CHAPTER XVI

LARVAL DEVELOPMENT AND METAMORPHOSIS

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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

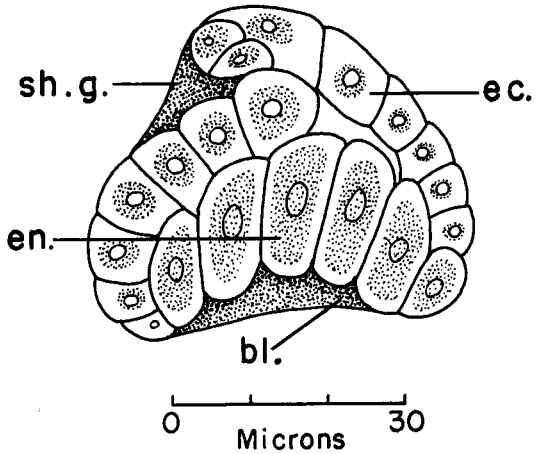

FIGURE 333.—Optical section of an early stage of development of the larva of *O. edulis* according to Horst. Reproduced from Pelseneer, 1906. bl.—blastopore; en.—endoderm; ec.—ectoderm; sh.g.—rudimentary shell gland.

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

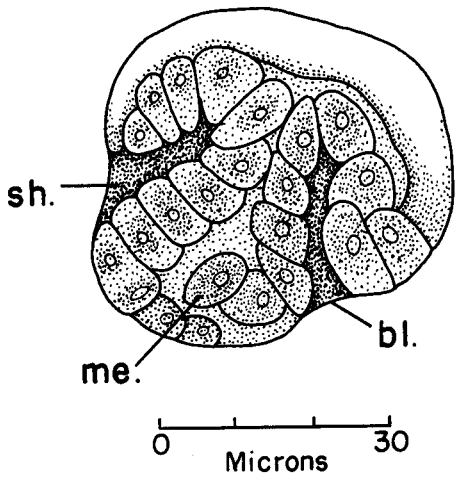

FIGURE 334.—Optical section of the gastrula stage of development of the larva of *O. edulis* according to Horst. Reproduced from Pelseneer, 1906. bl.—blastopore; me.—mesodermic cells; sh.—rudiment of shell.

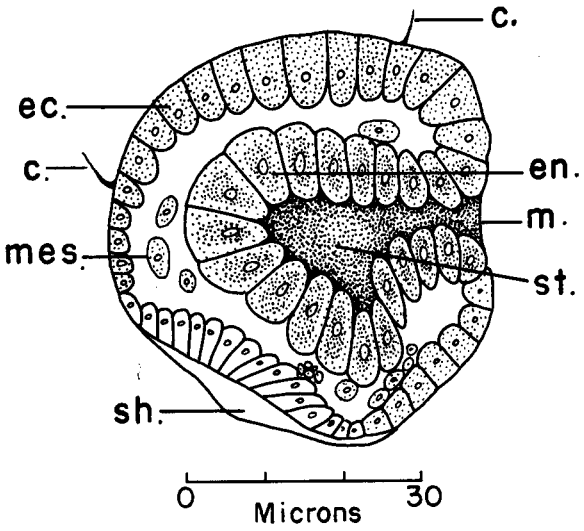

FIGURE 335.—Optical section of the trochophore larva of *O. edulis* according to Horst, 1883. Reproduced from Pelseneer, 1906. c.—cilia; ec.—ectoderm; en.—endoderm; m.—mouth; mes.—mesodermic cells; sh.—shell; st.—stomach.

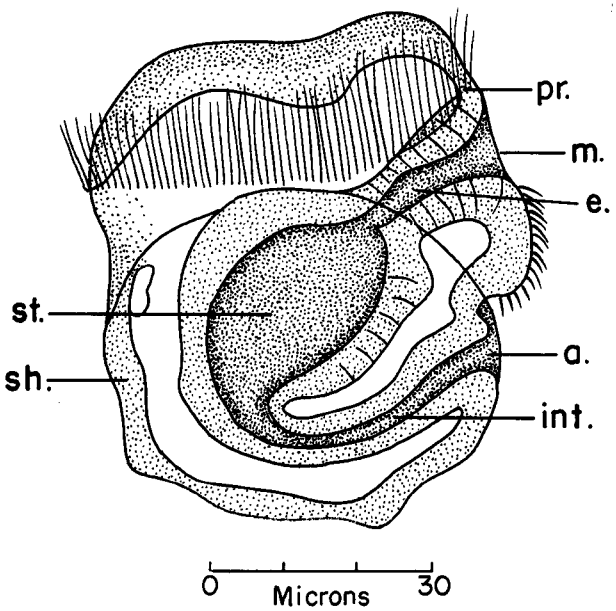

FIGURE 336.—Trochophore of *O. edulis* according to Horst, 1883. Reproduced from Pelseneer, 1906. a.—anus; e.—esophagus; int.—intestine; m.—mouth; st.—stomach; sh.—shell; pr.—prototroch.

