

The structure and development of the female internal reproductive system in six European species of Ephemeroptera

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Abstract. The anatomy, histology and development of ovaries and oviducts were studied in six European species (*Cloeon dipterum*, *Ecdyonurus torrentis*, *Oligoneuriella rhenana*, *Caenis robusta*, *Paraleptophlebia submarginata* and *Ephemerella danica*). There are paired ovaries situated along the alimentary canal extending from thorax (even from head in *Caenis*) to the sixth abdominal segment. Ovarioles differentiate in younger larvae (e.g. up to about 10th instar). Previtellarium and first egg chambers of vitellarium are formed in half-grown larvae where last oogonia disappear from the germarium. Further egg chambers and the first yolk granules (beginning of vitellogenesis) occur in older larvae (e.g. up to approximately 13th—17th instar). Vitellogenesis is finished and chorion secretion starts in larvae of the last 2—3 instars before moulting into subimago. The descent of mature 4—7 oocytes into extended oviducts (meiosis occurs after the secretion of chorion) and complete disintegration of germarium and previtellarium was observed in mature larvae. In *Ecdyonurus*, *Paraleptophlebia* and *Ephemerella* conspicuous shape changes of mature eggs probably connected with water loss were observed during subimaginal and adult stage. Only 1—2 oocytes mature in ovarioles of *Cloeon dipterum* and mature eggs with very thin chorion do not usually descend into oviductus; these modifications of oogenesis are due to ovoviviparity.

The classical monograph on mayfly reproductive system by PALMÉN (1884) dealing with female gonads of 7 European genera also summarizes the data obtained by earlier authors (e.g. EATON, 1867; JOLY, 1877a; JOLY & JOLY, 1876; VAYSSIÈRE, 1882) in some Palaearctic genera. In later papers (DRENKELFORT, 1910; HEINER, 1915; VAYSSIÈRE, 1890, 1934, 1937; BRINCK, 1957, and others) the gonads, their ducts and openings together with female external genitalia were described in more than 10 further genera. Despite relatively extensive knowledge of mayfly reproductive system from the descriptive anatomy viewpoint, the development and histology of the ovaries remained practically unknown. Although BRANDT (1878) and PALMÉN (1884) had already described the ovarioles as panoistic, the structure of the ovaries was mentioned briefly by NEEDHAM, TRAVER & HSU (1935) for the first time. They studied mostly the Nearctic genus *Stenonema*. CODREANU (1939) deals with histological changes of the gonads in larvae infested by a parasitic chironomid *Symbiocladius*. The structure of the ovaries in adults of *Coloburiscus humeralis* (WALK.) is described by WISELY (1965). This information is summarized by MATSUDA (1976).

While there are no data concerning the germinal cells the structure of the chorion is an exception. BENGTTSSON (1913) described the eggs of species from 13 Holarctic genera and DEGRANGE (1960) the eggs of most European species. Detailed studies of chorionic structures and their classification were published by KOSS (1968) and KOSS & EDMUNDS (1974). Great attention has been paid to the European ovoviviparous species *Cloeon dipterum* (L.) (JOLY, 1877b;

CAUSARD, 1896; COGGI, 1897; HEYMONS, 1897); however, these studies contain mostly a base description of this phenomenon. BERNHARD (1907) and DEGRANGE (1959) studied also the embryogenesis of this species. The fecundity of mayflies, now known in more than 100 species, is summarized by CLIFFORD & BOERGER (1974).

This paper describes the anatomical and histological structure of the female gonads in six Palaearctic genera, which represent different morphological and ecological types within the recent Ephemeroptera.

MATERIAL AND METHODS

Contemporaneous with a study of mayfly testes (SOLDÁN, 1979), the structure and development of mayfly ovaries were studied in all developmental stages (larvae, subimagos, adults — total number of 285 specimens) of the following European species: *Cloeon dipterum* (LINNÉ, 1761) (Baetidae); *Ecdyonurus torrentis* KIMMINS, 1942 (Heptageniidae); *Oligoneuriella rhenana* (IMHOFF, 1852) (Oligoneuriidae); *Caenis robusta* EATON, 1884 (Caenidae); *Paraleptophlebia submarginata* (STEPHENS, 1835) (Leptophlebiidae), and *Ephemera danica* (MÜLLER, 1764) (Ephemeridae) which represent different morphological and life cycle types (for further details and for localities see SOLDÁN, 1979). Larval stages are classified according to LEHMKUHL (1970).

The same methods were used in the present study as in that dealing with male reproductive system (observation under phase and interference contrast microscope after fast green staining; paraplast sections stained with hematoxylin and eosin after fixation in Bouin or Carnoy). Measurements of oogonia, growing oocytes and mature eggs were taken by means of an ocular micrometer from sections and squashes. Sections and preparations were examined and microphotographs taken with a Zetopan microscope.

THE STRUCTURE OF THE OVARIES AND OVIDUCTS

The ovaries and peritoneal tissues

There is a pair of ovaries situated along the alimentary canal in mayflies. As with testes, the position of ovaries can be dorsal (*C. dipterum*), lateral (*E. torrentis*, *O. rhenana*, *P. submarginata*, *C. robusta*) or ventrolateral (*E. danica*) to the alimentary canal. Ovaries in mature larvae, subimagos and adults may be contiguous dorsally at the midline. The position of the ovaries to the alimentary canal and the peritoneal structures described below do not substantially change during the larval development from younger to older larvae. In the older larvae (growth of ovaries complete) ovaries extend either from metathorax (*O. rhenana*, *C. dipterum*, *E. torrentis* — in the former species ovaries need not be produced to thorax at all, in the two latter species ovaries may be produced into mesothorax), or from mesothorax (*E. danica*) or prothorax (*P. submarginata*) or even from head (*C. robusta*) to the posterior margin of the sixth abdominal segment. Apical part of ovary in thorax or in the first abdominal segment is rounded or bluntly pointed (*E. torrentis*, *E. danica*, *O. rhenana*), pointed (*C. dipterum*) or produced into a narrow pointed projection (*P. submarginata*, *C. robusta*). Contrary to testes, the ovaries are usually compressed, especially in older and mature larvae. They are cylindrical or subcylindrical, with only slight bilateral flattening only in *C. dipterum* and *O. rhenana*.

Each ovary consists of a large number of ovarioles which envelope the oviducts from above and on the sides. Similarly to testes, the number of ovarioles is not constant in individuals of the same species or even in individuals of the same population. Differences in ovariole number between left and right ovary occur as well. Relatively high number of ovarioles was found

in *P. submarginata*, *E. danica* and *C. robusta* (one ovary consists of 250 to 500 ovarioles), less numerous are ovarioles in *O. rhenana*, *E. torrentis* (approx. 150—300 ovarioles) and in *C. dipterum* (approx. 90—200 ovarioles). Fewer ovarioles was observed in relatively smaller specimens of the summer generation of bivoltine species (*C. dipterum*, *C. robusta*).

Ovarioles are cylindrical and elongated in older and half grown larvae. They are usually oblique to oviduct, directed cranially (*P. submarginata*, *E. danica*, *C. robusta*), slightly oblique or situated at right angles to the oviduct in *E. torrentis*, *C. dipterum* and *O. rhenana*. No differences in the size of ovarioles were observed, ovarioles differ only in the length of the vitellarium and in number of contained growing oocytes.

The peritoneal membrane is a thin, slightly stainable tissue covering ovaries and lateral oviducts. The nuclei of cells and cell boundaries were not found. Peritoneal membrane is richly interwoven with tracheae and fused with peritoneal epithelium where these tissue touch. Conspicuous, light brown pigmented tracheae are apparent in *C. dipterum*. Peritoneal epithelium forms the walls of ovarioles enveloping the whole ovariole up to the oviduct. Cytoplasmatic projections fixing peritoneal epithelium on the germarium were not observed. The walls of ovarioles are completely separated, formed by a syncytical tissue with hardly distinguishable nuclei (Pl. I, Figs. 1, 2).*

Terminal filaments (Pl. II, Fig. 5) are present in all ovarioles, fixing the ovary to other tissues in abdomen and partly to the endoskeleton in thorax and head. Terminal filaments consist of strong, long fibres of basophil plasma oriented longitudinally. The rounded nuclei with indistinct nucleoli (Pl. II, Fig. 5) are deposited in the syncytial plasmatic core. They are distributed evenly and a group of nuclei is apparent near the base of terminal filament. The suspensory ligament formed by a bundle of terminal filaments at apex of ovary is of the same structure.

