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STUDIES ON THE SHRIMP *CARIDINA LAEVIS* (HELLER)

II. THE REPRODUCTIVE SYSTEM

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THIS paper is the second part of the series dealing with the anatomy of the freshwater shrimp *Caridina laevis.* A paper on the Digestive system has already been published (Pillai 1960).

METHODS

The morphology of the reproductive system was studied mostly by dissecting anaesthetised specimens under the stereoscopic binocular microscope. In addition to Bouin's fluid, Zenker formol, Formol saline and Carnoy's fluid were used as fixatives and Heidenhain's iron haematoxylin, Heidenhain's Azan as stains and Mallory's connective tissue stain as counter stain. Squash technique was employed for studying the chromosome number using aceto-orcein as stain-fixative (La Cour, 1941). Growth of the ovary was studied on live animals through the transparent cuticle.

External sex differentiation

The coxopodites of the pleopods get coalesced with the sternal plates in males with the result that the two appendages of the same segment originate close togetEer. In the females they are far apart, providing space for the accommodation of eggs during spawning. In the male (Fig. 2) the exopodite of the first pair of pleopods is longer and extends to the base of the third maxilliped of the same side, while in the female (Fig. 1) it extends only upto the coxa of the third walking leg. **The** endopbdite in the male is short, blunt, almost completely fringed with setae **and** runs obliquely towards the front whereas in the female it is narrow, acute, long, almost bare and directed posteriorly. While the two endopodites on either side cross each other in the female they do not even touch in male. The first pair of pleopods do not develop appendix interna in both the sexes. The second pair of pleopods in the female is similar to those behind. In the male (Fig. 3) it is markedly distinctive bearing the appendix masculina between the endopodite and the appendix interna. Thtappendix masculina is armed on its mesial side with two rows of short and jointed spines.

1. MALE REPRODUCTIVE SYSTEM

Morphology of testis and vas deferens

The testis (Fig. 4) consists of two simple tubes with a precardiac connection. Its anterior part is slightly convoluted. The vas deferens, arising from the anterior one-third of the testis passes backwards, gets folded on itself and is again reflected

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back. The ultimate portion is superficial and runs below the last branchiae to open out on the inner side of the last walking leg.

Histology of testis (Fig. 5)

The testis is enveloped by an outer epithelium with flattened nuclei below which is the general germinal layer. Only the cells of one side of the germinal epithelium actively form the germinal ridge. The cells of the germinal ridge have large diffuse nuclei with more than one nucleolus. In the precardiac portion of the testis, this ridge almost fills the cavity of the testis. It is here that the peak of transformation of spermatocytes into spermatids and spermatozoa is noticed. Concurrent with the formation of the spermatocytes with large diffuse nuclei, smaller nuclei with variable shapes and indistinct cytoplasmic boundaries have also been observed to be formed inside the germinal ridge. Though the details of the spermatogenesis have not been studied, certain observations have been made about these cells, the so-called nutritive cells. Their nuceli which may be spherical, oval, elongated or irregular, are highly chromophilic and are found only in the vicinity of the primary, spermatocytes. Invariably these cells are situated towards the periphery of the testicular wall, nearer to the blood that surrounds the testis and it is possible that they serve a nutritive function. There are various views regarding the origin of these cells. Earlier workers held that the nutritive cells gave rise to primary spermatocytes (Grobben, 1878 and Hermann, 1890). On the contrary it is believed that the nutritive cells are derived from disintegrating spermatocytes (Sabatier, 1885) and more concrete evidence was given by Keppen (1906) on *Astacus fluviatilis* and Ratnavathy (1941) on *Clibanarius olivaceous.* The average number of nutritive cells and the average number of other cells (spermatocytes and spermatids) in a part of the testis in *Caridina* were observed to be almost the same. If there is a later transformation of the spermatocytes into nutritive cells by disintegration, there should have been an increase in the average count of nutritive cells. It appears that each germinal cell divides mitotically, the products being a nutritive cell and a primary spermatocyte.