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

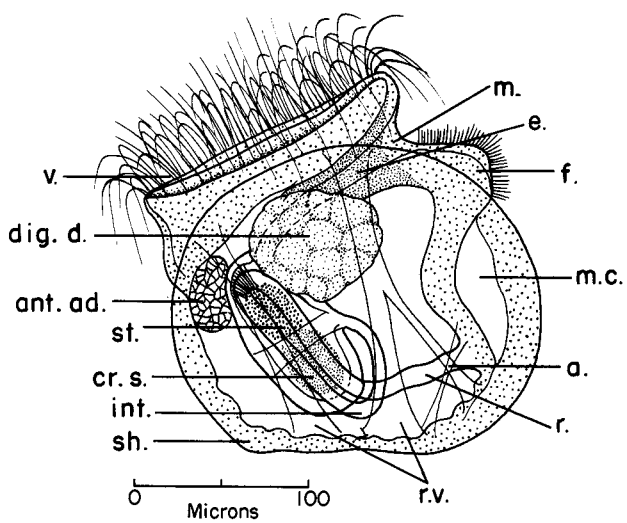

FIGURE 337.—Early free-swimming veliger of *O. edulis*. From Yonge, 1926. a.—anus; ant.ad.—anterior adductor muscle; cr.s.—crystalline style sac; dig.d.—digestive diverticula; e.—esophagus; f.—rudiment of foot; int.—intestine; m.—mouth; m.c.—mantle cavity; r.—rectum; r.v.—velar retractor muscles; sh.—shell;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