The ovarioles are joined to oviducts directly, i.e. no connective tissues forming pedicel are developed. Each ovariole joins the distal, slightly broadened part of respective oviduct (Pl. I, Fig. 4) which is analogous to calyx. This tissue is formed by syncytial mesodermal cells with scattered flat nuclei and the cell boundaries scarcely visible.

Germinal cells and follicular tissues

The cavities of ovarioles are filled up by germinal cells and follicular cells later forming follicular tissue. Since ovarioles of mayflies are of the panoistic (atrophic) type, no trophocytes or other trophic tissues occur (Pl. I, Fig. 3; Pl. II, Figs. 6, 7, 8).

Oogonia which differentiate from the primordial cells probably during embryogenesis or the first larval instar are found usually only at the top of germarium. They do not substantially differ from spermatogonia, being spherical with large, well stainable nuclei and slightly basophil cytoplasm. The nuclei contain dense faintly granular chromatin and hardly distinguishable nucleoli (Pl. I, Figs. 1, 2). Oogonia are about 4—6 μm in size.

Oocytes are found first in a thin layer at the lowest part of the germarium. They undergo a gradual development in vitellarium — the oogenesis. Although many systems of classification of oocytes according to their

* Plates I—VII will be found at the end of this issue.

morphological character and size have been published the situation varies from species to species and no particular classification has general acceptance. The oocytes can be simply divided as follows: germarial oocytes, previtellogenetic oocytes, oocytes undergoing vitellogenesis, and oocytes with chorion. Although meiosis was not observed, the oocytes in the germarium, previtellarium and vitellarium can be presumed to be oocytes I. Since the chromosomes of oocytes before starting of vitellogenesis are in prophase I, meiosis is presumably complete in oocytes provided with the chorion (oocytes II), as is usual in most insect orders investigated.

The zone of oocytes follows the zone of oogonia in the germarium. Germarial oocytes are all of the same size and shape — a thin ring of slightly basophil cytoplasm, with a large, rounded nucleus (Pl. I, Figs. 1—3). Conspicuous and well stainable nucleolus (macula germinativa) is apparent on sectioned material, indistinguishable in phase or interference contrast (Pl. II, Fig. 6; Pl. III, Fig. 9). Oocytes in the germarium are about 9—12 μm in size (approx. 2—3 times larger than oogonia) and they differ from oogonia in much less intensively stainable nucleus (Pl. I, Figs. 1, 2). They have no visible connections with trophic tissues, i.e. follicular cells dispersed among them.

Oocytes in previtellarium (Pl. I, Fig. 3; Pl. II, Fig. 9) are of the same structure as germinal oocytes. After reaching twice the size of other oocytes in the germarium they are surrounded by the follicular cells and they align themselves linearly at the basis of the ovariole (Pl. II, Fig. 7; Pl. III, Fig. 10). The nucleoli of their nuclei (maculae germinativae) gradually disappear (Pl. IV, Fig. 14). The original cytoplasm is presented only on the periphery, and around the nucleus a specious layer of ooplasm is formed. The growing nucleus (sometimes called germinal vesicle) is distinctly distinguished from ooplasm (Pl. III, Figs. 9, 10).

Aligned oocytes constitute the vitellarium where the vitellogenesis takes place. The oocytes in an advanced stage of previtellogenesis or at the beginning of vitellogenesis are considerably larger than those in germarium (50—100 μm in size) and the first yolk granules in their ooplasm begin to appear (Pl. II, Fig. 7; Pl. III, Figs. 10, 11). Yolk bodies gradually cover the nucleus and fill up the ooplasm (Pl. III, Figs. 11, 12). The originally oval nucleus is now spherical and is shifted to the peripheral position (Pl. III, Fig. 12). The first differences in shape were observed in oocytes during vitellogenesis in genera investigated: growing oocytes of *E. torrentis* and *O. rhenana* are spherical, those of *E. danica* and *P. submarginata* are oval and slightly elongated. The shape of growing oocytes resembles that of mature eggs (Pl. II, Fig. 7; Pl. III, Fig. 11).

The oocytes in the last period of oogenesis — the chorion formation — reach the dimension of a fully matured egg (Pl. IV, Figs. 15, 16; Pl. VI, Figs. 22, 24). They are still surrounded by follicular epithelium and their ooplasm is fully filled with yolk granules which form yolk polyhedron on sections (Pl. V, Figs. 15, 16; Pl. VI, Fig. 24). Mature eggs (Pl. VI, Figs. 22, 23; Pl. VII, Figs. 25—30) do not differ in the arrangement of nucleus or ooplasm from distal oocytes in the vitellarium. Neither chromosomes of nucleolar emitted bodies were observed. With the exception of *E. torrentis*, the chorionic structures (arrangement of chorion, micropyle, adhesive layer

and polar caps) have been described in all the species investigated (cf. BENGTSSON, 1913; GRANDI, 1947; DEGRANGE, 1960).

Follicular epithelium forms the mesodermal wall of the vitellarium. This epithelium consists of follicular cells which start to organize around the growing oocyte in previtellarium. The epithelium consists originally of 2—3 layers of cells, unilayered tissue is formed distally in the vitellarium. The follicular cells are at first very similar to prefollicular cells occurring in the germarium — they are spindle-shaped with distinct cell boundaries, elliptic nuclei with small chromatine granules and cytoplasm slightly basophil. Follicular cells gradually change their shape. They are cuboidal in follicles at the very beginning of vitellogenesis (Pl. IV, Figs. 13, 14), flat or squamous at the end of vitellogenesis (Pl. IV, Figs. 15, 16). The cell boundaries gradually disappear, the epithelial wall undergoes histolysis, pycnotic nuclei break down into small intensively basophil droplets disappearing in the basophil cytoplasm. The empty follicles collapse immediately after ovulation. No remnants of follicular tissues (corpus luteum) were observed in subimagos and adults. Interfollicular cells are arranged similarly to follicular ones and undergo the same developmental changes.

The oviducts

Oviducts of mayfly ovaries are formed by simple mesodermal ducts and variously arranged ectodermal openings. Mesodermal portion of oviducts (oviducti laterales are well separated and never joined to form a common oviduct (oviductus communis). The oviducts are simple and tubiform, usually 2—3 times broader than male ducts. They run from the apex of ovary to the posterior margin of 7th abdominal segment where they can be slightly expanded and bent medially (*E. torrentis*, *O. rhenana*, *P. submarginata*). The walls of the ducts are formed by an inner layer of epithelium and an outer layer of circular muscles (Pl. I, Fig. 4), both layers being approximately equal in width. The cells of inner epithelium are roundish or spherical with relatively large nuclei and distinct nucleoli. The outer muscular layer is of a syncitial character and considerably enlarged in subimagos and adults. The nuclei are very small and hardly distinguishable.

There is a great diversity in the arrangement of adult external genitalia and duct openings within the genera investigated: the oviducts simply open in an unmodified intersegmental membrane between sterna VII and VIII, sternum VII is not produced caudad (*C. dipterum*, *E. danica*); a short subgenital plate covering a pouch-like unpaired vestibulum is developed (*O. rhenana*); subgenital plate cover a vestibulum with a sac-shaped seminal receptacle and copulatory pouches (*E. torrentis*); the posterior margin of sternum VII is sclerotized, assisting oviposition (*P. submarginata*) (for more details see PALMÉN, 1884; GRANDI, 1947, 1955; BRINCK, 1957). The duct openings of *C. robusta* are non-functional, oviposition is only possible by rupture of intersegmental membranes between sterna VII and VIII.

