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VOLUME REGULATION IN EGGS, LARVAE AND
ADULTS OF A BRACKISH-WATER POLYCHAETE,
DIOPATRA 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 $29.5 \pm 0.5^\circ \text{C}$. 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).

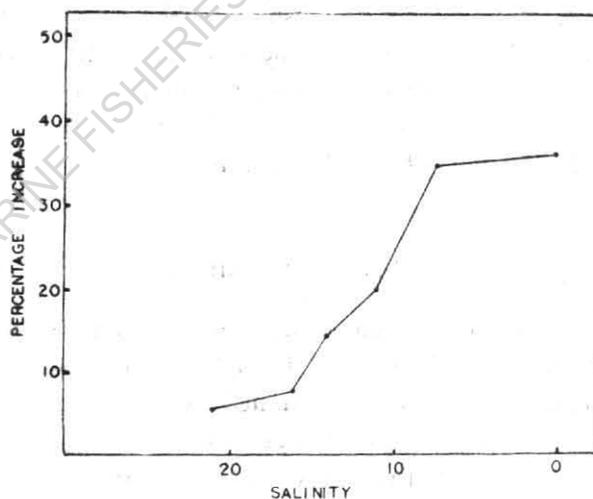


FIG. 1. Eggs—Increase in volume in different hypotonic media.

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.

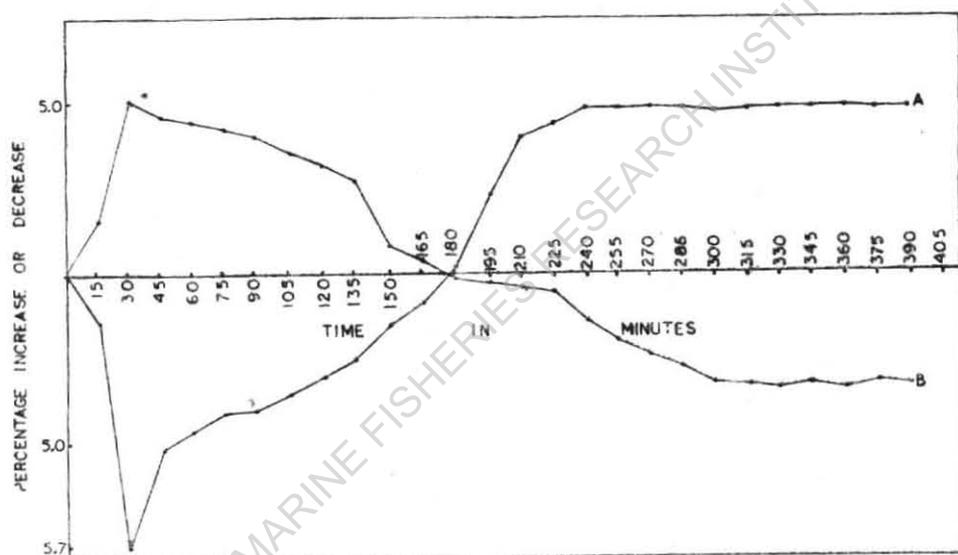


FIG. 2. Eggs—Volume change in hypotonic (B: 16.34‰) and in hypertonic (A: 27.39‰) media during different intervals.

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								Larvae								Adults			
	Without jelly				With jelly				Metatrochophores				Nectochaetae				Adults			
	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	17	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
9.22	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% Disintegration	
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 4½ 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 4½ 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.

