# ON THE NEPHRIDIA OF NEREIDAE IN RELATION TO HABITAT.

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CONTENTS



## 1. INTRODUCTION.

Beadle (1937) has suggested that in *Nereis diversicolor*, which is able to withstand lowering of the salinity of the external medium, the nephridia may be responsible for the necessary osmotic adjustments. A similar relationship between the thoracic nephridia and the ability to survive low salinity conditions has been shown to exist in *sabella pavonina* by Ewer and Ewer (1943). Since closely allied species differ widely in their osmotic behaviour and therefore in their ability to thrive in waters of low and varying salinity it was thought that an examination of the nephridia in such species would be of interest, as it might reveal whether the nephridia which perform osmoregulatory functions show correlated anatomical variations. Hitherto work on nephridia of polychaetes was carried out without reference to the external medium. In the following study an attempt is made to compare the nephridia of allied species of Nereidae from the sea and brackish water to find if any relation exists between the nephridia and the salinity of the external medium.

## 2. MATERIAL AND METHODS.

The material for this study consists of the following Nereids: *Lycastis indica* Southern, *Nereis chilkaensis* Southern and *Pereinereis nuntia* Savigny. *Lycastis indica* was collected from the brackish waters in the vicinity of Madras where it is abundantly distributed. In this habitat there is a wide seasonal fluctuation in salinity. (Panikkar and Aiyar, 1937). *Lycastis indica* occurs very close to the sea as well as in the inner reaches of the brackish water area where the water is almost fresh. In the laboratory it has been kept for months in fresh water in a healthy condition. Though it shows a marked tolerance to salinity changes it has not so far been encountered in the sea. *Nereis chilkaensis* was taken from the Madras harbour where it occurs in large numbers in the midst of sedentary organisms which are attached to the buoys and boulders. The harbour is an artificial enclosure of about quarter of a square mile in area enclosed by concrete breakwaters. The

salinity of the water here may be slightly less than that of the open sea (Aiyar and Panikkar, 1937). *Nereis chilkaensis* also occurs in the Adyar backwater but is confined to a region close to the bar where the salinity is comparatively high. It would appear that its ability to tolerate changes in salinity is limited. *Perinereis nuntia* was obtained from the Madras beach near the harbour. The worms are found under stones between tide marks. There are no records of this species from waters less saline than the sea. From its distribution and occurrence it would appear that it is exclusively marine and cannot withstand dilution of the external medium.

In the following investigation both fresh and sectioned materials were studied. The worms were narcotised by gradual addition of alcohol to the water in which they were kept. A number of fixatives such as Bouin's fluid, Duboscq Bouin, Zenker's fluid and Susa were tried. Fixation with Bouin's fluid overnight gave successful results. Sections were cut 8 to 10 $\mu$ thick and stained in Heidenhain's haematoxylin.

For experimental purposes the specimens of *Lycastis* were brought up to higher salinity conditions by the gradual addition of sea water. In about ten days the worms were acclimatised to sea water, in which they remained vigorous thereafter indefinitely. No special attempt was made to feed them. After the animals had thus been kept for a week, they were fixed at periodical intervals. The fixatives used were the same as for normal animals and the sections were cut 8 to 10 $\mu$ thick and stained in Heidenhain's haematoxylin. In attempting to estimate the relative size of the nephridium the following procedure was adopted. The length of the worm was measured and this divided by the number of segments was taken as the average length of a single segment. The girth of the worm at different points was measured and the average obtained was assumed to be the girth of a single segment. These two readings together with the average height of the segment would give a measure of the cubic capacity of a single segment. The size of the body of the nephridium was arrived at by taking a number of readings and striking an average of what may be called the length, breadth and height of the nephridium as seen in sections. From the above readings the index of volume of the segment and that of the nephridium are calculated and the ratio between these two values in the species studied are compared. Measurements of the size of the nephridium taken from the fixed sections may differ slightly from those of the living animals due to shrinkage in the process of fixation. But as the object is to arrive at the relative sizes of the nephridium of the three species studied, any shrinkage due to fixatives may not vitiate the values found. Further from the measurements of the segment and the nephridium that could be made and by the nature of the organs measured an exact quantitative computation is difficult to make. But as only an expression of the relative sizes of the nephridium and the segment is sought, the above procedure would appear to be justified. The size of the nephridium was also compared with the weight of the animal both wet and dry. For taking the dry weight, the worms were kept in a hot air oven at a temperature of 105°C. The animals were weighed at intervals till constant readings are obtained.

## 3. Nephridia in Nereidae.

### (*a*) *Previous Work:*

Our knowledge of the nephridium of *Nereis* is based largely on the detailed account given by Goodrich (1893, 1900, 1945) who observed that it is of the metanephridial type without any connection with a coelomostome. The passage of the genital products is not through the nephridium nor by way of coelomoducts which have lost the function of serving as genital conduits. The method of escape of the reproductive elements in a number of Nereids is still obscure, although it is known that in those with an epitokous phase the body wall splits up for the discharge of

the genital products. A point of interest in regard to the nephridia is the blood supply they receive and the significance of the same. Cosmovici (1879) figured a network of blood vessels surrounding the nephridium in a number of Polychaetes. It would appear that there is considerable quantitative variation in regard to the blood supply to the nephridium even among closely allied species (Gamble and Ashworth, 1900). As in the case of the blood supply the size of the nephridium also appears to vary considerably. With a few exceptions papers on nephridia do not contain details about the size of the nephridium.

