

FERTILIZATION IN THE PARASITIC COPEPOD,  
LERNÆOPODA EDWARDSII OLSSON.<sup>1</sup>

NATHAN FASTEN.

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GENERAL REMARKS.

Wright (1882), in discussing reproduction in the parasitic copepod *Achtheres micropropteri* deprecates the fact that so little is known of the exact method of fertilization in the parasitic copepods, and adds that "a thorough examination of the male reproductive apparatus of the Lernæopodidæ is very desirable for the purpose of elucidating the formation of the spermatophores in the parasitic Copepoda." Since Wright's work no one has published any results along this line. It, therefore, seemed advisable to investigate this problem a little more fully.

The following studies were made on the parasitic copepod *Lernæopoda edwardsii* Olsson which infests the gills of the brook trout *Salvelinus fontinalis*. The writer, while in the service of the Wisconsin Fish Commission, during the summer of 1912, secured a lot of fine stages of this organism in copulation, from the time the male attaches itself to the female until the actual fixation of the spermatophores near the genital pores of the female's body. Many mature males and females were also secured.

The material was fixed in a 5 per cent. solution of corrosive-acetic for eighteen hours and was then washed in running water for a day. Selected stages were stained in toto in borax-carmin for thirty-six hours, and the excess of stain was removed in water or in very weak acid-alcohol. The material was next

<sup>1</sup> From the Zoölogical Laboratories, University of Wisconsin.

passed through the various grades of alcohol, being left in each for about ten hours, and after being cleared in xylol was finally mounted in balsam.

Many mature males, females, and copulating individuals were also imbedded in paraffine for sectioning. These were cut into frontal, sagittal and transverse sections, ranging from 3-6  $\mu$  in thickness, and stained either in Delafield's hæmatoxylin and eosin or in Heidenhain's iron-alum hæmatoxylin and acid fuchsin. The latter stains gave the finest results and were utilized almost exclusively.

#### THE MALE REPRODUCTIVE ORGANS.

The male reproductive organs of *Lernæopoda edwardsii* are, in the main, similar to those of the free living copepods, as described by Gruber (1879). They are paired, and are located in the posterior half of the body (Figs. 1-2, *t.*, *v.d.*, and *sp.*). These organs lie in the space between the intestine and the lateral body wall, and consist of three main parts: (*a*) the testis (Figs. 1-2, *t*), (*b*) the coiled vas deferens (*v.d.*), and (*c*) the spermatophores (*sp.*). The testis is sac-like in appearance, with its anterior end projecting into a tube-like structure that ends blindly. When a section of the testis is examined under the microscope, three distinct zones can be recognized which correspond very closely to the three zones in the testis of *Diaptomus* sp., described by Ishikawa (1891). The anterior portion is the formative zone, and consists of spermatogonial stages. The middle region is the zone of growing spermatogonial cells, and lastly, the posterior region represents the maturation zone. Here the testis is in active proliferation and is filled with great numbers of spermatozoa. Each testis unites with the vas deferens posteriorly.

The vas deferens is an elongated coiled structure composed of three parts. The first portion of the vas deferens (Figs. 3-4, *v.d. 1*), lies nearest the lateral body wall of the copepod, and receives the spermatozoa from the testis. The second branch (*v.d. 2*), is a thin, tube-like structure that makes its way from the lower margin of the preceding portion (*v.d. 1*) and then coils up diagonally behind it, to a point slightly above the terminal region of the testis. This is best seen in Fig. 4. It then coils forward as the third lobe of the vas deferens (*v.d. 3*). This runs along

posteriorly to a slit-like opening located in the extreme lower margin of the male's body. This opening is the ejaculatory pore (Figs. 3-6, *e.p.*), through which the spermatophore is extruded.

