CHAPTER XVI

LARVAL DEVELOPMENT AND METAMORPHOSIS

The anatomical structure of an oyster larva is known primarily from works on the development of *O. edulis* by Horst (1883), Huxley (1883), Dantan (1917), and Erdmann (1935). Fragmentary information regarding other species is found in publications of Stafford (1913) on *O. lurida;* Prytherch (1934) on *C. virginica* and Fujita (1934); on *C. gigas.* Larval histology is described in a comprehensive paper by Erdmann (1935), and fate of larval organs in the metamorphosis of *O. edulis* is discussed by Cole (1938b).

The voluminous literature on the ecology and biology of oyster larvae of *O. edulis* and other species has been reviewed by Korringa (1941) in a lengthy publication which places emphasis on spawning and the setting of oysters. An abundance of ecological data found in the reports of Federal, State, and private organizations concerned with the conservation and management of oyster bottoms, deals mainly with the time of appearance and setting of oyster larvae. Relatively little is known about the factors which control the life and behavior of the larvae, and only a few studies have been made in recent years on larval physiology, nutritive requirements, and metabolism. However, advances in the technique of artificial rearing of oyster larvae from fertilized eggs (Loosanoff and Davis, 1963a, 1963b) now make it possible to obtain a continuous supply of larvae of known age regardless of the season of the year. This advantage may stimulate future studies of larval physiology.

ANATOMY OF TROCHOPHORE AND VELIGER

The slightly flattened embryo which forms at the completion of cleavage does not increase in bulk during embryonic development and is about 40μ to 50μ along its dorso-ventral dimension, about the same size as the egg. The two polar bodies may still be attached to some of the embryos and a tuft of robust cilia marks their anterior ends. The larva, which at this moment begins to swim, is called trochophore from the Greek "trochos," a wheel: and "phero," to bear.

The formation of the trochophore results from the epiboly, i.e., the multiplication of small ectodermic cells, their arrangement around the single and much larger macromere, and invagination of the endoderm. At the early stage of larval development, described by Horst (1883) for *O. edulis,* the invagination of endodermic cells (fig. 333, en.) marks the position of the blastopore, bl., (from the Greek "blastos," bud, and "poros," passage). a channel which leads to the archenteron (the cavity of the gastrula). A small invagination of the ectodermic cells at the animal pole of the larva indicates the location of a saddlelike shell gland (sh.g.), which at the later stages gives rise to the larval shell called prodissoconch (from the Greek "pro," before, "dissos," double, and "kongchē," conch or shell).

The invagination of the blastopore (fig. 334, bl.) becomes deeper and narrower; the mesoderm, me., is formed; and the shell gland, sh., increases in size. At the trochophore stage (fig. 335) the blastopore is closed and the mouth, m., is formed above it; the ectodermic cells, ec., develop cilia and are now called the trochoblasts. They form a ciliated ring or prototroch, which functions as an organ of swimming. The position of the prototroch is indicated in fig. 335 by two ectodermic cells, c., with cilia.

As the development of the trochophore advances, the prototroch forms a ciliated crown at

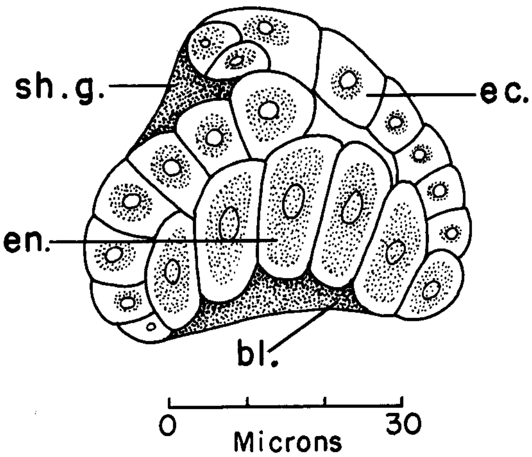

FIGURE 333.—Optical section of an early stage of development of the larva of *O. edulis* according to Horst. Reproduced from Pelseneer, 1906. bl.—blastopore; en.—endoderm; ec.—ectoderm; sh.g.—rudimentary shell gland.

the ventral side of the larva (fig. 336, pr.). The digestive system consists of the mouth (m.) surrounded by ciliated lobes; large stomach (st.); relatively short intestine (int.); and anus (a.). The thickened central part surrounded by the prototroch is considered to be a rudiment of the cephalic ganglion. The larval shell (sh.) covers a considerable part of the body and is formed into right and left oval valves of equal size and shape joined at the dorsal side of the larva. At the beginning of larval life the beating of the cilia of the prototroch is sporadic and disorganized. Within the next 15 to 20 minutes the larva first rotates around its dorsoventral axis and swims with the ciliated crown directed forward and up toward the surface of the water. The trochophore stage of *C. virginica* is short; in the laboratory at 22° to 24° C. it lasts no longer than 48 hours and in some instances only 24 hours.