Histology of vas deferens (Fig. 7)

. The vas deferens has three layers, an outermost fibrous coat, a layer of circular muscle fibres and an inner glandular epithelium whose cells become columnar at places. The wall of the terminal portion of the vas deferens is thicker because of the interpolation of a sheet of longitudinal muscle fibres around the epithelial cells. \ The epithelium of the first limb of the vas deferens is uniform and less columnar. The cells of the glandular epithelium have large nuclei and the free ends have amoeboid appearance. Large vacuoles are observed in these incorporating the secretory products. Where the first limb gets folded on itself and proceeds as the second limb, a ridge of about dozen cells the ' typhlosole' makes its appearance on its upper side, projecting into the cavity of the duct. The cells are taller (36 *n)* with oval nuclei and have secretory vacuoles in their cytoplasm. The ' typhlosole' shifts to the lateral side in the second limb of the vas deferens and passes into the third limb becoming less and less conspicuous at its distal part. It is interesting to note that the cells have an affinity for nuclear dyes, a condition which has also been observed in the sand crab, *Hippa pacifica* (Matthews, 1956).

Cilia as found in the deferent ducts of Crustacea (Fasten, 1917 and King, 1948) were not observed in the vas deferens of *Caridina*, except in the region of the typhlosole, where they resemble flagella although no basal granules have been observed. The flagella assist the expulsion of the sperm mass.