Each spermatophore (Figs. 1-6, *sp.*) originates in the third branch of the vas deferens. It is pear-shaped in form, and in the living organism is of a pale yellowish color. In Figs. 5-7 the structure can best be studied. The spermatogonial pouch, filled with spermatozoa (*sz.*), is enclosed by an outer wall of chitin (*ch.*). Immediately behind this there is a thin layer of cement-like substance (*c.*), that stains intensely black in Heidenhain's iron-alum hæmatoxylin. In the center of the spermatophore is a spherical pouch filled with a similar cement (*c.*) as is evident from the similarity of its staining reaction. This cement substance of the spermatophore appears to be continuous with the small sphere of cement found in the loop-like enlargement of the vas deferens (see Fig. 3, *v.d.* 3).

When the spermatozoa are mature, they are discharged into the first branch of the vas deferens. From here they pass into the second lobe, and finally, they migrate into the canal situated in the center of the third part of the vas deferens. This is shown in Fig. 5, where the canal (*can.*) conducts the spermatozoa into the spermatophoral pouch, located between the central sphere of cement and the outer cement wall. Here the sperm (Figs. 6-7, *sz.*) are stored until copulation occurs.

#### THE FEMALE REPRODUCTIVE ORGANS.

The mature female is much bigger than the male, being about three times as long as the latter. Fig. 1 shows this size difference. The sex organs of the female lie dorso-laterally within the abdomen, between the intestine and the body wall. The ovaries (Figs. 1 and 9, *ov.*), are paired in character, and each gives rise to a slender oviduct (Figs. 1 and 9, *od.*), which makes its way around the intestine to the posterior portion of the body, where it opens to the exterior. Both oviducts unite slightly above their terminations into a broad pouch that later becomes the spermatheca and its accessories (Figs. 9 and 11, *s.*). From this pouch, two slender tubes are developed that open posteriorly through the genital pores (Figs. 1 and 8, *g.p.*). Also, within each oviduct,

a small, spiral gland can be distinguished (Figs. 1 and 9, *c. g.*), which, under the microscope shows strong powers of refracting light. These structures later develop into the cement glands of the adult (Figs. 10-11, *c.g.*).

#### FERTILIZATION.

About two and a half or three weeks after the attachment of *Lernaeopoda edwardsii* to the brook-trout, the copepod is ready to copulate. In two earlier papers (Fasten, 1912 and 1913), the attachment of the parasite to the host was discussed. As already stated, the mature male is very much smaller than the female. In order to fertilize the female, the male must release his hold on the gill of the brook-trout, and attach himself to the lower extremity of the female's body, in the neighborhood of the genital pores. This is accomplished in the following manner. When mature, the male, though still attached, makes circling movements with his body, thus coming in contact with a female. As soon as this occurs, the male clasps her with his maxillipeds, and at the same time, withdraws his second maxillæ from the filament of attachment to the gill. Then he moves towards the female's genital pores, and places himself in position for fertilization. The position assumed is shown in Fig. 1. These observations are similar to those made by Wilson (1911), on the copepod *Achtheres ambloplitis* Kellicott, infecting the rock bass.

When once in position, the male brings forward the posterior region of his body to a position near the genital pores. The spermatophores are then ejected through the ejaculatory pores, and by the aid of his free maxillæ the male attaches them near the genital openings of the female (see Fig. 8). The cement found in the spermatophores is the substance that makes them adhere tightly. As soon as they are attached, the spermatozoa wander through the genital pores and are conducted into the spermatheca, where they are stored until the eggs are mature for fertilization. When the spermatozoa have all migrated into the spermatheca, the spermatophores collapse, and become transparent, shell-like, yellowish spheres (Figs. 9-10, *sp.*).

The female may be fertilized more than once. As many as six spermatophores were found clinging to the genital pores of

some of the females, showing that these females have, in all probability, been fertilized three times. After fertilization, the male drops off from the female's body and dies. The female however, grows enormously in size. During this growth period, the ovaries develop and produce a great many eggs. The cement gland also develops completely, and from each side of the extreme lower margin of the abdomen, an egg-sac grows out. The oviducts communicate with these egg-sacs directly. Figure 10 shows the abdomen of an adult female, with the eggs (*o.*), the oviducts (*od.*), the cement glands (*c.g.*), the spermatheca (*s.*), the spermatophores (*sp.*), and the egg-sacs (*e.s.*).