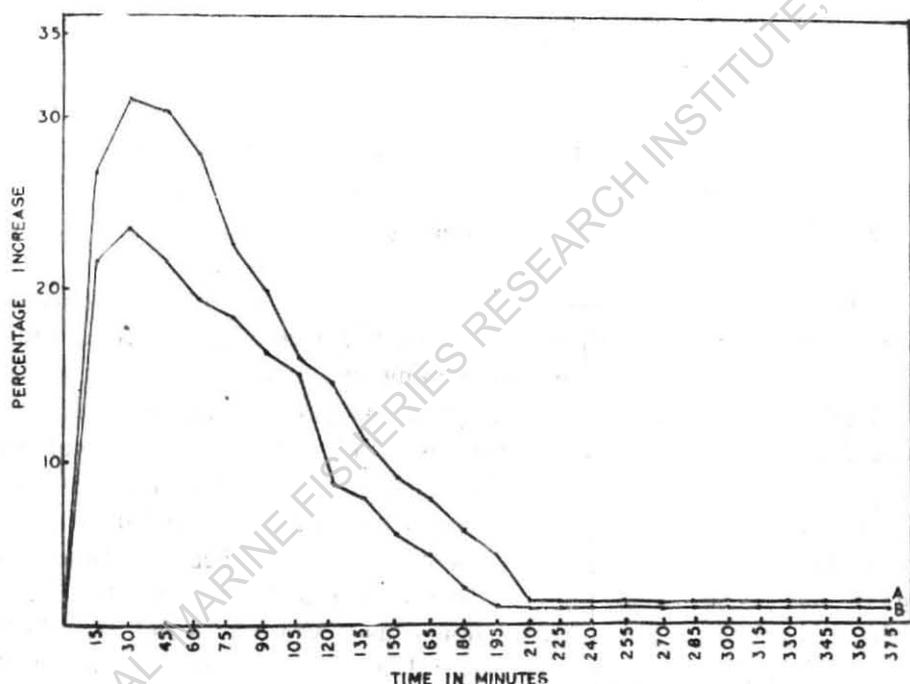


FIG. 3. Metatrochophores—Volume changes in two hypotonic media (A: 19‰; B: 21‰) during varying intervals.

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.

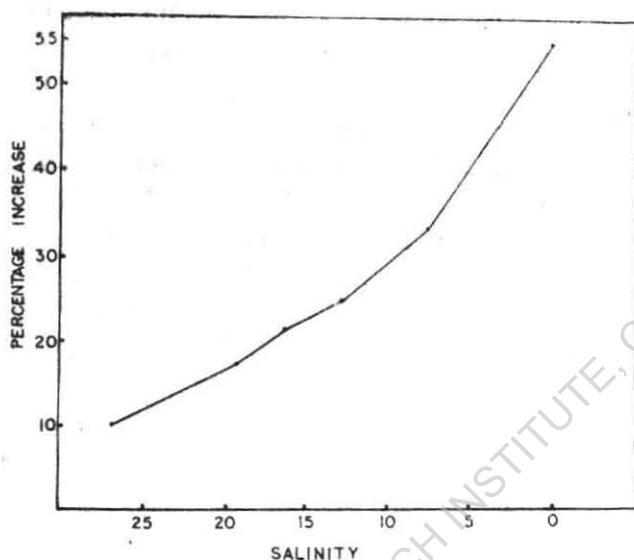


FIG. 4. Nectochaetae—Increase in volume in different hypotonic media.

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.

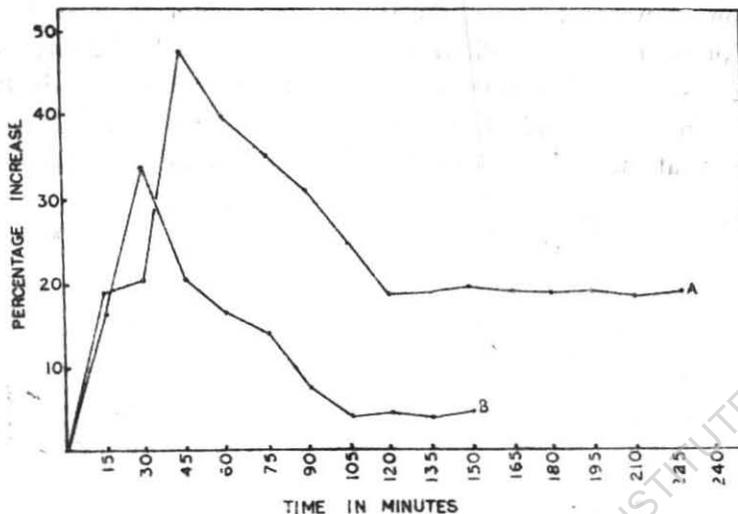


FIG. 5. *Nectochaetae*—Volume changes in two hypotonic media (A: 16.64‰; B: 20.64‰) during different intervals.

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.

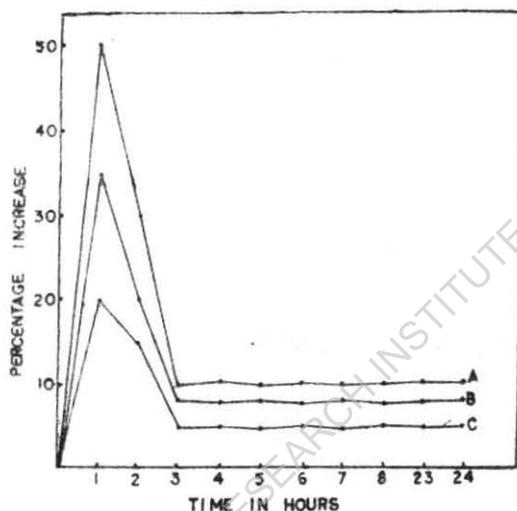


FIG. 6. Adults—Volume changes in three hypotonic media (A: 8.62‰; B: 13.7‰; C: 20.72‰) during different intervals.

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 graveyi* 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 latipes*, 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.

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VII. REFERENCES

- Beadie, 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 Protonocn 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 D₂O and H₂O," *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.

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- 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.

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