine invertebrates.

J. exp. Biol., 16:387-397.

——— 1941 The function and metabolism of calcium in the invertebrata.

Biol. Rev., 16:106-133.

——— 1949 Ionic regulation in some marine invertebrates.

J. exp. Biol., 26:182-200.

——— 1953 Further studies on ionic regulation in marine invertebrates.

Ibid., 30:277-296.

——— 1957 Osmotic and Ionic Regulation in Aquatic Invertebrates, IN Recent Advances in Invertebrate Physiology, Ed. B.T. Scheer, University of Oregon Publications, 1957.

——— 1960 Osmotic and Ionic Regulation. Chapter 9., in The Physiology of Crustacea, Vol.1., (Ed.) T.H. Watermann. pp.317-339., Academic Press, New York & London, pp.xvii + 670.

———, & Webb, D.A. 1939 The micro-extimation of sodium, potassium, calcium, magnesium, chloride and sulphate in sea water and the body fluids of marine animals.

J. exp. Biol., 16:155-177.

Roe, J.H., Epstein, T.H., & Goldstein, N.P. 1949 A photometric method for the determination of inulin in plasma and urine.

J. biol. Chem., 178:839-845.

Sayles, L.P. 1935 The effects of salinity changes on body weight and survival of Nereis virens.

Biol. Bull., Woods Hole, 69:233-244.

Schallek, W. 1949 The glycogen content of some invertebrate nerves.

Ibid., 97:252-253.

Schlieper, C. 1929a Uber die Einwirkung niederer salzkonzentration auf marine organismen.

Z. vergl. Physiol., 9:478-514.

——— 1929b Neue Versuche uber die osmoregulation wasserlebender Tiere.

Sitzungsberich d. Ges. Z. Beford. d. gesamt. Naturwis. Marburg, 64:6-

——— 1930 Die Osmoregulation wasserlebender Tiere.

Biol. Rev., 5:309-356.

——— 1935 Neuere Ergebnisse und Probleme aus dem gebiet der osmoregulation wasserlebender Tiere.

Biol. Rev., 10:334-360.

——— 1955 Uber die physiologischen Wirkungen des Brackwassers (Nach Versuchen an der Miesmuschel Mytilus edulis).

Kieler Meresf., 11:22-23.

Schneider, G. 1899 Uber Phagocytose und Exkretion bei den Annliden.

Z. wiss. Zool., 66:497-520.

Schwabe, E. 1933 Uber die osmoregulation verschiedener Krebse (Malacostracean).

Z. vergl. Physiol., 19:183-235.

Sendroy, J. 1937 Microdetermination of chloride in biological fluids with solid silver iodate.

J. biol. Chem., 120:305-403.

Shaw, J. 1955 Ionic regulation and water balance in the aquatic larva of Sialis lutaria.

J. exp. Biol., 32:353-382.

——— 1955a A simple procedure for the study of Ionic regulation in small animals.

Ibid., 32:321-329.

——— 1955b The permeability and structure of the cuticle of the aquatic larva of Sialis lutaria.

Ibid., 32:330-352.

——— 1955a Ionic regulation in the muscle fibres of Carcinus maenas. I. The electrolyte composition of single fibres.

Ibid., 32:383-396.

——— 1955b Idem: II. The effect of reduced blood concentration.

Ibid., 32:664-680.

——— 1958a Osmoregulation in the muscle fibres of Carcinus maenas.

Ibid., 35:902-919.

Shaw, J. 1958b Further studies on ionic regulation in the muscle fibres of Carcinus maenas.

Ibid., 35:920-929.

——— 1958 Sodium uptake by the crayfish.

Nature, London, 182:1105-1106.

——— 1959 The absorption of sodium ions by the crayfish, Astacus pallipes Leverboullet. I. The effect of external and internal sodium concentrations.

J. exp. Biol., 36:126-144.

——— 1959a Solute and water balance in the muscle fibres of the East African fresh water crab, Potamon niloticus (M. Edw.).

Ibid., 36:145-156.

——— 1959b Salt and water balance in the East African fresh water crab, Potamon niloticus (M.Edw.).

Ibid., 36:157-176.

——— 1960a The absorption of sodium ions by the cray fish, Astacus pallipes Leverboullet. II. The effect of the external anion.

J. exp. Biol., 37:534-547.

——— 1960b Idem., III. The effect of other cations in the external solutions.

Ibid., 37:548-556.

——— 1960c The absorption of chloride inons by the crayfish, Astacus pallipes Leverboullet.

Ibid., 37:557-572.

Shaw, J. 1960c The Mechanisms of Osmoregulation. IN Comparative Biochemistry, Vol.2, Ed. M. Florkin & H.S.Mason, Academic Press, New York.

_____, & Sutcliff, D.W. 1961 Studies on sodium balance in Gammarus dubeni and Gammarus pulex. J. exp. Biol., 38:1-16.

Simpson, G.G., Roe, A., & Lowentin, R.C. 1960 "Quantitative Zoology", Harcourt, Brace & Company, New York, pp.vii + 440.

Smith, R.I. 1950 Embryonic development in the viviparous nereid polychaete, Neanthes lighti Hartman. J. Morph., 87:417-466.

_____ 1953 The distribution of the polychaete Neanthes lighti in the Salinas River Estuary, California, in relation to salinity, 1948-1952. Biol. Bull. Woods Hole., 105:335-347.

_____ 1955a On the distribution of Nereis diversicolor in relation to salinity in the vicinity of Tvarminne, Finland, and the Isefjord, Denmark. Ibid., 108:326-345.

_____ 1955b Comparison of the level of chloride regulation by Nereis diversicolor in different parts of its geographical range. Ibid., 109:453-474.

_____ 1955c Salinity variation in interstitial water of sand at Kames Bay, Millport, with reference to the distribution of Nereis diversicolor. J. Mar. biol. Ass. U.K., 34:33-46.

Smith, R.I. 1956 The ecology of the Tamar Estuary. VII. Observations on the interstitial salinity of intidal muds in the estuarine habitat of Nereis diversicolor.

Ibid., 31:81-104.

——— 1957 A note on the tolerance of low salinities by nereid polychaetes and its relation to temperature and reproductive habit.

Anns. Biol., 33:93-107.

——— 1959 Physiological and Ecological problems of brackish waters in Marine Biology. Proceedings of the Twentieth Annual Biology Colloquium Oregon State College, April 1959, pp.52-89.

——— 1963a On the occurrence of Nereis (Neanthes) succinea at the Kristineberg Zoological station, Sweeden, and its recent northward spread.

Arkiv. Zool., 15:437-441.

——— 1963b A comparison of salt loss rate in three species of brackish-water Nereid polychaetes.

Biol. Bull. Woods Hole, 124:

Smith, R.I. 1964 D_2O uptake rate in two brackish-water Nereid Polychaetes.

Biol. Bull., Woods Hole, 126: 142-149.

Southern, R. 1931 Fauna of the Chilka Lake: Polychaet

Mem. Indian. Mus., 5:565-659.

Stephensen, J. 1930 "The Oligochaeta", Clarendon Press, Oxford.

Strunk, C. 1932 Urea, Uric acid in coelomic fluid, Arenicola.

Zool. Jb. allgem. Zool., 52: 216-222.

Sukumaran, S. 1961 Osmoregulation in Clibanarius padavensis De Man, under heterosmotic conditions.

Proc. Ind. Acad. Sci., 54:145-151.

*Tarussov, B. 1927 Uber den Einfluss der osmotischen Bedingungen auf die oxydationsgeschwindigkeit.

Zurn. Esper. Biol. i. Med., 6:229-

Topping, F.L., & Fuller, J.F. 1942 The accommodation of some marine invertebrates to reduced osmotic pressures.

Biol. Bull., Woods Hole, 82: 372-384.

Troll, W., & Cannan, R.K. 1953 A modified photometric ninhydrin method for the analysis of amino and imino acids.

J. biol. Chem., 200:803-811.

Verwey, J. 1957 A plea for the study of temperature influence on osmotic regulation.

Anne. Biol., 33:129-149.

Webb, D.A. 1940 Ionic regulation in Carcinus maenas.

Proc. roy. Soc. (B), 129:107-136.

Wells, G.P. 1937 Studies on the physiology of Arenicola marina L. I. The pacemaker role of the oesophagus, and the action of adrenaline and acetylcholine.

J. exp. Biol., 14:117-

Wells, G.P. 1950 Spotaneous activity cycles in Polychaete worms.

Symp. Soc. exp. Biol., 4:127-

_____, & Ledingham, I.C. 1940a Physiological effects of a hypotonic environment. I. The action of hypotonic salines on isolated rhythmic preparations from polychaete worms (Arenicola marina, Nereis diversicolor, Perinereis cultrifera).

J. exp. Biol., 17:337-352.

_____, & Ledingham, I.C. 1940b Studies on the physiology of Arenicola marina L. II. Accommodation to magnesium concentration in the isolated extrovert.

Ibid., 17:353-363.

_____, & Ledingham, I.C. 1942 Idem., III. The potassium relations of the isolated extrovert (with remarks on the effects of varying the lever weight).

Ibid., 19:176-185.

_____, Ledingham, I.C., & Gregory, M. 1940 Physiological effects of a hypotonic environment. II. Shock effects and accommodation in cilia (Pleurobrachia, Mytilus, Arenicola) following sudden salinity change.

Ibid., 17:378-385.

Welsh, J.H., & Smith, R.I. 1960 "Laboratory Excercises in Invertebrate Physiology", Burgess Publishing Co., Minneapolis, pp.viii + 169.

Werntz, H.O. 1963 Osmotic regulation in marine and fresh water Gammarids (Amphipods).

Biol. Bull., Woods Hole, 124: 225-239.

Wesenberg-Lund, E. 1958 Lesser Antillean Polychaetes, chiefly from brackish water, with a survey and a bibliography of fresh and brackish water polychaetes.

Studies on the Fauna of Curacao and other Caribbean Islands, 8 (30):1-41.

Widmann, E. 1936 Osmoregulation bei einheimischen und Feuchtluft- Crustacean.

Z. wiss. Zool., 147:132-169.

Wigglesworth, V.B. 1938 The regulation of osmotic pressure and chloride concentration in the haemolymph of mosquito larvae

J. exp. Biol., 15:235-247.

Wikgren, B.J. 1953 Osmotic regulation in some aquatic animals with special reference to the influence of temperature.

Acta. Zool., Fennica, 71:1-102.

Wilber, C.G. 1948 Uric acid in body fluids of marine invertebrate animals.

J. cell. comp. Physiol., 31:107-10

_____ 1948 Glucose content of the body fluid in marine animals.

J. biol. Chem., 173:141-143.

_____, & MacDonald, E.S. 1950 Glucose metabolism in marine animals.

Sci. & Tech., 1:7-11.

Willem, V., & Minne, A. 1900 Recherches sur l'excretion chez quelques Annelides.

Mem. Acad. Sci. Belgiques, 58: 1-73.

Zenkevich, L.A. 1938a The influence of Caspian and Black Sea water of different concentrations upon some common Black Sea Invertebrates. Part I. Survival and body weight changes (In Russian).

Zoologitscheskij Journ., 17: 845-876.

——— 1938b Idem., Part II. The changes of internal salinity (In Russian).

Ibid., 17:976-1002.

——— 1958 Caspian and Aral Seas. IN Treatise on Marine Ecology and Paleoecology, Vol.1, Ecology. Mem., 67, Geol. Soc. America, 891-918.

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* Not referred to in original.

APPENDICES.

APPENDIX 1.

Studies on the Osmotic Properties in Eggs and Larvae of a Brackish water Polychaete, *Marphysa gravelyi* Southern.

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STUDIES ON THE OSMOTIC PROPERTIES OF THE EGGS AND LARVÆ OF A BRACKISH-WATER POLYCHÆTE, *MARPHYSA GRAVELYI* SOUTHERN

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INTRODUCTION

WHILE the osmotic behaviour of marine polychætes has been studied by several authors (Schlieper, 1929; Ellis, 1933, 1937; Beadle, 1934, 1937, 1943; Bethe, 1934; and Adolph, 1936), the osmotic properties of their eggs and larvæ have received no attention. McClendon (1910 *a*, *b*), R. S. Lillie (1916, 18), Runnstrom (1925), Luke and McCutcheon (1925–26), Bialaszewicz (1927), Needham (1930), Euphrussi and Rapkine (1928), Needham and Needham (1930 *a*, *b*), Ranzi (1930) and Northrop (1926–27) have performed experiments on the osmotic properties of the eggs of sea urchins and of Sepia among the invertebrates, but have not investigated the behaviour of the larvæ and adults of these forms. An investigation of the osmotic properties of the eggs, of larvæ and of the adult of the same species may throw light on the adaptations of species to different habitats during their whole life cycle. In the present paper an account of the behaviour of eggs and larvæ of the brackish water polychæte, *Marphysa gravelyi* Southern is given and will be followed later by a study of the osmotic regulations of the adult.

MATERIAL AND METHODS

North of the Zoology Laboratory (Madras University) where the present investigations were carried on is the River Cooum. Except in the monsoons the river does not flow into the sea because of the formation of a sand bar at its mouth. By virtue of the formation of the sand bar at the mouth, about 100 yards up the river, the bed of the river is sandy and beyond it, it becomes gradually soft and clayey. The salinity of the waters at the mouth is almost that of seawater and decreases as one travels up the river into the interior. In the vicinity of the clayey region the salinity of the water ranges between 20 $^{o}/_{oo}$ and 34 $^{o}/_{oo}$ in the different months of the year, it being the minimum during the rainy season and highest in the hotter months. Thus there are fluctuations in the degrees of salinity all the year round. Here

may be seen during February to September, and in lesser numbers in the months of December to February (Aiyar, 1931), numerous pear-shaped masses of jelly which are the egg-cases of *Marphysa gravelyi*. Embedded in this jelly are large numbers of eggs, distributed evenly throughout the spawn. These egg-cases are firmly rooted in the soft mud by long stalks. Since the soil is soft, they can, however, be dug out easily. Such egg-cases were collected carefully and brought to the Laboratory along with the water. They were left in glass tanks and samples of eggs were removed from the cocoon from time to time for study. An account of the development has already been given by Aiyar (1931). As an adaptation to its habitat, the *trochophore* stage in the development of *Marphysa* is eliminated and even the earlier stages of development are undergone within the jelly. It is only when a stage which corresponds to the initial *metatrochophore* stage is reached, after a period of $3\frac{1}{2}$ days, that the larvæ come out of the jelly and swim about in the medium. Such larvæ were collected for experimentation.

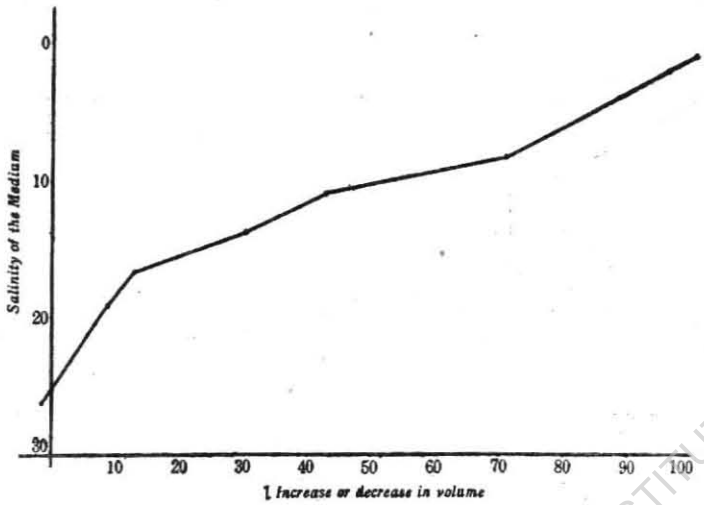
The object of the present investigation being the determination of the effects of different concentrations on the volume changes in the eggs and larvæ, it was necessary to measure their volumes. The method suggested by Weil and Pantin (1931 *a*, *b*) was followed in determining the diameter and area of the eggs and larvæ respectively. The diameter was measured direct by making use of an ordinary ocular-micrometer. Instead of a ghost-micrometer a net-micrometer was used in the eye-piece and the areas of the larvæ were directly read off. For every such determination eye-piece $\times 5$ and objective $\times 40$ were used and the magnification kept constant. Since the eggs are spherical, the volumes of the eggs were calculated by making use of the formula $1/6\ 22/7\ d^3$ as suggested by Krogh (1939). The volume of each egg and larva recorded in this paper represents the mean volume of six readings. For experimentation the eggs and larvæ from a single spawn were used.

A. *Experiments on Eggs*

Experiment I.—*Hypotonic Media and its Effect on Volume Changes in the Eggs*

(*a*) *Increase in Volume in Different Hypotonic Media.*—Six eggs of a single spawn were isolated from the jelly and the diameter was first measured and the volume calculated. They were transferred to petri dishes containing sea water of a particular strength for 30 minutes. At the end of which the diameter of each egg was read and the volume calculated.

In a similar way the experiment was repeated with different concentrations of the medium. Graph 1 indicates that the increase in volume is



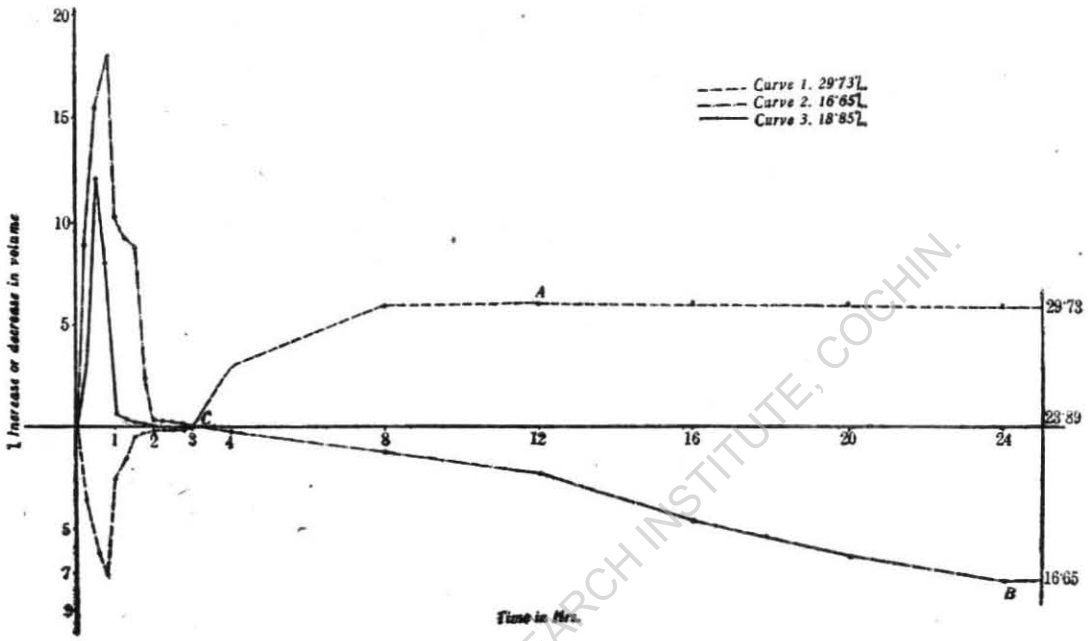
GRAPH 1. Increas in volume in different Hypotonic Media

proportional to the dilution of the medium used. This observation agrees with the results of Lillie (1916) and Runnstrom (1925) on the eggs of *Arbacia* and *Paracentrotus lividus* respectively.