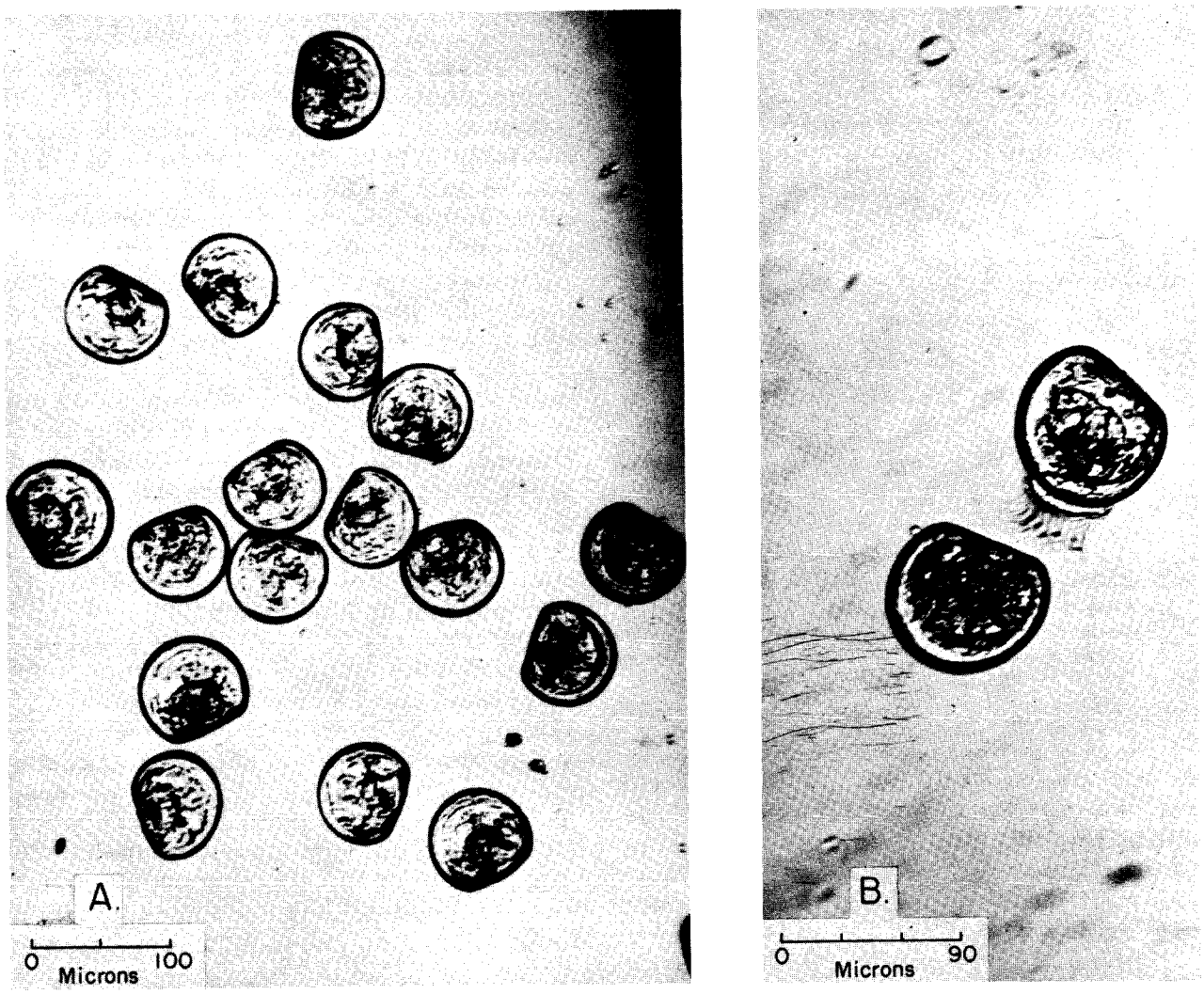

FIGURE 338.—Photomicrographs of early straight-hinge live larvae of *C. virginica*. A—larvae resting on bottom, shells closed; B—the slightly narcotized larva (upper part) has its velum protruding from the shell; the lower larva has closed its shell and withdrawn its velum.

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

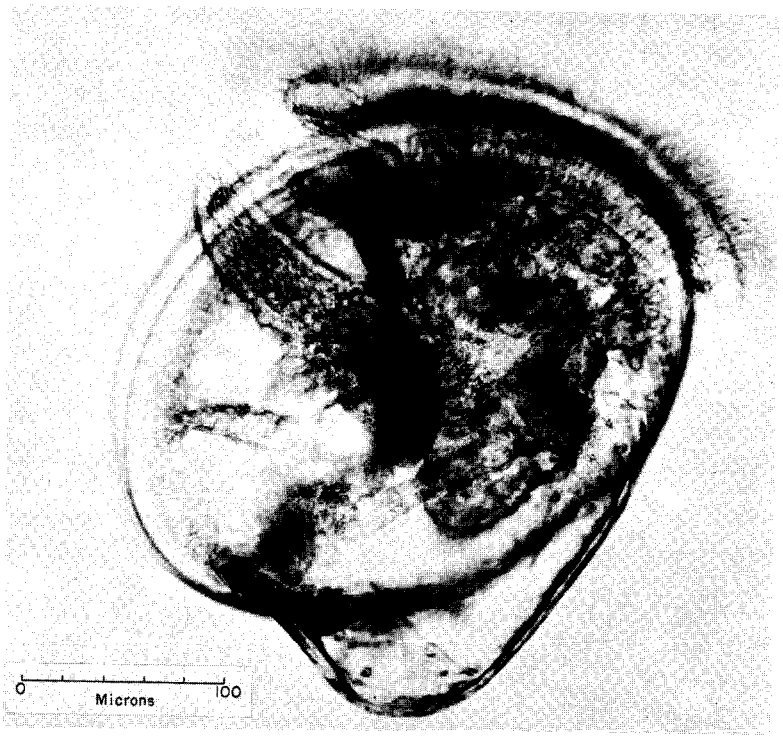

FIGURE 339.—Photomicrograph of live, slightly narcotized umbo larva of *C. virginica*.