The next stage is known as veliger (from the Latin "velum", veil; and "gerere", to carry). A detailed account of the structure and development of bivalve veliger was made by Meisenheimer (1901) for *Dreissensia polymorpha.* MacBride (1914) stated that the development of larvae of *Pecten, Teredo, Pholas, Cardium* and *Ostrea* (including *Crassostrea*) is virtually identical with that of *Dreissensia.* The early larval stages of these forms are so similar that their recognition in plankton samples cannot be made with confidence until their larval shells have been developed. The structure of an early veliger of *O. edulis,* described by Yonge (1926, 1960), is similar to that of *C. virginica* and *C. gigas.* The description given below is based primarily on publications by Yonge (1960) and Erdmann (1935) on *O. edulis.*

The veliger (fig. 337) is a highly complex organism containing several larval organs which disappear with the end of free-swimming life. The most conspicuous among the larval structures is the velum, v., which is formed by an outgrowth of the lateral parts of the prototroch area in two semicircular folds or lobes bearing large cilia along their margins. The prototroch thus develops at

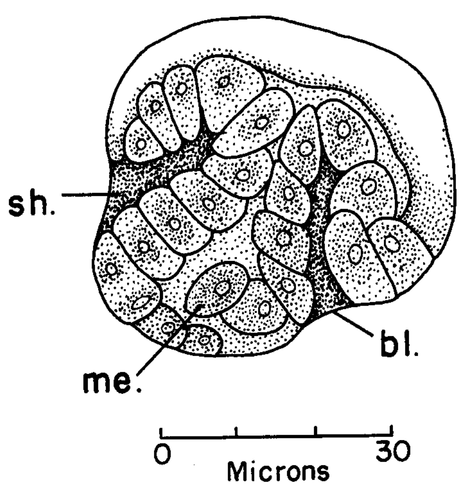

FIGURE 334.—Optical section of the gastrula stage of development of the larva of *O. edulis* according to Horst. Reproduced from Pelseneer, 1906. bl.—blastopore; me.—mesodermic cells; sh.—rudiment of shell.

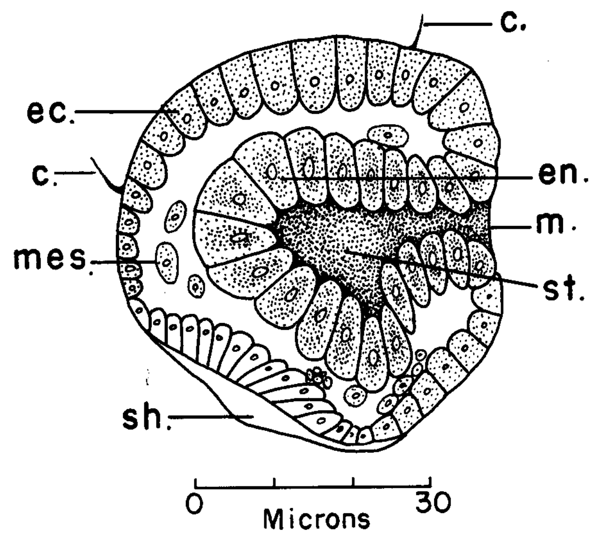

FIGURE 335.—Optical section of the trochophore larva of *O. edulis* according to Horst, 1883. Reproduced from Pelseneer, 1906. c.—cilia; ec.—ectoderm; en.—endoderm; m.—mouth; mes.—mesodermic cells; sh.—shell; st.—stomach.

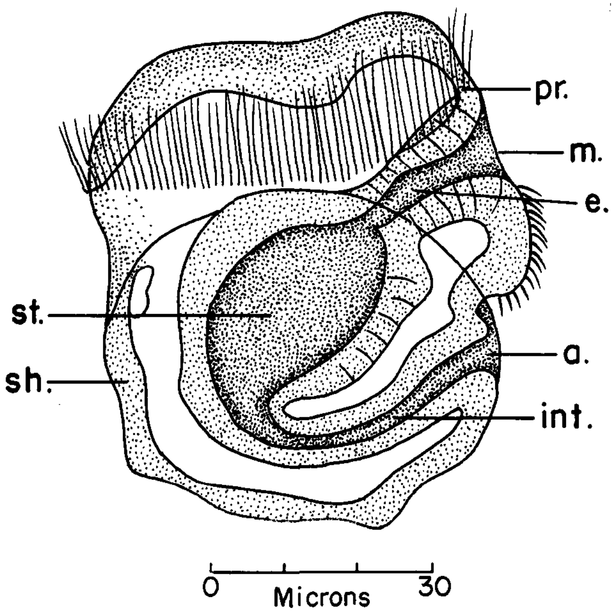

FIGURE 336.—Trochophore of *O. edulis* according to Horst, 1883. Reproduced from Pelseneer, 1906. a.—anus; e.—esophagus; int.—intestine; m.—mouth; st.—stomach; sh.—shell; pr.—prototroch.

the veliger stage into a powerful organ for swimming. During swimming the velum projects between the valves of the shell. It is highly contractile and at the slightest disturbance is withdrawn between the valves by several velar retractor muscles (r.v.), which are attached to the velum and are anchored at the opposite end to the shell.