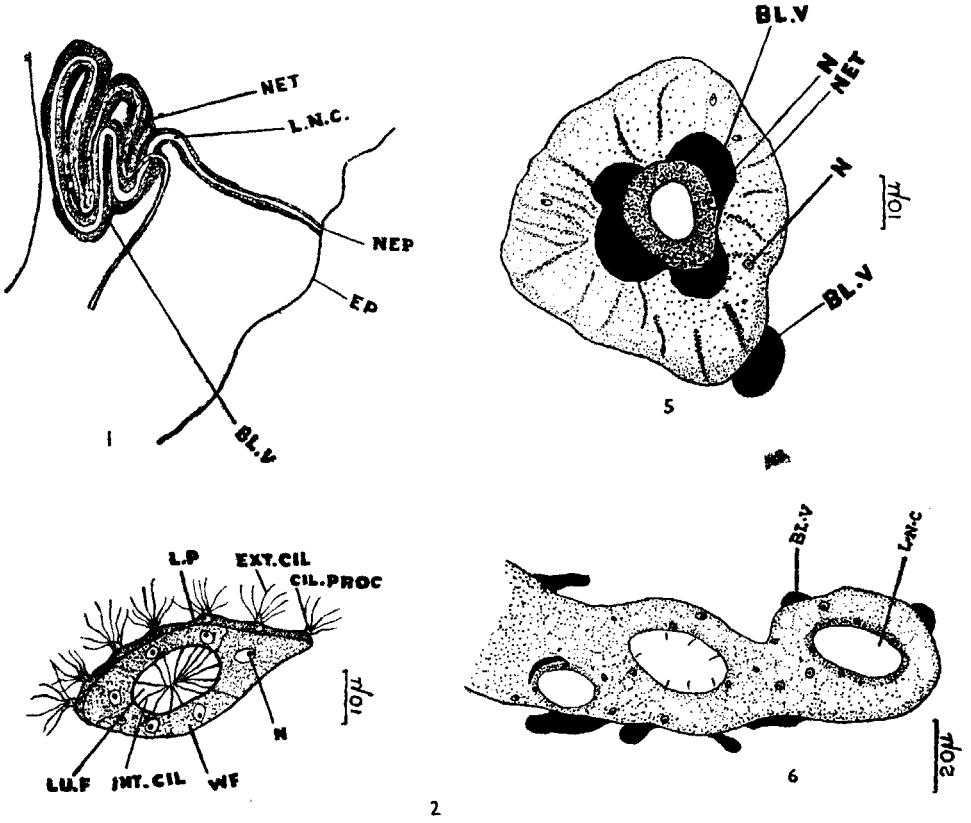
(*b*) *Nephridia of Lycastis indica:*

The nephridia in *Lycastis indica* lie on either side of the gut in the body cavity. Figure 1 shows a diagrammatic section of the nephridium. It consists, as in *Nereis*, of a pre-septal funnel (not shown in the above figure) and a post-septal body through which runs the nephridial canal. Unlike in *Nereis*, the nephridial canal is disposed in the form of distinct loops held together by connective tissue similar to the nephridium of Oligochaeta. The body of the nephridium consisting of the loops of the nephridial canal, together with the connective tissue enveloping them, extends dorso-ventrally from about the level of the dorsal blood vessel to a point slightly above the ventral blood vessel. The last part of the nephridial canal emerges free on the ventral side of the body of the nephridium and runs to the nephridiopore on the ventral epidermis by the side of the ventral longitudinal muscle. In conformity with the typical plan in annelids, the nephridium occupies parts of two consecutive segments. It begins with a pre-septal funnel from which leads a canal to the post-septal part. The canal on entering the body of the nephridium becomes continuous with the ciliated canal running inside. The ciliated canal courses dorsalwards making several coils. At the dorsal end it turns ventralwards forming a loop and then passes into the next division which is characterised by the absence of cilia. The non-ciliated canal after reaching the ventral edge of the body of the nephridium turns again dorsalwards and runs parallel to the ciliated canal. Dorsally it again turns inwards forming an inner loop. Before it emerges free from the body of the nephridium, it forms another loop. The different parts of the canal and the relation between them can be seen in figure 1 and (Ph.m. 1 and 2).

The nephrostome is funnel-shaped, the lip of the funnel being folded back as in *Nereis diversicolor* (Goodrich, 1893). Fig. 2, shows a transverse section across the margin of the funnel. From the free edge of the funnel as well as from the reflected surface of the margin of the funnel finger-shaped processes arise carrying numerous cilia. The internal surface of the funnel carries long prominent cilia which beat towards the lumen of the funnel. The walls of the funnel are formed of large cells with prominent nuclei. The cell limits are not clear and the cytoplasm is granular. There is a prominent inner border to the funnel cells. The funnel is covered externally by coelomic epithelium.

Leading from the funnel is the first part of the nephridial canal which is continuous with the post-septal part of the nephridium (Fig. 3). The lumen of the canal is narrow and not ciliated. The bounding cells are small with granular cytoplasm. This canal on entering the body of the nephridium is continued into the next part which is ciliated (Fig. 4), the cilia being long and prominent. The cytoplasm of the canal cells is granular and no cell limits are visible. The ciliated canal runs dorsalwards forming the middle loop of the nephridium. The succeeding part of the canal is non-ciliated and is of considerable length. It runs through the inner and outer loops of the nephridium. There is a well-marked inner boundary layer which forms a lining to its lumen. The nuclei of the bounding cells are large with prominent nucleoli. A feature of this part of the nephridial canal is the presence of blood capillaries which run all round the canal closely

investing its lumen (Fig. 5, Ph.m. 1 and 2). In transverse sections the blood capillaries are seen forming a ring round the canal. After coursing through the inner