(*b*) *Volume Changes in Hypotonic Media during Different Intervals.*— 400 Eggs taken from a single spawn were left in a petri-dish containing hypotonic medium of a known concentration. The volumes of six eggs were determined for a period of 3½ hours at intervals of every 15 minutes. The figures recorded indicate that the volume increases in about 30 to 45 minutes and decreases subsequently (Curve 3, Graph 2). The eggs reach their original volumes irrespective of the strength of the medium within 3½ hours.

(*c*) *Volume Changes in Hypotonic Media during Different Intervals.*— Five to six hundred eggs were immersed in a medium of a particular concentration (16·65‰) and the volumes of six eggs were determined after an interval of every 15 minutes for a period of 3 hours and later after intervals of 4 hours for a period of 40 hours. Curve 2 in Graph (2) shows the results of such an experiment. They indicate that the eggs increase in volume within the first half hour and then begin to decrease in volume. The volume shrinks to the original level in 3½ hours but the decrease continues for about 24 hours till the egg is 92·6% of the original size. There is no shrinkage beyond this upto a period of 40 hours.

During this decrease in volume obviously due to loss of salts, nearly 41·2% of the eggs died within 24 hours and about 50% died in 40 hours. It is quite probable that the initial increase in volume was due to the higher concentration of the egg and later due to loss of salts as well as water there



GRAPH 2. Volume Changes in Hypotonic and Hypertonic Media during different intervals

was shrinkage of volume. The differences in the rate and amounts in loss of salts essential for normal well being, probably account for the mortality of 50% of the eggs—for by 96 hours all the eggs died (*vide infra*).

(*d*) *Volume Changes in Hypertonic Media during Different Intervals.*— 200 Eggs were immersed in a hypertonic medium of 29·73‰ and the volumes of six eggs were determined after an interval of every 15 minutes for a period of 3 hours and later after intervals of 4 hours for a period of 32 hours. Curve 1, Graph (2) shows the results of such an experiment. The readings recorded indicate that the volumes decrease at first and reach the maximum in 45 minutes and subsequently the volumes increase. The volume increases to the original level in 3 hours and the increase continues for about 12 hours till the egg is 106·2% of the original size. There is no increase in volume beyond this upto a period of 32 hours.

During this increase in volume, obviously due to uptake of salts and water, nearly 41·6% of the eggs died within 12 hours and about 75% died in 32 hours. It is quite probable that due to the higher concentration of the external medium, there is an initial decrease in volume and later due to uptake of water as well as salts from the medium there is an increase in volume. The differences in the rates of diffusion of salts and water into the egg probably account for the different percentages of mortality and also the final volume being greater than the original.

A comparison of the Curves 1 and 2 in Graph (2) suggests that as at A and B the eggs may be isotonic with the two different media used, it is probable that C gives the osmotic concentration of the egg.

EXPERIMENT II.—*Effect of Hypotonic Media on the Development : Eggs*

(*a*) *On Eggs without Jelly.*—Numerous eggs removed from the jelly were exposed to different salinities. After every 24 hours of such exposure, 100 eggs from each lot were taken and the number of eggs that were dead* were counted. Column A in Table I gives the results of such a series of experiments. The figures indicate a rise in mortality correlated with a prolongation

TABLE I

Effect of Hypotonic Media on Development—On Eggs without jelly, with jelly and larvæ

Concentration of the medium ‰	A				B			C			
	Egg—Without Jelly				Egg—With Jelly			Larva			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Distilled water	..	..	..	..	5%	7%	nil	..	..	..	..
11·40	..	..	..	..	3%	6%	nil	..	..	..	..
14·45	..	..	..	..	..	..	..	100%	100%	100%	100%
15·39	..	..	..	..	2%	5%	nil	..	..	..	..
15·65	..	..	..	..	..	..	..	53%	51%	53%	53%
16·82	..	..	..	..	..	..	..	24%	22%	22%	23%
17·50	..	..	..	..	..	..	..	2%	nil	nil	nil
17·78	33·3%	86·6%	88·3%	100%	..	..	..	..	..	..	..
17·86	..	..	..	..	6%	7%	nil	..	..	..	..
18·42	10·0%	83·3%	84·3%	100%	..	..	..	..	..	..	..
20·63	..	..	..	..	3%	3%	nil	..	..	..	..
20·65	..	..	..	..	..	..	..	2%	nil	nil	nil
21·88	..	..	..	..	..	..	..	2%	nil	nil	nil
22·18	..	..	..	..	..	..	..	1%	nil	nil	nil
24·33	..	..	..	..	4%	3%	nil	..	..	..	..
30·12	6·6%	66·6%	100·0%	100%	..	..	..	..	..	..	..
31·05	..	..	..	..	..	..	..	1%	nil	nil	nil
31·11	..	..	..	..	nil	nil	nil	..	..	..	..
31·30	..	..	..	..	..	..	..	nil	nil	nil	nil
33·42	..	..	..	..	..	..	..	nil	nil	nil	nil

of exposure, till all the eggs died after 96 hours. The table also indicates that the mortality increases in media of lower salinity.

(*b*) *On Eggs with Jelly.*—A single, complete and uninjured egg case was allowed to develop in each of the different media of known salinities. After

* Complete cessation of the setting movement of the yolk indicated the outset of death.

a duration of every 24 hours the number of eggs that were dead were counted. It is evident from Column B in Table (I) that the rate of mortality is comparatively low and at the end of 72 hours all the eggs hatched out into larvæ irrespective of the salinity of the outside media in which they were left.

B. *Experiments on Larvæ (Initial Metatrochophore)*

EXPERIMENT I.—*Hypotonic Media and Its Effect on Volume Changes*

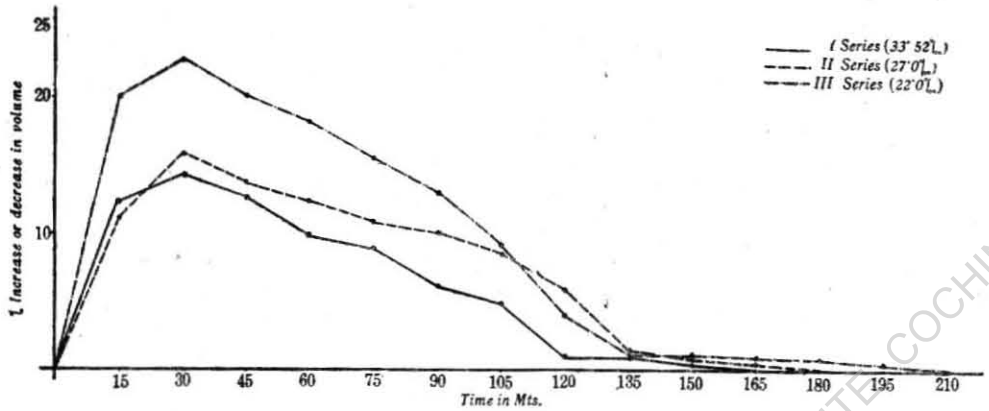
(*a*) *Increase in Volume in Different Hypotonic Media.*—The volumes of the larvæ hatched in the laboratory were first determined. Six of them were left in each of the seven petri dishes containing different concentrations of brackish water of the natural habitat of the worm. The volumes of these larvæ were measured after 30 minutes in each case so as to determine the effect of the different media on the volumes of the larvæ. Table II shows that in and beyond a salinity of 14·43 °/₀₀ the larvæ swell and disintegrate.

Concentration of the medium ‰	Laivae Initial mean volume 72C μ V	%
Distilled water	DISINTEGRATES	
7·43 ..	..	..
9·43 ..	DISINTEGRATES	
10·83 ..	..	..
13·31 ..	..	..
14·43 ..	DISINTEGRATES	
16·72 ..	..	..
17·76 ..	91·33	24·0
19·20 ..	..	..
21·10 ..	88·33	22·7
26·01 ..	..	..
26·10 ..	84·50	17·4
34·14 ..	81·66	13·4

(*b*) *Volume Changes in Hypotonic Media during Different Intervals.*—A number of larvæ were exposed to hypotonic media of different concentrations graded from 33·52 °/₀₀ to 22 °/₀₀ in order to determine their behaviour at different intervals. They were exposed for 3½ hours and the volume measured at an interval of every 15 minutes. Graph (3) shows that in all cases the original volume was regained after a duration of 3½ hours and that the larvæ are capable of reaching osmotic equilibrium in the different concentrations of the media.

EXPERIMENT II.—*Hypotonic Media and Their Effects on Development : Larvæ*

A number of larvæ of same age were allowed to continue their development in nine different grades of hypotonic media of known salinities in order to test their effect on the development of the larvæ. Controls were also



GRAPH 3. Volume Changes in Hypotonic Media during different intervals

maintained. After every 24 hours the rate of mortality was calculated and Column C in Table I shows the readings of such a series of experiments. The experiments were continued for a period of 96 hours at the end of which period all the larvæ developed into the next stage. The low rate of mortality bears testimony to the fact that the larvæ continued their development irrespective of the low salinity of the media in which they were allowed to develop. It is further evident that larvæ are not able to tolerate a medium which falls below a salinity of 14·45 °/₀₀.

DISCUSSION

The investigations of Loeb and Westeneys (1915) on the eggs of *Fundulus heteroclitus* are of interest since they found that the eggs of this marine fish can develop and hatch in distilled water. They conclude that the protoplasmic membrane of these eggs is impermeable to salts and almost impermeable to water. Ramult (1925) who studied the influence of salt solutions upon the development of *Daphnia* eggs, Gray (1920, 1932) and Krogh and Ussing (1937) who studied the eggs of *Salmo*, a fresh water trout, have come to a similar conclusion since they found a normal and healthy development in all of them due to the impermeable membrane, conserving and protecting the internal concentration. The envelopes of the eggs of the present form show that they are permeable both to water and salts and exposure for about 24 hours to a hypotonic medium of 17·78 °/₀₀ proves harmful for the normal development of about 33·3% of the eggs. If the exposure is for 48 hours the percentage of mortality of the eggs rises to 86·6%, until at the end of 96 hours all are dead. In a state of nature, however, the eggs are covered with jelly and changes in salinity through long periods of exposure do not affect the development of eggs. Therefore it can be concluded that the

envelop of jelly protects the eggs and serves the same purposes as the non-permeable egg coats of animals without jelly.

In the light of the observations made by August Krogh, Agnes Krogh and Wernstedt (1938) on the osmotic behaviour of the eggs of *Pleuronectes flesus* and *Crenilabrus exoletus*, teleosteon fish, the conclusion that the eggs of the present form are permeable both to salts and water seems to be justifiable. The behaviour of the eggs, which these authors studied, show that they decrease in diameter in water of 25–34 $^{o}/_{oo}$ at first, but increased to the original later. The eggs of the Polychæte, *Marphysa gravelyi*, also decreased in diameter in water of 29·73 $^{o}/_{oo}$ in the first 45 minutes but increased to the original in 3 hours, suggesting that they are permeable both to salts and water. Needham (1930), Ephrussi and Rapkine (1928) who studied the osmotic behaviour of the eggs of *Strongylocentrotus lividus* during their development stress the fact that they absorb large amounts of salts from the surrounding water. Ranzi (1930) observes that the eggs of *Sepia officinalis* increase in weight, ash content and water content during development. But in the present form shrinkage in volume due to loss of water and salts is seen. It is quite probable that due to the loss of salts and water which are quite essential for a normal and healthy development the eggs die and the rise in the percentage of mortality is directly proportional to the length of exposure. But the initial increase in volume in the eggs of the present form is probably due to the fact that the rate of inflow of water is greater than the rate of loss of salts, which indicates that the eggs must have had a greater concentration at the beginning. Similar conclusions were arrived at by Lillie (1916, 1918), Northrop (1926–27) and Runnstrom (1925) who observed an increase in the volumes of the eggs of echinoderms when they were subjected to the effect of different hypotonic media.

The experiments regarding the effect of different hypotonic media on the volume changes of the larvæ suggest that when they are exposed to different diluted media there is an inflow of water through the skin. But when they are exposed for a long time, they show a recovery of the original volume, indicating loss of water probably through the excretory organs. Since nephredia of the protonephridial type are already developed at this stage of the larvæ, it can be supposed that the excretory organs are responsible for osmo-regulation. Westblad (1922) who came to a similar conclusion, emphasises the fact that the flame cell system in the turbellaria is mainly concerned with osmo-regulation. Herfs (1922) by observing the rate of pulsation of flame cell system in rotifers and trematodes in solutions of different osmotic pressure, has obtained more direct evidence of their osmo-regulatory action. In the light of the above observations it is justifiable to

conclude that the recovery of the original volume, through the excretion of the water taken up during swelling, and the maintanance of a constant internal concentration, must be due to the activity of the protonephridial organs in the larvæ.

Krogh and Ussing (1937) further observe that the production of impermeable plasma membranes is probably a general mechanism for the protection of eggs in fresh water against osmotic swelling, a protection which can be dispensed with when mechanisms for the excretion of water become functional in the embryo. The observations of Ikeda (1937 *a*) on the eggs of *Oryzias latipes*, a fish living in fresh water and brackish waters of Japan, furnish another example of this kind. Since in the present form there is already a larval kidney (of the type of protonephredia) functioning, the larvæ seem to have dispensed with the protection afforded by the jelly. The almost insignificant low rate of mortality of the larvæ when they are exposed to different hypotonic media further strengthens the conclusion that the larvæ at this stage are able to maintain a steady internal concentration due to the presence of the functional kidneys which begin to actively excrete water.

The different modes of behaviour as exhibited by the eggs and larvæ when they are exposed to the effects of similar hypotonic media, further suggest that the eggs can scarcely be supposed to possess mechanisms specially adapted for invasion of water with fluctuating salinities, whereas the larvæ are endowed with well-developed excretory organs which serve as the osmoregulatory mechanism, as evidenced by the present investigations.

Summary

1. Experiments regarding the effects of the hypotonic and hypertonic media on the volume changes are described and the conclusion that the eggs are passive as far as the transport of the salts and water are concerned is arrived at.

2. The rate of mortality observed when eggs with jelly and without jelly were subjected to hypotonic media is recorded and the conclusion that the envelop of jelly acts in the same way as an impermeable membrane in the case of eggs of other animals is drawn.

3. The effects of hypotonic media on the volume of larvæ is described and the role played by the excretory organs and their importance in osmoregulation is indicated.

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BIBLIOGRAPHY

1. Adolph, E. F. .. "Differential permeability to water and osmotic exchanges in the marine worm *phascolosma*," *J. Cel. Comp. Phisol.*, 1936, **9**, 117–35.

2. Aiyar, R. G. .. "An account of the development and breeding habits of brackish-water polychæte worm of the genus *Marphysa*," *Jour. Lin. Soc. Zool.*, 1931, **37**, 387–403.

3. Beadle, L. C. .. (*a*) "Osmotic regulation in Gunda ulvae," *J. Exp. Biol.*, 1934, **11**, 382–96.

(*b*) "Adaptations to changes of salinity in the Polychætes. I. Control of body volume and body fluid concentration in *Nereis diversicolor*," *Ibid.*, 1937, **14**, 56–70.

(*c*) "Osmotic regulation and the faunas of inland waters," *Biol. Rev.*, 1943, **18**, 172–83.

*4. Bethe, A. .. "Die salz-und Wassendurchlassigkeit der korperoberflachen verschiedener Seetiere in ihrem gegenseitigen Verhaltnis," *Pflug. Arch. ges. Physiol.*, 1934, **234**, 629–44.

*5. Bialaszewicz, K. .. "Contributions a'l'e'tude de la composition minerale des cellules-oeufs," *Pubbl. Staz. Zool. Napoli.*, 1927, **8**, 355–69.

6. Ellis, W. G. .. (*a*) "Calcium and the resistance of Nereis to brackish-water," *Nature*, Lond., 1935, **132**, 748.

(*b*) "The water and electrolyte exchange of *Nereis diversicolor*," *J. Exp. Biol.*, 1937, **14**, 340–50.

7. Euphrussi, B. and Rapkine, L. .. *Ann. Physiol. Physicochim. Biol.*, 1928, **4**, 386.

8. Gray, J. .. (*a*) "The relation of the animal cell to electrolytes. I. A physiological study of the egg of trout," *J. Physiol.*, 1920, **53**, 308–19.

(*b*) "The osmotic properties of the eggs of the trout (*Salmo fario*)," *J. Exp. Biol.*, 1932, **9**, 277–99.

*9. Herfs, A. .. "Die pulsierende Vakuole der Protozoen, ein Schutzorgan gegen Aussussung. Studien uber Anpassung der Organismen an das Leben im Susswasser," *Arch. Protistenk*, 1922, **44**, 227–60.

10. Ikeda, Y. .. "Effect of sodium and potassium salts on the rate of development in *Oryzias latipes*," *J. Fac. Sci. Tokyo Imp. Univ.*, 1937 *a.*, **4**, 307–12.

11. Krogh, A. .. *Osmotic Regulations in Aquatic Animals*, Camb. Univ. Press, 1939.

12. Krogh, A. and Ussing, H. H. "A note on the permiability of trout eggs to D_2O and H_2O," *J. Exp. Biol.*, 1937, **14**, 35–37.

13. Krogh, A. Agnes Krogh and Wernstedt, C. — "The salt concentration in the tissues of some marine animals," *Skand. Arch. Physiol.*, 1938, **80**, 214–22.

14. Loeb, J. and Westeneys, H. — "Note on the apparent change of the osmotic pressure of cell contents with the osmotic properties of the surrounding solution," *J. Biol. Chem.*, 1915, **23**, 157–62.

15. Luke, B. and McCutcheon — "The effect of hydrogen ion concentration and swelling of cells," *J. Gen. Physiol.*, 1925–26, **9**, 709.

16. Lillie, R. S. .. (*a*) Increase of permeability to water following normal and artificial activation in sea urchin eggs," *Amer. Jour. Physiol.*, 1916, **40**, 249–66.

(*b*) "The increase or permeability to water in fertilised sea urchin eggs and the influence of cyanide and anæsthetics upon this change," *Ibid.*, 1918, **45**, 406–30.

17. McClendon, J. F. .. "Electrolytic experiments showing increase in permeability of the egg to ions at the beginning of development," *Science*, 1910 *a*, **32**, 122–24.

(*b*) "Further proofs of the increase in permeability of the sea urchin egg to electrolytes at the beginning of development," *Ibid.*, 1910 *b*, **32**, 317–18.