For examination of the velum, the larvae should be narcotized with menthol, chloral hydrate, or other narcotics, and made transparent with glycerol. The larvae can be satisfactorily narcotized in a small dish by placing tiny crystals of menthol on the surface of the water and allowing them to relax before giving additional crystals. When narcosis appears to be complete, glycerol should be added slowly, drop by drop, to avoid disturbing the larvae and causing them to contract. The method is tedious, time-consuming, and requires a great deal of patience.

Large cilia around the margin of the velum are for swimming; small cilia (not shown in fig. 337), covering the base of the velum carry food particles toward the mouth (m.). A relatively long esophagus (e.) leads to a barrellike stomach (st.), which is in close contact with the glandular structure of digestive diverticula (dig. d.). The crystalline style sac (cr. s.) is at the lower part of the stomach. The intestine (int.) emerging from the stomach makes a single loop and continues into the rectum (r.); the anus (a.) opens into the mantle cavity (m.c.). The foot rudiment (f.) appears as a ciliated outgrowth of the body under the mouth and reaches its full development toward the end of larval life. The anterior adductor muscle (ant. ad.), destined to disappear in older larvae, is conspicuous; the posterior adductor has not yet developed.

The early larvae of *C. virginica* found in plankton samples or developed in the laboratory are oval-shaped and slightly asymmetrical. Because the hinge side of their shells is straight, they are called straight-hinge larvae or D-shaped larvae. Rees (1950) refers to this stage as Prodissoconch I. Dimensions of the larvae vary from 70μ to 75μ in length, i.e., parallel to the hinge side, and from 60μ to 68μ in height, with the greatest distance at a right angle to the hinge side. The prodissoconchs of *C. virginica* are shown in the photomicrographs in fig. 338.

Major changes take place in the appearance and structure of the larva as it grows, reaches its full development, and becomes ready to set. The advanced stages of larval development are called by various descriptive names referring to the most conspicuous morphological change of each stage: umboned larva, eyed larva, adult, and mature larva. The latter expression is frequently used

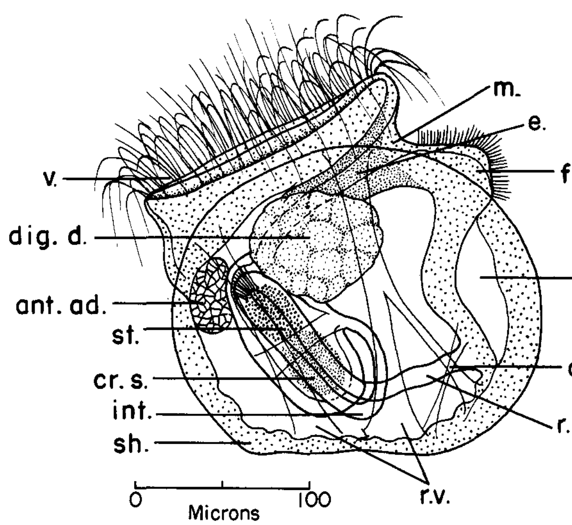

FIGURE 337.—Early free-swimming veliger of *O. edulis*. From Yonge, 1926. a.—anus; ant.ad.—anterior adductor muscle; cr.s.—crystalline style sac; dig.d.—digestive diverticula; e.—esophagus; f.—rudiment of foot; int.—intestine; m.—mouth; m.c.—mantle cavity; r.—rectum; r.v.—velar retractor muscles; sh.—shell; st.—stomach; v.—velum.