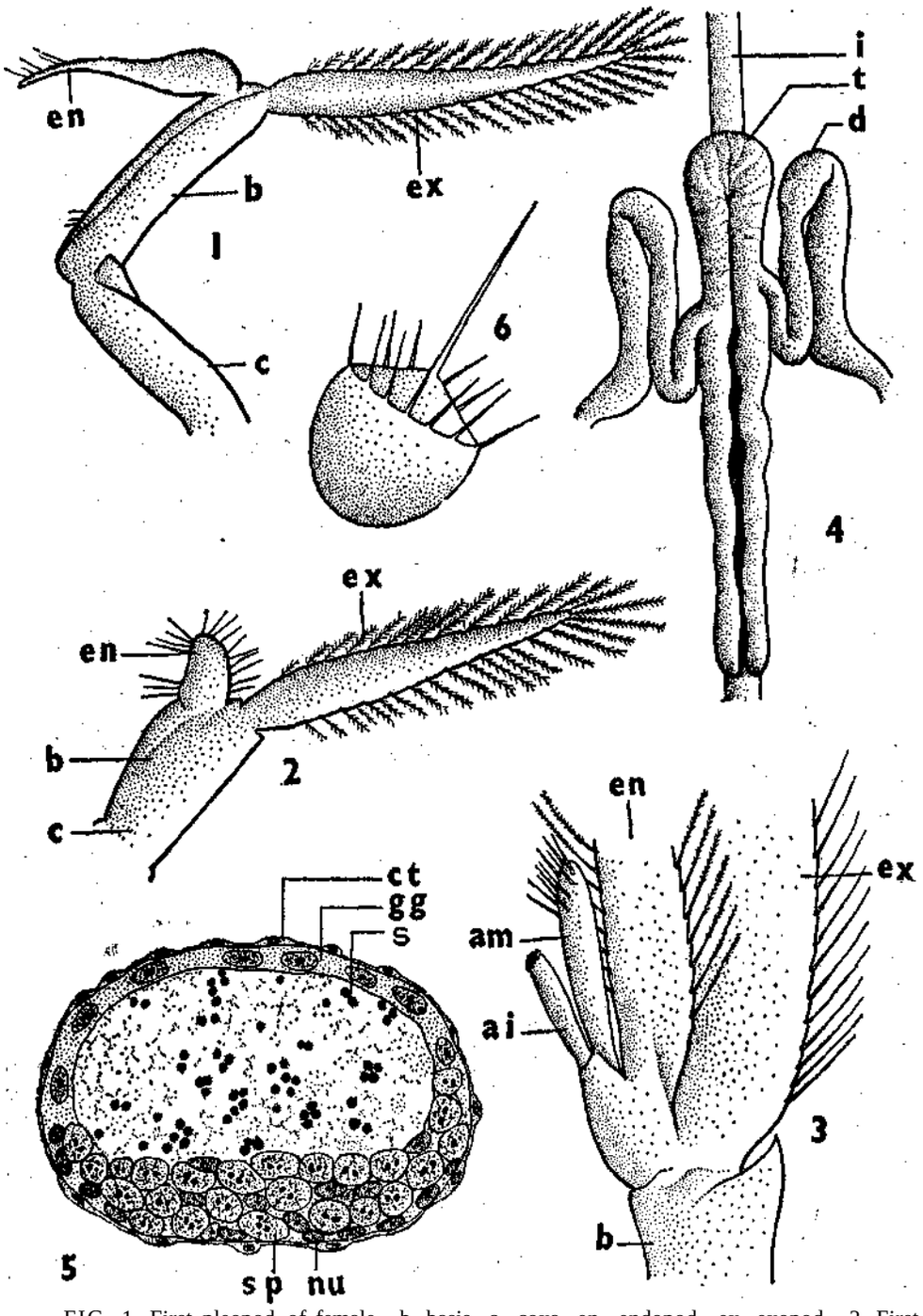

FIG. 1. First pleopod of female, b. basis, c. coxa, en. endopod, ex. exopod. 2. First pleopod of male. 3. Second pleopod of male. ai. appendix interna, am. appendix masculina. 4. Dorsal view of testis and vas deferens, d. vas deferens, i. intestine, t. testis. 5. Cross section through posterior part of testis, ct. connective tissue, gg. general germinal layer, nu. nucleus of nutritive cells, s. sperm, sp. nucleus of spermatocyte. 6. Lateral view of the sperm.

Under the phase contrast microscope the epithelium of the vas deferens shows that cells of the typhlosole have secretory vacuoles containing a secretion, secretion A, towards their distal ends. The rest of the cells also show vacuoles containing a secretion, secretion B, but of a different nature as is shown by its different staining reactions. Secretion A does not stain so intensely as secretion B in Mallory's connective tissue stain but takes a light blue colour in Heidenhain's iron haematoxylin, while secretion B does not. Secretion B is stainable deep blue in Mallory's triple stain while secretion A is not. The secretion B binds the sperms inside the first limb of the vas deferens. The secretion A of the typhlosole pushes this mass to the opposite side and gradually envelops it. Vitally stained and dis* sected vas deferens shows a simple, string-like spermatophore.

The development and expulsion of the spermatozoa into the deferent ducts do not appear to be a continuous process but are periodical. The nature and the secretory phase of the cells lining the first limb of the vas deferens do bear a close relationship to the stage of activity of the testis. In undernourished individuals the process of spermatogenesis is slackened, the sperm mass in the duct is thinner and the cells of the vas deferens smaller. The cells of the typhlosole have also a reduced size indicating a slackening of their secretory activity. A more or less similar appearance is presented as soon as the peak of their secretory activity is over. The cells shrink and clefts are formed among them.

The spermatozoa (Fig. 6) are extremely small, measuring about 2.8 /* across. Under the phase contrast microscope, the live spermatozoon appears like a hemisphere, at the top of which is a cone. It resembles that *of Palaemon lamarrei* in the possession of a long apical spine (Nath, 1937). In addition to this, eleven shorter spines radiate outwards all round from the junction between the cone and the hemisphere. In smears, a peripheral nuclear vesicle has also been observed.

2. THE FEMALE REPRODUCTIVE SYSTEM

With regard to the gross structure of the ovary, *Caridina* resembles *Palaemon malcolmsonii* (Patwardhan, 1937) andP.Wae (Parameswaran, 1953).

Morphology of the ovary and oviduct (Fig. 8)

The ovary consists of two longitudinal columns lying in the cephalothorax and extending into the abdomen. Anteriorly the two columns are joined together. In a mature female the ovary extends from the anterior one-fourth of the carapace (measuring from the base of the rostrum) to more than three-fourths of the third abdominal segment. The oviducts arise as thin-walled tubes from the ventral side of the ovary, running downwards between the ventral muscles and the thoracic wall. They open to the outside of the inner face of the coxa of the first pair of walking legs. The genital opening in a mature female is circular. Its anterior lip bears a row of about 8-10 stout bristles and is guarded by a hinge-like transparent cuticular operculum. During ovulation the eggs coming down the oviduct push the operculum outwards and are guided backwards by the bristles.