THE DEVELOPMENT OF THE FEMALE REPRODUCTIVE SYSTEM

Newly hatched larvae

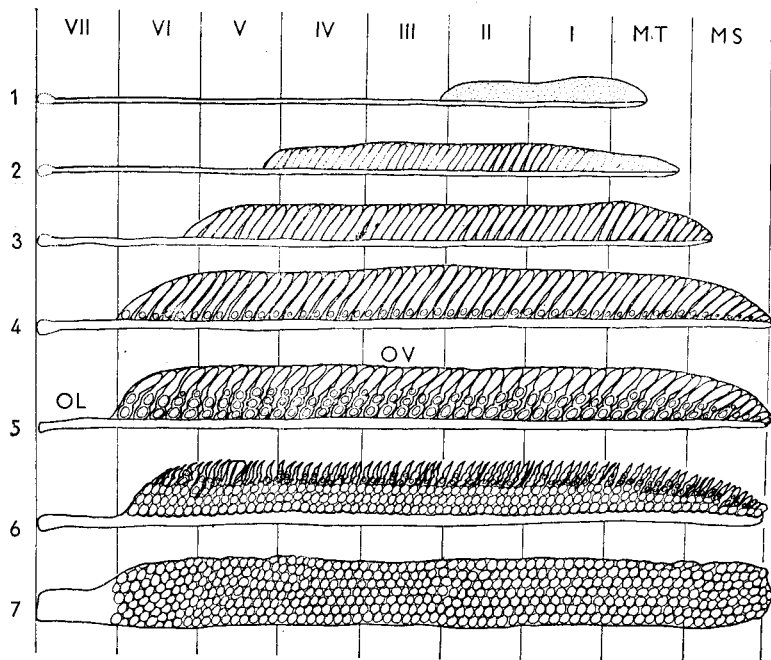
The internal reproductive system is represented by a pair of elongated groups of germinal cells. These cell groups, each consisting of several hundred

cells (morphologically not distinguishable from oogonia), are situated in segments I—II. The follicles are not discernable in sections and no histological evidence of oviducts were found.

Younger larvae

(Figs. 1, 2, 8, 9; Pl. I, Figs. 1—3)

In younger larvae the ovaries are elongated, subcylindrical, hyaline whitish opaque organs situated in the segments I—III or I—IV. The ovary of younger larvae may intrude into the metathorax (*P. submarginata*, *E. danica*),



Figs. 1—7: The development of the internal female reproductive system in Ephemeroptera (sketch): 1 — ovary of younger larva (approx. 2nd—6th instar). 2 — ovary of younger larva (approx. 5th—10th instar). 3 — ovary of half-grown larva (approx. 10th—13th instar). 4 — ovary of half-grown larva (about 15th instar). 5 — ovary of older larva (about 2—3 instars before subimaginal moulting). 6 — ovary of mature larva (beginning of last instar). 7 — ovary of subimago. MS — mesothorax. MT — metathorax. I, II, III, IV, V, VI, VII — abdominal segments I—VII. OV — ovarioles. OL — lateral oviducts. Ovary and ducts in lateral view.

even slightly into the mesothorax (*C. robusta*). Ovarioles are differentiated and they gradually become apparent on the surface of ovary in the same way as testicular follicles (Figs. 1, 2). Ovaries of young larvae are very similar to testes and they can be distinguished only by the position of ducts (oviducts in segments I—VII, male ducts in segments I—IX).

With the exception of the apical portion of ovary all ovarioles are differentiated approximately up to the 5th—6th instar. The whole of the ovariole is filled up with germarium containing spermatogonia and spermatocytes. In even younger larvae the ovarioles begin to increase in size and

in addition to distal zone of oogonia and oocytes (Pl. I, Figs. 1, 2) the previtellarium containing growing oocytes (Pl. I, Fig. 3) is being formed. The proximal oocytes are soon surrounded by follicular cells (in winter species during winter months). Terminal filaments as well as the suspensory ligament at the apex of ovary are easily recognizable. Oviducts are formed by a pair of solid strands extending from the apex of ovary to the posterior margin of sternum VII. They are narrow and simple connected with a pair of hollow ampules situated in segment VII. No fundaments of ectodermal portion of ducts are developed in younger larvae.

Half grown larvae

(Figs. 3, 4, 10, 11; Pl. I, Fig. 4; Pl. II, Fig. 5; Pl. III, Fig. 9; Pl. IV, Figs. 13, 14)

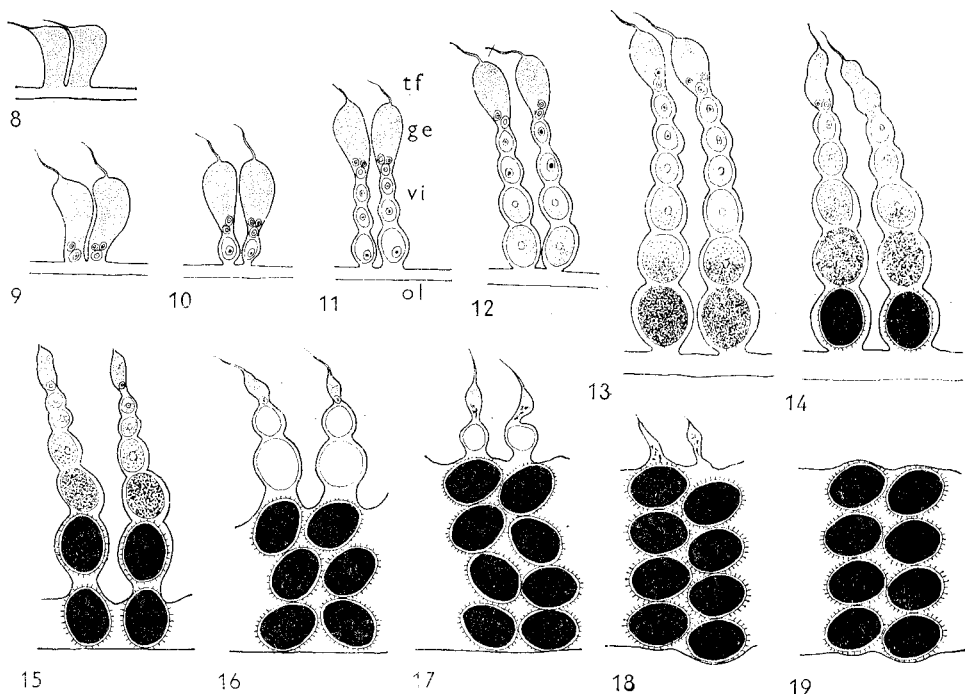
The ovaries are situated in the thorax (usually extruding into metathorax or even into meso- and prothorax in *P. submarginata* and *C. robusta*) and abdominal segments I—IV. Ovarioles are differentiated in all abdominal segments. In about the middle of the larval stage only a very narrow layer of oogonia remaining at the apex of germarium or oogonia disappear entirely. Germarium is rounded and well developed, filled up with the oocytes. Two or three distal oocytes (nearest to the oviduct) considerably increase in size and align themselves linearly, forming the vitellarium. Originally multilayered follicular epithelium becomes only unilayered. The oocytes forming vitellarium reach about 25—40 μm in size, i.e. they are approximately 10 times smaller than mature eggs. Ovarioles elongate and become more oblique to oviduct. These changes are apparent especially in *P. submarginata* and *C. robusta* where the ovary as a whole is bilaterally flattened and tongue-shaped in thorax.

Even in half-grown larvae (in winter species at the end of winter) the vitellarium containing about 3—5 oocytes is fully developed, but usually no yolk granules appear in the ooplasm of distal oocyte. Distal oocytes are about $1/4$ — $1/3$ as large as the mature eggs. The vitellarium in *C. dipterum* is relatively very short, formed only by 1—2 growing oocytes; ovaries of this species remain cylindrical or subcylindrical and ovarioles are situated nearly at right angles to the oviduct. Oviducts show only slight changes in half-grown larvae. They are usually moderately enlarged at the apex of the ovary and in abdominal segment VII and connected with a pair of hollow ampules which form the anlage of vestibulum in *E. torrentis*, *O. rhenena* and *P. submarginata*.

Older larvae

(Figs. 5, 12—15; Pl. II, Figs. 6—8; Pl. III, Figs. 10—12; Pl. V, Figs. 17—20; Pl. VI, Figs. 21—23)

The growth of ovaries and oviducts is completed in older larvae (larvae from about 13th instar with fully developed larval characters). All ovarioles are produced and easily distinguishable. The vitellogenesis begins in oocytes reaching about half the size of the mature eggs in vitellarium (in winter species at the beginning of spring). Yolk granules appear first in the ooplasm of distal oocyte and gradually superimpose on the nucleus. Oocytes continue growing and the vitellarium elongates considerably. At the beginning of vitellogenesis the vitellarium contains about 4—7 oocytes, the germarium is still apparent and filled with oocytes in the previtellogenetic stage. The distal



Figs. 8—19: The oogenesis in *Ephemeroptera* (sketch): 8, 9 — ovarioles (always two ovarioles drawn) and duct of younger larva (differentiation of follicles, forming of previtellarium). 10, 11 — ovarioles and duct of half-grown larva (forming of first egg chamber, forming of vitellarium). 12—15 — ovarioles and duct of older larva (beginning of vitellogenesis, secretion of chorion in distal follicles) 16—18 — ovarioles and extended duct mature of larva (ovulation, degeneration of germarium and proximal oocytes). 19 — oviduct of subimago filled with mature eggs. tf — terminal filament. ge — germarium. vi — vitellarium. ol — oviduct. Germarium and oocytes with yolk dotted, mature eggs blackened.

oocyte mature first, the chorion is formed by the secretion of follicular cells (in winter species during spring). The descent of mature oocytes (eggs with chorion) starts even in older larvae but only one or two oocytes reach the oviduct. Germarium of these larvae is considerably smaller and oocytes inside as well as those in previtellarium start to degenerate. These changes proceed very slowly in *C. dipterum*. Usually (in most ovarioles) only a single oocyte is provided with very thin chorion; descent of mature eggs does not occur in older larvae of this species.