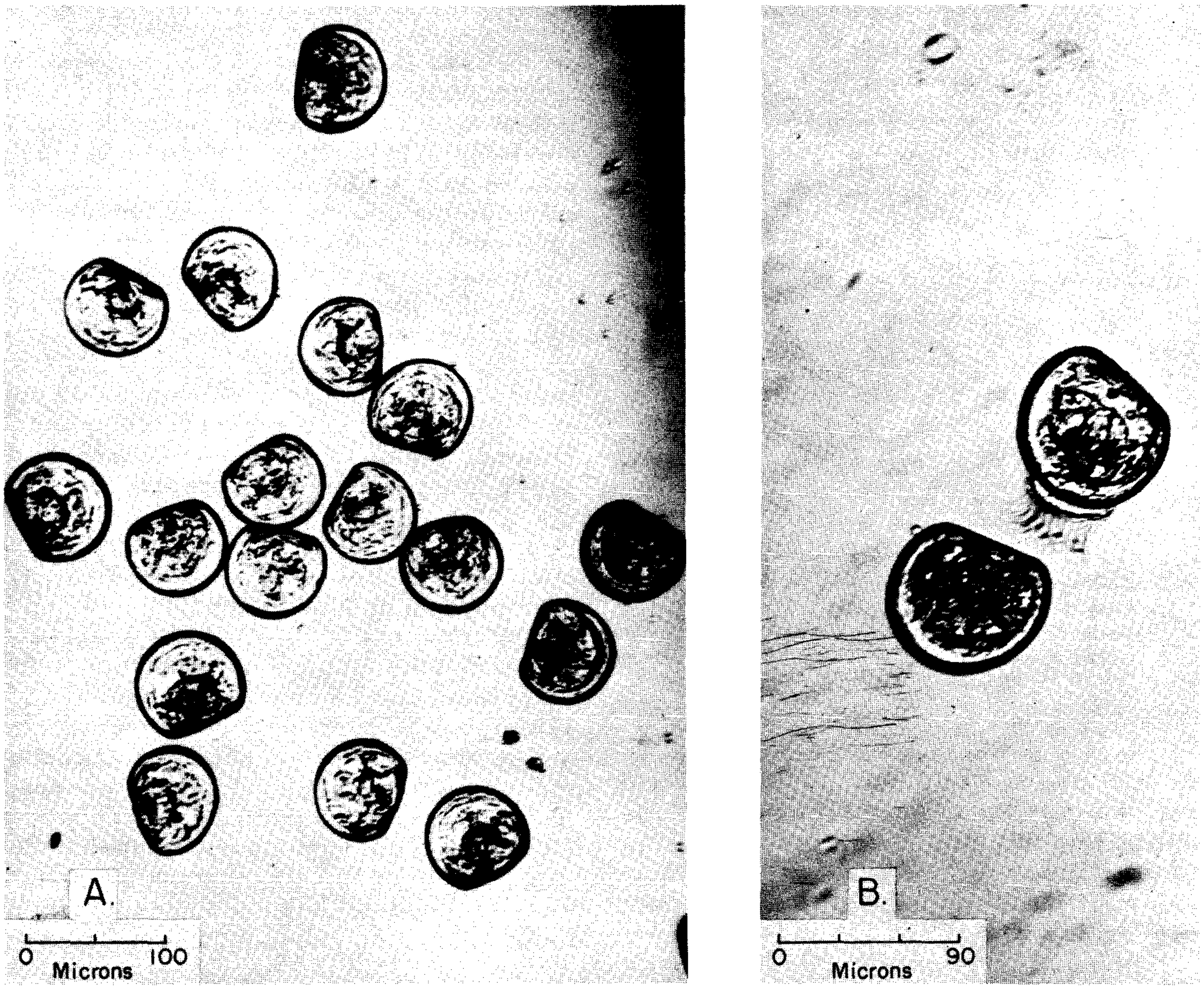

FIGURE 338.—Photomicrographs of early straight-hinge live larvae of *C. virginica*. A—larvae resting on bottom, shells closed; B—the slightly narcotized larva (upper part) has its velum protruding from the shell; the lower larva has closed its shell and withdrawn its velum.

by English-speaking oyster biologists in spite of the obvious contradiction in applying the adjectives adult and mature to larval stages. The term "velichoncha" proposed by Werner (1940) and adopted by Rees (1950) refers to the advanced stages of development of bivalve larvae, but the expression is not generally used in malacological literature. The name "pediveliger" was proposed by Carriker (1961) to designate the "swimming-creeping" stage of clam larva, *Mercenaria* (*Venus*) *mercenaria*. The term deserves to be accepted in malacological literature because it indicates the major character, i.e., the presence of a foot, and is applicable to many bivalve species, including oysters, in which a larval foot appears during the planktonic period.

As the larva grows its valves become deeper and almost circular. The hinge develops two bulgings or umbones, the one on the left side larger than its opposite number. At these stages the umbones bend toward the posterior end of the shell, which at this time has pronounced concentric rings, is heavy, and obscures the organs under it. In swimming the umbo larva protrudes its large ciliated foot forward. The larvae of *C. virginica* now have diameters of more than 300 μ in both length and height. The photomicrograph in figure 339 shows the side view of the advanced