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

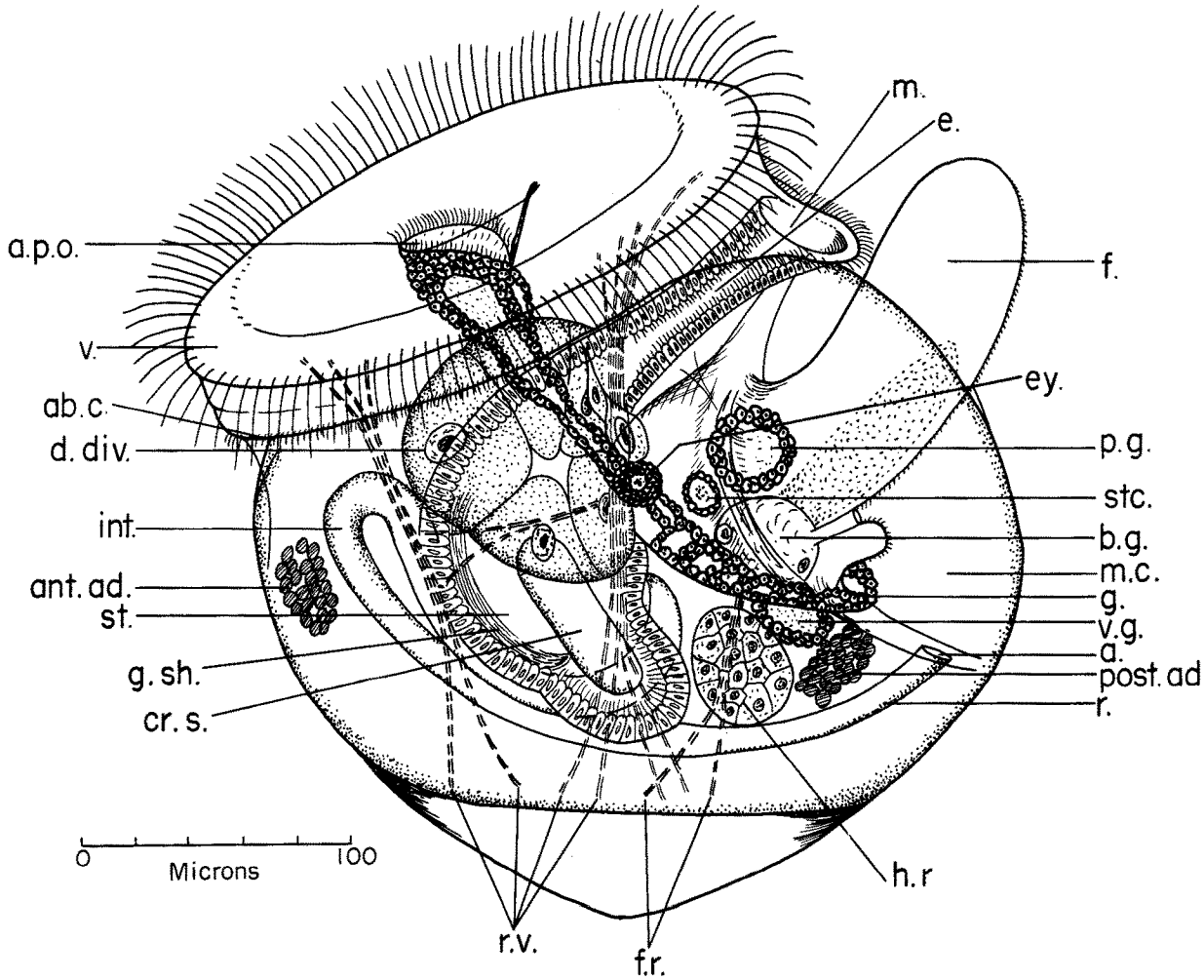

FIGURE 340.—Fully developed larva of *O. edulis* viewed from the right side with velum and foot at the ventral side in the uppermost position, typical for swimming. According to Erdmann, 1935. a.—anus; ab.c.—aboral belt of cilia; ant.ad.—anterior adductor muscle; a.p.o.—apical sense organ and ganglion; 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

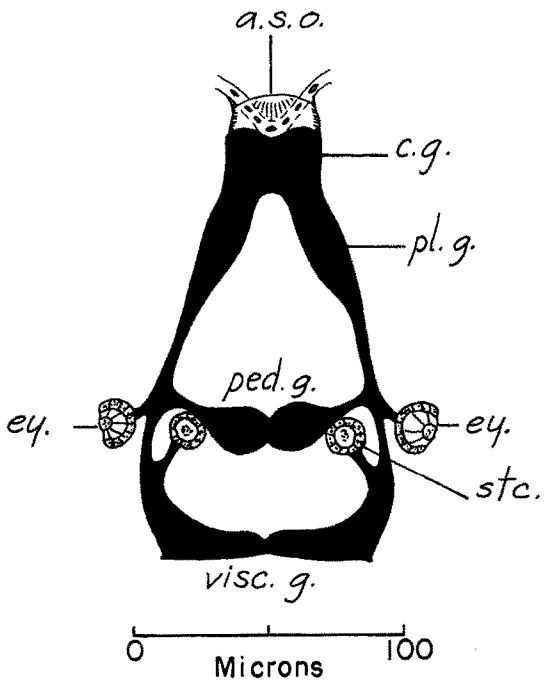

FIGURE 341.—Diagram of the nervous system and sense organs of fully developed larva of *O. edulis*. According to Erdmann, 1935. a.s.o.—apical sense organ; c.g.—cerebral ganglion; ey.—eye; ped.g.—pedal ganglia; pl.g.—pleural ganglion; stc.—statocyst; visc.g.—visceral ganglia.