Histology of ovary (Fig. 9)

The ovary is bounded by a layer of epithelium which is continuous with that of the wall of the oviduct. The cells forming this general epithelium are flattened with small, round nuclei. Below this there is another layer of cells which at places extends into the cavity of the ovary as numerous shelf-like invaginations. In whole

mounts of the ovary, their nuclei have been observed to undergo amitosis without any corresponding cytoplasmic division. This layer invests the developing oocytes forming follicles. In a medium sized ovary fixed in a weak solution of chromic acid, the formative region or the germogen is seen as a pale whitish streak on the lateral ventral aspect of each lobe. These streaks shift to the lower side and meet together anteriorly. The exact shape and position of the germogen is subject to variation depending upon the tension exerted by the developing ova on the ovarian wall. The germogen does not develop an invagination as in *Palaemon idae* (Parameswaran, 1953) but appears as a thickened region of the inner epithelium as in *Eupagurus* (Jackson, 1913) and *Clibanarius* (Kamalaveni, 1949).

The germogen is most active anteriorly and by far the greater number of oocytes are formed here. In a cross section of the anterior part of the ovary, the germogen appears as a multi-nucleate protoplasmic mass. The cytoplasm is granular and devoid of vacuoles. The nuclei are small and compact with usually a few clumps of chromatin. The primary oogonia derived from these have large diffuse nuclei each with a pronounced reticulum and about three chromatin clumps. The oogonium during oogenesis, divides to form an oocyte and a cluster of nutritive cells or nurse cells (Fig. 10). The number of nurse cells has been noticed to be five. Earlier in development there are clusters of five nuclei arranged in a mass of cytoplasm. These represent the nuclei of an oocyte and four nutritive cells, all derived from one oogonium. The oocyte probably divides further forming another sister cell which'}6irts the other cluster of four nurse cells. In *Deilephila euphofbiae* (Insecta) Schneider (1917) has described a similar derivation of the nutritive cells. The cytoplasm of the nurse cells seems to be confluent at this stage. The nuclei exhibit a reticulum and the chromatin bodies are not discernible. Chromosomes of the nurse cells have been observed at about this stage but in sections it has not been possible to count them. It is possible that the nurse cells have the diploid and the oocytes, the haploid number of chromosomes as this condition is seen *Triops* (Longhurst, 1955). The oocyte eventually outgrows the nurse cells. The ultimate fate of the nurse cells have eluded investigation but based on the fact that the fragments of the nuclear system of the nurse cells have sometimes been detected inside the growing oocytes, there is reason to believe that they are absorbed into the ovum. But conclusive evidence on this point is lacking.

As the oocyte grows, the number of follicle cells investing it also increases. During this process the cytoplasm of the ovum is seen to develop vacuoles which finally are charged With yolk. These vacuoles shift towards the periphery at a slow pace. Developing oocytes cut and stained in Heidenhain's Azan demonstrate certain black staining granules which might represent the Golgi system (Thomas, 1948). Such bodies have been observed inside the dark cells of the digestive diverticula also (Pillai, 1960).

The wall of the oviduct is made up of two layers of cells, an outer epithelium, continuous with the outer epithelium of the ovary and an inner-more columnar layer. The inner layer is glandular and in a mature female becomes markedly larger. The cytoplasm becomes granular and vacuolated and the nucleus with; a very conspicuous nucleolus assumes a basal position.

The growth of the ovary (Fig. 13)

A very young ovary (Fig. 13, 1) stretches from just in front of the heart to the Second abdominal segment. The two lobes are uniformly thin and pale like the testis, with which it is easily confused at this stage* In the next four days (Fig. 18, 2)

it grows and extends to the hind limit of the second abdominal segment. On the outer side of each lobe the germogen appears as a longitudinal streak from which an inner row of cells becomes differentiated. These cells are large and pale yellowish. The two lobes touch each other in the first abdominal segment. In another four days, the ovary enlarges (Fig. 13, 3) and develops two to three rows of cells. The innermost row contains the largest ova which are green due to the deposition of yolk while the others are smaller and yellowish. The lobes of the ovary now press against each other in the middle line and extend upto the hind extremity of the second abdominal segment. The peripheral layer of the oocytes appears whitish. The outer two rows of cells also begin to secrete yolk, enlarge and become green. The ovary elongates further and develops a flexion below the heart. Within the next six or seven days it extends from three-fourths of the cephalothorax to more than half (Fig. 13, 4) the third abdominal segment. Some of the cells now shift downwards and hence the linear arrangement of the ova disrupts. The lobes are not usually of the same length and in the majority of cases examined, the left is longer. The outline of the egg cell becomes rounded and more defined. Ovarian flexion is more pronounced and all the ova now. become alike in size and colour. They stream towards the anterior region. Posteriorly the lobes almost touch the fourth abdominal segment and anteriorly extend to more than three-fourths of the cephalothorax. The approximate minimum length of the animal at this stage is 13 mm. Within another day or two the ova are ready for extrusion and fertilization. Before mating the animal, as is well known, rndergoes ecdysis and acquires the breeding dress, mainly in the form ovigerous setae.