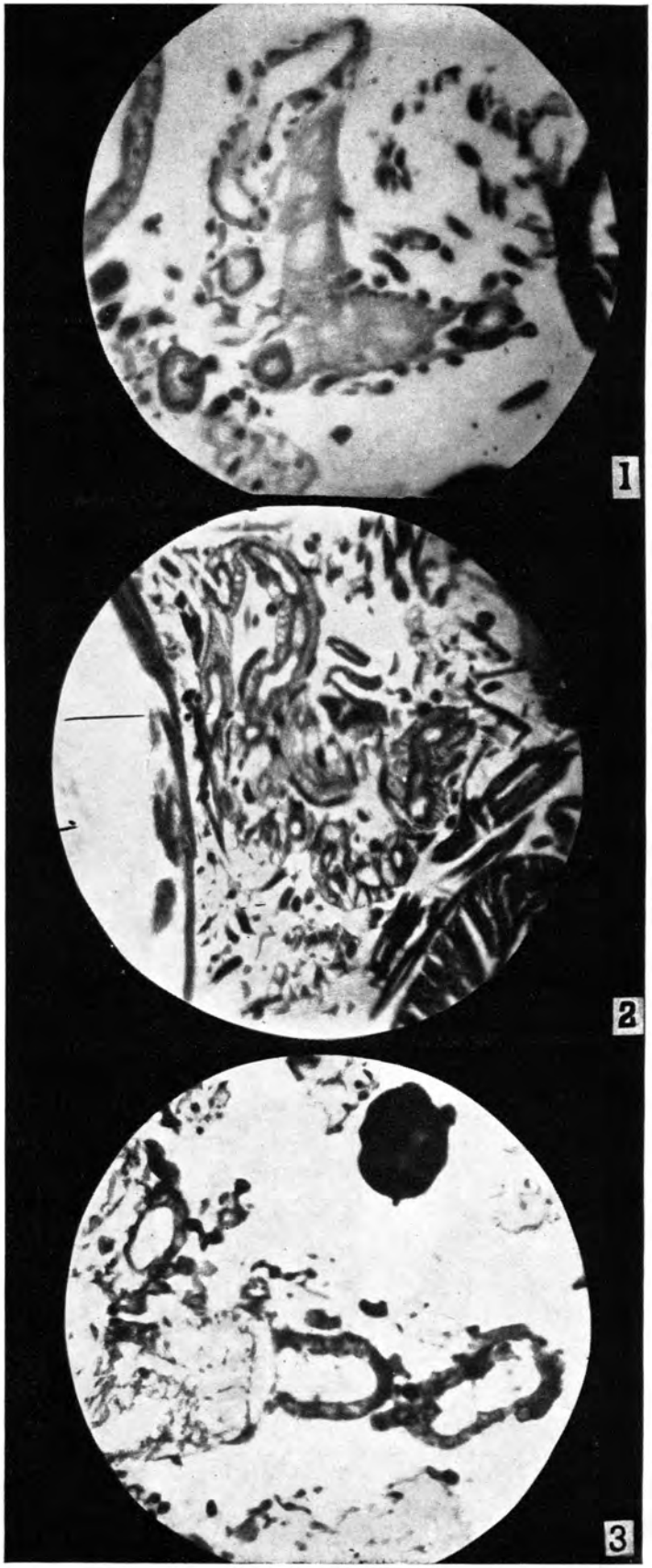



*Lycastis indica:*

FIG. 1. An optical section of the nephridium (diagrammatic) showing the different regions of the canal. The nephridiostome is not shown in the figure.
FIG. 2. A transverse section through the nephridiostome.
FIG. 5. A transverse section through the wider non-ciliated part of the nephridial canal showing the position of blood capillaries.
FIG. 6. A section through a part of the nephridium of a worm acclimatised to sea-water.

and outer loops of the nephridium the canal emerges out of the body of the nephridium and runs towards the lateral border of the ventral longitudinal muscle where it opens by the nephridiopore.

The connective tissue which envelopes the nephridial canal is much vacuolated (Fig. 5). The nuclei of these cells are small and loosely arranged fibrils of various degrees of thickness are seen in the connective tissue and also in the cytoplasm of the cells of the canal. They pass circularly round the non-ciliated part of the canal. The appearance and distribution of these fibrils are similar to those in *Nereis diversicolor*. It is not known what function they serve in Polychaetes. They recall the so-called resistance fibrillae known to be of wide occurrence in the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nephridia of Oligochaeta, where it has been suggested that they function in preventing a too extreme compression of the walls of the nephridial canal (Stephenson, 1930).

The blood supply to the nephridium is highly developed. It comes from a lateral vessel which arises as a branch from the ventral longitudinal vessel. This vessel runs to the gill, and close to the body of the nephridium gives off a number of branches one behind the other to the nephridium. Each such branch divides forming a cluster of twigs which spread over the nephridium. The capillaries penetrate the connective tissue enclosing the nephridial canal and come to lie in close association with the distal part of the nephridial canal (Fig. 5, Ph.m. 1 and 2). In this position the blood capillaries are separated from the lumen of the nephridial canal by the narrow cells lining the canal. Another feature of the vascular supply to the nephridium is the presence of blind-ending capillaries with minute dilatations on them (Ph.m. 1). These do not appear to have been described so far, in the Nereidae. The ampullae, as these dilatations have been called, are of wide occurrence in many Oligochaetes (Stephenson, 1930) and have also been observed in a few Polychaetes. Benham (1891) noted them in the blood vessels supplying the nephridium in species of *Arenicola*. Their functional significance is obscure. Gegenbaur (as quoted by Benham, 1891) thought that they are connected with the reproductive function. Claparédé (1869) figured them as being filled with corpuscles which might suggest that they subserve the excretory function. The blind-ending capillaries have been noted in relation to the nephridia in widely separated species such as *Marphysa sanguinea* (Fuchs, 1907) and *Lanice conchilega* (Meyer, 1888). It is possible that they occur more widely than at present known. Their occurrence appears to be somewhat erratic being found in one species and absent in other closely related species. Ewer (1941) noted them on the segmental organ of *Travesia forbesii* but Brown (1938) who worked on the allied *Ophelia cluthensis* failed to see them. Ewer discusses at some length the possible function of the blind-ending capillaries and concludes that in the absence of a more complete knowledge of the mechanics and physiology of circulation, their significance, if any, must remain a matter for speculation.

Tables I and II show the relation between the size of the nephridium and the size of the animal. An attempt is here made to compare the size of the nephridium with the body weight as well as with the cubic capacity of a single average segment.

TABLE I.

| | Worm. | | | | | Nephridium | | |
|---|---|---|---|---|---|---|---|---|
| No. | Length of the worm. mm. | No. of segments. | Length of a segment. mm. | Breadth of a segment. mm. | Height of a segment. mm. | Length of the body. $\mu$ | Breadth of the body. $\mu$ | Height of the body. $\mu$ |
| 1 | 56 | 95 | ·568 | 1·50 | ·929 | 190 | 146 | 269 |
| 2 | 89 | 130 | ·685 | 1·75 | ·888 | 240 | 170 | 296 |
| 3 | 82 | 125 | ·655 | 1·60 | ·942 | 210 | 152 | 310 |
| 4 | 102 | 126 | ·806 | 2·50 | 1·211 | 290 | 272 | 471 |
| 5 | 54 | 75 | ·720 | 2·20 | ·915 | 270 | 238 | 444 |
| 6 | 130 | 148 | ·878 | 2·60 | ·942 | 310 | 242 | 256 |

Table I gives the dimensions of a single segment and the size of the nephridium. Table II shows the relation between the body weight both dry and wet weight and the size of the nephridium.