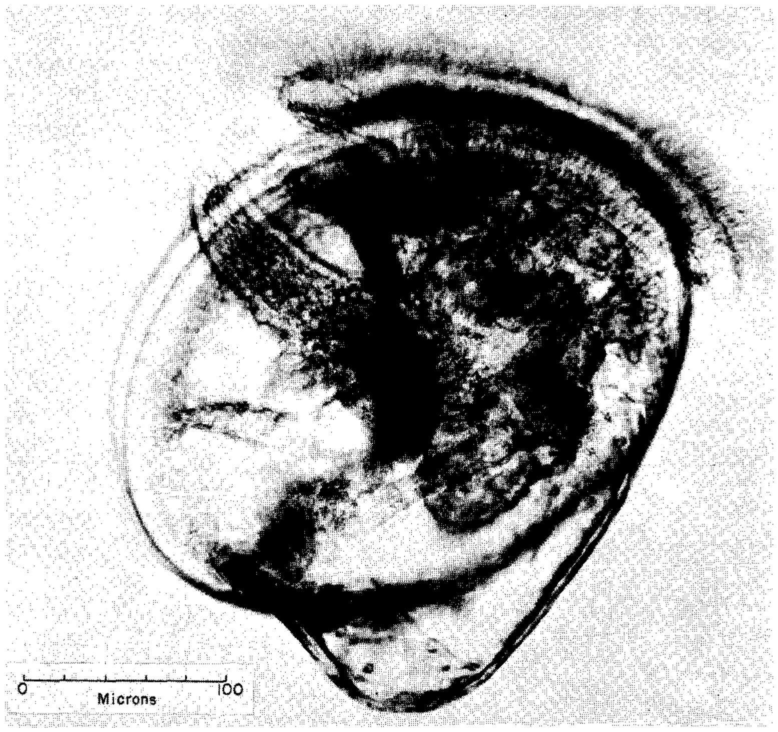

FIGURE 339.—Photomicrograph of live, slightly narcotized umbo larva of *C. virginica*.

umbo larva of this species, slightly narcotized to reduce its movements.

The anatomy of fully developed oyster larva is known primarily from the work of Erdmann (1935) on *O. edulis*. Figure 340, reproduced from his publication, shows the velum (v.) with a crown of powerful cilia arranged in a preoral ciliated circle, and a ciliated aboral belt or zone covered with small cilia (ab.c.).

Four pairs of velar retractors (r.v.) withdraw the velum. The muscle bands consist of bundles of cross-striated fibers along the dorsal side of the body. The cross striation of the velar retractors of oyster larva is typical for rapidly contracting muscles. In swimming the veliger rapidly changes the degree of expansion and the position of the velum, and withdraws the organ with great rapidity when the valves begin to close. The striated muscles in the larva indicate the high degree of specialization of larval organs needed for the organism to function effectively. The muscles of an adult oyster are nonstriated. Their contractions are relatively slow and do not require the mechanism typical for the rapid movements of the free-swimming organism. The apical sense organ (a.p.o.) ("Scheitelorgan", according to Erdmann) and cerebral ganglion occupy a central position in the crown of the velum. The function of the apical organ is not known.

A new feature in the larval anatomy, not present at earlier stages, is a well-developed foot (f.) covered with strong cilia. The foot is highly contractile and can be withdrawn by its retractor muscle (f.r.). A byssus gland (b.g.) with a small duct opening into the mantle cavity (m.c.) is located at the base of the foot. Both the foot and the gland are typical larval structures which disappear after performing their function during the attachment. Two muscles, the anterior and posterior adductors (ant.ad. and post.ad.), close the valves. The mouth (m.) is surrounded by a ciliated ridge which develops into the labial palps. The esophagus (e.) leads to the stomach (st.), part of which is covered with the gastric shield (g.sh.). The crystalline style sac (cr.s.) and digestive tubules have greatly increased in size, and the ciliary motion inside them is accelerated. The intestinal tract (int.) forms a loop and ends in the rectum (r.), which has an anal (a.) opening into the mantle cavity (m.c.). The rudiment of heart and kidney is represented by a group of cells (h.r.) shown in figure 340 above the