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

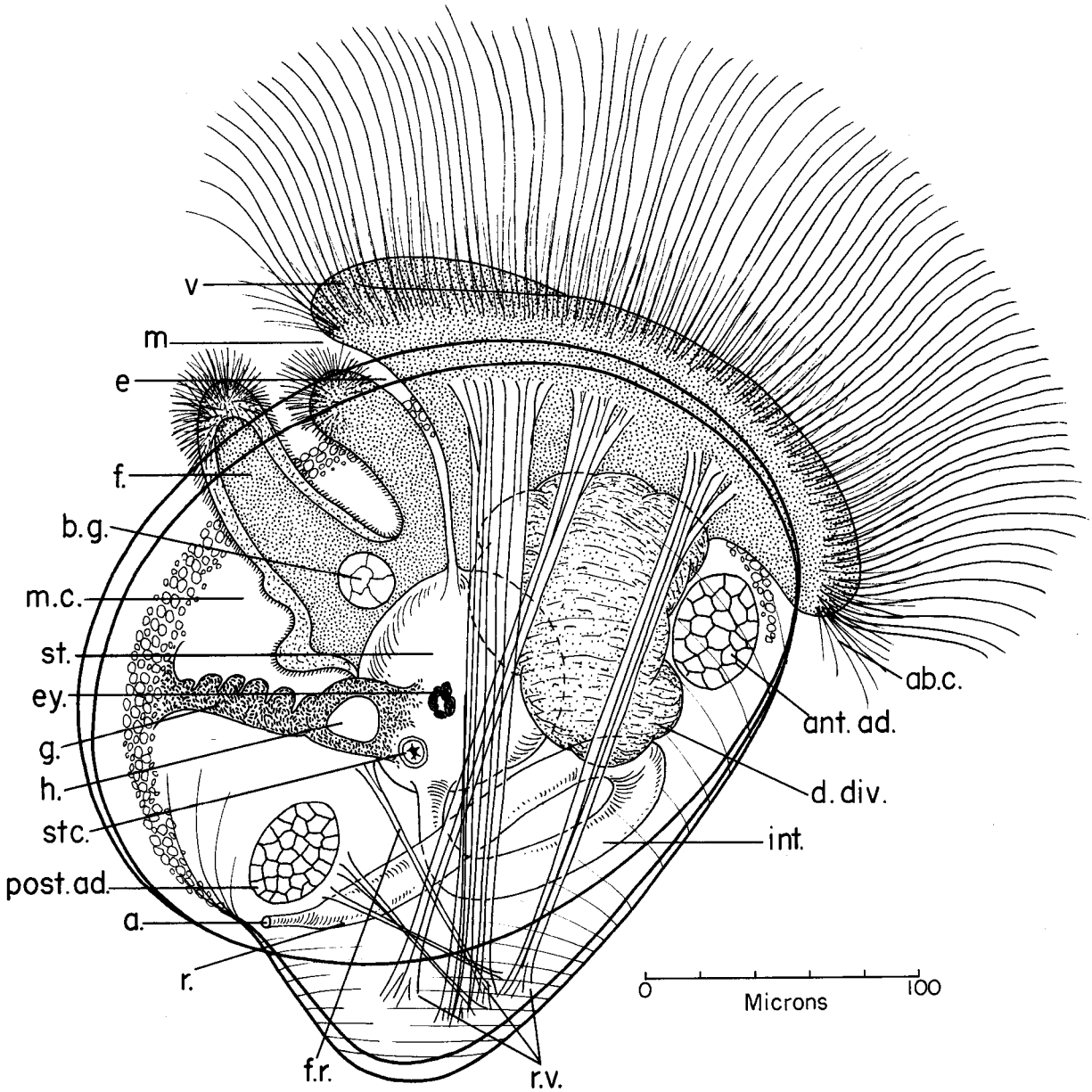

FIGURE 342.—Optical section of fully developed larva (pediveliger) of *C. virginica* viewed from the left side, in swimming position. Composite drawing from a number of photomicrographs of live, slightly narcotized larvae and whole mounts in glycerol. a.—anus; ab.c.—aboral circle of cilia; ant.ad.—anterior 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

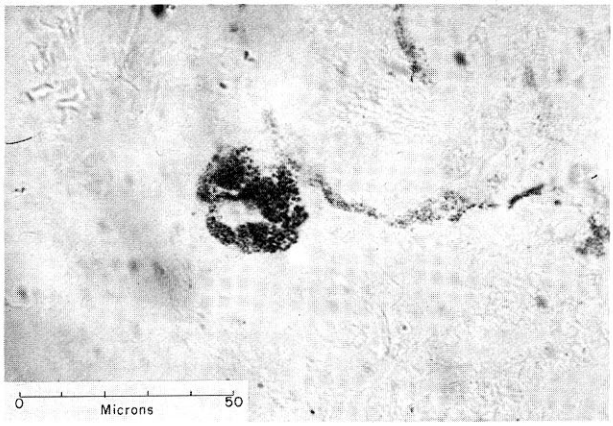

FIGURE 343.—Photomicrograph of a tangential, slightly slanted section of the larval eye of *C. virginica* preserved in osmic acid.