18. Needham, J. .. "On the penetration of marine organisms into fresh water," *Biol. Zbl.*, 1930, **50**, 504–09.

19. Needham, J. and Needham Dorothy .. (*a*) "Nitrogen-excretion in Selachian ontogeny," *Brit. J. Exp. Biol.*, 1930, **7**, 7–18.

(*b*) "On phosphorous metabolism in embryonic life. I. Invertebrate eggs," *Ibid.*, 1930, **7**, 317–48.

20. Northrop, J. N. .. "Kinetics of the swelling of cells and tissues," *J. Gen. Physiol.*, 1926–27, **11**, 43.

21. Ramult, M. .. "Development and resisting power of Cladocera embryos in the solutions of certain inorganic salts," *Bull. Inst. Acd. Sci. Cracovie.*, 1925, 135–94.

*22. Ranzi, S. .. "La'ccrescimento dell' embrione dei cefalopodi," *Arch. Entw-Mech. Org.*, 1930, **121**, 345–65.

23. Runnstrom, J. .. "Ueber den Einfluss des Kalimmangels auf das secigelie. Experimentelle Beitrage zur Kenntuiss des Plasmabaues, der Teilung und der Determination des Eies," *Publ. Staz. Zool. Napoli.*, 1925, **6** (1).

24. Schlieper, C. .. "Ueber die Ein wirkung niederer salzkonzentrationen auf marine organismen," *Z. Vergl. Physiol.*, 1929, **9**, 478–514.

25. Weil, E. and Pantin, C. F. A. (*a*) "The adaptation of *Gunda ulvae* to salinity. I. The Environment," *J. Exp. Biol.*, 1931 *a*, **8**, 63–72.

(*b*) "The adaptation of *Gunda ulvae* to salinity. II. The Water exchange," *Ibid.*, 1931 *b*, **8**, 73–81.

*26. Westblad, E. .. "Zur Physiologie der Turbellarien. II. Die Exkretion," *Lunds. Univ. Arsskr.*, 1922, **18**, No. 6.

* Not referred to in the original.

APPENDIX 2.

Salinity Tolerance and Volume Regulation in four species of Polychaetes.

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SALINITY TOLERANCE AND VOLUME REGULATION IN FOUR SPECIES OF POLYCHAETES

BY

B. KRISHNAMOORTHI

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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 erimita*, *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).

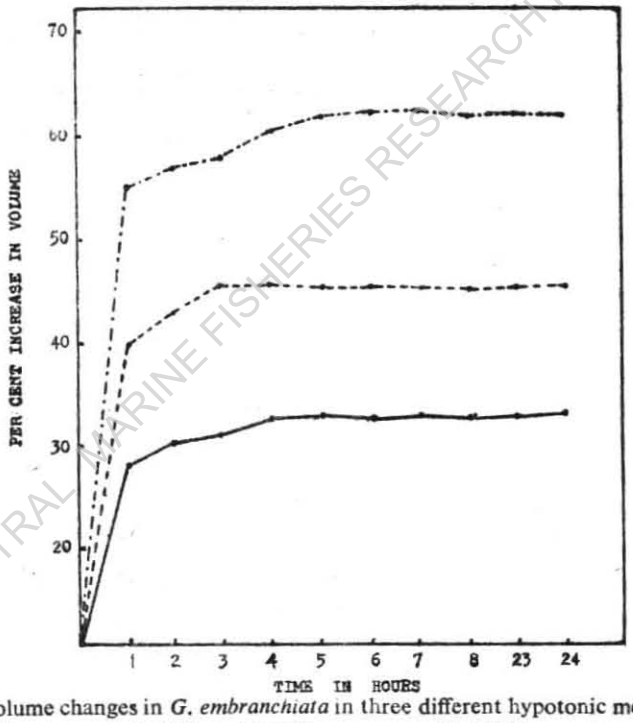

FIG. 1. Volume changes in *G. embranchiata* in three different hypotonic media during different intervals. (·—·—·—· 8·62‰; ·—— 13·70‰; ·—— 20·72‰.)

Experiment III. Volume Regulation in Hypotonic Media in O. ermita.—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 experi-

mental 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

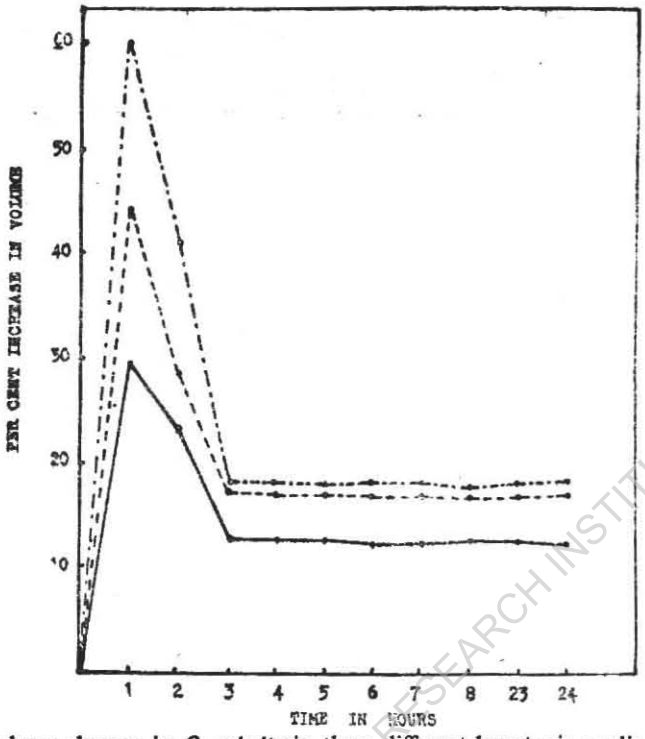

FIG. 2. Volume changes in *O. erimita* in three different hypotonic media during different intervals. (·—·—·—· 8·62‰; — — 17·30‰; ——— 20·72‰.)

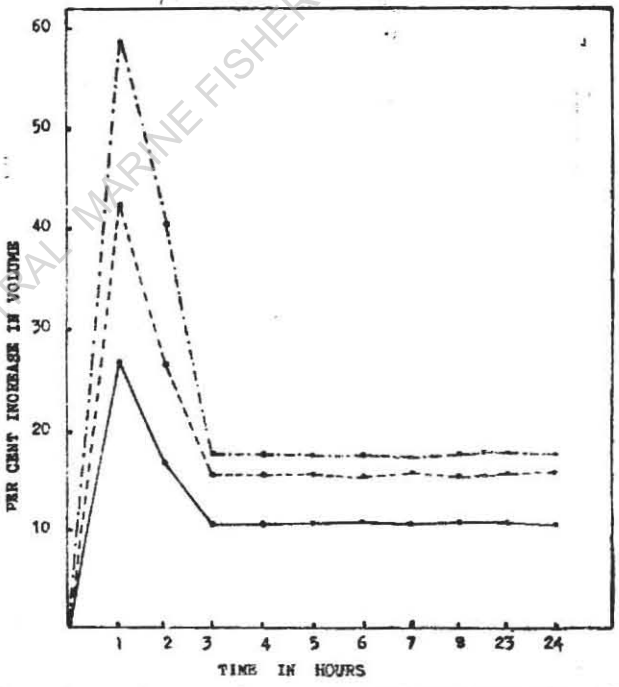

FIG. 3. Volume changes in *L. medusa* in three different hypotonic media during different intervals. (·—·—·—· 8·62‰; — — 13·70‰; ——— 20·72‰.)

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).

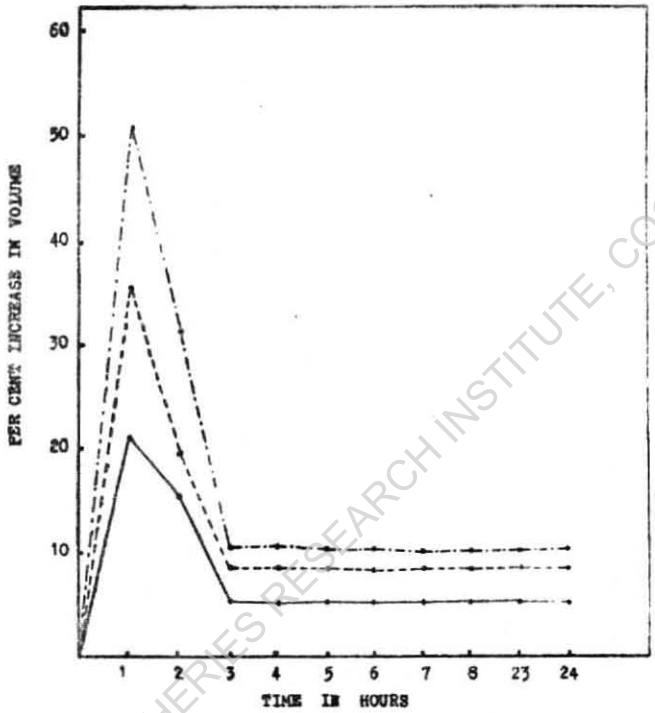

FIG. 4. Volume changes in *C. insecta* in three different hypotonic media during different intervals. (•—•—•—• 8·62‰; 13·70‰; •——• 20·72‰; ←– – –→)

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. ertmita*; 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. ertmita*.

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. ertmita* 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. ertmita* showed the highest initial increase and the lowest subsequent decrease. *L. medusa* ranked in between *O. ertmita* 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. ertmita* 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. ertmita*, the responses of this worm to osmotic stresses were thus different from those exhibited by either *O. ertmita* 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. ertmita* 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. ermita* 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. ermita* 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. ermita*, *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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References

Beadle, L. C. .. "Adaptation to changes of salinity in the polychaetes. I. Control of body volume and of body fluid concentration in *Nereis diversicolor*," *J. Exp. Biol.*, 1937, **14**, 56–70.

Ewer, D. W. and Ewer, E. F. .. "Osmotic regulation in *Sabella pavonina*," *Nature*, London, 1943, **152**, 598–99.

Jørgensen, C. B. and Dales, R. P. "The regulation of volume and osmotic regulation in some nereid polychaetes," *Physio. Comp. et Oecol.*, 1957, **4**, 357–74.

Krishnan, G. .. "On the nephridia of Nereidae in relation to habitat," *Proc. Nat. Inst. Sci. India*, 1952, **18**, 241–55.

Krishnamoorthi, B. .. "Studies on the Nephridia of Polychaetes," *M.Sc. Thesis of the University of Madras*, 1951 (unpublished).

——— and Krishnaswamy, S. .. "Activity of *Marphysa gravelyi* Southern (polychaeta) under heterosmotic conditions, 1962 (in press).

Lowndes, A. G. .. "The displacement method of weighing aquatic animals," *J. Mar. Biol. Ass.*, U.K., 1942, **25**, 555–74.

Prosser, C. L. (Edr.) .. *Comparative Animal Physiology*, Saunders, Philadelphia and London, 1950, pp. 888.

Sayles, L. P. .. "The effect of salinity changes on body weight and survival of *Nereis virens*," *Biol. Bull. Wood's Hole*, 1935, **69**, 233–44.

Schlieper, C. .. "Die osmoregulation wasserlibender Tiere," *Biol. Rev.*, 1930, **5**, 309–56.

Topping, F. L. and Fuller, J. L. "The accommodation of some marine invertebrates to reduced osmotic pressures," *Biol. Bull.*, 1942, **82**, 370–84.

APPENDIX 2.

Activity of *Nereis virens* Southern (Polychaeta) under heterosmotic conditions, (WITH S. Krishnaswamy).

Proc. Indian Acad. Sci., 57: 22 - 27, (1963).

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ACTIVITY OF *MARPHYSA GRAVELYI* SOUTHERN (POLYCHAETA) UNDER HETEROSMOTIC CONDITIONS

BY

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ACTIVITY OF *MARPHYSA GRAVELYI* SOUTHERN (POLYCHAETA) UNDER HETEROSMOTIC CONDITIONS

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Received September 20, 1962

INTRODUCTION

Marphysa gravelyi Southern is a common polychaete which occurs in the muddy substratum of the brackish-water regions of Madras, where salinities fluctuate over a wide range (Panikkar and Aiyar, 1937). In the laboratory under experimental conditions the worm is able to tolerate dilutions of sea-water ranging from 20–70% without any ill-effects (Krishnamoorthi, 1951). An attempt was made to see the extent to which the tissues of the animal would tolerate sea-water dilutions. Similar studies have been reported by Wells and Ledingham (1940). A knowledge of the toleration of the tissues to hypo- as well as hypertonic media, will help in understanding the importance of the constancy of an internal fluid and its regulation.

MATERIAL AND METHODS

Marphysa gravelyi was collected in the brackish-water regions of the Adyar estuary. The worms were washed in the medium and kept in water collected from the estuary. Survival rate was good and the worms remained in a healthy condition for over a week. The estuarine water at the time of collection was of the same strength as 30% sea-water (salinity of sea-water: 32·0‰).

The preparation for recording the activity consisted of a small strip of the animal 3·2 cm. long. The head was removed and the first few anterior segments were utilised. One end of the worm was pinned on to a piece of cork and the other end was connected to an isotonic lever. The preparation was exposed to dilutions of sea-water in a glass tube of 30 ml. capacity. The movements were recorded in a kymograph drum, rotating at a very slow speed of 1·5 cm. per minute for 5–6 hours. The preparation was exposed to a constant volume of the experimental medium for a period of only 15 minutes so that the shock effects of the change in medium rather than slow acclimatisation were studied.

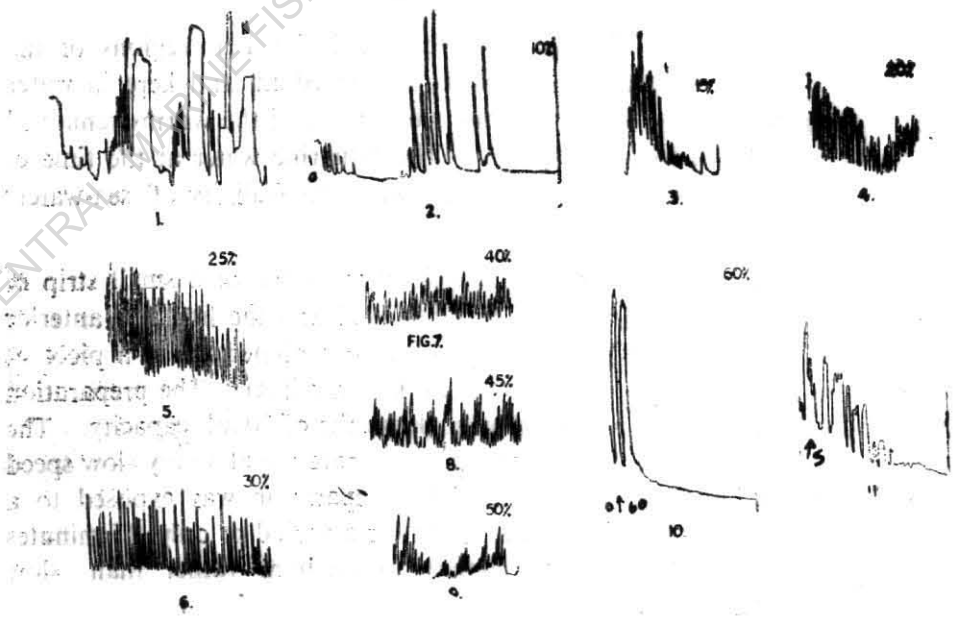
RESULTS

1. *Effect of Hypo- and Hypertonic Media*

Whole worms (Fig. 1) as well as bits of worm (Fig. 6) exhibited spontaneous activity which was maintained for long periods of time. No rhythmicity could be recognised in the activity. In normal medium there is a continuous peristalsis going on, there being rapid contractions and relaxations of the body muscles which is clear from the traces.

When the medium (30% sea-water) is replaced with 25% and 20% sea-water, there is an increase in activity which is maintained for several hours (Figs. 4 and 5). The preparation exhibits very rapid contractions of the body. There is a definite drop in the height attained indicating that the preparation is in a slightly relaxed condition. In 15% sea-water it is very vigorous to begin with but trails off at the end of a few minutes (Fig. 3). When the medium is changed and replaced with 10% sea-water, there is very little activity at first, but becomes more active at the end of a few minutes only to remain quiescent again (Fig. 2). If replaced with distilled water there is practically no activity. In all the hypotonic media below 20% the markings do not touch the base line indicating of the preparation being always in a slightly contracted condition.

The preparation, when transferred to hypertonic media of sea-water diluted to 40–45% and 50%, continued to be active (Figs. 7, 8, 9). In 60%,



FIGS. 1–11.

70% and higher concentrations the worm contracts and remains so (Fig. 10). When sucrose or urea is added to the preparation in 30% sea-water, there is a sudden decrease in activity suggestive of the worms responding to osmotic stress which seems to be the main factor affecting the behaviour of the worm (Fig. 11).

2. *Effect of Ions*

Preliminary experiments conducted in the laboratory show that there is a loss of salts from the worm in hypotonic medium as indicated by change in conductivity (Table I). It was therefore felt desirable to study the effect of various ions so as to see how they affect the activity of the animal.

TABLE I

Time	Reading at 1 hr. intervals in dilutions of sea-water*						
	10%	20%	30%	40%	50%	60%	70%
Initial ..	$0{\cdot}60\times10^{4}$	$1{\cdot}23\times10^{4}$	$1{\cdot}70\times10^{4}$	$2{\cdot}20\times10^{4}$	$2{\cdot}60\times10^{4}$	$3{\cdot}10\times10^{4}$	$3{\cdot}90\times10^{4}$
1 hr. ..	$0{\cdot}65\times10^{4}$	$1{\cdot}25\times10^{4}$	$1{\cdot}70\times10^{4}$	$2{\cdot}20\times10^{4}$	$2{\cdot}60\times10^{4}$	$3{\cdot}10\times10^{4}$	$3{\cdot}90\times10^{4}$
2 hrs. ..	$0{\cdot}68\times10^{4}$	$1{\cdot}25\times10^{4}$	$1{\cdot}70\times10^{4}$	$2{\cdot}14\times10^{4}$	$2{\cdot}55\times10^{4}$	$3{\cdot}10\times10^{4}$	..
3 hrs. ..	$0{\cdot}67\times10^{4}$	$1{\cdot}27\times10^{4}$	$1{\cdot}71\times10^{4}$	$2{\cdot}19\times10^{4}$	$2{\cdot}58\times10^{4}$	$2{\cdot}90\times10^{4}$	..
4 hrs. ..	$0{\cdot}63\times10^{4}$	$1{\cdot}25\times10^{4}$	$1{\cdot}68\times10^{4}$	$2{\cdot}21\times10^{4}$	$2{\cdot}59\times10^{4}$	$3{\cdot}10\times10^{4}$	..