TABLE II.

| Worm. | | | | | | | Nephridium. | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Length of the worm. mm. | No. of segments | Girth of a segment. mm. | Wet weight. gm. | Dry weight. gm. | % of water. | Length of the body. $\mu$ | Breadth of the body. $\mu$ | Height of the body. $\mu$ |
| 1 | 56 | 95 | 1·50 | ·300 | ·045 | 85·00 | 190 | 146 | 269 |
| 2 | 89 | 130 | 1·75 | ·550 | ·062 | 88·73 | 240 | 170 | 296 |
| 3 | 82 | 125 | 1·60 | ·505 | ·060 | 88·12 | 210 | 152 | 310 |
| 4 | 102 | 126 | 2·50 | ·755 | ·082 | 89·14 | 290 | 272 | 471 |
| 5 | 54 | 75 | 2·20 | ·558 | ·041 | 92·65 | 270 | 238 | 441 |
| 6 | 130 | 148 | 2·60 | 1·050 | ·105 | 90·00 | 310 | 242 | 256 |

It is seen that the percentage of water varies between 85 and 92. A comparison of these values with those relating to the specimens acclimatised to sea-water is interesting. Table III shows the values for the weight of animals which have been acclimatised to sea-water.

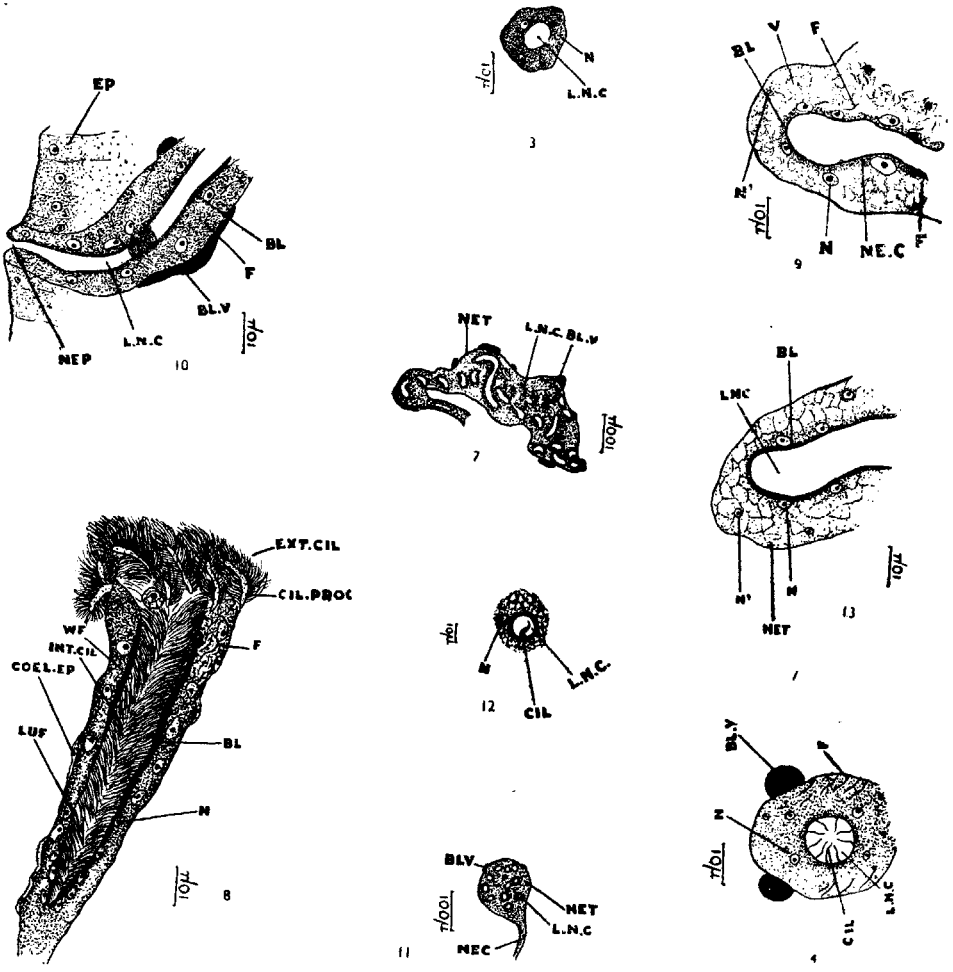
TABLE III.

| No. | Length of the worm. mm. | No. of segments. | Girth of a segment. mm. | Wet weight. gm. | Dry weight. gm. | % of water. |
|---|---|---|---|---|---|---|
| 1 | 48 | 78 | 1·50 | ·165 | ·030 | 81·81 |
| 2 | 50 | 80 | 1·45 | ·150 | ·023 | 84·67 |
| 3 | 45 | 75 | 1·40 | ·125 | ·030 | 76·00 |
| 4 | 110 | 135 | 2·50 | ·475 | ·065 | 86·31 |
| 5 | 99·5 | 132 | 2·55 | ·455 | ·060 | 84·62 |

It is seen that there is a fall in weight of the animals compared to those living in their normal habitat, i.e. fresh water. The change in weight on transference to sea-water might indicate an upset of the osmotic equilibrium due to a change in the concentration of the external medium resulting in water movements between the body fluids and the external medium as has been noted in a number of animals. Panikkar (1941) pointed out that *Leander serratus* and *Palaemonetes varians* when transferred from dilute to concentrated sea-water showed a fall in weight which is due to the escape of water to the exterior.