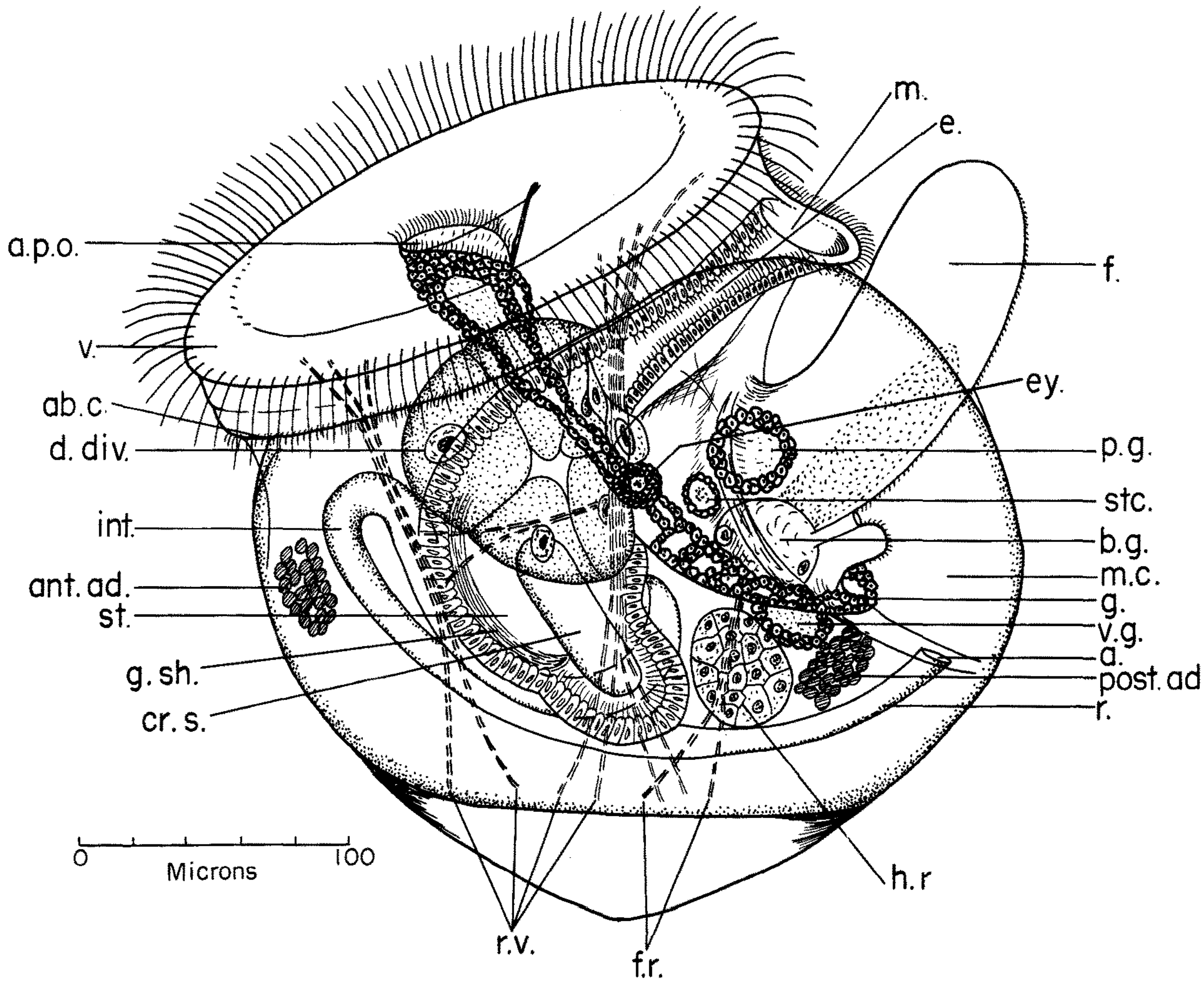

FIGURE 340.—Fully developed larva of *O. edulis* viewed from the right side with velum and foot at the ventral side in the uppermost position, typical for swimming. According to Erdmann, 1935. a.—anus; ab.c.—aboral belt of cilia; ant.ad.—anterior adductor muscle; a.p.o.—apical sense organ and ganglion; b.g.—byssus gland; cr.s.—crystalline style sac; d.div.—digestive diverticula; e.—esophagus; ey.—eye; f.—foot; f.r.—foot retractor muscles; g.—gill rudiment; g.sh.—gastric shield; h.r.—heart and kidney rudiment; int.—intestine; m.—mouth; m.c.—mantle cavity; p.g.—pedal ganglion; post.ad.—posterior adductor muscle; r.—rectum; r.v.—velar retractor muscles; st.—stomach; stc.—statocyst; v.—velum; v.g.—visceral ganglion.

rectum. The gill rudiment (g.), located between the base of the foot and heart rudiment, consists of a series of short, tubular channels. The pedal ganglia (p.g.), a round structure at the base of the foot, disappear with the dissolution of the foot. The visceral ganglion (v.g.) appears in its permanent position at the ventral side of the posterior adductor. The larval sense organs comprise a pair of statocysts (stc.) in the foot tissue and a pair of dark pigmented eyes (ey.) which develop toward the end of larval life. Their presence in the free-swimming larvae indicates the approaching of setting and metamorphosis.

The nervous system of the larva, shown diagrammatically in figure 341, is more complex than that of the adult oyster. It contains the pedal ganglia (ped.g.), which are absent in the adult; the pleural ganglia are present as a separate structure and are connected to the statocysts (syc.) and eyes, which disappear without a trace during metamorphosis. The visceral ganglia (visc.g.) of the larvae are less conspicuous than in the adult. All these organs are obviously necessary to a free-swimming organism, and some of them disappear with the loss of locomotion and the change to a sedentary mode of living.