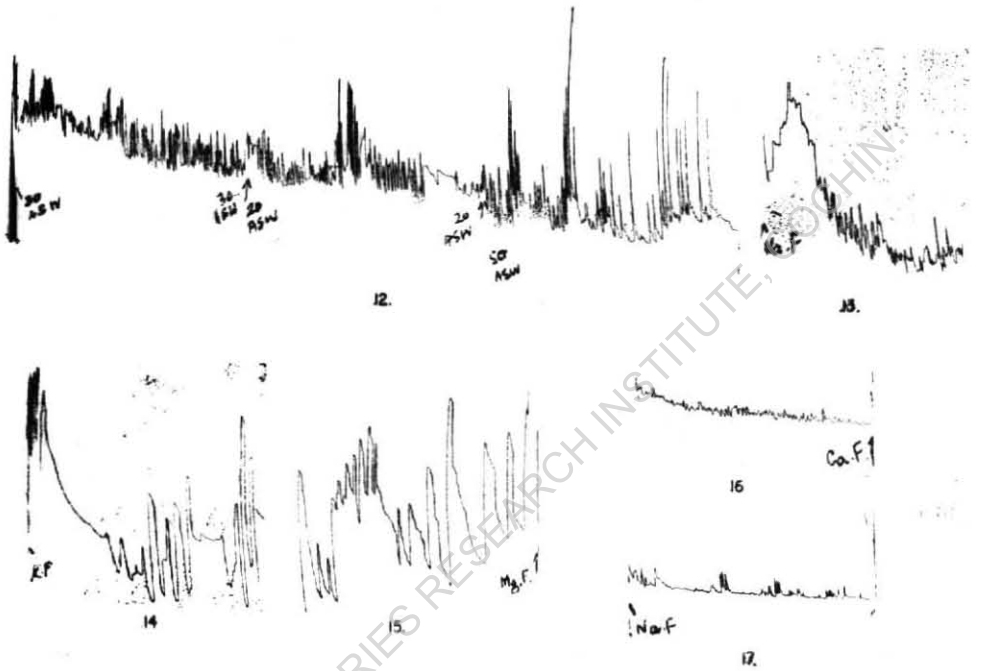
* All values are in μ mhos taken with a Mullard Conductivity Bridge and a dip-type of electrode.

Artificial sea-water prepared according to the formula given by Pantin (1948) and diluted to 30% also elicited responses comparable to normal medium. The preparation remained active and healthy for several hours (Fig. 12). The activity in artificial sea-water without the particular ion is shown in Table II. When worms first treated with Ca^{+} free sea-water, were subjected to Na^{+} free water, they showed reduced activity (Fig. 17).

TABLE II

Ion	Remarks
Na^{+} free	Activity present (Fig. 13)
K^{+} free	Activity as in Na^{+} free (Fig. 14)
Ca^{+} free	Completely contracted but activity present (Fig. 16)
Mg^{+} free	Complete relaxation. Considerable activity present (Fig. 15)

From Table II it would be clear that in K^+, Na^+ and Ca^+ free ions the activity is maintained. In Mg^+ free sea-water the animal shows greater activity as evident from the traces (Fig. 15).



FIGS. 12-17.

REMARKS

The results reported in the present paper clearly show that *Marphysa gravelyi* Southern tolerates sea-water dilutions ranging from 20 to 70%, while the muscles are active only in ranges from 20 to 50%. Thirty per cent. seems to be the optimal salinity where the animals exhibit considerable activity. Wells and Ledingham (1940) observed a similar behaviour in four species of polychaetes. The results presented, however, cannot be compared with the results obtained by Wells and Ledingham (*loc. cit.*) as only the shock effects and not the prolonged gradual acclimation have been studied here. The body volume changes very rapidly on transferring to hypotonic media. Therefore, there is very little " damping effect " due to body integument. That the animal becomes very active is very interesting. In hypotonic media the spontaneous activity is quite evident and shows that the muscles are capable of working under conditions of reduced salinity up to a point. The effects of ions clearly show that Na^+, K^+ are essential. Absence of Ca^+ produces very little contraction. In the absence of magnesium ions the preparation remains very active. Wells and Ledingham (*loc. cit.*) found that the high Mg^+ con-

centration depresses the activity whereas low Mg^+ content increases the activity. The present experiments have shown clearly that there is likely to be a regulation of the essential ions by the worm as indicated by the retention of activity in very low concentrations of sea-water and also from the results obtained with ions. Presence of Na^+, K^+ and Ca^+ appears to be essential for a proper functioning. It will be interesting to study the ionic regulation in this animal to see if any particular ion is regulated.

Summary

1. Whole worms as well as body wall preparations of *Marphysa gravelyi* exhibit spontaneous activity in full strength as well as diluted sea-water.

2. The preparation is very active in sea-water dilutions from 20 to 50%.

3. In Na^+, K^+, Ca^+ free sea-water activity is present. In Mg^+ free sea-water, the preparation appears to be completely relaxed.

4. While the whole worms tolerate sea-water dilutions ranging from 20 to 70% the muscle preparation appears to tolerate only from 20 to 50% sea-water.

References

Krishnamoorthi, B. .. Studies on the Nephridia of Polychaetes. *M.Sc. Thesis of the University of Madras*, 1951 (unpublished).

Panikkar, N. K. and Aiyar, R. G. "The brackish-water fauna of Madras," *Proc. Ind. Acad. Sci.*, 1937, **6**, 286-337.

Pantin, C. F. A. .. *Notes on Microscopical Techniques for Zoologists*, Cambridge, 1948.

Wells, G. P. and Ledingham, I. C. "Studies on the physiology of *Arenicola*—I and II," *J. Exp. Biol.*, 1940, **17**, 337-63.

APPENDIX 4.

On the Gross Morphology and Histology of nephridia in four species of Polychaetes.

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GROSS MORPHOLOGY AND HISTOLOGY OF NEPHRIDIA IN FOUR SPECIES OF POLYCHAETES

By

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GROSS MORPHOLOGY AND HISTOLOGY OF NEPHRIDIA IN FOUR SPECIES OF POLYCHAETES

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I. Introduction

In a recent paper (Krishnamoorthi, 1962) it was shown that the four polychaetes studied, *viz.*, *Glycera embranchiata* Ranganathan, *Onuphis eremita* [redacted], *Loimia medusa* Savigny and *Clymene insecta* Ehlers, exhibited differences in their capacities for volume regulation when subjected to stresses of anisotonic media. Jurgens (1935), Beadle (1937) and Ewer and Ewer (1943) have brought evidence of the relative importance of excretory organs in the volume regulation of the polychaetes they had studied. It is known that the excretory organs exhibit differences in size and structure in nearly related species occurring in marine and brackish-water habitats as has been shown in fishes (Marshall and Smith, 1930; Nash, 1931); in Turbellaria (Westblad, 1922); in Crustacea (Marchal, 1892; Schlieper and Herrmann, 1930; Schwabe, 1933; Peters, 1935) and in Polychaetes (Krishnan, 1952). It appeared, therefore, that a knowledge of the anatomy and histology of the Nephridia of the above four species would throw some light to account for the differences in their abilities for volume regulation and their distribution in a brackish-water environment.

II. Material and Methods

The polychaetes studied were obtained from the brackish-water regions of Adyar, Madras, as well as the shores of Madras. While *C. insecta* and *L. medusa* were taken from the brackish-waters, *G. embranchiata* and *O. eremita* were taken from the intertidal zones of the Madras coast. The worms were collected and brought to the laboratory in earthen pots immediately. They were narcotised to ensure an extended condition with Chloral Hydrate and Menthol before fixing them. Gradual addition of 30% alcohol was also found suitable for narcotisation. Bouin's fluid, Duboscq Bouin, Zenker's fluid and Susa were found most suitable for fixation. Sections were cut

on a Spencer's Rotary Microtome to 4 to 7 μ thickness and were stained with Haemotoxylin or Borax Carmine which gave excellent results. All diagrams were made with the aid of a Camera Lucida. An ordinary micrometer was used for measurements recorded, keeping the magnification 5×8 constant.

III. (A) NEPHRIDIA IN *O. ermita* [redacted]

(i) *Previous work*

Ehlers (1864) was the first to study the nephridia in Eunicidae. But it was Goodrich (1900, 1945) who gave a detailed account of the nephridia of Eunicidae. Fage (1906) added to this knowledge describing the nephridia in some more genera. Aiyar (1933) confirmed the mixed nature of the nephridium in *Marphysa gravelyi* Southern, a common Eunicid of Madras.

(ii) *Structure*

The nephridia in *O. ermita*, as in all Eunicidae, occur a pair per segment except in the few anterior and posterior segments where there are none at all. It is trumpet shaped with a wide nephrostome and a tapering nephridial canal which becomes the narrowest before opening out by the nephridiopore (Fig. 1). Each nephridium occupies a position lateral to the longitudinal muscle and ventral to the pigment gland. It starts from the outer edge of the ventral longitudinal muscle and following its contour, runs outwards. At the level of the circular layer of muscles, it pierces through it as well as the epidermis to open by the nephridiopore at the base of the neuropodium

The broad *nephrostome*, with the anterior lip very close to the intersegmental septum and the posterior lip freely hanging into the segment, is cup-shaped (Fig. 2). The concavity of the cup is turned towards the coelomic space. It gradually narrows down till it becomes continuous with the nephridial canal. The walls of the nephrostome are made up of a single layer of similar cells 10 μ in length, with uniformly granulated and transparent cytoplasm. But the cell limits are not clear. Each cell bears a number of cilia packed together and overhanging the lumen of the nephrostome. Nuclei are excentric being towards the proximal end (Fig. 3). The nephrostome, except where it opens into the coelomic cavity, is surrounded immediately by the coelomic epithelium and an outer much vacuolated connective tissue.

The commencement of the *nephridial canal* from the narrower end of the nephrostome is not well demarkated. Cross-sections and longitudinal sections (Figs. 4 and 5) reveal that the walls of the nephridial canal are made up of a single layer of cubical cells 2 μ in length with not very distinct cell

limits. In addition to small granules, bright refringent bodies and vacuoles are present in the cells. The lumen of the canal is of uniform size all through its entire length except at the region of the nehpridiopore where it becomes constricted before opening to the outside. The nephridial canal, as the nephrostome, is surrounded immediately by the coelomic epithelium and an outer much vacuolated connective tissue.

At the time of maturity the nephridia take on the function of the genital ducts. In a gravid worm the nephrostome becomes much enlarged (Fig. 6) so also the nephridial canal to facilitate the passage of the genital products. Such a condition could be noticed only in the nephridia of the posterior segments and perhaps only the posterior segments are concerned in this process, while the nephridia of the anterior segments continue their excretory function. Similar observations in *Eunice* sp. have been made by Goodrich (1900).

III. (B) Nephridia in *Loimia medusa* Savigny

(i) *Previous work*

We owe our knowledge of nephridia in Terebellids to Milne Edwards (1838), Keferstein (1862), Cunningham (1887 *a* and *b*), Schneider (1899), Cosmovice (1880) and Hessle (1917).

(ii) *Structure*

In *L. medusa* there are three pairs of nephridia—one in the cehpalic region consisting of the first three segments and the other two in the trunk region made up of the rest of the segments. The first pair is located in the III segment while the second and the third pairs are disposed inter segmentally between the VI–VII segments and VII–VIII segments respectively. A diaphragm demarkates, at their region of the IV–V segments, the cephalic and the trunk regions (Fig. 7). This division of the body and the arrangement of the nephridia in *L. medusa* recalls the description of nephridia in *Pectinaria belgica* (Cunningham, 1887) which, however, has one more pair in the trunk region.

(*a*) *Nephridia of the Cephalic Region.*—Commencing from the nephrostome which is situated near the gut, the first part of the nephridial canal is very narrow and runs dorsalwards to open into a wider part which is longer and runs straight outwards, after an initial twist, to the parapodia of the segment II, to open out by the nephridiopore on an elevated papilla (Figs. 8 and 9).

The *nephrostome* is spherical and globular in shape with a wide slit in the middle by which it opens into the coelomic cavity near the gut (Fig. 10).

The wall of the nephrostome is formed of a single layer of cells, 6 to 7 μ long with indistinct cell boundaries. All the cells of the inner wall of the nephrostome carry a number of cilia measuring 15 μ long directed towards the nephridial canal. The lumen of the nephrostome is broad and does not decrease in size. Further there is no trace of either coelomic epithelium or connective tissue surrounding the nephrostome. Unlike in *Lanice conchilega* and *Arenicola* (Cunningham, 1887) the nephrostome is not provided with digitate processes.

In a nephridium of 1140 μ long, the first and the narrower part of the *nephridial canal*, 20 μ long, commences its course a little excentrically from the nephrostome (Fig. 10). Its wall is one cell thick (Fig. 11). The cells have indistinct boundaries but have prominent deeply staining nuclei. The cells bear cilia 8–10 μ long. The cytoplasm is uniformly granular. Vacuoles occur but rarely. Cellular inclusions like the refringent bodies are absent. It opens into the wider part of the nephridial canal.

The wider part measuring 1120 μ in length is also one cell thick (Fig. 12). The cells are 8 μ long and have better differentiated boundaries and centrally placed prominent nucleus. They bear longer cilia measuring about 20 μ long.

The cytoplasm is more granular towards the periphery of the cells and contains large refringent bodies. Hence this part of the nephridial canal is darker in colour than the rest. As Schneider (1899) describing such a condition in the nephridial canal in *Pectinaria hyperborae*, *Terebellides stromii*, *Polymnia nebulosa* and *Polymnia nidensis* considers the cells engaged in active removal of excretory products, this dark-coloured part of the nephridial canal in *L. medusa* may also be capable of a similar function.

(*b*) *Nephridia of the trunk region.*—These nephridia (Fig. 13) differ from the cephalic nephridia in the absence of the narrow region of the nephridial canal so that the lumen is wide throughout—fit for the passage of the gonadial cells. Nevertheless, there is considerable similarity in the nephrostome being spherical and globular; the nephridial canal opening out on an elevated papilla; the cells of the wall of the canal having refringent bodies. It is probable that they discharge excretory function with equal efficiency. There is no common chamber or tube connecting all the nephridia as in *Pectinaria belgica* (Cunningham, 1887).

III. (C) NEPHRIDIA IN *Glycera embranchiata* RANGANATHAN

(i) *Previous work*

Nephridia in *G. unicornis*, *G. siphonostoma* and *G. convolutus* have been described (Goodrich, 1898). Fage (1906) has described the nephridia in *G. alba* and *G. tessellata*. Ranganathan (1942) created the species and gave a brief description of the nephridium.

(ii) *Structure*

The nephridia in this form, as in all other Glycerids, are of the proto-nephromixial type (Fig. 14) and occur in all segments excepting a few anterior and posterior ones. Each nephridium consists of two parts, the proto-nephridium and the coelomoduct. The protonephridium is a large sac-like swelling provided with characteristic solenocytes; and leads into a narrow duct opening to the exterior. The coelomoduct is modified to form a funnel-shaped ciliated organ leading into a phagocytal sac (Goodrich, 1898). Composed of these parts each nephridium is intersegmental in position, the major portion being in front of the septum and the post-septal part being only of the duct, opening at the base of the parapodium.

The *protonephridium* is without an opening into the coelomic cavity and consists of a sac-like nephridial swelling and the nephridial duct. The wall of the nephridial swelling is of a single layer of syncytial cells of granular character, enclosing a large cavity into which the solenocytes open. Each solenocyte is a spherical mass of protoplasm which narrows into the proximal transparent part attached to the nephridial swelling (Fig. 15). This narrow stalk-like portion is hollowed into a tube which dilates into a large intercellular cavity within the lumen of the cell (Fig. 16). The nucleus is situated at the spherical end.

The *nephridial duct* is the narrow outward extension of this sac extending from the level of the septum to the ventral region of the parapodia where it opens to the exterior by a narrow circular nephridiopore. The walls of the duct, as in the nephridial swelling, are syncytial with scattered nuclei and uniformly granular cytoplasm (Fig. 17). The lumen of the duct is continuous with the cavity of the nephridial swelling and contain excretory products.

The presence of the excretory products in the lumen of the nephridial duct suggests their passage into it by the action of the solenocytes which probably absorb them from the coelomic fluid. Similar observations have been made by Goodrich (1898) in *Glycera siphonostoma* and by Fage (1906) in *Glycera tessellata*.

The *coelomoduct* part of the nephridium is an extremely short funnel-shaped *ciliated organ* dilating into the *phagocytal sac*. The opening at the free end of the funnel-like ciliated organ, is a wide transverse slit bounded by thick upper and lower lips. The upper lip has a transverse groove on its dorsal surface running parallel to the edge. The cavity within the ciliated organ is lined by numerous cilia which by their movements drive the excretory products along with the coelomic fluid into the phagocytal sac. The lips consists of cuboid cells 8 μ long.

The *phagocytal sac* is longer and wider than the ciliated organ. It is a thick-walled sac. Communicating in front with the ciliated organ, it is continued behind into a short blind tube which ends posteriorly to the septum. The cells forming the wall are cuboid and 12 μ long, whereas those of the blind tube are much smaller. In *Glycera siphonostoma* and *Glycera unicornis* (Goodrich, 1898) there are two blind caecae which increase the surface of this phagocytal sac. The large cuboid cells of this sac are phagocytal in character. The excretory bodies wafted into the sac along with the coelomic fluid are ingested by the cells of the sac. As there is no external outlet, it is not clear how these bodies are disposed of. Goodrich's (1945) suggestion that these bodies may be later digested by the cells and the waste matter may be passed through the walls of the protonephridial duct and thus reach the outside, seems likely.

III. (D) NEPHRIDIA IN *Clymene insecta* EHLERS

(*i*) *Structure*

There are four pairs of nephridia occurring in segments VI, VII, VIII and IX and each nephridium is of the mixonephridial type and extends the whole length of the segment. It is looped and consists of a nephrostome and two limbs of the nephridial canal, an outer limb running close to the body wall and an inner limb away from it. The inner limb opens out by the nephridiopore at a distance approximately 1/3 the length of the segment from the septum (Fig. 18). Invariably the third pair of nephridia is the largest in size.

The *nephrostome*, which is funnel-shaped, has a transverse and elliptical opening, the axis being 96 μ long and consists of two lips. The upper lip of the opening is closely attached to the septum while the lower lip hangs freely in the coelomic cavity. Its walls are made up of a single layer of uniform cubical cells. Each cell, 8 μ long, has a spherical centrally placed nucleus. The surrounding cytoplasm is granular, granulation being more at the proximal end. Cellular inclusion like the refringent bodies are absent (Fig. 19).

Arising from each cell and hanging into the lumen are a number of long cilia measuring about 15 μ. The lumen is of uniform size throughout the length of the nephrostome and is full of waste matter probably of a nitrogenous nature. The outer side of the nephrostome is covered by an extension of the coelomic epithelium which, however, stops short of the lips. The nuclei are uniformly distributed in this layer. Surrounding it is the loose and much vacuolated connective tissue which serves to fix the nephrostome to the wall of the coelomic cavity where the nuclei are not distributed uniformly.

Though the opening is larger and elliptical in form the two lips appear always apart. The inflow of the coelomic fluid is thus uninterrupted.