The importance of the nephridia in the regulation of the water balance of these worms is indicated in the changes that have been observed in the nephridia of specimens acclimatised to sea-water. Sections prepared of specimens after a fortnight's stay in sea-water show that the blood supply to the nephridium has undergone a marked diminution as seen from the appearance of the blood capillaries of the nephridium. This is in contrast to what is seen in the sections of nephridia of the controls which had been in fresh water. In the latter the capillaries form a conspicuous feature. The ampullae and the capillaries that surround the lumen of the nephridial canal show a bulged out appearance being filled with blood. On the other hand, in the test animals the capillaries become shrunk and shrivelled due possibly to the diminished flow of blood in them (Fig. 6 and Ph.m. 3). Some of the smaller vessels do not show at all possibly due to complete collapse. This is

the case in regard to the vessels surrounding the distal part of the nephridial canal so well seen in the sections of the controls. The possibility of such an appearance being an histological artefact is ruled out by the circumstance that in all the test animals sectioned, this feature is invariably seen, whereas in not a single specimen of the controls prepared by an identical technique is a similar condition met with.




*Lycastis indica:*

FIG. 3. A transverse section of the nephridial canal immediately following the funnel.
FIG. 4. A transverse section through the ciliated part of the nephridial canal.

*Nereis chilkaensis:*

FIG. 7. A section passing through the body of the nephridium.
FIG. 8. The nephridiostome as seen in a longitudinal section showing the ciliated processes.
FIG. 9. A longitudinal section through the non-ciliated part of the nephridial canal inside the body of the nephridium.
FIG. 10. A section through the last part of the nephridial canal and the nephridiopore.

*Perinereis nuntia:*

FIG. 11. A section passing through the body of the nephridium.
FIG. 12. A transverse section through the ciliated part of the nephridial canal.
FIG. 13. A longitudinal section through the wider non-ciliated part of the nephridial canal.

The reduced blood supply to the nephridium in animals acclimatised to sea-water might indicate that the nephridia in these are probably doing less osmotic work than in the normal forms living in fresh water.

(*c*) *Nephridia of Nereis chilkaensis:*

The nephridia are arranged in pairs in all segments excepting a few segments at either end of the worm. Each nephridium lies at the entrance to the parapodial cavity between the ventral cirrus and the lateral border of the ventral longitudinal muscles and has an elongated oval-shaped body made up of a mass of connective tissue cells. The channel for the passage of the excretory fluid is a long coiled canal which runs a tortuous course in the body of the nephridium (Fig. 7). The canal emerges free from the body of the nephridium on its ventral side and runs towards the body cavity ventrolaterally to open to the exterior by the nephridiopore which is a small opening in the ventral epidermis on the outer side of the lateral border of the ventral longitudinal muscles. Dorsally, the nephridial canal is continuous with a narrow duct which runs a winding course towards the body cavity where it opens in front of the anterior septum by a funnel-shaped nephrostome (Fig. 8) which carries a number of finger-shaped processes along the edge of the funnel. These processes are short and stumpy and more or less resemble those of *Nereis diversicolor*. But the lip of the funnel is not reflected and the finger-shaped processes are confined to the free margin of the funnel. The cells of the funnel are large and vesicular with prominent spherical or oval nuclei which contain in addition to a deeply staining nucleolus smaller nucleolar bodies. The cell boundaries are not clear and the cytoplasm is granular. A prominent feature of these cells is the dark staining inner border which forms a lining to the funnel. Fibrillar structures are clearly seen in the cytoplasm of these cells similar to those found in the body of the nephridium. The outer surface of the funnel is covered with coelomic epithelium. In some of the preparations a few cells are seen adhering to the margin of the funnel. The condition is similar to that observed in many Oligochaetes where such have been shown to be coelomic corpuscles in the process of giving up the excretory products or getting disintegrated to be finally thrown out to the exterior through the nephridium (Stephenson, 1930).

The first part of the nephridial canal leading from the funnel is of narrow lumen. The cells bordering the lumen are flattened, and without cilia. The canal on entering the body of the nephridium is ciliated for some length. The arrangement of the cilia appears to vary in different regions of this part of the canal. The cell limits are absent, and the nuclei appear at intervals. After coiling about within the body of the nephridium, the ciliated part passes into the next division which is characterised by the absence of cilia (Fig. 9). This part of the canal is considerably longer than the preceding division and has a wider lumen. The lining cells are larger in size, and vacuolated. The cells are also distinguished by a well-marked inner boundary layer which stains dark with iron haematoxylin and by the occurrence of fibrillar structures in the cytoplasm. The canal leaves the body of the nephridium ventrally and runs directly to the nephridiopore which is a small opening on the ventral epidermis. Close to the nephridiopore as seen in sections are two large nuclei one on each side at the terminal part of the nephridial canal (Fig. 10). The connective tissue which invests a greater part of the nephridial canal consists of cells which are vacuolated. The degree of vacuolation varies in different regions of the body of the nephridium.

The blood supply to the nephridium seems to be less developed than in *Lycastis*. Blood comes from a lateral branch of the ventral blood vessel, which on entering the parapodial cavity gives off a bunch of fine branches which further subdivide and spread over the nephridium. The blood capillaries lie between the coelomic epithelium and the connective tissue. The blood from the nephridium is returned to a lateral vessel which comes from the parapodium and joins the dorsal

longitudinal vessel. The blood vessels are not only fewer but they differ markedly in their position relative to the nephridial canal. The blind-ending capillaries and the ampullae so well seen in *Lycastis* are absent in this species. Table IV shows the size of the nephridium relative to that of the segment in which it lies.

TABLE IV.

| Worm. | | | | | | Nephridium. | | |
|---|---|---|---|---|---|---|---|---|
| No. | Length of the worm. mm. | No. of segments. | Length of a segment. mm. | Breadth of a segment. mm. | Height of a segment. mm. | Length of the body. $\mu$ | Breadth of the body. $\mu$ | Height of the body. $\mu$ |
| 1 | 35 | 66 | ·530 | 1·20 | ·646 | 104 | 128 | 81 |
| 2 | 33 | 65 | ·509 | 1·00 | ·566 | 96 | 120 | 81 |
| 3 | 48 | 72 | ·667 | 1·50 | ·980 | 144 | 187 | 108 |
| 4 | 38 | 60 | ·633 | 1·10 | ·781 | 128 | 140 | 67 |
| 5 | 36 | 64 | ·563 | 1·20 | ·727 | 104 | 162 | 94 |
| 6 | 30 | 52 | ·577 | 1·20 | ·781 | 96 | 135 | 108 |