The anatomy of the fully developed larva of *C. virginica* is similar to that of *O. edulis*. Figure

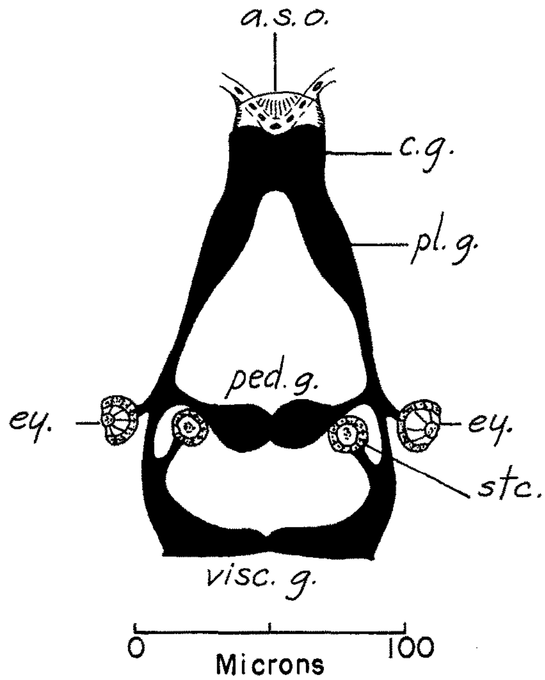

FIGURE 341.—Diagram of the nervous system and sense organs of fully developed larva of *O. edulis*. According to Erdmann, 1935. a.s.o.—apical sense organ; c.g.—cerebral ganglion; ey.—eye; ped.g.—pedal ganglia; pl.g.—pleural ganglion; stc.—statocyst; visc.g.—visceral ganglia.

342 shows the structure of the larva as it appears in the narcotized live specimen. The drawing is a composite from a number of photographs of live larvae taken with the microscope magnification of about 100 X, and from examination under higher power of specimens mounted in glycerin jelly. Only the organs visible under these conditions are shown in this illustration. The larvae were at the last stage of development, over 300μ in height, with eyes (ey.) and a well-developed foot (f.). The velum was large with long cilia at the top and a row of shorter ones forming an aboral circle (ab.c.) at the base. The apical organ could not be seen in the whole mount preparations. The retractors of the velum (r.v.) were well developed. As in *O. edulis* they consisted of rapidly contracting bands of striated fibers. When the velum is completely withdrawn within the shell cavity, the valves close and the larva drops to the bottom. In a contracted state the different organs become undistinguishable. The well-developed foot (f.) contains a large byssus gland (b.g.). During swimming it protrudes between the valves and is kept in the direction of swimming. The tip of the foot frequently turns right or left and up and down while the larva is swimming. This behavior suggests that it serves to orient the movements. At the last phase of larval life the foot is used for crawling over the hard surface where the young oyster will finally attach itself. The funnel-shaped mouth (m.) leads to a narrow and long esophagus (e.), which opens into a barrellike stomach (st.) partially surrounded with massive and dark digestive diverticula (d.div.). The intestinal loop (int.) and rectum (r.) are similar to those described for *O. edulis*. Both adductor muscles (ant.ad. and post.ad.) are well developed. The gill rudiment (g.) appears as a strand of cells in the mantle cavity, and the beating of the heart (h.), located between the stomach and the posterior adductor, can be seen in live specimens. At 24° C. the beating of the heart is rapid, varying from 80 to 100 pulsations per minute.

Food apparently is gathered by the ciliary mechanism of the velum, and small food particles can be observed entering the esophagus and moving inside the stomach where they are rotated by the ciliary epithelium. The ciliated apparatus of the gills has not yet fully developed, and food is gathered only by the aboral circle of the velum (ab.c.) and by the labial palps around the mouth. The statocysts (stc.) and the eye (ey.) are well formed. In a tangential section the eye of *C. virginica* appears as a transparent lens surrounded by a circle of darkly pigmented cells (fig. 343). The dark band is a short branch of a nerve leading to the eye.

The highly developed ciliary mechanism of the velum and the rapidly contracting velar retractors are essential to the life of a free-swimming larva. Their structure appears to be better developed than those of the muscles and ciliary epithelium of adult oysters. Electron microscopy reveals that the ciliated cells of the velum have a highly complex system of basal bodies and rootlets with distinct periodicity (fig. 344). Intercommunication between adjacent cilia through the basal bodies and their branches provides a system for the coordination of ciliary motion. The complexity of the ultrastructure conforms to the complexity of the ciliary activity of the velum, making it possible for the larva to swim in any direction, to turn around, or instantaneously to stop ciliary activity. The ciliated cells of the velum are very large; their surface is covered with microvilli, and