than it does at the straight-hinge stage. When the larva attaches to the substratum, the velum and the foot are no longer needed. Their disappearance marks the transition from free-swimming to a sedentary mode of life. Garstang (1929) expresses the correct opinion that larval organs should be regarded as an adaptation to the condition of life during development and need not affect the organization of the adult. His charming book on larval forms (Garstang, 1951) summarizes in a somewhat unorthodox way the ideas and theories concerning the significance of various larval forms in the evolution of aquatic animals.

MORPHOLOGY OF LARVAL SHELL

The morphology of the larval shell differs from that of an adult oyster primarily in the greater

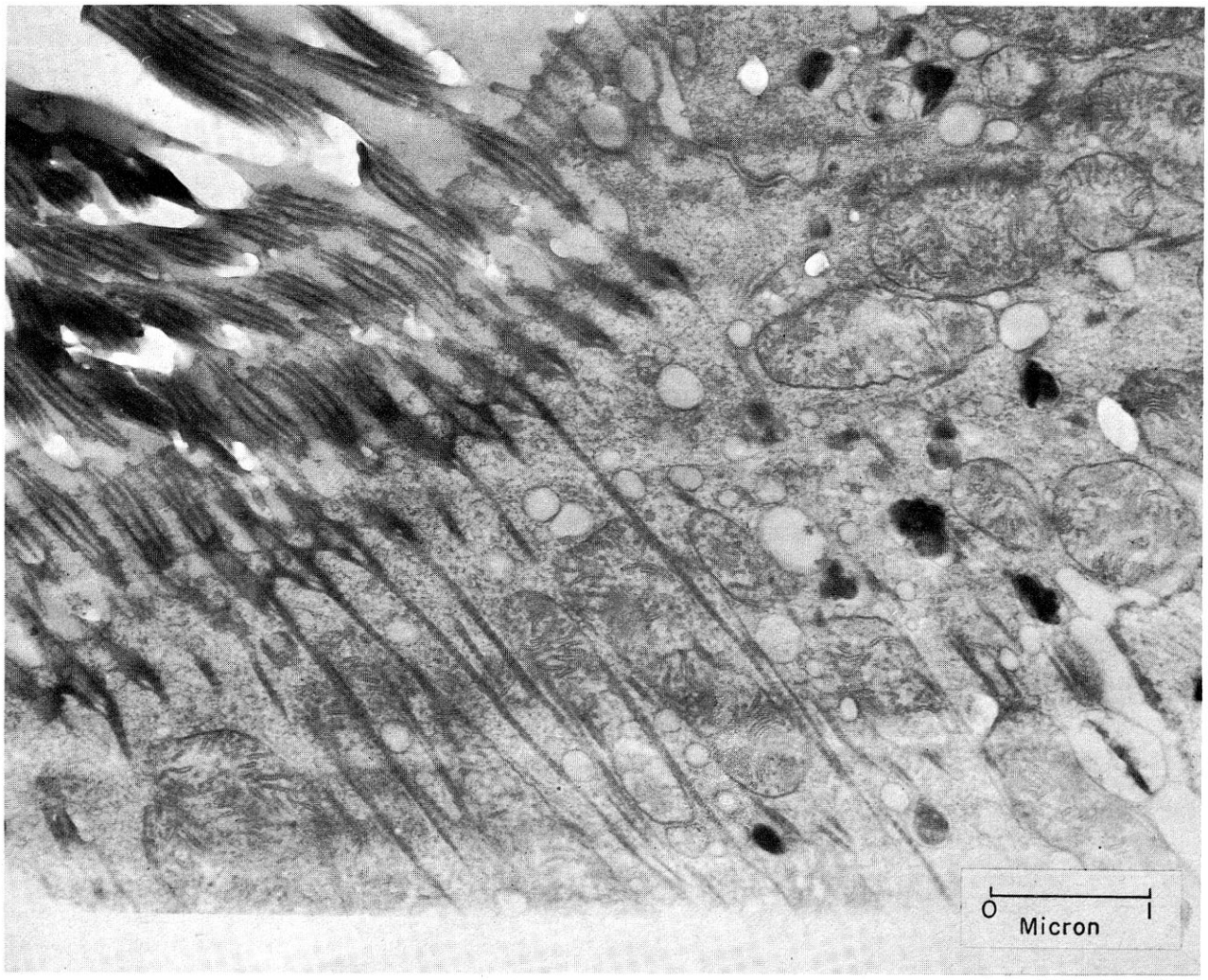

FIGURE 344.—Electron micrograph of a tangential section of a portion of a ciliated cell of the velum of the larva of *C. virginica*.