TABLE V.

| Worm. | | | | | | | Nephridium. | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Length of the worm. mm. | No. of segments. | Girth of a segment. mm. | Wet weight worm. gm. | Dry weight worm. gm. | % of water. | Length of the body. $\mu$ | Breadth of the body. $\mu$ | Height of the body. $\mu$ |
| 1 | 35 | 66 | 1·20 | ·100 | 0·028 | 72·00 | 104 | 128 | 81 |
| 2 | 33 | 65 | 1·00 | ·120 | 0·032 | 71·67 | 96 | 120 | 81 |
| 3 | 48 | 72 | 1·50 | ·300 | 0·045 | 85·00 | 144 | 140 | 108 |
| 4 | 38 | 60 | 1·10 | ·085 | 0·025 | 70·59 | 128 | 140 | 67 |
| 5 | 34 | 64 | 1·20 | ·175 | 0·041 | 76·57 | 104 | 162 | 94 |
| 6 | 30 | 52 | 1·20 | ·125 | 0·027 | 78·40 | 96 | 135 | 108 |

Comparing these values with the corresponding ones in *Lycastis* it will be seen that the nephridium in *Nereis chilkaensis* is smaller relatively to the size of the segment. The relation between the size of the nephridium and the size of the animal on the basis of the body weight is given in Table V. In the same table are given dry weights of the animal. *Nereis chilkaensis* unlike *Lycastis indica* is not able to survive in fresh water. Acclimatisation experiments on this species show that the degree of tolerance to changes in the salinity of the external medium is limited. But compared with many of its marine relatives, it shows a certain endurance to changes in the salinity.

*(d) Nephridia of Perinereis nuntia:*

The nephridia of *Perinereis nuntia* resemble in their lay out and general features of their structure those of *Nereis chilkaensis*. As in the latter the nephridia occur segmentally at the entrance to the parapodial cavity. Fig. 11 shows a section

through the body of the nephridium. It is somewhat oval in outline and is formed of a mass of connective tissue through which the nephridial canal winds in a complicated manner. The histological features of the connective tissue present a close similarity to the condition seen in other Nereidae. In the nephridial canal the same divisions as were made out in *Nereis chilkaensis* could be seen. Fig. 12 shows a section through the ciliated part of the canal and Fig. 13 shows a section through the wider non-ciliated part of the nephridial canal. The blood supply to the nephridium is poorly developed. The delicate twigs of vessels on the nephridium so well seen in *Nereis chilkaensis* are here markedly reduced. A few small vessels that are seen on the nephridium arise as in the allied species of *Nereis* from a branch vessel which supplies the parapodium. The blind-ending capillaries and the ampulla are absent.

Table VI gives the dimensions of the body of the nephridium relative to the size of an average segment of the animal. From the values obtained it is seen that the size of the nephridium is smaller compared to that of *Nereis chilkaensis* and *Lycastis indica.*

TABLE VI.

| | Worm. | | | | | Nephridium. | | |
|---|---|---|---|---|---|---|---|---|
| No. | Length of the worm. mm. | No. of segments. | Length of a segment. mm. | Breadth of a segment. mm. | Height of a segment. mm. | Length of the body. μ | Breadth of the body. μ | Height of the body. μ |
| 1 | 64 | 85 | ·753 | 2·80 | 1·890 | 96 | 94 | 188 |
| 2 | 50 | 58 | ·862 | 2·00 | 1·080 | 80 | 81 | 162 |
| 3 | 58 | 65 | ·892 | 2·50 | 1·620 | 96 | 81 | 135 |
| 4 | 40 | 62 | ·645 | 1·00 | 0·942 | 72 | 67 | 107 |
| 5 | 36 | 64 | ·563 | 0·80 | 0·673 | 72 | 61 | 94 |

In Table VII are given the size of the nephridium in relation to the body weight of the animal.

TABLE VII.

| | Worm. | | | | | | Nephridium. | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Length of the worm. mm. | No. of segments. | Girth of a segment. mm. | Wet weight worm. gm. | Dry weight worm. gm. | % of water. | Length of the body. μ | Breadth of the body. μ | Height of the body. μ |
| 1 | 64 | 85 | 2·80 | ·425 | ·095 | 77·65 | 96 | 94 | 188 |
| 2 | 50 | 58 | 2·00 | ·282 | ·062 | 78·01 | 80 | 81 | 162 |
| 3 | 58 | 65 | 2·50 | ·180 | ·048 | 73·33 | 96 | 81 | 135 |
| 4 | 40 | 62 | 1·00 | ·155 | ·025 | 83·87 | 72 | 67 | 107 |
| 5 | 36 | 64 | 0·50 | ·055 | ·010 | 81·82 | 72 | 61 | 94 |

## 4. COMPARISON OF THE NEPHRIDIA.

The nephridia in all the three species examined are disposed metamerically in all segments excepting a few anterior and posterior segments. In *Nereis chilkaensis*

and *Perinereis nuntia* the nephridium is situated ventro-laterally at the entrance to the parapodial cavity, while in *Lycastis indica* the nephridium is more centrally located in the body cavity on either side of the alimentary canal. The histological features of the canal and the connective tissue enveloping the canal are similar in all the three species. The nephrostome occupies the same position and shows similar structural details except that in *Lycastis indica* the margin of the funnel is reflected and processes carrying cilia arise from the reflected surface also. A notable difference between the nephridia of *Lycastis indica* and of *Nereis* apart from size, is in regard to blood supply. *Nereis chilkaensis* shows comparatively a richer blood supply than *Perinereis nuntia*. The same blood vessel supplies the nephridium in *Lycastis* and *Nereis* but *Lycastis* differs from the two allied species of Nereidae not only in the profuse blood supply but also in the manner of distribution of the blood capillaries and in the presence of dilatations on some of the capillaries. The penetration of capillaries into the connective tissue so as to come in close proximity with the nephridial canal is significant. The part played by the nephridium in all the three species is apparently the same but it appears to differ quantitatively. The nephridium in *Lycastis indica* appears to be an elaboration of the structure met with in *Nereis chilkaensis* and *Perinereis nuntia*.

Although the nephridia are similar in structure in all the three species, they show marked differences in size.