Oviducts in older larvae are expanded (approximately 2—3 times broader than those in half-grown larvae) from the apex of ovary to the 7th segment. The anlagen of vestibulum are well recognizable.

Mature larvae

(Figs. 6, 16—18; Pl. IV, Figs. 15—16; Pl. VI, Fig. 24)

Developmental changes of mayfly ovaries are characterized by disintegration of germarium and proximal portion of vitellarium and gradual expansion of the oviducts which is connected with the descent of mature eggs. The changes of the position and shape of ovaries are very slight. The apex of ovary usually becomes produced into a pointed protuberance in

thorax which is squeezed between indirect thoracic muscles. This pointed projection is produced even into the head in *C. robusta*. The ovaries become cylindrical or subcylindrical again because the alimentary canal is now empty and non-functional. Several hours before subimaginal moulting the ovaries are converted into sac-like elongated bodies filled with the mature eggs and filling the whole abdominal cavity even in segment VII.

The descent of mature eggs, i.e. those after chorion and adhesive layer secretion, which have started in the older larvae, proceeds very rapidly in mature larvae and the oviducts enlarge remarkably. The mechanism of the descent of mature eggs could be compared with the turning inside out of a finger (ovariole) of a rubber glove (oviduct) which is inflated at the same time (cf. Figs. 15–18). The vitellarium containing mature oocytes is pulled, probably by contractions of oviduct muscles, inside the dilating oviduct. Approximately 4–8 oocytes mature in each ovariole. Oocytes of all the ovarioles mature at the same time. Germarium, previtellarium and about 1–3 enlarged oocytes in vitellarium (proximal portion) are not pulled inside the oviducts but change in a membraneous syncytial formation with well apparent terminal filaments. The nuclei of germinal cells disintegrate, yolk resorptions appear in ooplasm of degenerating oocytes in vitellarium. These tissues disappear entirely before subimaginal moulting.

In *C. dipterum* the eggs usually do not descend into the oviducts although the oviducts dilate. The distal oocytes can be more or less pulled into oviducts in some females. They remain (at least partially) in the membraneous remnants of ovarioles where only 1–2 oocytes mature. The germarium degenerates in the same way as in the other species examined.

Subimagoes and adults

(Figs. 7, 19; Pl. VII, Figs. 25–30)

No changes in the ovaries were observed in the extremely short-lived species *O. rhenana* and *C. robusta*, nor did the ovaries change substantially in the other species investigated or in *C. dipterum*. In the relatively long-lived species *E. torrentis*, *P. submarginata* and *E. danica* the conspicuous shape changes of mature eggs occur during the subimaginal stage. The most intensive changes were found in *P. submarginata*. While in the mature larvae the surface of the exochorion is nearly smooth, transversal grooves appear on eggs in young subimagoes. These changes deform the egg shape in subimagoes before imaginal moulting. Similar shape changes of mature eggs were found also in *E. torrentis* and *E. danica* where originally oval or nearly spherical eggs gradually change into irregular polyhedrons. Contrary to newly moulted subimagoes mature eggs descend into the oviducts in segment VII and to ectodermal portions of duct openings in adults. The shape changes of eggs disappear after oviposition.

No shape changes of eggs were found in *C. dipterum*; the eggs or even newly hatched larvae descend into dilated oviducts in older adults (adult females can survive 10–14 days).

DISCUSSION AND CONCLUSIONS

The results obtained in six European genera investigated fully agree with those obtained by NEEDHAM et al. (1935). These authors first mentioned the mechanism of ovulation and developmental changes of oviducts which are

probably common within the Ephemeroptera. WISELY (1963) observed similar phenomena in adult *Coloburiscus*. The anatomical arrangement of ovaries in species investigated corresponds to that of the genera *Baetis*, *Ephemerella*, *Siphonurus*, *Parametetus* and *Leptophlebia* described by BRINCK (1957). Certain differences, which are undoubtedly due to ovoviviparity, were found only in *Cloeon dipterum*. The development of ectodermal portions of ducts is practically the same as in the other genera (cf. QUADRI, 1940; BRINCK, 1957; GRANDI, 1947, 1955).

As already pointed out by several authors, no accessory organs of female gonads (spermatheca, paragenital glands etc.) occur in the Ephemeroptera. Despite the absence of any accessory glands, the adhesive layers are present in a large number of genera (DEGRANGE, 1960; KOSS, 1968; KOSS & EDMUNDS, 1974). There is no histological evidence that the epithelial cells of oviducts are responsible for secretion of an adhesive layer, nor do the follicular cells represent an active secretory tissue. According to KOSS (1970) the adhesive layers of mature eggs have to be produced only by follicular cells of the distal follicle after the chorion formation. The results obtained in *E. danica* support this hypothesis, because descending oocytes have been already provided with the adhesive layers.

The female internal reproductive system of mayflies is of a very primitive character within the pterygote insects having the same plesiomorphic (separation of oviducts) and apomorphic (multiplying of ovarioles which have lost their metameric arrangement) characters as the male gonads (SOLDÁN, 1979). Moreover, the female gonads are produced to the meso- and prothorax (even to the head in the genus *Caenis*) and the posterior portion of the oviducts can be fused in some families (Heptageniidae, some Leptophlebiidae — cf. GRANDI, 1955; BRINCK, 1957; PLESKOT, 1956). In this respect, the ovaries seem to be "more advanced anagenetically" than the testes.

While all the species investigated exhibit a certain uniformity in most details of their structure, quite different development of ovary and type of oogenesis were found in *C. dipterum* (only 1—2 oocytes in vitellarium which do not descend into oviducts). Taking into account the number of ovarioles, these data fully agree with the total fecundity of the female being 400—600 newly hatched nymphs (DEGRANGE, 1959, 1960). Similar type of oogenesis and similar development of ovaries probably occur in several Nearctic species of the genus *Callibaetis* (Baetidae) which are also ovoviviparous (cf. BERNER, 1941; EDMUNDS, 1945). The oogenesis of oviparous species of these genera (e.g. *Cloeon simile* ETN.) does not differ substantially from that of remaining genera investigated. The oogenesis and development of ovaries can be supposed to be principally the same in all recent oviparous genera. A quite different type of oogenesis was found in the oviparous Nearctic genus *Dolania* (Behningiidae). Although the ovaries possess about 200 ovarioles, only about 80—100 eggs are laid. In spite of apparent oviparity, probably the only oocyte matures in the vitellarium. Moreover, some of the ovarioles do not produce any eggs (unpublished results).

Similarly to spermatogenesis, oogenesis in Ephemeroptera takes place evidently only in one wave. In the same ovariole there are never found oogonia simultaneously with the oocytes undergoing vitellogenesis as is usual in insects that reproduce several times. Otherwise the oogenesis (forming

of germarium and vitellarium, vitellogenesis) is realized in the usual way. The descent of mature eggs into the oviducts is worth our attention. Contrary to most insect orders ovulation starts simultaneously in all ovarioles and all mature oocytes ovulate gradually before the beginning of oviposition. The descent of all oocytes leads to enormous extension of oviducts (they are about 10—15 times broader than those of younger larvae — Figs. 1—7). With the exception of mature eggs and ducts extensive degeneration of all germinal and connective tissues of ovary occurs in the last instar. Even the oocytes, which have started vitellogenesis, are resorbed. This process can be easily compared with an extensive degeneration of testicular follicles. Contrary to oogenesis, all male germinal cells are utilized and only remaining tissues (including ducts) become shrunken and degenerate. The resorption of over-numbered oocytes during oogenesis is probably due to the capacity of abdominal cavity and, of course, to humoral factors.