The eggs are discharged into the oviducts, and are then passed down posteriorly, where they are fertilized by the spermatozoa which are stored in the spermatheca. Then they are coated with a layer of cement from the cement glands, and finally pass into the egg-sacs, where they develop into larvæ. The cement coating hardens into one of the egg covers. Fig. 11 shows the relation of the spermatheca (*s.*) to the oviducts (*od.*). The spermatheca is filled with great numbers of ripe spermatozoa.

These observations on the structure of the reproductive apparatus of the adult female corroborate those of Miculicich (1905), who worked with Lernæopodidæ of the genus *Brachiella*.

I wish to express my thanks to Professors M. F. Guyer and A. S. Pearse for reading this paper and for their helpful suggestions.

#### SUMMARY.

1. About two and a half or three weeks after the attachment of *Lernæopoda edwardsii* to the brook-trout, the parasite is mature for fertilization.

2. The mature male is about one third as long as the mature female. The reproductive organs of the male are paired, and are located in the posterior region of the body, between the intestine and the body wall. They consist of a testis, a coiled vas deferens, and a spermatophore.

3. The female reproductive apparatus is also paired in character and is located within the abdomen, between the intestine and the body wall. The ovaries give rise to slender oviducts, which open at the lower extremity of the abdomen. Slightly

above their terminations, the oviducts combine to form the spermatheca, which opens to the exterior through the two genital pores. Within each oviduct a spiral gland can be seen, which later develops into the cement gland.

4. In order to effect fertilization, the male makes circling movements while still attached to the host, thereby meeting a female. Then the male releases his hold on the host, attaches himself to the female, and moves down posteriorly in the vicinity of the genital pores.

5. The male bends his abdomen upward towards the female, and extrudes the spermatophores from the reproductive organs. These he manipulates with his second maxillæ and soon attaches them about the genital pores of the female.

6. The spermatozoa wander from the spermatophores into the spermatheca of the female and are stored until the eggs are ripe enough to undergo fertilization.

7. The eggs pass down the oviducts when mature, and are fertilized by the stored spermatozoa as they pass the spermatheca. The embryos pass into the external egg-sacs, where they develop into larvæ.

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EXPLANATION OF PLATES.

All drawings were made with the aid of the camera-lucida. The magnification is given after the description of each figure.