complexity of the hinge apparatus of the prodissoconch. The hinge ensures the exact closure of the valves and prevents them from sliding on each other under uneven pressure. Consequently, as the larva grows the hinge apparatus increases in strength and complexity. According to Bernard (1898), who made an extensive study of the ontogeny and morphology of larval shells of bivalves, the straight part of the dorsal shell margin thickens to form a provinculum (from the Latin "pro", before, and "vinculum", bond or band) or primitive hinge. The provinculum (by definition) always bears teeth or is shaped into toothlike projections which fit into the corresponding gaps of the opposite valve.

On the basis of the hinge structure Rees (1950) proposed a system of classification of bivalve larvae that greatly facilitates their recognition in plankton samples (fig. 345). He postulated that each superfamily of bivalves has a distinct type of larval hinge; that the shape of the hinge is typical as a generic and species characteristic; and that the texture of the larval shell can be used in certain cases in the recognition of a species. In the families Pteriacea and Ostreacea the hinge apparatus consists of a series of small, uniform teeth (taxodont teeth) in the central portion of the strip and a few larger rectangular teeth with clear gaps between them (fig. 346) at the posterior section. The distinguishing features of the species of these families is the absence of lateral and special teeth and of flanges, i.e., the thick edges of the valves on both sides of the provinculum. The ligament lies between the posterior rectangular teeth and the taxodont strip (Bernard, 1898; Borisiak, 1909). In *O. edulis* there are some large

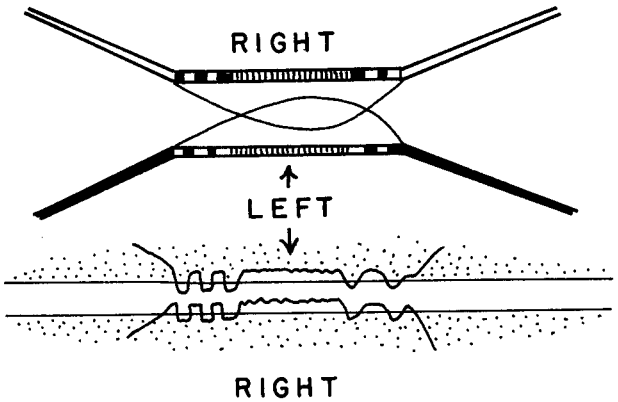

FIGURE 345.—Type of hinge of the families Ostreacea and Pteriacea. Redrawn from Rees, 1950.

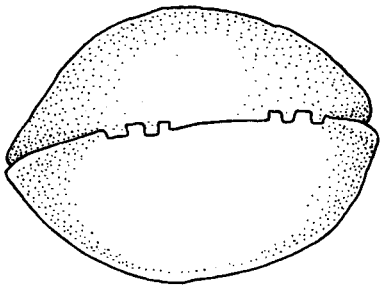

FIGURE 346.—Drawing of a 5- to 6-day-old prodissoconch of *C. virginica*, 70 μ long, examined from the dorsal side.

corrugations anterior to the taxodont teeth (Rees, 1950), but their taxonomic value is doubtful.

Differing arrangements and numbers of taxodont teeth in the shells of various species of oyster larvae are used for their identification. The straight-hinge line of a 5- to 6-day-old larva of *C. virginica* grown in laboratory culture has two groups of rectangular teeth that can be clearly seen by examining the shell from the dorsal side (fig. 346). At this stage there is only a slight difference between the upper (right) and lower (left) valves. The difference becomes more pronounced as the larva reaches the umbo stage.

In a series of papers Ranson (1943, pp. 52–58) attempted to establish the classification of all adult Ostreacea on the basis of the fully developed prodissoconchs. Essentially this work was based on the investigations published long ago by Bernard (1898) and Borisiak (1909). Ranson (1960) separates the oysters into three genera: *Pycnodonte*, *Crassostrea*, and *Ostrea*. Each genus, according to his data, is determined by the character of the final prodissoconch hinge and the position of the ligament in relation to the hinge. He concludes his paper with the statement that "as far as the Ostreidae are concerned, the species can now be established on a firm basis, which so far had never been done by studying the adult." The list published by Ranson includes 5 species of *Pycnodonte*, 12 species of *Crassostrea*, and 19 species of *Ostrea*. Unfortunately the diagnosis is given only for each genus without descriptions of taxonomic characters which are shown by the illustrations. The drawings referring to the five species found in the waters of the United States are reproduced in figures 347 through 351. Ranson's text does not include the larvae of *C. gigas* or *O. equestris*.