The shape changes of mature eggs which can be observed during the stage of subimago represent an unusual phenomenon. These changes being more apparent especially in relatively long-lived species (*E. torrentis*, *P. submarginata*, *E. danica*) occur probably in all genera with the exception of ovoviviparous species. Similar changes have been mentioned by BENGTSSON (1913), NEEDHAM et al. (1935) and DEGRANGE (1960). Two possible explanations of this phenomenon are considered here. (i) The shape changes of eggs are due to a water loss during subimaginal stage that represents a serious problem in long-lived mayflies. Weighing of subimagos of *E. danica* showed that the males lost 10—15% of body weight and the females as much as 20—25% (cf. BURKS, 1953). Since all the organs of males and females are developed in the same way and water loss from the rest of the testis is minimal, the reduction of body weight in females must originate from the eggs. Moreover, the eggs quickly lose their deformations after oviposition. (ii) The eggs are deformed only in a passive way. They arrange themselves in dilated oviducts economically so that each egg occupies a minimal area (irregular polyhedrons). The former explanation is more probable in short-lived species (minimal water loss, usually bad fliers), the latter in long-lived species (relatively high water loss, usually good fliers).

The fecundity of mayflies is really amazing, ranging from approximately 100 eggs in *Dolania* (Behningiidae) to 12000 eggs in *Palingenia* (Palingeniidae). Average fecundity is about 2000—6000 eggs per female (see review by CLIFFORD & BOERGER, 1974). This very high fecundity (with the exception of some social Hymenoptera the highest within the insects) is enabled mainly by the anatomical arrangement of the ovaries and by the unique mechanism of ovulation (distal oocytes ovulate simultaneously).

Another characteristic of oogenesis (and spermatogenesis as well) is that it is entirely shifted into the larval stage. The first mature eggs can be found in older larvae, and almost all female germinal cells are in the stage of mature egg in the last larval instar. This conspicuous shifting of spermatogenesis and oogenesis undoubtedly indicates a certain tendency to neoteny. Moreover, females of some genera mate and lay the eggs in the subimaginal stage or their moulting to adults remains imperfect (e.g. Palingeniidae, Polymitarcidae). These tendencies are more apparent in extremely short-lived species in adult stage (*O. rhenana*, *C. robusta*). It is generally regarded as a rule in Ephemeroptera that the shorter the subimaginal and imaginal

stages the earlier oogenesis (and spermatogenesis) is finished. Contrary to spermatogenesis (mature spermatozoa in mature larvae) finishing of meiosis is terminated fully in adults. Although meiosis was not observed in detail it is probably finished in mature oocytes after secretion of chorion or even in adults after fertilization (cf. BOHLE, 1969). These facts could explain the conspicuously high incidence of gynandromorphs in connection with parthenogenesis (common in most families — DEGRANGE, 1960) and dispermy (BOHLE, 1969) so that supernumerary spermatozoon might fuse with egg nucleus after beginning of parthenogenetic embryogenesis (cleavage).

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Строение и развитие внутренних органов размножения самок шести европейских видов поденок

Анатомия, гистология, оварии, зародышесые клетки, яйцеводы, оогенез

Резюме. Изучалась анатомия, гистология и развитие оварий и яйцепроводов у шести европейских видов поденок (*Cloeon dipterum*, *Ecdyonurus torrentis*, *Oligoneuriella rhenana*, *Caenis robusta*, *Paraleptophlebia submarginata* и *Ephemera danica*). Парные оварии расположены вдоль пищеварительного канала от груди (у *Caenis* даже от головы) по 6-ой сегмент брюшка. Овариолы дифференцируются у младших личинок (т. е. примерно до 10-ого возраста). Превителлярный и первые яйцевые мешки вителлярия образуются у среднеразвитых личинок, у которых из герматия исчезают последние оогонии. Развитие дальнейших яйцевых мешков и отложение первых зерен желтка (т. е. начало вителлогенеза) имеют место у старших личинок (т. е. примерно в 13-ом по 1-ый возраст). У 2—3 последних возрастов перед линькой в субимago заканчивается вителлогенез и начинается выделение хориона. Выход 4—7 ооцитов в растянутой яйцевод (мейоз имеет место после выделения хориона) и полное распадение герматия и превителлярия наблюдалось у зрелых личинок. У *Ecdyonurus*, *Paraleptophlebia* и *Ephemera* на стадии субимago и имаги наблюдались значительные изменения формы зрелых яиц, которые, повидимому, связаны с потерей воды. В опвариолах *Cloeon dipterum* созревают лишь по 1—2 яйцам. Зрелые яйца с очень тонким хорионом не выходят в яйцеводы. Это видоизменение оогенеза связано с яйцеживорождением.

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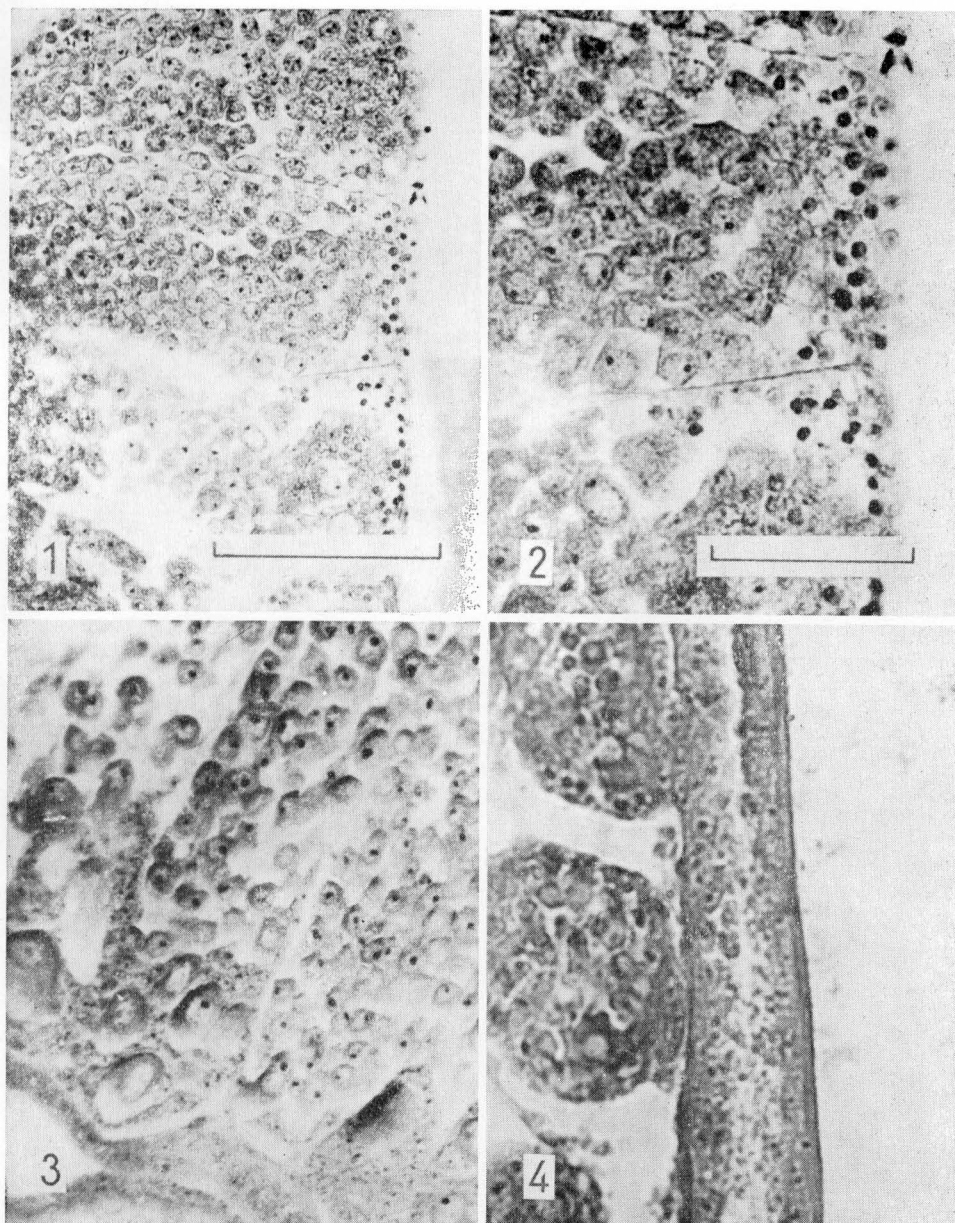


PLATE I, 1-4: 1 — *Oligoneuriella rhenana*, younger larva, section through ovarioles with oocytes and apical layer of oogonia. 2 — the same, detail of oogonia. 3 — *Ephemera danica*, younger larva, section through ovariole, formation of previtellarium. 4 — *Cloeon dipterum*, half-grown larva, longitudinal section of oviduct. 1 — Harris haematoxylin, eosin, scale 100 μ m. 2 — the same, scale 50 μ m.