The *nephridial canal* is a narrow tube commencing from the narrow end of the funnel-shaped nephrostome. It is 1·2 mm. long with a cnstriction beyond 1·1 mm., and it bends about it. The walls of both the limbs are made up of a single layer of uniform cubical cells each having a size of 10 μ (Fig. 20). The outer longer limb is darker in colour, has granular cytoplasm and contains refringent bodies (Fig. 21). The cells of the nephridial canal contain vacuoles, also indicating water elimination. Arising from each cell of both the limbs are number of hair-like cilia which in a living worm can be seen actively beating away from the nephrostome. They hang freely into the lumen. Surrounding the limbs and very close to them lies the coelomic epithelium with its uniformly distributed nuclei. Outside the coelomic epithalium and surrounding the limbs is the connective tissue with its scattered nuclei.

IV. Blood Supply to Nephridia

The blood supply, the nephridia of Polychaetes receive, deserves attention because the amount of blood supply is not only a measure of the degree of metabolic activity but also throws light on the nature of their activity. In the Eunicid, *O. ertmita*, the nephridia receive blood by a branch of the ventral blood vessel as in *Eunice* sp. (Goodrich, 1900). The main branch of the ventral vessel supplies the parapodia as well as the branchia and before it proceeds to the parapodia and the branchiae proper, it gives a subsidiary branch to the nephridium which breaks into capillaries on the nephridial body and is brought back by the general circulation of the blood to the epidermis. Several of the capillaries end blind within the nephridia as in *Marphysa sanguinea*, also an Eunicid (Fuchs, 1906). The nephridia of *L. medusa* are supplied by a vessel directly from the ventral blood vessel as in *Terebella conchilega* (Cunningham, 1887), and contain blind-ending

capillaries. As far as can be studied from sections the blood supply in *C. insecta* and *G. embranchiata* also is similar to the other forms studied.

TABLE A

Blood supply in the four species of Polychaetes studied

Genera	Number of blind-ending capillaries in the worms						
	1st	2nd	3rd	4th	5th	6th	Average
Glycera ..	12	12	11	12	12	12	11·83
Onuphis ..	16	16	16	15	16	15	15·66
Loimia ..	16	17	16	16	17	17	16·50
Clymene ..	23	23	23	22	22	23	22·66

However, it is evident (Table A) that the number of blind-ending capillaries vary in the different forms. If the number of such capillaries (which are obviously of importance because the blood they bring is undoubtedly irrigating the nephridial tissue) be counted and used as an index of the degree of vascularisation, we will have a basis for the comparison of different types of nephridia, and their grade of renal activity, as has been suggested by Jaquet (1885), Meyer (1888), Cosmovice (1880) and Ewer (1941) for the polychaetes studied.

V. EXTENT OF THE EXCRETORY SURFACE OF THE NEPHRIDIUM RELATIVE TO THE SIZE OF THE WORM

Evidence from the study of the structure of the nephridia of different polychaetes go to show that since the nephrostome does not take part in the actual process of excretion, except to aid in the collection of nitrogenous waste matter, the nephridial canal, lying between the nephrostome and the external opening, must be responsible for the different renal processes. The length of the canal, implying the greater number of cells, may therefore be an index of the excretory capacity of the nephridium of any animal. In order to arrive at some value likely to be constant for different genera of polychaetes, the ratio between the length of the nephridial canal to the length of the worm was determined in the different forms studied and tabulated (Tables I, II, and III). Such a ratio was not derivable for *Glycera embranchiata* because

the cells of the wall of the nephridial canal are of syncytial nature and measurements are not likely to be accurate. It is evident from the tables that in *C. insecta* the ratio is higher than in either *O. erƒmita* or *L. medusa*. Such a grading on the basis of the excretory capacity tallies with their powers of tolerating dilution of the media and migration up the river (Krishnamoorthi, 1962). Whereas *C. insecta* is a pronouncedly euryhaline form, both *O. erƒmita* and *L. medusa* are stenohaline. Such a correlation between kidney structure and the habitat has been studied in Crustacea (Grobben, 1881; Schwabe, 1933; Panikkar, 1941); in fishes (Marshall and Smith, 1930) and in Polychaetes (Krishnan, 1952).

TABLE 1

Onuphis erƒmita—*Extent of the excretory surface relative to the length of the worm*

No.	Length of the worm mm.	No. of segments	Length of each cell of the nephridial canal μ	No. of such cells	Length of the nephridial canal μ	Length of the excretory surface μ
1	252	250	2	68	136	68000
2	248	232	2	64	128	59392
3	264	258	2	65	130	67080
4	238	220	2	63	126	55440
5	210	200	2	63	126	50400
6	272	265	2	66	132	67960
Average	247					61212

The ratio between the length of the excretory surface and the length of the worm: 61212: 247000 :: 0·247 : 1.

VI. REMARKS

Among the polychaetes studied, three, viz., *O. erƒmita*, *L. medusa* and *C. insecta*, possess nephridia of the mixonephridial type, while in *G. embranchiata* it is of the protonephromixial type with solenocytes performing the

function of excretion. Even among the former three forms, in *O. ermita* and in *L. medusa* the nephridia are simple in structure and small in size compared to that of *C. insecta* which has a nephridium with the nephridial canal long and bent on itself. While in *O. ermita* and in *G. embranchiata* there are a pair to each of the segment except in the anterior and posterior few segments where they are absent, in *L. medusa* there are only three pairs and in *C. insecta* four pairs. Further in the blood supply they receive and in the ratio of the excretory surface to the length of the worm they differ. While in *C. insecta* the nephridia receive the maximum blood supply (22·60 units) with the maximum excretory surface (1 : 0·310) in *O. ermita* the blood supply is 15·66 units and the excretory surface (ratio) is 1 : 0·247 and in *L. medusa* they are respectively 6·50 units and 1 : 0·225. In *G. embranchiata* the blood supply is 11·83 units. These differences could perhaps be attributed to their capacities for osmotic regulation as reflected by volume regulation and related to their habitats.

TABLE II

Loimia medusa—*Extent of the excretory surface relative to the length of the worm*

No.	Length of the worm mm.	No. of segments	Length of each cell of the nephridial canal μ	No. of such cells	Length of the nephridial canal μ	Length of the excretory surface μ
1	30	3	8	140	1120	6720
2	32	3	8	142	1136	6816
3	30	3	8	142	1136	6816
4	32	3	8	140	1120	6720
5	30	3	8	140	1120	6720
6	30	3	8	140	1120	6720
Average	31					6752

The ratio between the length of the excretory surface and the length of the worm: 6752 : 31000 :: 0·225 : 1.

TABLE III

Clymene insecta—*Extent of the Excretory surface relative to the length of the worm*

No.	Length of the worm mm.	No. of segments	Length of each cell of the nephridial canal μ	No. of such cells	Length of the nephridial canal μ	Length of the excretory surface μ
1	25	4	10	111	1110	8880
2	26	4	10	109	1090	8720
3	24	4	10	110	1100	8800
4	25	4	10	109	1090	8720
5	25	4	10	110	1100	8800
6	24	4	10	110	1100	8800
Average	25					8780

The ratio between the length of the excretory surface and the length of the worm: 8780 : 25000 : : 0·31 : 1.

It was argued in an earlier paper (Krishnamoorthi, 1962) that *C. insecta* showed better powers of volume regulation and consequently greater penetration into the brackish-water zones of Adyar, Madras. From the present investigations have emerged out further evidences to justify the role and importance of nephridia which may have enabled these polychaetes for better adjustments to a changing environment and to account for their distribution. The excretory surface is the maximum in *C. insecta* compared with those of either *O. ertmita* or *L. medusa*, implying that the nephridia are bigger in the former species than in the latter two species. A bigger kidney is certainly an advantage to meet the demands of baling out of water that enters in against an osmotic gradient. It further helps in keeping down the swelling of the animal to the minimum required for the maintenance of a constant internal environment for the smooth functioning of the body tissues and other organ systems. *L. medusa* and *O. ertmita*, possessing as they do smaller nephridia, have limited powers of regulation. *G. embranchiata* none at all. Grobben (1881), Schwabe (1933) and Krishnan (1952), examining a number of crusta-

ceans or polychaetes belonging to same families and genera, have shown that in the stenohaline forms the kidney is the smallest while in freshwater forms it is the biggest, the brackish-water forms ranking in between the two. In the light of the above observations, it appears reasonable to conclude that the relatively large size of the nephridia in *C. insecta* is a definite advantage which has enabled this polychaete a greater penetration into the brackish-water zones and better adaptation for volume regulation calculated to meet the demands of a fluctuating environment. *O. ertmita*, *L. medusa* and *G. embranchiata* with smaller nephridia are confined to the mouth of the brackish-water regions characterised by stable conditions. From the above it also appears reasonable to suppose that the expenditure of energy would be greater, the higher the grade of adaptation and the greater the excretory effort. The importance of vascularisation of the nephridia viewed in this light needs no emphasis. Of the polychaetes studied the blood supply the nephridia receive in *C. insecta* is the maximum, with *L. medusa*, *O. ertmita* and *G. embranchiata* following in the order mentioned. This is only to be expected since *C. insecta* alone is found in the upper reaches of the brackish-water regions while the other polychaetes have hardly passed beyond the limits of the mouth of the brackish-water regions. Krishnan (1952) has made similar observations in the three Nereids he has studied.

VII. SUMMARY

1. The anatomy and histology of four species of polychaetes, *viz.*, *O. ertmita*, *L. medusa*, *G. embranchiata* and *C. insecta* are given.

2. The nephridia in *O. ertmita*, *L. medusa* and in *C. insecta* are of the mixonephridial type while in *G. embranchiata* it is of protonephromixial type with simple solenocytes performing the function of excretion. There is a pair to each segment in the former two species excepting in the few anterior and posterior segments. But in *L. medusa* there are only three pairs and in *C. insecta* four pairs.

3. The blood supply to the nephridia and the extent of the excretory surface to the length of the worm are given. Both in blood supply and in the excretory surface *C. insecta* showed the maximum development. *L. medusa*, *O. ertmita* and *G. embranchiata* showed lesser grades of development in the order mentioned.

4. The differences in the blood supply the nephridia receive and in the extent of the excretory surface to the length of the worm have been argued as due to the different capacities for osmotic regulation as reflected by volume

regulation. Taking this a measure their distribution in a brackish-water environment has been explained.

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IX. References

Aiyar, R. G. .. "On the anatomy of *Marphysa gravelyi* Southern." *Rec. Indian Mus.*, Calcutta, 1933, **35,** 287–323.

Beadle, L. C. .. "Adaptation to changes of salinity in the Polychaetes. Control of body volume and of body fluid concentration in *Nereis diversicolor*," *Jour. Exp. Biol.*, 1937, **14,** 56–70.

Cosmovici, L. C. .. "Glandes, genitales et organes segmentaires des Annelides Polychetes," *Arch. Zool. exp. gen., Paris,* 1880, **8,** 232–372.

Cunningham, J. R. .. "Some points in the anatomy of Polychaeta," *Micr. Sci. Lond. Quart. Jour.*, 1887, 28, 239–277.

——————— .. "Nephridia of *Lanice conchilega*," *Soc. Edinb. Soc., Proc.*, 1887, **14,** 238.

Ehlers, E. .. *Die Borstenwurmer nach systematischen und anatomischen Untersuchungen dargestellt.* Leipzig, Wilhelm Engelmann, 1864-68, pp. xxx-748.

Ewer, D. W. .. "The blood system of *Sabella* and *Spirographis*," *Micr. Sci. Lond. Quar. Jour.*, 1941, **82,** 587.

——— and Ewer, F. R. .. "Osmotic regulation in *Sabella pavonina*," *Nature*, London, 1943, 152, 598-99.

Fage, L. .. "Recherches sur les organes segemnataires des Annelides Polychetes," *Sci. Nat. Zool. Paris, Ann.*, 1906, **3,** 261-410.

Fuchs, K. .. "Die Topograhie des Blutgefas systems der Chaetopodon," *Jena. Z. Naturw.*, 1906, **42,** 374-484.

Goodrich, E. S. .. "On the nephridia of Polychaeta. Part I. On *Hesione, Tyrrhena* and *Nephthys*," *Micr. Sci. Lond. Quar. Jour.*, 1897, **40,** 185-96.

——————— .. "On the nephridia of Polychaela, Part II. *Glycera* and *Goniada*," *Ibid.*, 1898, **41,** 439–57.

——————— .. "The study of nephridia and genital duct since 1895," *Ibid.*, 1945, **86,** 113–392.

Grobben, C. .. "Die Antennendruse der Crustacean," *Arb. Zool. Inst. Univ. Wien.*, 1881, **3**.

Heasle, C. .. "Zur Kenntnis der terebellomorphen Polychaetes," *Zool. Bidr.*, Uppsala, 1917, **5**, 39–258.

Jaquet, M. .. "Recherches sur le systeme vasculaire des Anneles," *Zool. Stat. Neapel. Mitt.*, 1886, **6**, 297–398.

Jurgens, Otto .. "Die Wechselbeziehungen von blutkeislauf, Atmug und osmoregulation bei Polychaeten *Nereis diversicolor* O.F. Muller.," *Zool. Jahrb. Abt. Allg. Zool.*, 1935, **55**, 1–46.

Keferstein, W. .. "Untersuchungen uber niedere seethiere," *Zeits. Wiss. Zool.*, Leipzig, 1862, **12**, 1–147.

Krishnan, G. .. "On the nephridia of Nereidae in relation to habitat," *Proc. Nat. Inst. Sci.*, India, 1952, **18**, 241–55.

Krishnamoorthi, B. .. "Salinity tolerance and volume regulation in four species of Polychaetes," *Proc. Ind. Acad. Sci.*, 1962, **56**, 363–71.

Marchal, P. .. "Recherches anatomiques et physiologiques sur l'appareil excreteur des crestacees decapodes," *Arch. Zool. exp. gen. Sci.*, 1892, **10**, 57–275.

Marshall, E. K. and Smith, H. W. .. "The glomerular development of the vertebrate kidney in relation to habitat," *Biol. Bull.*, 1930, **59**, 135–53.

Meyer, E. .. "Studien uber des Korperbau der Anneliden," *Zool. Stat. Neapel. Mitt.*, 1888, **8**, 462–662.

Milne Edwards, H. .. "Recherches pour servir a l'histoire de la circulation du sand chez les Annelides," *Ann. Sci. Nat.*, Paris, 1839, **10**, 193–221.

Nash, J. .. "The number and size of glomeruli in the kidney of fishes with observations on the morphology of the renal tubules of fishes," *Amer. Jour. Anat.*, 1931, **47**, 425–39.

Panikkar, N. K. .. "Osmotic regulation in some palaemonid prawns," *J. Mar. biol. Ass., U.K.*, 1941, **25**, 317–59.

Peters, H. .. "Uber den Einfluss der Salzgehaltes in Aussen medium auf den Bau und die Funktion der Exkeretionsorgane dekapoden crustaceen. (Nach Untersuchungen an *Potamobius fluviatilis* und *Homarus vulgaris*)," *Zeitschr. Morpholiokol Tiere*, 1935, **30**, 355–81.

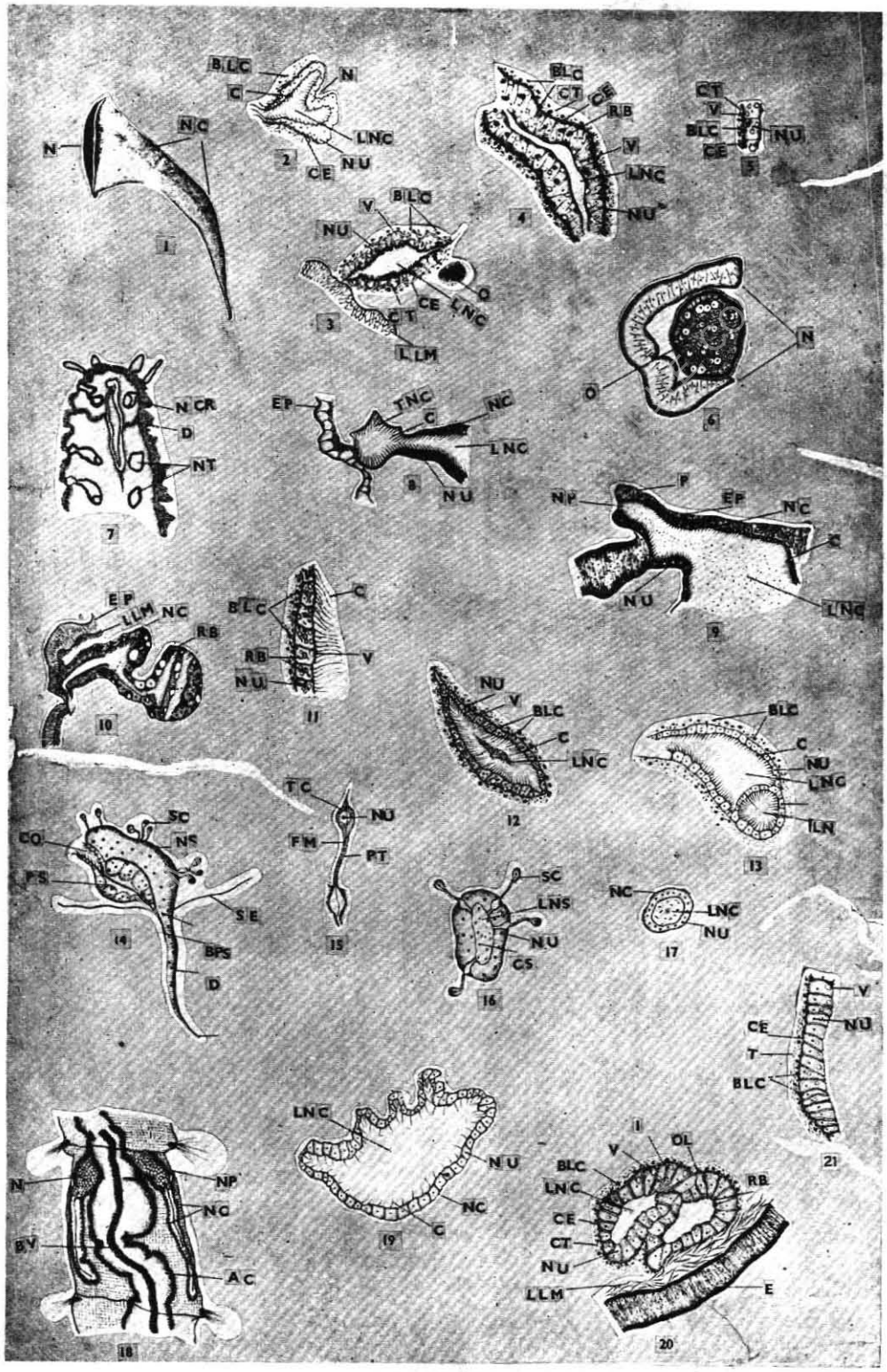
Ranganathan, V. .. *Studies on Polychaetes.* M.Sc. Thesis of the University of Madras, 1942 (Unpublished).