Chromosomes (Figs. 11 and 12).

Squash preparations have shown that the diploid number (2n) of chromosomes is 64. Most of the chromosomes are thick and rod-shaped with subterminal centromeres. A few are relatively longer, with median or submedian constrictions. The chromosomes regularly form 32 bivalents as may be seen from the metaphase of the first reduction division of the primary spermatocyte. Embryos also yield good chromosome pictures. Eggs were dissected approximately at about 126 hours after the initiation of cleavage. The egg membranes and the mass of the yolk were teased off in normal saline and the embryonic portion was squashed. This also revealed the diploid number of 64 chromosomes.

3. BREEDING HABITS AND BREEDING

Copulation

Caridina generally mates only in darkness. However, fully mature specimens kept in the aquaria were observed to mate in shaded blue light. Copulation, followed by spawning, continues to take place for 4 or 5 days after they are captured from the field. Individuals kept for more than this period lose their vigour in the aquarium and seldom spawn. Mating usually takes place between 3 a.m. and 6 a.m. The moulted female before mating is lethargic and staggers on the bottom of the aquarium. A mature male in the vicinity on passing its feelers over the moulted female, stands still, its antennae alone hovering about the female. Then it approaches and clasps the female. He applies his ventral side close against the ventral part of the female and effects a few quick convulsive movements.

A female specimen which was fixed at the time of extrusion of eggs revealed a few sperms inside the ultimate portion of the oviduct. How the nonmotile sperms ascend the oviduct has not been understood. During the process of mating, the