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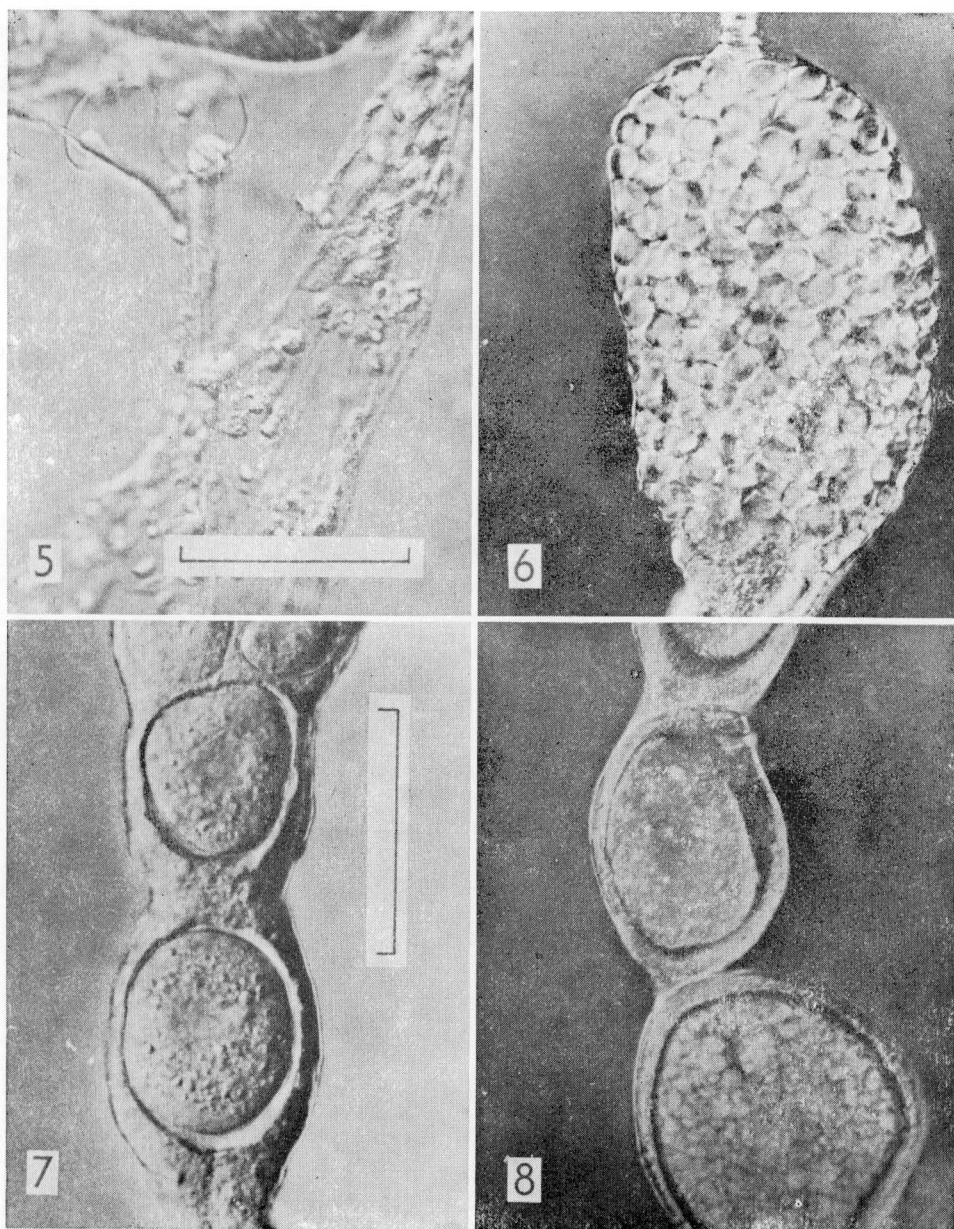


PLATE II, 5-8: 5 — *Ecdyonurus torrentis*, half-grown larva, terminal filaments. 6 — *Oligoneuriella rhenana*, older larva, germarium. 7 — the same, previtellarium and first egg chambers. 8 — the same, vitellarium. 5 — interference phase contrast, scale 20 μ m. 6-8 — the same, scale 40 μ m.

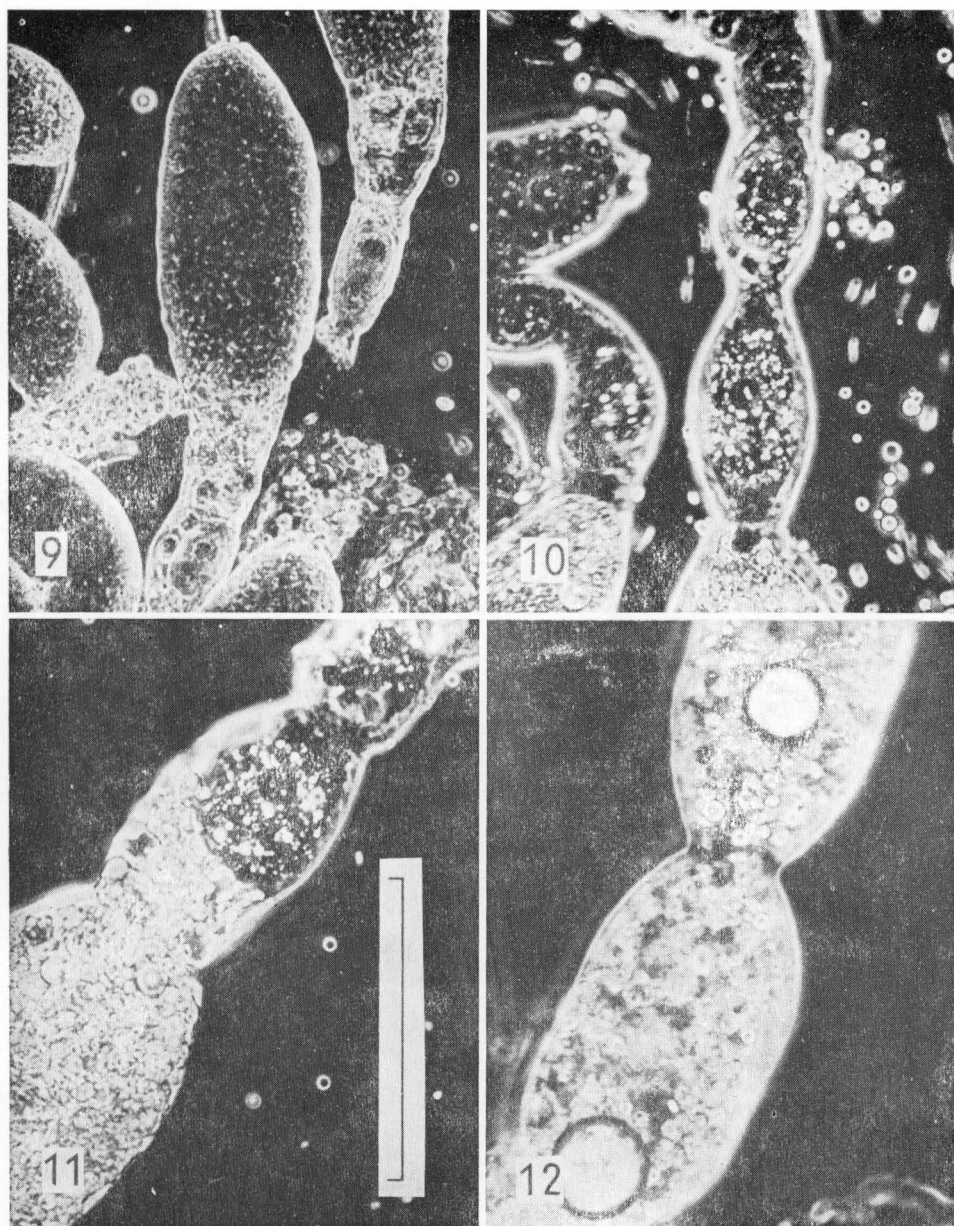


PLATE III, 9-12: 12 — *Ephemera danica*, half-grown larva, whole ovariole. 10 — the same, first egg chambers. 11 — the same, beginning of vitellogenesis (older larva). 12 — postvitellogenic follicles (older larva). 9-12 — anoptral phase contrast, scale 50 μ m.