Schlieper, C. and Herrmann, F. "Beziehungen zwischen Bau und Function bei den Exkretion-sorganen dekapoden crustaceen," *Zool. Jahrb. Abt. Anat. Ontog.*, 1930, **52**, 624–30.

Schneider, G. .. "Uber Phagocytose und Exkretion bei den Anneliden," *Zeits. Wiss. Zool.*, Leipzig, 1899, **66**, 497–520.

Schwabe, E. .. "Uber die osmoregulation verschiedener Kresbe (Malaco-straceen)," *Zeitschr. vergl. Physiol.*, 1933, **19**, 183–235.

Westblad, E. .. "Zur Physiologie dur Turbellarien," *Lunds Univ. Arsskr.*, 1922, **18**, 1–120.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FIGS. 1–21

EXPLANATION OF PLATE X

Onuphis eremita

FIG. 1. Diagrammatic representation of a single whole nephridium (× 400).
FIG. 2. Longitudinal section through the nephrostome of the nephridium (×450).
FIG. 3. Cross-section through the nephridial canal of the nephridium (×450).
FIG. 4. Longitudinal section through the nephridial canal of the nephridium (×400).
FIG. 5. Section of a single cell of the nephridial canal (×1,350).
FIG. 6. Cross-section through the nephrostome during the maturation period (×400).

Loimia medusa

FIG. 7. Longitudinal section through the anterior 8 segments showing the distribution of the 3 pairs of nephridia (×40).
FIG. 8. Longitudinal section through the terminal part of the nephridial canal just before opening to the exterior (×200).
FIG. 9. Longitudinal section through the terminal part of the nephridial canal of the nephridium to show the opening to the exterior by the nephridiopore on an elevated papillae (×400).
FIG. 10. Longitudinal section of a single whole nephridium of the cephalic region (× 200).
FIG. 11. Longitudinal section through the nephridial canal of the nephridium (× 900).
FIG. 12. Cross-section through the nephridial canal (× 280).
FIG. 13. Longitudinal section through the nephridium of the trunk region (× 200).

Glycera embranchiata

FIG. 14. Diagrammatic representation to show the composite nature of the nephridium (× 400).
FIG. 15. Longitudinal section through a solenocyte (× 900).
FIG. 16. Cross-section through the nephridial swelling showing the opening of the solenocytes into the lumen of the nephridial swelling (× 450).
FIG. 17. Cross-section through the nephridial canal (× 450).

Clymene insecta

FIG. 18. Longitudinal sections through a segment showing the location of nephridia (× 400).
FIG. 19. Cross-section through the nephrostome of the nephridium (× 450).
FIG. 20. Longitudinal section of the wall of the nephridial canal (× 450).
FIG. 21. Cross-section through the two limbs of the nephridium (×200).

ABBREVIATIONS USED

AC, Alimentary canal; BC, Blind ending capillaries; BV, Blood vessel; C, Cilia; CE., Coelomic epithelium; CO, Ciliated organ; CS, Concrements; CT, Connective tissue; D, Diaphragm; EP, Epidermis; EM, Flagellum; IL, Inner limb; LLM, Longitudinal layer of muscles; LN, Lumen of nephrostome; LNC, Lumen of the nephridial canal; LNS, Lumen of the nephridial swelling; N, Nephrostome; NC, Nephridial duct; NP, Nephridiopore; NS, Nephridial swellirg; NT, Nephridia of the trunk region; NU, Nucleus; NCR, Nephridia of the cephalic region; O, Ovum ; OL, Outer limb; P, Papilla; PS, Phagocytal sac; PT, Proximal tube; RB, Refringent bodies; SC, Solonocytes; SE, Septum; TC, Terminal chamber; TNC, Terminal part of the nephridial canal; V, Vacuoles.

APPENDIX 5.

Volume Regulation in Eggs, Larvae and Adults of a Brackish water Polychaete, *Dicnatra variabilis* (Southern).

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VOLUME REGULATION IN EGGS, LARVAE AND ADULTS OF A BRACKISH-WATER POLYCHAETE, *DIOPATRA VARIABILIS* (SOUTHERN)

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I. Introduction

Attendant upon the stresses of an anisotonic and fluctuating environment, a brackish-water organism acquires many adaptations that contribute to its successful colonisation. In the brackish-water zones of Adyar, Madras, *Diopatra variabilis* (Southern), a polychaete, occurs in large numbers along with *Marphysa gravelyi* Southern. Although the osmotic properties of the eggs and larvae of the latter species have been studied (Krishnamoorthi, 1951) and the life-history of the former species has been reported (Krishnan, 1936), very little is known of the osmotic behaviour of the eggs, larvae and the adults of *D. variabilis* Southern under heterosmotic conditions. The importance of such studies for a fuller understanding of the adaptation of a species through ontogeny, to a fluctuating environment, has been more recently stressed (Beadle, 1957). With this object in view the following study was undertaken.

II. Material and Methods

The worms along with their tubes were collected from the brackish-water zones of Adyar, Madras, and brought to the laboratory in earthen pots. At the laboratory they were immediately transferred to clean glass troughs. The worms as well as the egg-cases were taken out, for experimentation, by cutting open the tubes. The jelly-like egg-case of *D. variabilis* is similar to that of *M. gravelyi* but is small in size and attached to the inside walls of the tube. A single egg-case would contain a few hundreds of spherical eggs. They were reared in the laboratory and the larvae so hatched were used for experiments. No special care was needed for culturing them. All experiments were performed at room temperature of $29 \cdot 5 \pm 0 \cdot 5^{\circ}$ C. All dilutions were of sea-water made up to the desired

concentration by the addition of distilled water. The method of Weil and Pantin (1931 *a* and *b*) as followed in an earlier paper (Krishnamoorthi, 1951), was adapted for the determination of volumes of eggs and larvae. But for determination of volume of the adults, the method of Lowndes (1941) was followed. All readings given are the averages of a minimum of six determinations. In all the experiments controls were run.

III. (A) EXPERIMENTS ON EGGS

1. *Effect of different hypotonic media.*—Six out of a lot of eggs isolated from a single spawn were left in each of the six hypotonic media of the following concentrations: 20·66‰, 16·34‰, 14·42‰, 11·06‰, 7·04‰ and distilled water, contained in six separate petri-dishes, after determining the initial volume of each egg. They were exposed for a period of 30 minutes, after which time the final volume of each egg was determined. A preliminary experiment had shown that the eggs attain the maximum volume by the end of 30 minutes. Figure 1 indicates the results of the above experiment. It may be seen that the final volume attained in each of the six dilutions was directly proportional to the concentration of the medium similar to the behaviour of the eggs of *M. gravelyi* (Krishnamoorthi, 1951) and the eggs of the sea urchin (Lillie, 1916, 1918; Northrop, 1926–27).

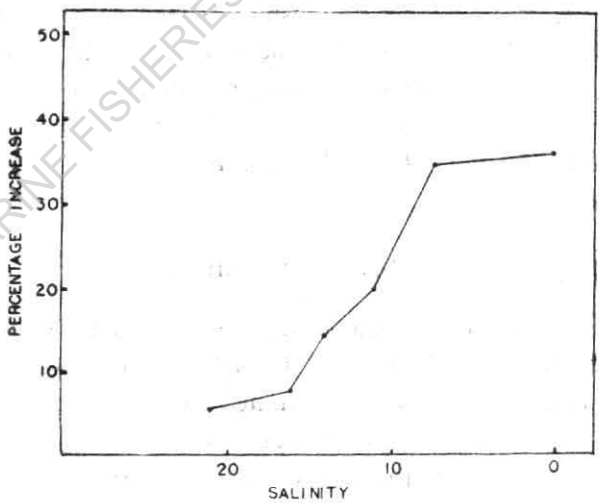

FIG. 1. Eggs—Increase in volume in different hypotonic media.

2. *Effect of a single hypotonic medium on volume during different intervals over a period of* 8 *hours.*—Out of a single spawn, a batch of 400 eggs of similar size was selected and after determining the initial volume of six eggs, the entire batch was exposed to the stresses of a hypotonic medium of strength

of 16·34‰ contained in a petri-dish. The volume of six eggs selected at random out of this batch was determined at intervals of 15 minutes, over a period of 8 hours. It may be seen (Fig. 2 *b*) that the eggs increased to the maximum volume at the end of 30 minutes, and thereafter began to decrease in volume. The decrease continued for about 5 hours, till the eggs were about 96·4% of the original volume. No further shrinkage was seen for a period of 8 hours, when the experiment was stopped. The initial increase in volume was perhaps due to the intake of water, the concentration of the eggs being higher than the surrounding medium. The later decrease in volume may be due to subsequent loss of salts and consequent lowering of the concentration of the eggs.

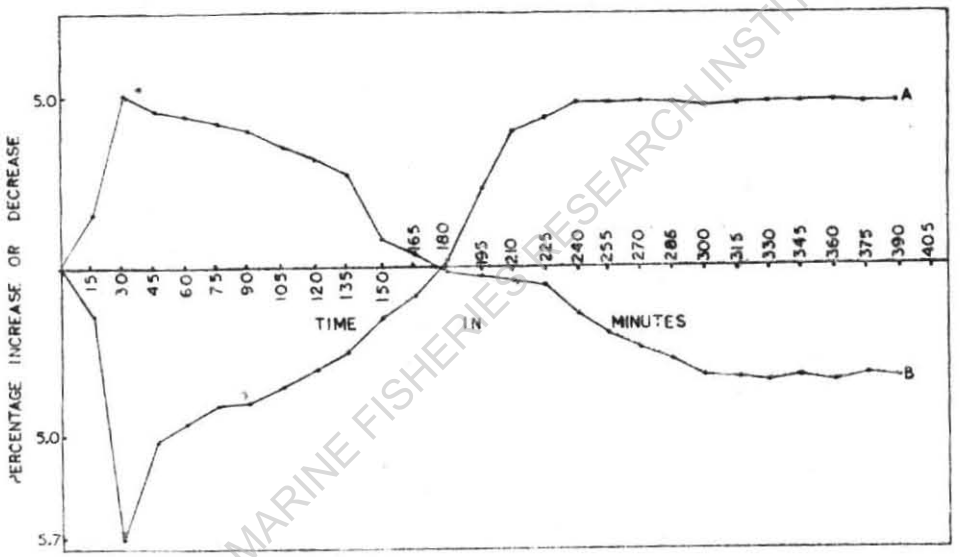

FIG. 2. Eggs—Volume change in hypotonic (B: 16·34‰) and in hypertonic (A: 27·39‰) media during different intervals.

3. *Effect of a single hypertonic medium on volume during different intervals over a period of* 8 *hours.*—From a single spawn, a batch of 600 eggs of equal size was isolated and from this batch, after determining the initial volume of each of the six eggs randomly selected, the rest were subjected to the rigours of a hypertonic medium of 27·39‰ contained in a petri-dish. The final volume attained at intervals of every 15 minutes, of each of the six eggs selected at random, was determined over a period of 8 hours (Fig. 2 *a*). It may be seen that the eggs to begin with shrank, the volume reaching the maximum decrease at the end of 30 minutes. Thereafter it continued to increase till the end of 4½ hours. No increase in volume was

TABLE I

Effect of hypotonic media on the development of eggs—with and without jelly; Larvae—metatrochophores and nectochaetae; and adults of Diopatra variabilis *(Southern)*

Concentration of medium ‰	Percentage rate of mortality after a period of																			
	Eggs								Larvæ								Adults			
	Without jelly				With jelly				Metatrochophores				Nectochaetae							
	3 Hrs.	6 Hrs.	9 Hrs.	12 Hrs.	3 Hrs.	6 Hrs.	9 Hrs.	12 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
	A				B				C				D				E			
26·62	..	..	..	..	..	..	..	..	Nil	Nil	Nil	Nil	..	..	..	..	..	..	..	..
25·17	..	..	..	..	..	..	..	..	..	..	..	..	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
24·78	..	..	..	..	..	..	..	..	Nil	Nil	Nil	Nil	..	..	..	..	..	..	..	..
24·22	10	25	70	96	1	1	2	2	..	..	..	..	..	..	..	..	..	..	..	..
22·28	..	..	..	..	..	..	..	..	..	..	..	..	Nil	Nil	Nil	Nil	..	..	..	..
20·49	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Nil	Nil	Nil	Nil
19·49	..	..	..	..	..	..	..	..	..	..	..	..	Nil	Nil	Nil	Nil	..	..	..	..
19·23	..	..	..	..	..	..	..	..	10	10	12	12	..	..	..	..	..	..	..	..
17·26	..	..	..	..	..	..	..	..	40	53	64	78	..	..	..	..	10	15	1[illegible]	20
16·83	16	30	76	96	2	3	3	3	..	..	..	..	..	..	..	..	..	..	..	..
16·02	..	..	..	..	..	..	..	..	..	..	..	..	Nil	1	1	2	..	..	..	..
15·42	..	..	..	..	..	..	..	..	58	61	77	92	..	..	..	..	..	..	..	..
12·34	24	48	86	98	5	5	5	6	..	..	..	..	..	..	..	..	23	32	48	67
10·80	..	..	..	..	..	..	..	..	60	65	80	98	..	..	..	..	..	..	..	..
10·54	..	..	..	..	..	..	..	..	..	..	..	..	15	38	72	94	..	..	..	..
8·22	35	58	90	98	5	5	5	5	..	..	..	..	..	..	..	..	..	..	..	..
7·80	..	..	..	..	..	..	..	..	..	..	..	..	50	63	82	98	52	60	73	98
Distilled water	100	..	..	..	6	6	7	7	100	..	..	..	100	..	..	..	100	..	..	..

noticed after this period till 8 hours, when further observations were stopped. The initial decrease in volume is probably due to the stress of a more concentrated external medium. Subsequently, due to inrush of salts and rise of concentration within the egg, the eggs perhaps increase in volume.

4. *Effect of different hypotonic media on the rate of mortality of eggs without jelly.*—Numerous eggs were carefully isolated from the jelly and left to continue their development in separate petri-dishes containing hypotonic media of different concentrations. After every 3 hours of such exposure, the number of eggs that were dead from out of 100 eggs selected from each of the concentration, was counted. The rate of mortality was not only proportional to the dilution but also increased with time, so much so 96% of them died even in a concentration of 24·22‰ (Table I, Col. A).

5. *Effect of different hypotonic media on the rate of mortality of eggs with jelly.*—A single uninjured cocoon collected from the tube of the animal was left in each of the different dilute media of known salinities. At the end of every 3 hours, the number of eggs that were dead in a lot of 100 eggs, were counted. It is evident that the rate of mortality is comparatively lower (Table I, Col. B) and the eggs continued their development even at the end of 12 hours, irrespective of the surrounding medium being dilute.

III. (B) Experiments on Larvae—*Metatrochophore* Stage

6. *Volume changes in different hypotonic media.*—The initial volumes of six randomly selected metatrochophore larvae, out of a batch of few hundreds, were determined. The rest were left in petri-dishes of sea-water diluted to desired strength. The volumes of six larvae selected at random belonging to each of the dilutions were determined at the end of 30 minutes. A preliminary experiment had shown that the larvae attained the maximum volume at the end of 30 minutes. It may be seen (Table II) that the increase in volume over the initial volume in each case was proportional to the dilution of the medium. It may also be seen that in dilutions less than 19·06‰, the larvae became bloated and broke up. The greater the dilution, the greater was the percentage of disintegration of the larvae.

7. *Volume changes in hypotonic media during varying intervals.*—Out of a batch of larvae hatched out in the laboratory, about 400 of them were left in two hypotonic media of 19·6‰ and 21‰ concentrations, after determining the initial volumes of six larvae selected at random out of this batch. The volumes of six larvae, also selected at random, from each of the concentrations were determined at intervals of every 15 minutes over a period of 6 hours. In both the dilutions the larvae attained the maximum volume

TABLE II

Increase in volume in different hypotonic media

Concentration of the medium ‰	Increase in volume after 30 minutes (initial mean volume: 90 c.μ)	
	Volume in c.μ	Percentage
26·28	92	2·22
22·32	96	6·66
21·02	112	24·44
19·06	120	33·33
17·74	10% Disintegration	
15·68	15% Distintegration	
10·92	20% Disintegration	
Distilled water	40% Disintegration	

(Fig. 3) at the end of 30 minutes. Also the increase in volume over the initial was higher, greater the dilution. They were 31·12% and 23·91% in the respective concentrations of 19·6‰ and 21‰. Thereafter, the volumes decreased gradually and steadily to reach their original volumes at the end of 4¼ hours and 4 hours respectively. The initial increase in volume was perhaps due to inrush of water and later due to loss of salts the volume is regained. The sharpness of descent in the behaviour of larvae in 21‰ dilution in contrast to the behaviour of the larvae in 19‰, especially during the first 2 hours of recovery, makes one believe that the organs of excretion may also work vigorously when the osmotic gradient is high.

8. *Effect of hypotonic media on the rate of mortality.*—In each of the seven different hypotonic media of concentrations 26·62, 24·78, 19·23, 17·26, 15·42, 10·80 parts per mille and distilled water, a number of larvae were allowed to continue their development. After every 24 hours the number of larvae dead out of a known number of larvae selected from the batch, was noted. The experiments were continued over a period of 96 hours, the time required for the metatrochophores to develop into the next nectochaete stage. The low rate of mortality (Table I, Col. C) as compared against

controls proved that the larvae were able to develop in media ranging between 10·80 to 26·62 parts per mille. It may also be seen that the rate of mortality in media of 19‰ and above was practically nil. This together with the results of the previous experiments go to prove that the larvae which attained osmotic equilibrium within $4\frac{1}{4}$ hours, continued to develop normally. Since 40% of the larvae survived in 10·8‰ for 24 hours, in 15·42‰ for 48 hours and in 17·26‰ for 72 hours, it helps to understand the endurance of the larvae in dilute media as also the ability of the larvae to survive sudden dilution in an estuarine environment if it lasts for brief periods.

FIG. 3. Metatrochophores—Volume changes in two hypotonic media (A: 19‰; B: 21‰) during varying intervals.

III. (C) EXPERIMENTS ON LARVAE—*Nectochaetae* STAGE

9. *Effect of different hypotonic media on volume.*—The initial volumes of six nectochaetae, chosen at random from a lot hatched in the laboratory, were first determined. Six more were left in each of the six petri-dishes containing different hypotonic concentrations of 25·17, 19·49, 16·42, 12·82, 7·60 parts per mille and distilled water. The volumes of these six were determined at the end of 30 minutes. It may be seen (Fig. 4) that the increase in volume was directly proportional to the dilution of the media.