*Abbreviations.*

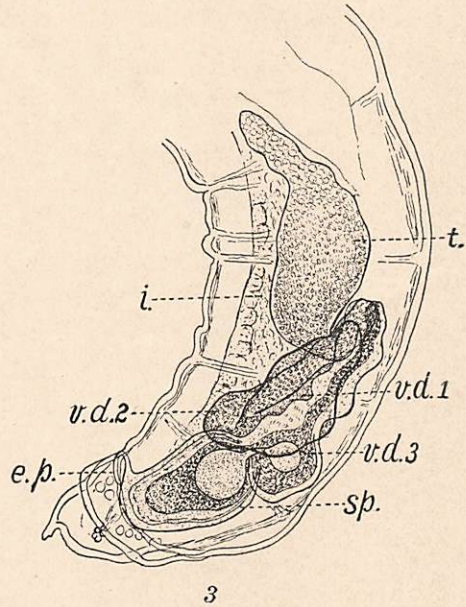
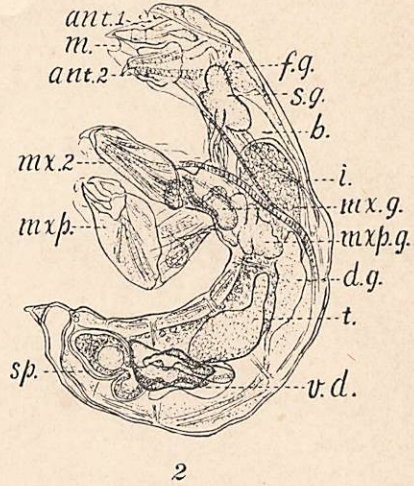
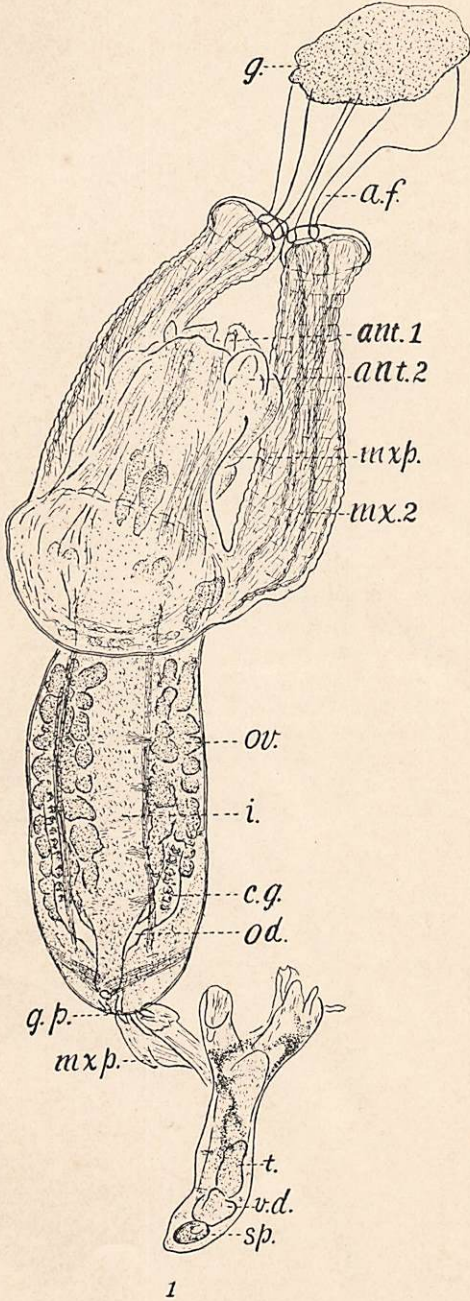
- a.f.* = attachment filament.
- ant. 1* = first antennæ.
- ant. 2* = second antennæ.
- b.* = brain.
- c.* = cement-like substance of spermatophores.
- can.* = canal of v. d. 3.
- c. g.* = cement gland.
- ch.* = chitinous covering of spermatophore.
- d. g.* = digestive gland.
- e. p.* = ejaculatory pore.
- e. s.* = egg-sacs.
- f. g.* = frontal gland.
- g.* = gill.
- g. p.* = genital pores.
- i.* = intestine.
- m.* = mouth.
- mx. 2* = second maxillæ.
- mx. g.* = maxillary gland.
- mxp.* = maxillipeds.
- mxp. g.* = maxillipedal gland.
- o.* = eggs.
- od.* = oviduct.
- ov.* = ovary.
- s.* = spermatheca.
- s. g.* = shell gland.
- sp.* = spermatophores.
- sz.* = spermatozoa.
- t.* = testis.
- v. d.* = vas deferens.
- v. d. 1* = first branch of vas deferens.
- v. d. 2* = second branch of vas deferens.
- v. d. 3* = third branch of vas deferens.

## PLATE I.

*Explanation of Figures.*

1. Male and female copepods in position for copulation. The female is attached to the gill (*g.*) by the funnel-like attachment filament (*a. f.*). The male is attached to the female near the genital pores (*g. p.*).  $\times 57$ .
2. Side view of a mature male showing the position of the reproductive organs (*t.*, *v. d.*, and *sp.*).  $\times 120$ .
3. Enlarged view of the reproductive organs of the male shown in Fig. 2.  $\times 218$ .