Table VIII gives the values of the index of volume of the nephridium relative to that of the segment. It will be seen that the average ratio between the index of volume of the nephridium and that of the segment shows marked differences in the three species. In Table IX are given the deviations from the average ratio and the square root of the deviations. The 'students' 't' test was applied to verify whether the differences noted above really exist in the species or whether they are only due to variations in sampling. It was found that for *Lycastis indica* and *Nereis chilkaensis* the value of 't' calculated from the observations made is 7·627 whereas the value of 't' at 1% level for 10 degrees of freedom is 3·169. Since the value obtained from the readings is higher it would appear that the differences in the ratio of the index volume of the segment and that of the nephridium between the two types studied exist in the species and are not due to errors in sampling. Similarly the readings obtained for *Lycastis indica* and *Perinereis nuntia* give the

TABLE VIII.

| | *Lycastis indica.* | | | *Nereis chilkaensis.* | | | *Perinereis nuntia.* | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Index of Vol. of segment. Cu. microns. | Index of Vol. of nephridium. Cu. microns. | Index of ratio of Vol. of S. & N. % | Index of Vol. of segment. Cu. microns. | Index of Vol. of nephridium. Cu. microns. | Index of ratio of Vol. of S. & N. % | Index of Vol. of segment. Cu. microns. | Index of Vol. of nephridium. Cu. microns. | Index of ratio of Vol. of S. & N. % |
| 1 | 0·791508 | ·007462 | 0·943 | ·410856 | ·001078 | 0·262 | 4·098730 | ·001697 | 0·041 |
| 2 | 1·037850 | ·012077 | 1·164 | ·287585 | ·000933 | 0·324 | 1·861920 | ·001050 | 0·056 |
| 3 | 0·987216 | ·009895 | 1·002 | ·980490 | ·002908 | 0·297 | 3·612600 | ·001050 | 0·029 |
| 4 | 2·440165 | ·037152 | 1·522 | ·537810 | ·001200 | 0·223 | 0·607590 | ·000516 | 0·085 |
| 5 | 1·449360 | ·028531 | 1·969 | ·491161 | ·001603 | 0·326 | 0·303119 | ·000413 | 0·136 |
| 6 | 2·106398 | ·019205 | 0·912 | ·540764 | ·001399 | 0·259 | .. | .. | .. |
| | Average ratio: | | 1·252 | | | 0·281 | | | 0·069 |

TABLE IX.

| | *Lycastis indica.* | | | *Nereis chilkaensis.* | | | *Perinereis nuntia.* | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Index of ratio of Vol. of S. & N. % | Deviation average. | Square root of deviation. | Index of ratio of Vol. of S. & N. % | Deviation average. | Square root of deviation. | Index of ratio of Vol. of S. & N. % | Deviation average. | Square root of deviation. |
| 1 | 0·943 | ·309 | ·095481 | ·262 | ·019 | ·000361 | ·041 | ·028 | ·000784 |
| 2 | 1·164 | ·088 | ·007744 | ·324 | — ·143 | ·020449 | ·056 | ·013 | ·000169 |
| 3 | 1·002 | ·250 | ·062500 | ·297 | — ·016 | ·000256 | ·029 | ·040 | ·001600 |
| 4 | 1·522 | — ·270 | ·072900 | ·223 | ·058 | ·003364 | ·085 | — ·016 | ·000256 |
| 5 | 1·969 | — ·717 | ·091909 | ·326 | — ·045 | ·002025 | ·136 | — ·067 | ·004489 |
| 6 | 0·912 | ·340 | ·115600 | ·259 | ·022 | ·000484 | .. | .. | .. |

value of 't' as 8·333 while 't' at 1% level for 9 degrees freedom is 3·250 and for *Nereis chilkaensis* and *Perinereis nuntia* the value of 't' is 5·436. So the hypothesis that there may not be any such differences in the corresponding values for the species can be rejected. From the above it seems justifiable to conclude that the nephridium in *Lycastis indica* is comparatively larger in size than that of *Nereis chilkaensis* and *Perinereis nuntia*.

## 5. DISCUSSION.

The differences in the size and blood supply of the nephridia of the above three species of Nereidae, are significant and can probably be explained with reference to their habitat. It has been seen that *Lycastis indica* though found in fresh water is able to live in waters of high salinity. *Nereis chilkaensis* shows a more restricted range of distribution. It can withstand a certain amount of dilution of the external medium enabling it to thrive in brackish water, while *Perinereis nuntia* is a purely marine species.

The question is how far the differences noted in the nephridia of these species are related to their ability to withstand variations in salinity of the external medium. The importance of excretory organs in enabling marine species to survive a lowering of salinity of the surrounding water is suggested by Beadle (1937) from his experiments on *Nereis diversicolor* in which circumstantial evidence points to an elimination by the nephridia of a fluid hypotonic to body fluids. In *Sabella pavonina* which can survive low salinity conditions Ewer and Ewer (1943) have shown the importance of the thoracic nephridia in osmoregulation as seen from the fact that while the normal worms regain their original weight when transferred to diluted media, those in which the thoracic nephridia have been removed failed to recover. In the light of the above instances it is likely that in *Lycastis indica* the nephridia function in osmoregulation in enabling the animal to thrive in brackish and fresh water. In this connection the observation of Grobben (1881) that the nephridia of fresh water annelids are comparatively larger than those of their equal sized relatives confined to the sea, appears to be significant. Similar differences in the relative sizes of the antennary glands of the fresh water amphipod *Gammarus pulex* and the marine *Gammarus locusta* have been observed by Schwabe (1933) who correlated the larger size of the gland in fresh water species with its importance in osmoregulation. The significance of the increase in size of the excretory organs in fresh water species would appear to be related to the need for the excretion of large quantities of water entering the body along the osmotic

gradient. That the nephridia eliminate water entering the body from the external medium is evident from the work of Bahl (1945) who showed that the urine produced by the earthworm *Pheretima posthuma* when kept in water comes largely from the water absorbed through the skin. It would appear, therefore, that when marine species penetrate to brackish and fresh water there is likely to be a copious production of urine due to an increased influx of water and a large sized excretory organ would be advantageous in the elimination of increased quantities of water entering the body. It appears reasonable to regard the relatively larger size of the nephridium of *Lycastis indica* as an adaptation for life in fresh and brackish water and the differences in the relative sizes of the nephridium of *Lycastis indica*, *Nereis chilkaensis* and *Perinereis nuntia* may be correlated with their relative ability to withstand low salinity conditions.