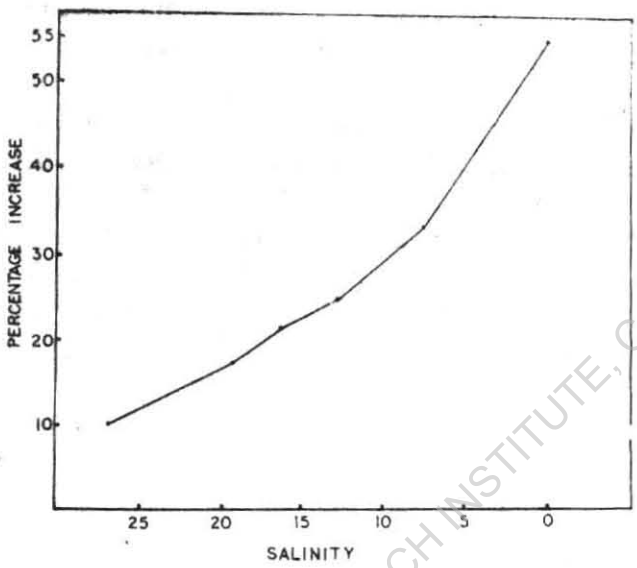

FIG. 4. Nectochaetae—Increase in volume in different hypotonic media.

10. *Effect of hypotonic media on volume during different intervals.*—About 400 larvae were exposed to hypotonic media of two concentrations of 16·64‰ and 20·64‰, after determining the initial volumes of six larvae randomly selected out of the lot. The changes in volumes of six larvae were followed during different intervals of 15 minutes, over a total period of 3 hours. The larvae in both the dilutions increased in volume reaching the maximum at the end of 30 minutes (Fig. 5) and then began to decrease in volume until at the end of 105 minutes, they reached a volume a little higher than the original volume. They continued to maintain this slightly higher volume over a period of 3 hours, and even at the end of a 24-hour period. The larvae were quite normal and healthy and continued their development. The extra amount of water in the body appeared not to impair their normal well-being or affect their development.

11. *Effect of different hypotonic media on larval mortality.*—A number of nectochaetae were exposed to each of the following media of concentrations: 25·17‰, 22·28‰, 19·49‰, 16·02‰, 10·54‰, 7·8‰ and distilled water. After every 24 hours the number of nectochaetae dead out of a batch of 100 larvae randomly selected from each of the concentrations, were determined for a period of 96 hours. Table I, Col. D, reveals the results of such an experiment. It may be seen that the nectochaetae tolerate a salinity upto 16·02‰, but if the osmotic gradient is higher than this, they are affected adversely and die till at the end of 96 hours not one survived.

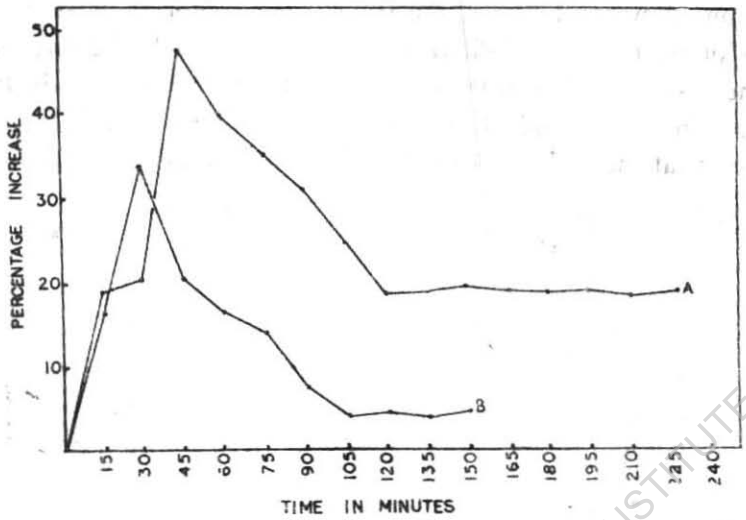

Fig. 5. Nectochaetae—Volume changes in two hypotonic media (A: 16·64‰; B: 20·64‰) during different intervals.

III. (D) Experiments on Adults

12. *Effect of hypotonic media on volume changes at different intervals.* —Out of a lot of worms collected from their natural habitat, six of them whose initial volumes had already been determined, were left in hypotonic media of the following concentrations: 8·62‰, 13·7‰ and 20·72‰. They were exposed over a period of 8 hours and their volumes measured at intervals of 1 hour. It may be seen that the worms reached in all the media the maximum volume at the end of the 1st hour and thereafter it declined. This decrease continued for a period of 4 hours when the new volume attained was higher than the initial volume of the worm. This volume was kept constant for over 8 hours and continued steady even for a period of 24 hours (Fig. 6). It may also be seen that both the maximum volume reached at the end of the 1st hour and the final volume attained at the end of 4 hours, were directly proportional to the dilution, *i.e.*, the greater the dilution the larger the volume.

13. *Effect of hypotonic media on survival.*—Numerous worms were exposed to each of the following six hypotonic media of concentrations: 25·17‰, 20·49‰, 17·36‰, 12·82‰, 7·6‰ and distilled water. In order to test their capacities for tolerance, the number of worms dead in a lot of 100 worms randomly selected from each concentration, was counted at the end of every 24 hours. The experiment was continued over a period of 96 hours. It may be seen (Table I, Col. E) that *D. variabilis* is able to survive

in dilutions down to 17·36‰, the percentage of mortality being only 20 at the end of 96 hours. In dilutions less than 17·36‰, the percentage mortality increased not only with increasing dilution but even with time, 67% of them dying at the end of 96 hours in a dilution of 12·82‰ and 52% in 17·6‰ even at the end of 24 hours. They do not survive in distilled water.

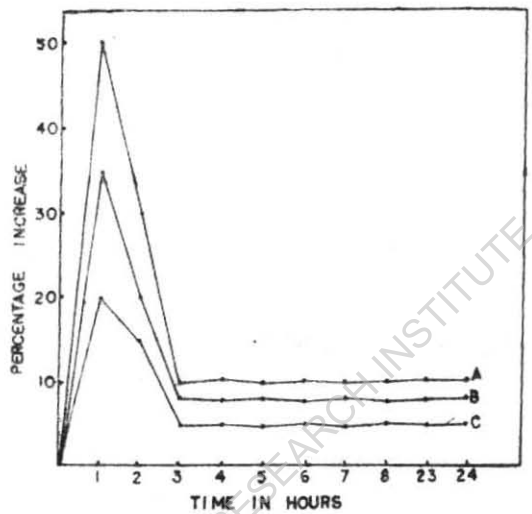

FIG. 6. Adults—Volume changes in three hypotonic media (A: 8·62‰; B: 13·7‰; C: 20·72‰) during different intervals.

IV. DISCUSSION

The experiments with the eggs, the larvae and the adults indicate that they increase in volume when subjected to stresses of hypotonic media. They reveal also that the higher the dilution the greater the increase in volume. The eggs isolated from their cocoons increased in their volume, the greater the dilution the greater the increase at the end of 30 minutes. Further, observations on the prolonged effects of a single hypotonic medium have also revealed that after a period of 30 minutes the volume decreased till about 5 hours reaching 96·4% of the original volume. In a hypertonic medium the reverse was true. The eggs at first decreased in volume reaching the maximum at the end of 30 minutes. But increased to 104·7% of the original volume in 4½ hours. The increase and the subsequent decrease in hypotonic media and the reverse condition in hypertonic media, argue that the eggs of *D. variabilis* are permeable both to salts and water as eggs of *Marphysa gravelyi* Southern also an Eunicid (Krishnamoorthi, 1951); and the eggs of *Strongylocentrotus lividus* (Needham, 1930; Euphrussi and Rapkine, 1928). The initial increase in volume in a hypotonic medium is

probably due to the fact that the rate of inflow of water is greater than the rate of loss of salts. The opposite may perhaps be true in a hypertonic medium. In other words, the eggs of *D. variabilis* isolated from the spawn jelly behave like osmometers passively increasing in volume in a hypotonic medium by absorption of water against an osmotic gradient or decreasing in volume in a hypertonic medium by loss of water due to greater osmotic pressure of the surrounding medium as reported in the eggs and larvae of a number of invertebrates (Krogh, 1939; Nicol, 1960). This probably accounts for their mortality which is directly proportional to the length of exposure and the dilution of the experimental media. Eggs denuded of jelly could not survive even in a medium of as high a salinity as 24·22 parts per mille, the mortality rate being 96% at the end of 12 hours. In lower dilutions the percentage rate of mortality was considerably high being 16% in 16·83‰, 24% in 12·34‰ and 35% in 8·22‰ even at the end of 3 hours. In distilled water all of them died at the end of 3 hours. With increasing time, the percentage mortality increased. But the eggs with jelly behaved differently when exposed to these salinities. Even in distilled water the rate of mortality was only 7% and that at the end of 12 hours. In higher concentrations the rates were much less, hardly 2% of them dying at the end of 12 hours in a medium with a salinity of 24·22‰. In this respect the eggs of *D. variabilis* resembled in their behaviour those of *M. gravelyi* Southern (Krishnamoorthi, 1951) and it may perhaps be concluded that the jelly affords protection against a fluctuating environment. Production of impermeable membranes is probably a general mechanism for the protection of eggs in fresh and brackish-waters against osmotic swelling as observed by Krogh and Ussing (1937). The jelly in *D. variabilis* perhaps acts like one ensuring adequate protection and climate for the normal development of the eggs in an environment of fluctuating salinities. The eggs of *Oryzias latipus*, a fish living in fresh and brackish-waters of Japan, furnishes another such example (Ikeda, 1937 *a*).

The behaviour of the larvae of both trochophore and the nectochaete stages, in various hypotonic media, was similar to that of the eggs without jelly. They increased in volume to begin with and subsequently decreased. The initial increase in volume may have been due to absorption of water against an osmotic gradient and the later decrease to subsequent loss of salts. Ionic regulation in the developmental stages of marine animals is not uncommon; (Needham, 1931; Krogh, 1939). But in the degree of tolerance of the hypotonic media and in the time taken to reach the final volume the metatrochophores differed from the nectochaetae; and both

from the eggs without jelly. The percentage increase in volume of metatrochophores was 33% even in a medium of as high a salinity as 19‰. For the nectochaetae the corresponding salinity was 7‰. While the metatrochophores disintegrated in media of lesser salinities the nectochaetae did not, reaching only 54% increase in distilled water. Accordingly the percentage of mortality of metatrochophores was considerably higher in media of lesser dilutions, 78% of them dying in a medium of salinity 17‰ at the end of 96 hours. The nectochaetae on the other hand reached as high a mortality as 98% at the end of 96 hours only in a dilution of 7‰. Further, the metatrochophores took 195 to 210 minutes to reach the original volume while the nectochaetae hardly required 105 to 120 minutes to reach the final volume. In both the stages there is already a larval kidney of the protonephridial type functioning. Perhaps the differences observed in the two developmental stages may be attributed to the possession of the larval kidneys which may be actively participating in keeping down the swelling of the larvae and in the retention of salts needed for a healthy development. Westblad (1922) (in Turbellaria) and Herfs (1922) (in Rotifers and Trematodes) have shown experimentally how the protonephridial kidney help in the osmoregulatory aspect of the work of the organs. This further argues that but for the precocious development of nephridia in the metatrochophores and the nectochaetae this brackish-water polychaete would not have survived the conditions obtained in an estuarine environment such as the Adyar estuary.

The behaviour of the adults under osmotic stresses of different hypotonic media was generally similar to that of the larvae and the eggs. They increased in volume due to inrush of water and later decreased in volume probably due to subsequent loss of salts. However, unlike the larvae and the eggs, they could survive dilute media of salinities ranging from 5 to 28‰ without loss of life. This may be due to the possession of a more efficient, fully grown kidney as observed in a number of polychaetes of that region (Krishnamoorthi, 1962, 1963). The excretory surface in *D. variabilis* is comparatively larger (Krishnamoorthi, 1951). While the ratio between the length of the excretory surface and the length of worm in *D. variabilis* was 0·35: 1, similar ratios in *L. medusa*, *O. erimita* and *C. insecta* (Krishnamoorthi, 1963) respectively were 0·225: 1; 0·247: 1 and 0·31: 1, arguing that a bigger kidney is advantageous in meeting the demands of baling out water absorbed against an osmotic gradient. Further, the vascularisation of the nephridia in *D. variabilis* was higher being 24·5 units (Krishnamoorthi, 1951), when compared to that in the other forms (Krishnamoorthi, 1963).

Higher vascularisation and bigger kidneys have equipped the adults of *D. variabilis* for better adjustments to hypotonic media than either the eggs or the larvae. Pre-larval and larval stages of *N. diversicolor* cultured in 7% salinity were sensitive to dilutions than the young worms (Bogucki, quoted by Beedle, 1957).

V. Summary

1. Experiments to study the effect of hypotonic and hypertonic media on eggs with and without jelly were performed as also on the development of the eggs. The eggs (without jelly) passively increased or decreased in volume respectively in hypo- or hypertonic media and behaved like osmometers. The rate of mortality was considerable in different dilutions and was a function of dilution and period of exposure to dilute media. But the eggs with jelly did not suffer much, only 2% of them dying even in the lowest dilution. It is presumed that the jelly may be providing the necessary barrier against a fluctuating environment for the healthy development of the eggs and loss of salts required for normal development and well-being.

2. The behaviour of the larvae was also similar to that of the eggs without jelly when subjected to the stresses of different hypotonic media. Both the metatrochophores and the nectochaetae increased in volume. But among themselves the rates of increase and mortality in various dilutions were different. It is argued that these differences may be due to the possession of a larval kidney which is already present at this stage in the development of *D. variabilis*.

3. The adults were similar in their behaviour, differing not only in the degree of swelling but also in the time taken to swell and in the rates of mortality. This is attributed to a more efficient kidney compared to the larval kidney.

VI. Acknowledgements

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VII. REFERENCES

Beadle, L. C. .. "Comparative physiology: Osmotic and ionic regulation in aquatic animals," *Ann. Rev. Physiol.*, 1957, **19**, 329–58.

Bogucki, M. .. *Polsk. Arch. Hydrobiol.*, 1954, **2** (15), 237–51.

Euphrussi, B. and Rapkine, L. .. "Composition chimique de l'oeuf doursin *Paracentrotus lividus* Lk., et ses variation au course du development," *Ann. Physiol. Physicochim. Biol.*, 1928, **4**, 386.

Herfs, A. .. "Die pusierende Vakuole der Protonoen ein Schutzorgen gegen Assussung. Studien uber Anpassung der Organismen au das Leven im Susswasser," *Arch. Protistenk.*, 1922, **44**, 227–60.

Ikeda, Y. .. "Effect of sodium and potassium salts on the rate of development in *Oryzias latipes*," *J. Fac. Sci. Tokyo Imp. Univ.*, 1937 *a*, **4**, 307–12.

Krishnan, G. .. "The development of *Diopatra variabilis* (Southern)," *Zeits. wiss. Zool. Leipzig*, 1936, **147**, 513–25.

Krishnamoorthi, B. .. "Studies on the Nephridia of Polychaetes," Thesis submitted for the M.Sc. degree of the University of Madras, 1951 (Unpublished).

———— .. "Studies on the osmotic properties of the eggs and larvae of a brackish-water polychaete, *Marphysa gravely* Southern," *Proc. Ind. Acad. Sci.*, 1951, **34**, 199–209.

———— .. "Salinity tolerance and volume regulation in four species of Polychaetes," *Ibid.*, 1962, **56**, 363–71.

———— .. "Gross morphology and histology of Nephridia in four species of Polychaetes," *Ibid.*, 1963, **57**, 195–209.

Krogh, A. .. *Osmotic Regulation in Aquatic Animals*, Camb. Univ. Press, 1939.

——— and Ussing, H. H. .. "A note on the permeability of trout eggs to D_2O and H_2O," *J. Exp. Biol.*, 1937, **14**, 35–37.

Lillie, R. S. .. "Increase of permeability to water following normal and a rtificial activation of seaurchin eggs," *Amer. Jour. Physiol.*, 1916, **40**, 249–66.

———— .. "The increase of permeability to water in fertilised sea urchin eggs and the influence of cyanide and anaesthetics upon this change," *Ibid.*, 1918, **45**, 406–30.

Lowndes, A. G. .. "The displacement method of weighing aquatic animals," *J. mar. biol. Ass. U.K.*, 1942, **25**, 555–72.

Needham, J. .. "On the penetration of marine organisms into freshwater," *Biol. Zbl.*, 1930, **50**, 504–09.

———— .. *Chemical Embryology*, 3 Vols., Camb. Univ. Press, 1931.

Nicol, J. A. C. .. *The Biology of Marine Animals*, Sir Isaac Pitman & Sons Ltd., London, pp. ix+707, 1961.

Northrop, J. N. .. "Kinetics of the swelling of cells and tissues," *J. Gen. Physiol.* 1926–27, **11,** 43.

Weil, E. and Pantin, C. F. A. .. "The adaptation of *Gunda ulvae* to salinity. I. The environment," *J. Exp. Biol.*, 1931 *a*, **8,** 63–72.

———— .. "The adaptation of *Gunda ulvae* to salinity. II. The water exchange," *Ibid.*, 1931 *b*, **8,** 73–81.

Westblad, E. .. "Zur Physiologie der Turbellarien. II. Die Exkretion, *Lunds. Univ. Arsskr.*, 1922, **18,** 1–120.

APPENDIX 6.

Chloride Regulation in *Marphysa gravelyi* Southern.

Curr. Sci., 32: 463-464, (1963c).

CHLORIDE REGULATION IN *MARPHYSA GRAVELYI* SOUTHERN

BY

B. KRISHNAMOORTHI

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CHLORIDE REGULATION IN *MARPHYSA GRAVELYI* SOUTHERN

Marphysa gravelyi Southern, an Eunicid polychæte, essentially a burrower, enjoys a wide distribution in the brackish-water regions of Madras, where the salinity of the medium varies widely from almost fresh to sea-water.[1] This worm tolerates sea-water dilutions to 20% and regulates its body volume better (unpublished data) than other polychætes found in similar habitats.[2] Further, isolated muscle preparations are active in dilute media ranging between 20% and 50% sea-water (34%).[3] A study of the blood chloride level at 27·5 ± 0·5° C., using the method of Sendroy[4] as modified by Robertson and Webb,[5] shows that the chloride ions are actively regulated as the following Table would show. The chloride levels are

TABLE I

Exp. No.	Salinity of experimental medium ‰	Mean chloride values of blood after 24 hrs. of exposure			
		gm./l.	S.d.	S.e.	No. of estimations
1	5·68	9·64	0·011	±0·005	3
2	8·52	10·68	0·029	±0·016	3
3	10·60	12·76	0·011	±0·004	8
4	14·60	14·39	0·014	±0·005	8
5	15·30	11·87	0·014	±0·004	12
6	16·60	13·94	0·007	±0·002	12
7	16·20	10·83	0·017	±0·003	8
8	18·40	14·09	Nil	Nil	4
9	19·50	14·84	0·035	±0·012	8
10	21·60	15·42	0·009	±0·003	12
11	23·00	14·09	0·012	±0·003	8
12	25·92	16·04	0·021	±0·006	12

always higher in the hyposmotic media and lower in hyperosmotic media and thus maintaining a steady level (Fig. 1). Such a regulation has not been reported in any polychæte so far, although similar work on *Nereis diversicolor* is available (Smith[6]). The mechanism underlying the regulation is being studied.