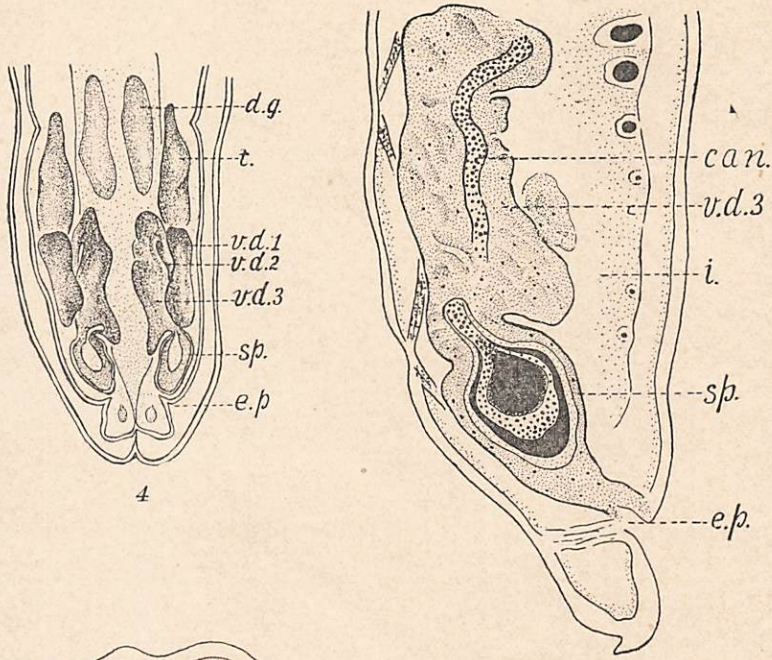




## PLATE II.

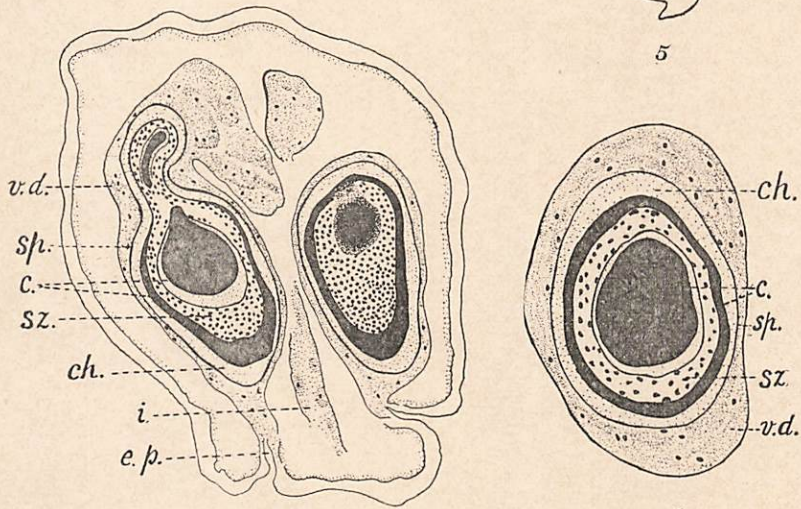
*Explanation of Figures.*

4. Dorsal view of the posterior extremity of a mature male, showing the position of the various parts of the reproductive organs.  $\times 144$ .
5. Sagittal section through the third lobe of the vas deferens and the spermatophore. The canal-like tube (*can.*), that conducts the spermatozoa is seen in *v.d.* 3.  $\times 560$ .
6. Frontal-section through the spermatophores, showing their structure.  $\times 800$ .
7. Cross-section through a spermatophore slightly above its central region, showing its structure.  $\times 1,240$ .



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## PLATE III.

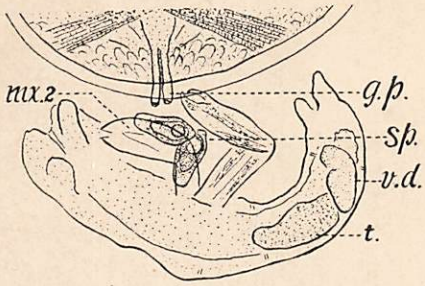
*Explanation of Figures.*

8. The process of fertilization. The male is here seen manipulating the extruded spermatophores (*sp.*) between his second maxillæ (*mx. 2*), in the vicinity of the genital pores (*g. p.*) of the female's body.  $\times 100$ .