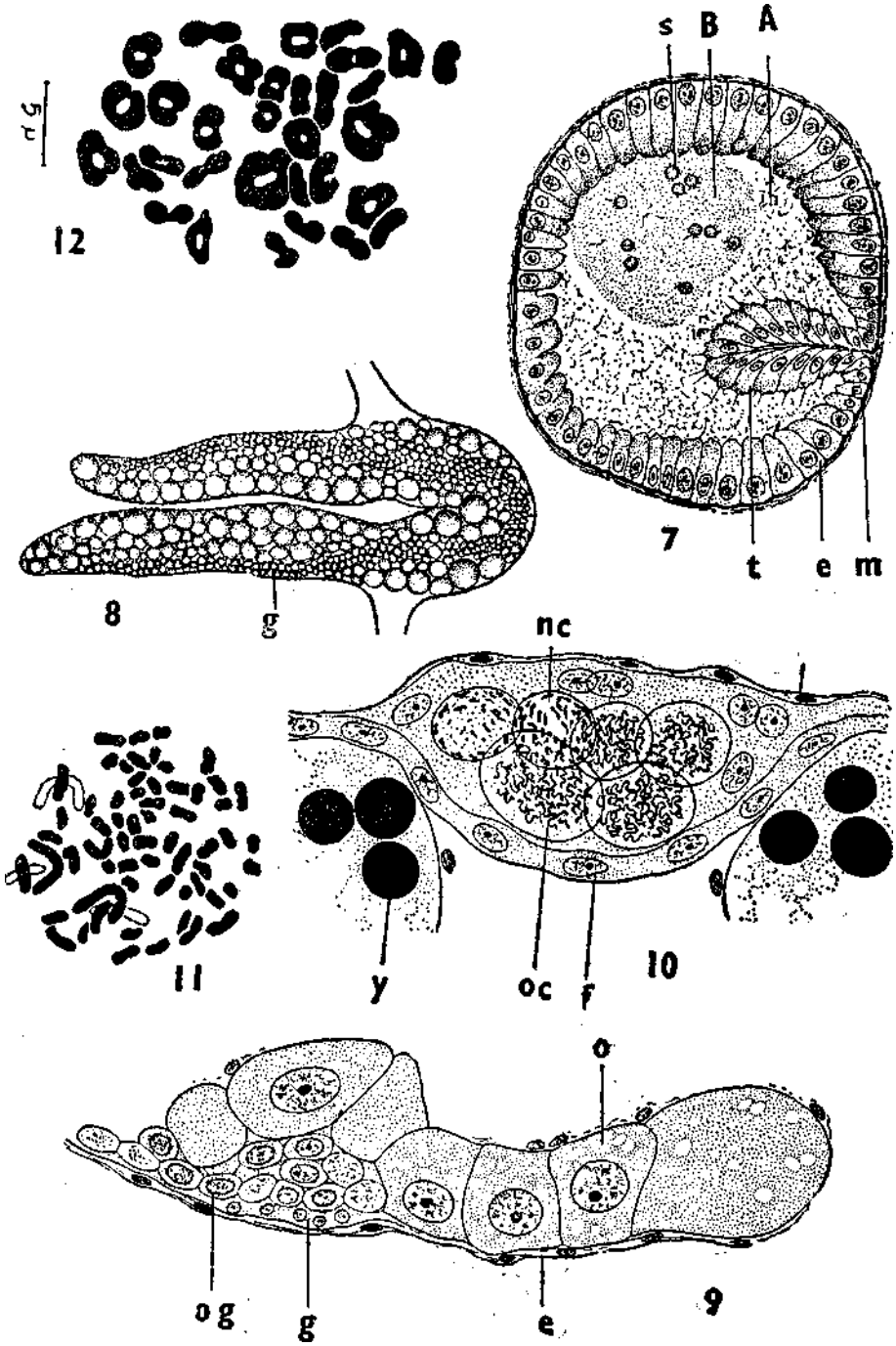

FIG. 7. Cross section through second limb of the vas deferens. A. secretion, A. B. secretion B,e. epithelium, m. fibrous coat with muscle layer, s. spermatozoa t. typhlosole. 8. Ventral view ofa growing ovary, g. germogen. 9. Section through germogen ofa mature ovary, e. epithelium, o. oocyte, og. oogonia. 10. Magnified view of a portion of germogen showing nurse cells. f. follicle cells, nc. nucleus of nurse cell, oc. nucleus of oocyte, y. yolk granules. 11. Polar view of metaphase of the equational division of the spermatogonia. 12. Polar view of the metaphase of the first reduction division of the primary spermatocyte.

sperm mass, as it emerges from the male genital opening is caught by the appendix masculina and is shoved into the female genital opening. The mass of sperms blocks this orifice and as the ova are squeezed out of the female genital opening, probably the contents of the oviduct are sucked upwards which result in a few spermatozoa also being drawn in.

Extrusion of eggs.

The process of extrusion of eggs commences soon after copulation and the first egg takes the maximum time of more than one minute to be extruded. The ova nearer to the oviducts are extruded first. Inside the ovary where the ova are pressing for space, the liberation of one ovum would produce a zone of low pressure towards where the other ova naturally get pushed breaking their incomplete follicular envelops. The diameter of the ova being larger than that of the oviduct, they get distorted considerably while streaming out. Since the number of eggs varies from 14 to 46, the time taken for the extrusion also is subject to variation. Normally it occupies from 40 to 50 minutes. The eggs are shovelled backwards by the endopodites of the first pair of pleopods. Eggs liberated first get cemented to the anterior pleopods ; later ones are passed on to the more posterior appendages. Eggs were seen to be carried exclusively by the first three pairs of pleopods in 89 % of the specimens examined, their number decreasing in an anteroposterior direction. The fourth pair of pleopods in 11 % alone were seen to carry one egg each. In all the cases these develop ovigerous setae unlike the fifth pair which do not develop them at all.