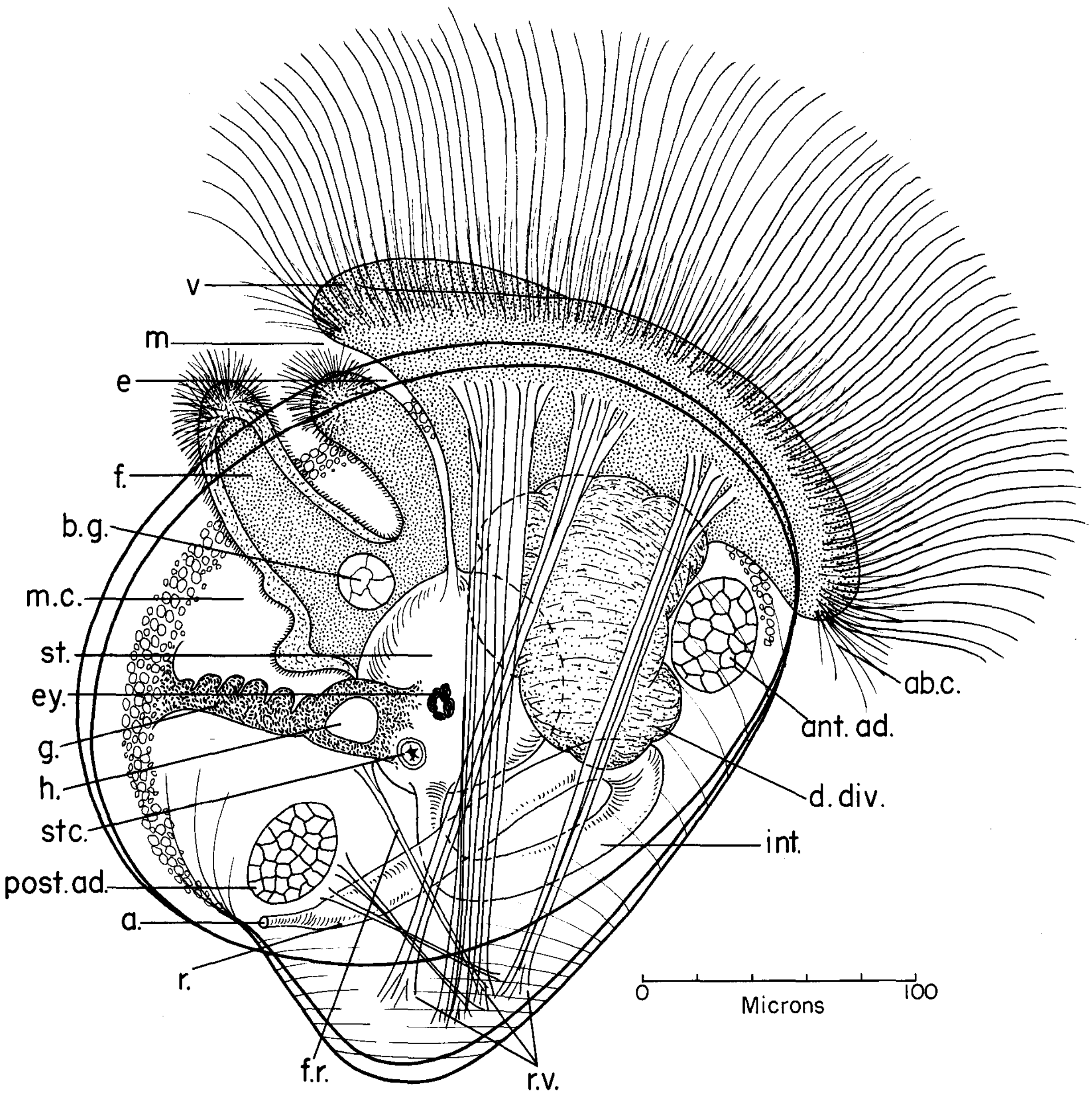

FIGURE 342.—Optical section of fully developed larva (pediveliger) of *C. virginica* viewed from the left side, in swimming position. Composite drawing from a number of photomicrographs of live, slightly narcotized larvae and whole mounts in glycerol. a.—anus; ab.c.—aboral circle of cilia; ant.ad.—anterior adductor muscle; b.g.—byssus gland; d.div.—digestive diverticula; e.—esophagus; ey.—eye; f.—foot; f.r.—foot retractor muscles; g.—gill rudiment; h.—heart; int.—intestine; m.—mouth; m.c.—mantle cavity; post.ad.—posterior adductor muscle; r.—rectum; r.v. velar retractor muscles; st.—stomach; stc.—statocysts; v.—velum.

they contain large oval mitochondria close to the rootlets.

The high degree of specialization of larval organs may be regarded as an adaptive organization of a free-swimming organism to its environment and may have no phylogenetic significance. The pelagic larva of a bivalve has a double task: to distribute the species and grow into an adult. The performance of these tasks requires the maintenance of an equilibrium between the locomotive efficiency and the weight to be carried; this maintenance is accomplished by the development of the velum. As the shell grows and becomes thicker and heavier, the task of swimming becomes more difficult, and the fully grown larva sinks to the bottom more rapidly and possibly more often