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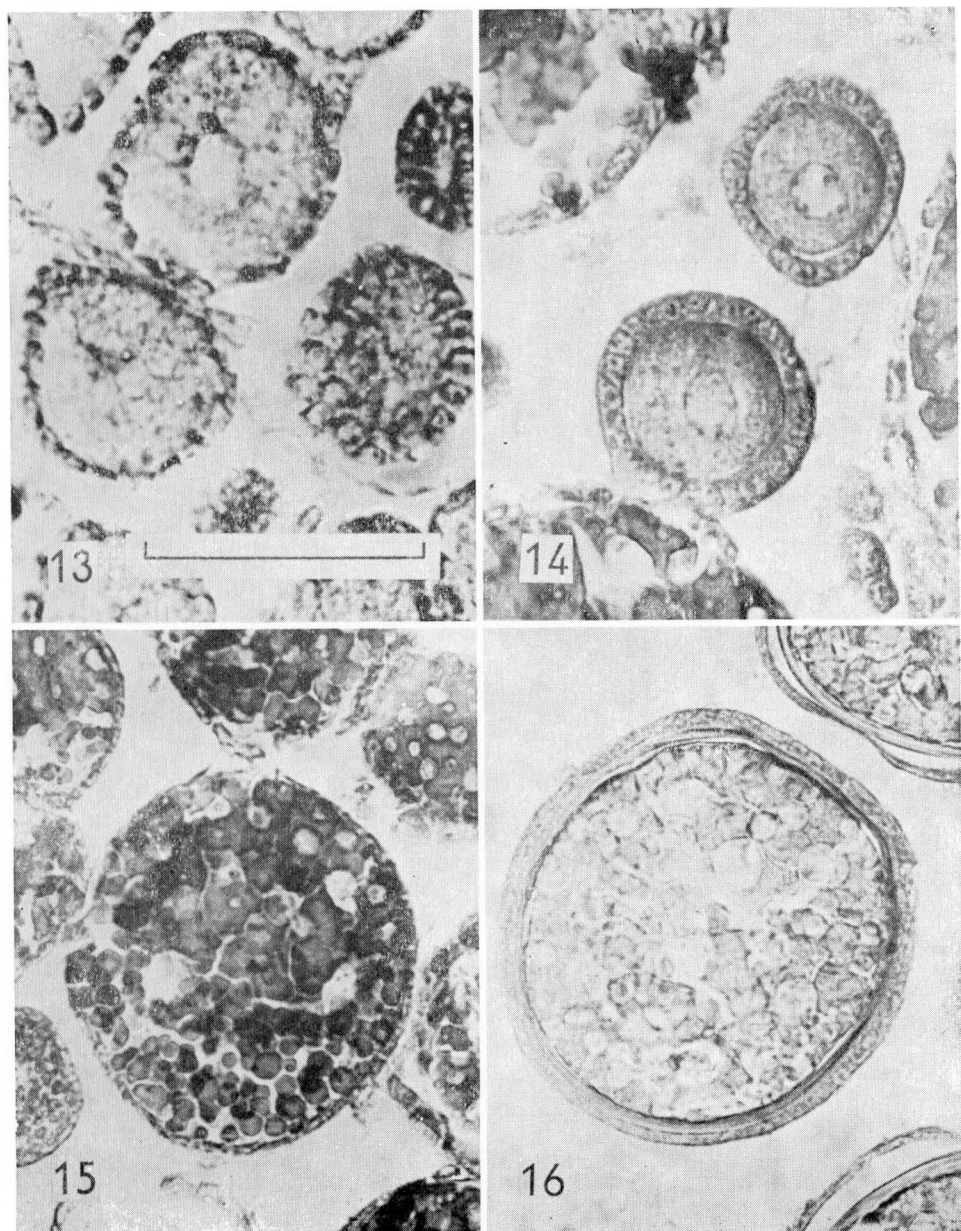


PLATE IV, 13-16: 13 — *Paraleptophlebia sulmarginata*, half-grown larva, section through first egg chamber. 14 — *Ephemera danica*, the same. 15 — *Caenis robusta*, mature larva, section through distal follicle. 17 — *Ecdyonurus torrentis*, the same, secretion of chorion. 13-16 — Harris haematoxin, erythrosin, metanil yellow, scale 60 μ m.

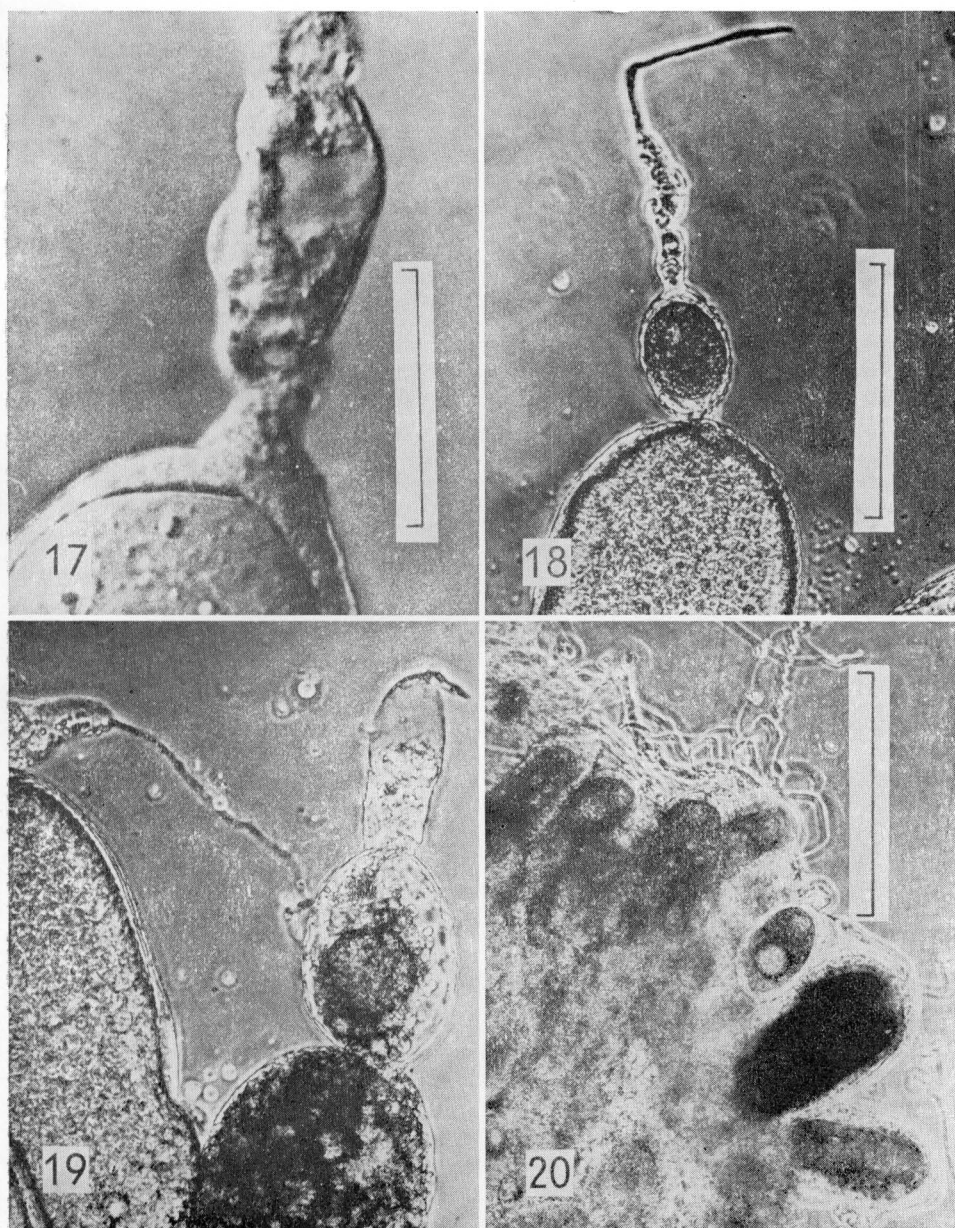


PLATE V, 17-20: 17 — *Paraleptophlebia submarginata*, older larva, detail of degenerating oocyte. 18, 19 — the same, degeneration of germarium and first egg chamber. 20 — the same, degeneration of apical portions of ovarioles and terminal filaments. 17 — phase contrast, scale 50 μ m. 18, 19 — the same, scale 100 μ m. 20 — the same, scale 200 μ m.