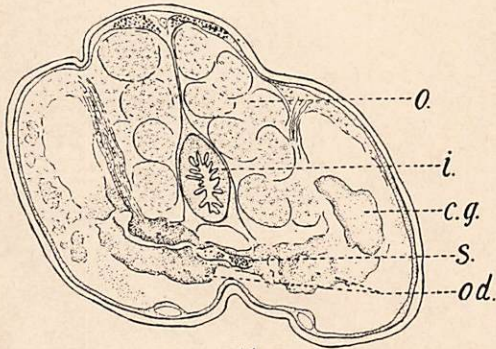
9. Side view of a young female after fertilization. The spermatophores (*sp.*), are attached near the genital pores.  $\times 76$ .

10. Ventral view of the abdomen of an adult female showing the relation of the reproductive organs to each other.  $\times 57$ .

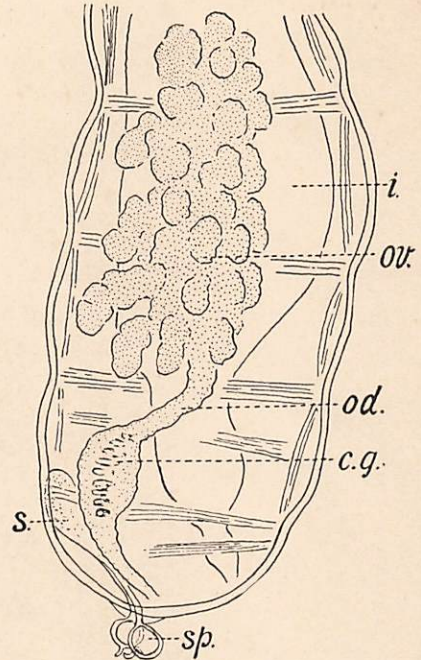
11. Cross-section through the abdomen of an adult female in the region of the spermatheca.  $\times 76$ .



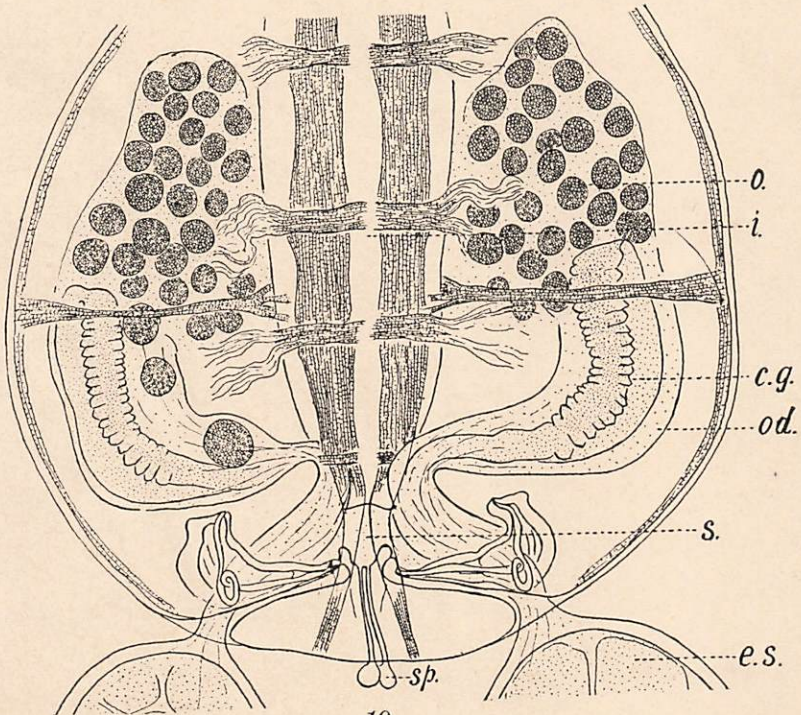
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