According to Forster (1951) the mating process appears to be a response to chemical stimuli rather than visual. It is only to an immediately moulted female that the male is aroused, the necessary stimulus being obtained while passing the olfactory antennular feelers over its body. The general mating behaviour of *C. laevis* also is broadly similar to that in other Caridea. The body of the male specimen is seen to be placed along the long axis of the female in *Caridina* while in *Crangon* the body assumes a U-shaped form and is placed transversely across the female (Lloyd and Yonge 1947). The sliding movements observed in *Crangon crangon* (Nouvel 1939) have not been observed in *Crangon vulgaris* (Lloyd and Yonge 1947). In *Caridina* certain convulsive movements have been observed instead which are probably for the expulsion of the spermatophoral mass. In *Crangon* spawning takes place two days after moulting irrespective of copulation (Lloyd and Yonge 1947). If copulation has taken place after moulting spawning is expedited and is brought about in 24 hours. Spawning takes place immediately after copulation if the specimen belongs to a small size group Nouvel (1939). In the larger ones it takes place after 24 hours and in the largest within 48 hours. It appears that there is a relationship between the size of the animal and the interval between copulation and spawning. And in *Caridina* which is smaller than the smallest mature female of *Crangon* spawning takes place immediately after copulation.

In most of the forms where the reproductive biology has been studied, spawning is clearly seasonal *[Crangon* Lloyd and Yonge (1947) and Meredith (1950-52) ; *Pandalus* Pike (1953) etc.] But the present data do not indicate any seasonal breeding in *Caridina.* Berried, females are obtained throughout the year, their number showing a slight increase soon after the Monsoon which fact has been reported by Nair (1949) also. The lack of a well defined breeding season in *Caridina* is possibly due to (1) the animals being inhabitants of ponds, ditches and other small freshwater bodies where environmental fluctuation is too varied to clearly mark the seasons and (2) the development and maturation of the ova and the spawning being

quicker than in other forms. In seven specimens where the growth and maturation were studied, the total number of days between one spawning and the other varied from 32 to 38. It may also be noticed that the eggs hatch out quicker. In the above seven specimens it varied from two weeks and a half to three. Nair (1949) has observed the period to be 17 days.

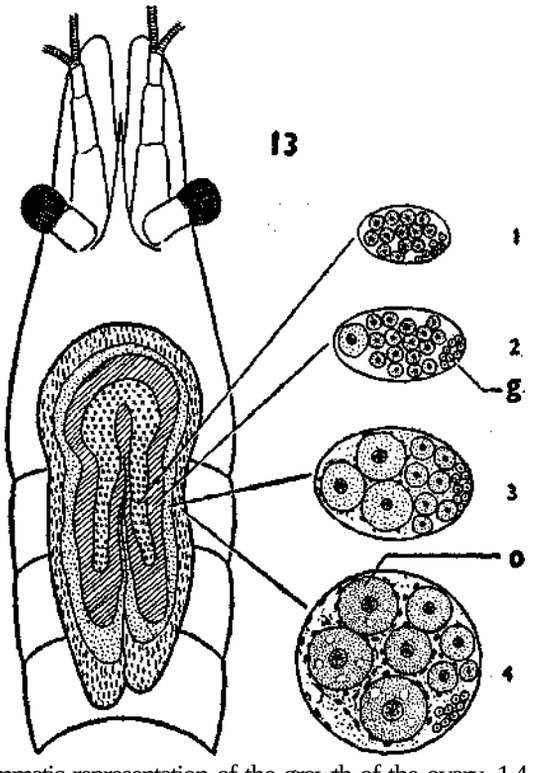

FIG. 13. Diagrammatic representation of the growth of the ovary. 1-4. cross section of the ovary at various stages indicated (not to scale).

SUMMARY

The generative organs are two simple tubes, connected together in front. Each germinal cell in the male appears to divide mitotically into a nutritive cell and a primary spermatocyte. The ' typhlosole ' and the rest of the glandular epithelium of the vas deferens each produces a secretion which binds and envelops the spermatozoa, resulting in the formation of a simple threadlike spermatophore. During oogenesis, the five nurse cells which are formed in association with the oocyte possibly are absorbed by the latter. The growth of the ovary has been traced and in a mature animal ready for fertilization it occupies three-fourths of the length of the cephalothorax apart from the first three abdominal segments. Copulation, observable in shaded light between 3 a.m. and 6 a.m. is a very quick process. Extrusion of eggs takes place soon after copulation and takes about 40 to 50 minutes. Squash preparations of the testis and embryo reveal the diploid number of chromosomes as 64.

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