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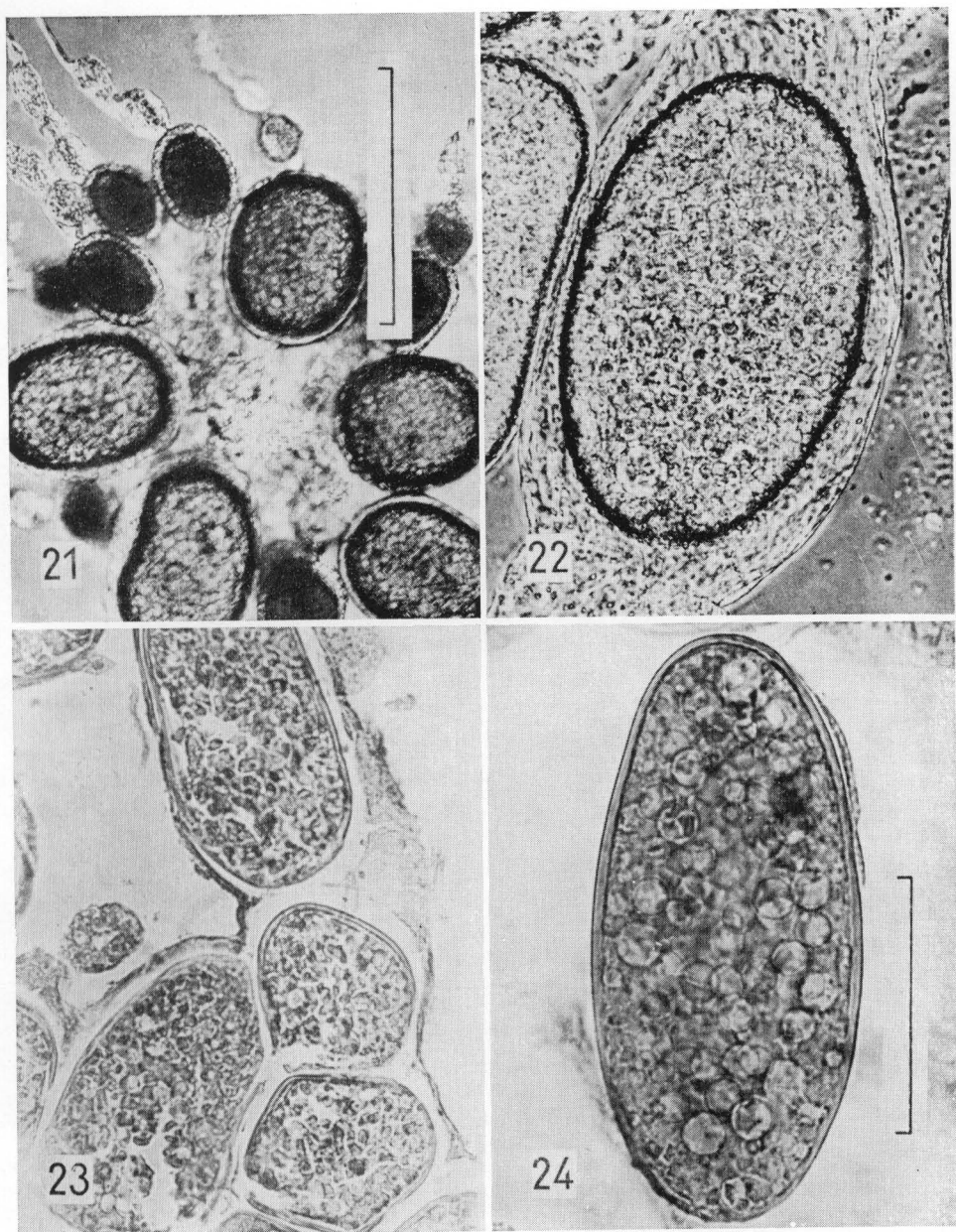


PLATE VI, 21-24: 21 — *Ecdyonurus torrentis*, mature larva, mature oocytes and degenerating apical portions of ovarioles. 22 — *Paraleptophlebia submarginata*, mature larvae, descending egg. 23 — *Ephemera danica*, mature larva, section through descending eggs and dilated oviduct. 24 — the same, section through mature eggs. 21, 22 — phase contrast. 23, 24 — Harris haematoxyline, eosin. 21, 23 — scale 170 μ m. 22, 24 — scale 100 μ m.

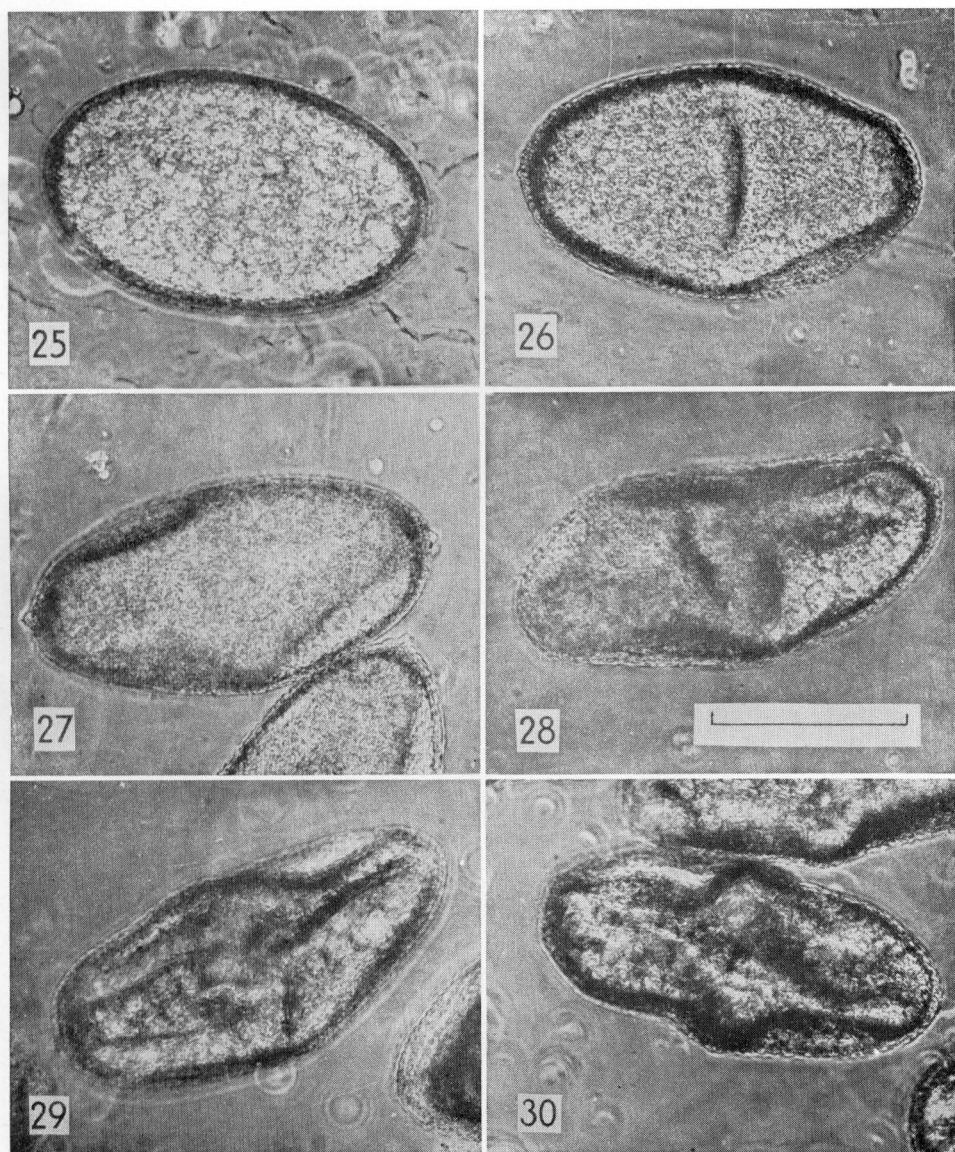


PLATE VII, 25—30: *Paraleptophlebia submarginata*, subimago, shape changes of mature eggs. 25 — unchanged eggs. 26, 27 — slightly deformed eggs. 28—30 — eggs deformed into irregular polyhedrons. 25—30 — phase, scale 100 μ m.