Similarly the differences in the vascularisation of the nephridia in the three species studied, appear to bear a relation to their habits. It is seen from the work of Bahl (1945) that the mechanism of excretion of the nephridium in the earthworm is similar to that of the vertebrate kidney involving filtration, reabsorption and chemical transformation. Picken (1936) has pointed out that in the crayfish and *Peripatus* there is a preliminary filtration from the blood to the nephridium, the process being assisted by the hydrostatic pressure of the blood. The amount and nature of blood-supply of the nephridium in *Lycastis indica* suggest that a filtration of fluid might take place from the blood into the lumen of the nephridial canal. Since nephridia appear to be intimately associated with the water balance it is probable that the differences in vascularisation observed in *Lycastis* and *Nereis* are related to the adaptations necessary to maintain an osmotic equilibrium with the external medium. *Lycastis indica* which normally inhabits fresh water probably excretes a relatively large quantity of water. The richer capillary supply to the nephridium would afford an advantageous juxtaposition of the blood in relation to the nephridial canal and facilitate the excretion of large quantities of water. The underlying assumption is that there takes place a filtration of fluid from the blood into the lumen of the nephridium. On this basis, the comparatively poor vascularisation in *Nereis chilkaensis* might be related to its marine habitat and the water cycle associated with such an environment. It would appear that in sea-water which is in osmotic equilibrium with the body fluids, a smaller quantity of water is excreted. This is suggested by another consideration. It has been observed that the blood supply to the nephridium undergoes a marked diminution when *Lycastis indica* is acclimatised to sea-water. The condition of the blood supply in *Perinereis nuntia* which is marine, is what might be expected in the light of the above observations. If the filtration theory is assumed the repeated branching of the capillaries in the nephridium of *Lycastis indica* renders the filtering surface extensive to facilitate a rapid and profuse excretion. It has also been seen that a feature of the blood supply to the nephridium in *Lycastis indica* is the occurrence of dilatations on the capillaries. Although their functional significance is not clear the presence of similar structures in Oligochaetes could be considered as indicating an adaptation for life in an 'Oligochaete medium'. Between the extremes of freshwater type of nephridium represented by *Lycastis indica* and a typical marine type as seen in *Perinereis nuntia*, intermediate types might be expected. Such a one is seen in *Nereis chilkaensis* which is a marine species capable of invading brackish water.

The differences in the size and the nature and amount of blood supply of the nephridium of *Lycastis* and *Nereis* are significant when considered in the light of the habits and distribution of these two genera. *Nereis* is essentially a marine genus though there are a few exceptions. The genus *Lycastis* comprises fourteen species so far known. Of these only the type species *Lycastis brevicornis* (recorded from the west coast of France) appears to be purely marine. Curiously enough this species has not been re-discovered. All the other known species are found in

brackish and fresh water or show a great tolerance to changes in salinity of the external medium. From the distribution of the genus it is clear that the various species are confined to tropical regions thus suggesting a close correlation between the habits of the genus and the structural adaptations in the nephridia as understood from an examination of *Lycastis indica*.

## 6. Summary.

1. The nephridia of three species of Nereidae taken from waters of different salinities, were studied. *Lycastis indica* is found in brackish and fresh water, *Nereis chilkaensis* in brackish water and the sea, and *Perinereis nuntia* is purely marine.

2. The nephridia of *Lycastis indica* lie in the body-cavity on either side of the gut: they are comparatively larger in size than those of the other two species. The nephridial canal is disposed in the form of loops and is considerably lengthened. The vascular supply is pronounced. The features in the blood supply are: (1) the occurrence of blood capillaries within the connective tissue surrounding the lumen of the distal part of the nephridial canal and (2) the presence of dilatations of the blood capillaries.

3. The nephridia of *Nereis chilkaensis* lie at the entrance to the parapodial cavity. The body of the nephridium is compact and smaller in size. It is formed of connective tissue through which runs a coiled nephridial canal. The blood-supply to the nephridium is not so rich as in *Lycastis indica*. The capillaries lie on the outside of the body of the nephridium.

4. The nephridia of *Perinereis nuntia* are comparatively smaller in size than those of the other two species and the nephridial blood vessels are poorly developed.

5. In specimens of *Lycastis* acclimatised to sea-water the blood supply to the nephridium undergoes a diminution as seen from the shrunken condition of the blood capillaries of the nephridium.

6. The nephridia of the three species examined above are compared and the variations in size and vascularisation are discussed in the light of their probable relation to osmoregulation.

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## 8. Explanation of Photomicrographs.

Fig. 1. A section through the body of the nephridium of *Lycastis indica*.
Fig. 2. A section passing through a different plane, of the nephridium in *Lycastis indica*.
Fig. 3. A section of the nephridium of *L. indica* which has been acclimatised to sea water showing the condition of the blood capillaries.

## 9. Key to Lettering.

| | |
|---|---|
| A | .. Dilatations of the capillaries. |
| BL | .. Boundary layer. |
| BL.V | .. Blood vessel. |
| CIL | .. Cilia. |
| CIL.PROC | .. Ciliated processes of the nephridiostome. |
| COEL.EP | .. Coelomic Epithelium. |
| EP | .. Ectoderm. |
| EXT.CIL | .. External cilia of the nephridiostome. |
| F | .. Fibrillae. |
| INT.CIL | .. Internal cilia of the nephridiostome. |
| L.P | .. Lip of the nephridiostome. |
| L.N.C | .. Lumen of the nephridial canal. |
| LUF | .. Lumen of the nephridiostome. |
| N | .. Nucleus. |
| N′ | .. Nucleus of the connective tissue cell. |
| NE.C | .. Nephridial canal. |
| NEP | .. Nephridiopore. |
| NET | .. Connective tissue. |
| WF | .. Wall of the nephridiostome. |
| V | .. Vacuoles in the connective tissue of the nephridium. |

* Not referred to in original.