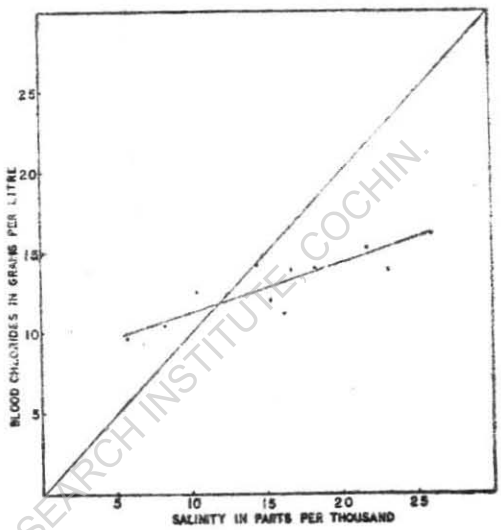

FIG. 1

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Zoological Res. Lab., B. KRISHNAMOORTHI.
University of Madras,
Chepauk, Madras-5, *April* 6, 1963.

1. Panikkar, N. K. and Aiyar, R. G., *Proc. Ind. Acad. Sci.*, 1937, **6**, 284.
2. Krishnamoorthi, B., *Ibid.*, 1962, **56**, 363.
3. — and Krishnaswamy, S., *Ibid.*, 1963, **57**, 83.
4. Sendroy, J., *J. biol. Chem.*, 1937, **120**, 335 & 405.
5. Robertson, J. D. and Webb, D. A., *J. Exp. Biol.*, 1939, **16**, 155.
6. Smith, R. I., *Biol. Bull.*, 1955 *c*, **109**, 453.

APPENDIX 7.

On the Distribution of Six Species of Polychaetes in the Adyar Estuary, Madras.

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On the Distribution of Six Species of Polychaetes in the Adyar Estuary, Madras

BY

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ON THE DISTRIBUTION OF SIX SPECIES OF POLYCHAETES IN THE ADYAR ESTUARY, MADRAS

By B. KRISHNAMOORTHI

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INTRODUCTION

MOST marine animals are stenohaline with a low range of tolerance of the differences in the salinity of external medium. Yet some have become euryhaline exhibiting a considerable range of tolerance, a few like *Heteromastus* sp., *Clistomastus* sp., (most capitellids), living as well in fresh water as in salt. Recent work has shown that the physiological independence and the emancipation from the limitations imposed by the changing environment in such homoiosmotic animals have been brought about by their ability to control the osmotic pressure of their body fluids by raising or lowering their internal concentration to suit the exigencies of the anisotonic media they inhabit. When such an investigation was extended to polychaetes, which are predominantly marine, it became apparent that they showed different types and grades of efficiency of the kidney (Krishnamoorthi, 1962) with regard to their osmoregulatory capacity and their tolerance to changes in the environment (Krishnamoorthi and Krishnaswamy, 1962 ; Krishnamoorthi, 1963). The tolerance to the external medium is of special significance to animals attempting to colonise and penetrate into brackish water habitats such as estuaries. With this object in view the present investigation was undertaken.

MATERIAL AND METHODS

The following polychaetes *Onuphis eremita*, *Glycera embranchiata*, *Loimia medusa*, *Diopatra variabilis*, *Clymene insecta* and *Marphysa gravelyi* were chosen for the study.

A series of Stations were located, as shown in the Map of the River Adyar (Fig. 1), along the banks of the River, covering as far as possible the range of brackish water conditions in the river. Weekly visits were paid to the Stations selected from where the material was collected. Samples of mud from both the bottom and the sides of the bank of the river where these polychaetes inhabit were collected. The procedure was as follows :

Areas of 1 sq.ft. were marked at different places and mud to a depth of 1 foot was dug out and brought to the laboratory where it was washed with fresh samples of brackish water in order to collect the worms from the sample of mud. Care was taken to see that the samples of mud were not mixed up with each other. From a lot of worms collected from each sample, the percentage of occurrence of each group of polychaetes was calculated and tabulated. The figures arrived at are the mean percentage of six samples. The same method was followed while determining the percentage of occurrence of polychaetes at all Stations.

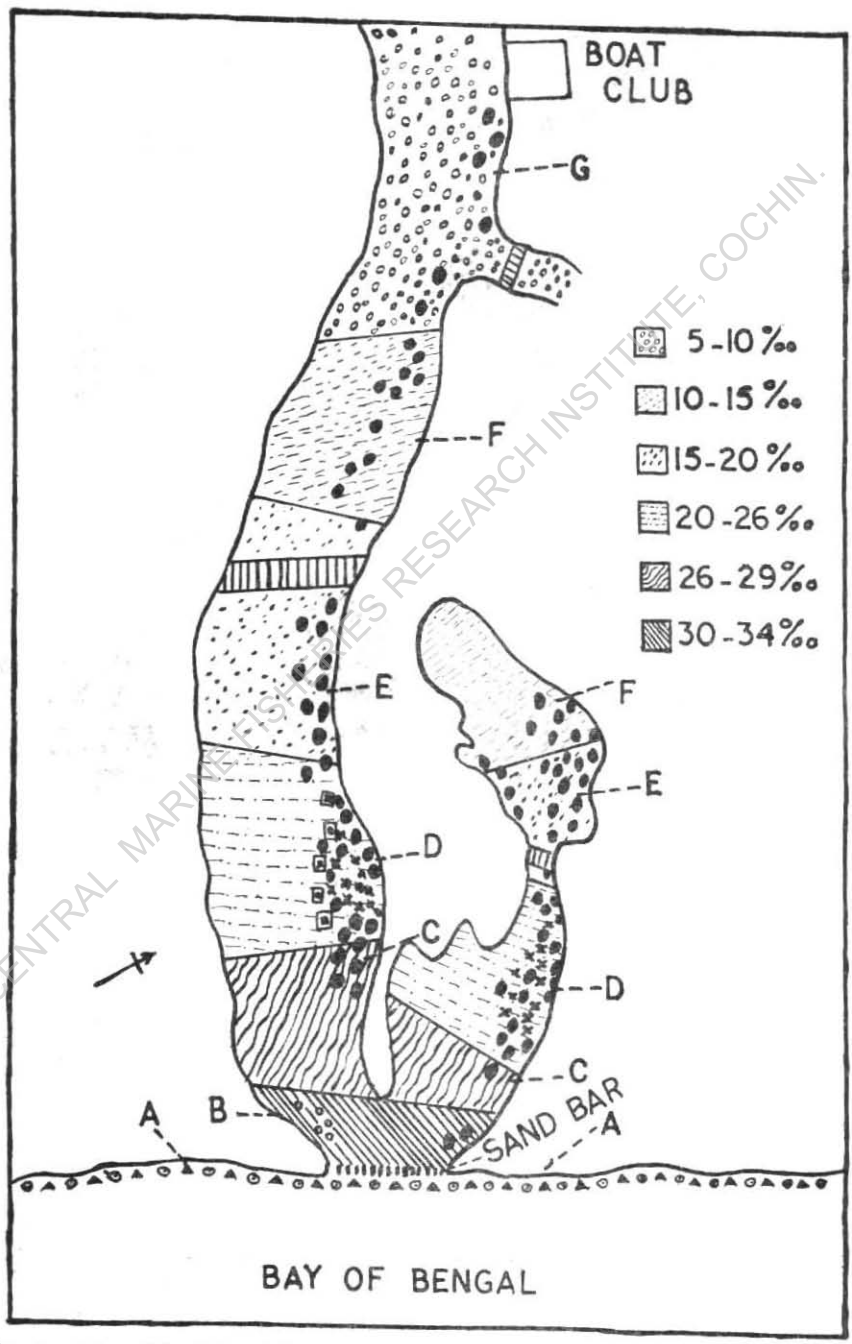

FIG. 1. Map of the River Adyar and the estuary showing the distribution of six species of polychaetes. (● *M. gravelyi* : × *D. variabilis* ; ▣ *C. insecta* : ○ *L. medusa* : ⊙ *G. embranchiata* and ▲ *O. eremita*).

RESULTS

It is evident from the figures (Table 1) that at Station A the salinity of the waters range between 30-34‰ and the predominating polychaetes in this region are *O. eremita* and *G. embranchiata*, none of the other genera being represented. They become scarce in other Stations, even at Station B, though the salinity range is between 30-34‰. But at Station B, *L. medusa* alone is well represnted it being absent at Station A. While *M. gravelyi* alone occurs at Station C, *D. variabilis* and *C. insecta* again become scarce, *M. gravelyi* alone predominating once again. From the above facts it was evident that while *M. gravelyi* had migrated to a greater extent, even to regions where the salinities range between 5-10‰, *D. variabilis* and *C. insecta* were restricted to regions of 20-26‰, and *O. eremita*, *G. embranchiata* and *L. medusa* never left a region with a salinity range between 30-34‰. It also argues that the salinity of the waters has been a barrier for these polychaetes in their migrations to regions of lower osmotic pressures.

TABLE 1

Distribution of various genera of polychaetes at different stations

Station	Salinity range. ‰	Percentage of occurrence of					
		Onuphis eremita	*Glycera embranchiata*	*Loimia medusa*	*Diopatra variabilis*	*Clymene insecta*	*Marphysa gravelyi*
A	30-34	52	48	nil	nil	nil	nil
B	30-34	nil	nil	94	nil	nil	6
C	26-29	nil	nil	nil	nil	nil	100
D	20-26	nil	nil	nil	33	31	36
E	15-20	nil	nil	nil	nil	nil	100
F	10-15	nil	nil	nil	nil	nil	nil
G	5-10	nil	nil	nil	nil	nil	nil

DISCUSSION

The facts favourable for the penetration of marine fauna into the mouths of rivers have been analysed by many workers. It has been maintained that temperature is an important factor (Panikkar & Aiyar, 1937; Broekema, 1941; Wickgren, 1953; Kinne, 1956b; Verwey, 1957). This may probably explain the abundance of marine fauna in the tropical brackish-waters (Panikkar, 1951). However, the importance of salinity as a factor in the distribution of animals cannot be minimised. Recently Veerabhadra Rao (1951) has stressed the effect of salinity on the spawning and development of a back-water Oyster. A similar observation was made by Remane (1934), Redeke (1933) and Topping & Fuller (1942) emphasising that salinity was an equally important factor. Viewed against this knowledge, in the distribution of *M. gravelyi*, *D. variabilis*, *C. insecta*, *L. medusa*, *G. embranchiata* and *O. eremita* in the brackish-water zones of Adyar,it may be seen that, the temperature remaining the same, the extent of penetration varies. The differences in the degree of migration of the polychaete genera studied appear to be accounted for on the basis of the different capacities of their nephridia for osmoregulation (Krishnan, 1952; Krishnamoorthi, 1962).

Other ecological factors such as the availability of suitable food, the prevalence of optimum percentage of oxygen for respiration and the nature of backwater flora and fauna congenial to the existence of these marine polychaetes also have to be considered. The importance of conditions favourable for the normal reproduction and safe development of these migrators cannot be minimised. In this connection the present study of these brackish-water polychaetes shows how the eggs are protected (Krishnamoorthi, 1951) ; how the early development abbreviated (Aiyar, 1931 ; Krishnan, 1936) and how the precocious development of the nephridium in the metatrochophore and nectochaetae is of a degree of osmoregulatory capacity sufficient to withstand sudden dilution of the medium (Krishnamoorthi, 1951).

The significance of the greater adaptability exhibited by *M. gravelyi* and lesser degrees of specialisation in the other forms is more pronounced when viewed in relation to the habits and distribution of the group as a whole. *M. gravelyi*, *D. variabilis*, and *C. insecta* are sharply contrasted from *O. eremita*, *L. medusa* and *G. embranchiata* in that the last mentioned three are purely marine in habitat. Of the eight species recorded so far in the genus *Onuphis*, *Onuphis bisipicta* from Indo-Malayan regions ; *O. dorsalis*, *O. verngreni*, *O. intermedia* and *O. fragalis* all from W. African and Angola coasts ; *O. litoralis* and *O. gorgonensis* from Panama and *O. investigators* from Arabian sea clearly indicate their distribution to marine habitats. Similarly *Loimia variegata* from Indo-Malayan regions ; *L. montagui* from the Pacific ; *L. minuta* off the coast of Florida ; *L. annulifilis* and *L. turgida* from East Indies show that they are confined to the sea. *Glycera tessellata* and *G. capitata* var. *benguellana* from the coast of S.W. Africa ; *G. rouxii* from China ; *G. unicornis* from Sweden ; *G. convoluta* var. *capensis* from S. Africa and *G. spadix* from Gulf of Davao are confined to the sea. Of the Genus *Diopatra*, *Diopatra neapolitana* alone has been recorded off the coast of France, while *D. cuprea*, *D. orientalis* and *D. striata* have been recorded from brackish-water. *Asychis plimmertonensis*, *Macroclymenella stewartensis* and *Nichomache* from Schmarda ; *Clymene tropica* from Panama ; *C. annandalei* from Amoy, China and *C. grossa* var. *newporti* from S. California show a similar distribution as the worms belonging to the genus *Diopatra*. The genus *Marphysa*, however shows a much varied and cosmopolitan distribution. Of the six species known in this genus, only *Marphysa mortenseni* and *M. sanguinea* from the Pacific have been recorded from sea, whereas *M. hentschei* from the brackish-waters of Brazil ; *M. sanguinea* var. *americana* from the Canal Zone of S. America where waters are less saline (Monro, 1933) ; *M. sinensis* and *M. orientalis* from brackish-waters of Amoy, China all go to prove their wide distribution, even to regions of less saline media. The second mentioned form, *M. sanguinea* has also been recorded from Wailupe Pond (Abbot, 1946) and its occurrence in an almost fresh water pond is significant because the same species has been recorded from the sea. Thus the wide distribution of *Marphysa* indicate a tendency towards an assumption of freshwater life. Although factors like temperature, especially in the tropics (Panikkar, 1951), availability of minerals of biological importance etc., have played a part in the migration of animals from a marine habitat to less dilute media, the importance of nephrida and their role in osmoregulation are too arresting to be neglected. The modifications undergone by the nephrida as a result of environmental factors they had to face during their migrations, are too true to be doubted. In this connection the observation of Pearse (1939) that ' Heredity gave the ability and environment provided the opportunity ' seems to be quite apt.

Summary

The distribution of six species of polychaetes viz., *M. gravelyi*, *D. variabilis*, *G. embranchiata*, *O. eremita*, *C. insecta* and *L. medusa* has been studied on the basis of percentage of occurrence at various stations in the Adyar estuary, Madras. Among the polychaetes studied *M. gravelyi* was found to exhibit the greatest penetration occurring in regions where salinities were as low as even 5-10 parts per mille. It is argued that besides factors like salinity, temperature, availability of food, protection to eggs, abbreviations of stages in the life history and precocious development of nephridia in the larvae, factors like capacities for tolerance of salinity and abilities for osmotic regulation as reflected in the grades of structural modifications of nephridia, may have played an equally important role in the successful establishment and distribution of these polychaetes in the estuary.

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References

Abbot, D. P. 1946. Some Polychaetous Annelids from a Hawaiian fish pond. *Univ. Hawaii Res. Publ.*, **23** : 1-24.

Aiyar, R. G. 1931. An account of the development and breeding habits of a brackish-water polychaete of the genus *Marphysa*. *Jour. Linn. Soc. London*, **37** : 398-404.

Broekema, M. M. M. 1941. Seasonal movements and the osmotic behaviour of the shrimp. *Crangon crangon*. *L. Arch. Neer. Zool.*, **6** : 1-100.

Kinne, O. 1956b. Über den Einfluss des Salzegenaltes und der Temperatur auf Wachstum Form und Vermehrung bei dem Hydroidopolypen *Cordylophora caspia* (Pallas), *Thecata*, *Clavidae*. I. Mitteilung über den Einfluss des salzgenaltes auf Wachstum und Entwicklung mariner, brackishcer und limnischer organismen. *Zool. Jahrb., Allg. Zool. W. phys.*, **66** : 565-638.

Krishnan, G. 1936. The development of *Diopatra variabilis* (Southern). *Zeits. wiss. Zool. Leipzig*, **147** : 513-525.

———. 1952. On the nephridia of Neridiae in relation to habitat. *Proc. Nat. Inst. Sci. India*, **18** : 241-255.

Krishnamoorthi, B. 1951. Studies on the osmotic properties of eggs and larvae of a brackish-water polychaete, *Marphysa gravelyi* Southern. *Proc. Indian Acad. Sci.*, **34** : 199-209.

———. 1962. Salinity tolerance and Volume regulation in four species of polychaetes. *Ibid.*, **56**(6) : 363-371.

———. 1963. Gross morphology and histology of nephridia in four species of polychaetes. *Ibid.*, **57**(3) : 195-209.

——— and Krishnaswamy, S. 1962. On the activity of *Marphysa gravelyi* Southern (Polychaeta) under heterosmotic conditions. *Ibid.*, **57** : 83-87.

Monro, C. C. A. 1933. On a collection of Polychaeta from Dry Tortugas, Florida. *Ann. Mag. Nat. Hist. London*, **12** : 244-269.

PANIKKAR, N. K., AND AIYAR, R. G. 1937. The brackish-water fauna of Madras. *Proc. Indian Acad. Sci.*, **6** : 284-336.

———. 1951. Physiological aspects of adaptation to estuarine conditions. *Proc. Indo-Pacific Fish. Counc.*, 2 Meeting, April, 1950, Cronulla, New S. Wales, Australia. Sec. II & III : 162-175.

PEARSE, A. S. 1939. *The Migrations of Animals from Sea to Land.* Univ. Press, Durham, North Carolina.

REDEKE, H. C. 1933. Uber die Jetzigen Stand Unserer Kenntnisse der Flora und Fauna des Brackwassers. *Verh. Iternat. Verein theor. angen. Limnol.*, **6** : 46-61.

REMANE, A. 1934. Die Brackwasser fauna. *Verh. Deutsch. Zool. Cesell., Zool. Anz., Suppl.*, **7** :34-74.

TOPPING, F. L., AND FULLER, J. L. 1942. The accommodation of some marine invertebrates to reduced osmotic pressures. *Biol. Bull.*, **82** : 372-384.

VEERABHADRA RAO, K. 1951. Observations on the probable effect of salinity on the spawning, development and setting of the Indian backwater Oyster, *Ostrea madrasensis* Preston. *Proc. Indian Acad. Sci.*, **33** : 231-256.

VERWEY, J. 1957. A plea for the study of temperature influence on osmotic regulation. *L' Anne. Biol.*, **33** : 129-149.

WICKGREN, B. J. 1953. Osmotic regulation in some aquatic animals with special reference to the influence of temperature. *Acta Zool. Fennica*, **71** : 1-102.