Ranson states also that the oysters can be correctly identified by the structure of the larval

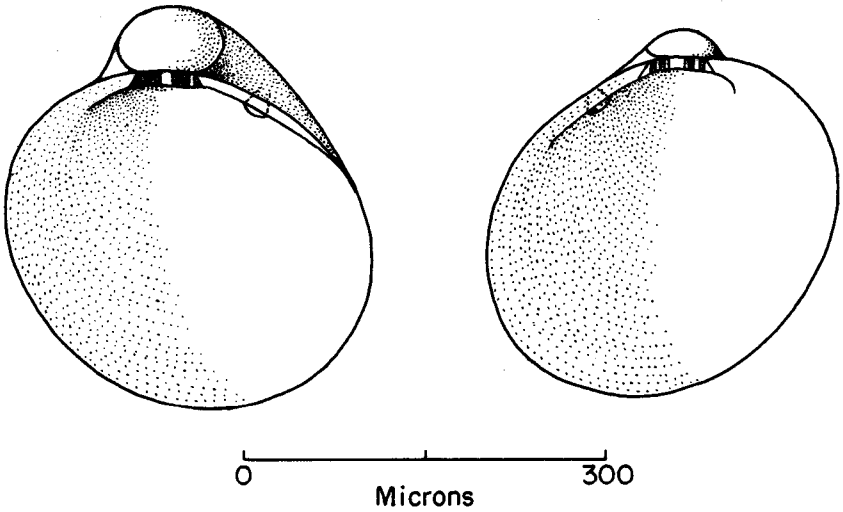

FIGURE 347.—Prodissoconch of *C. virginica* (Gmelin). Inner view of the valves. Left valve on the left and right valve on the right. Knoblike structure indicates the location of the ligament. From Ranson, 1960.

shells still visible on the shells of adults. Examination of the many shells of adult *C. virginica*, *C. gigas*, *C. rhizophorae*, and *O. equestris* in my collection did not reveal the structure of their larval shells, which in many instances appeared to be eroded or were missing. It is doubtful that Ranson's method of identification of adults by their larval shells will gain acceptance by taxonomists. Comparison of his illustrations of the closely related species, such as *C. virginica* and *C. rhizophorae*, indicates no significant differences between the two. On the other hand, his set of drawings of pelagic prodissoconchs may be useful for planktonologists, at least for separating the three genera of oyster larvae.

ATTACHMENT AND METAMORPHOSIS

Larval life ends when the oyster attaches itself to a substratum. This event is called setting, settlement, or spatfall; the different expressions are used interchangeably and are synonymous. The word setting is commonly used by American biologists and oyster growers; the expressions settlement and spatfall are more frequently found in Canadian and British publications. The term setting will be used throughout this text except in quotations from other authors.

The fully developed larva of *C. virginica* swims with its foot projecting between the valves. When the foot touches a solid surface, the larva

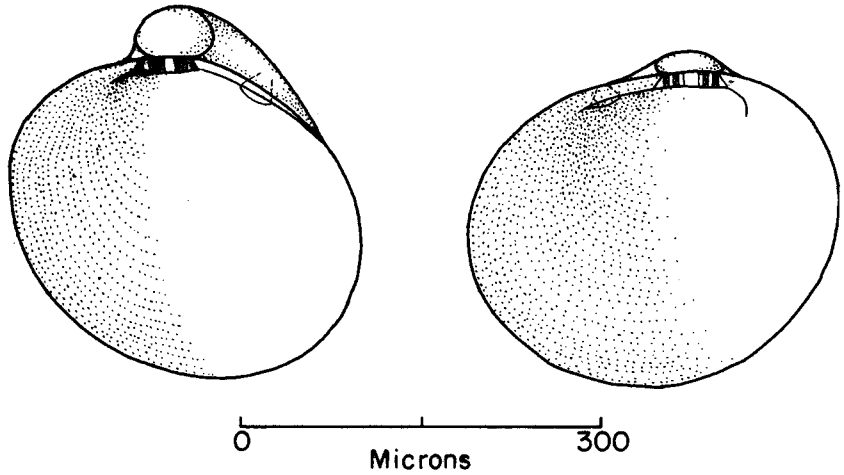

FIGURE 348.—Prodissoconch of *C. rhizophorae* (Guilding). Arrangement as in figure 347. From Ranson, 1960.

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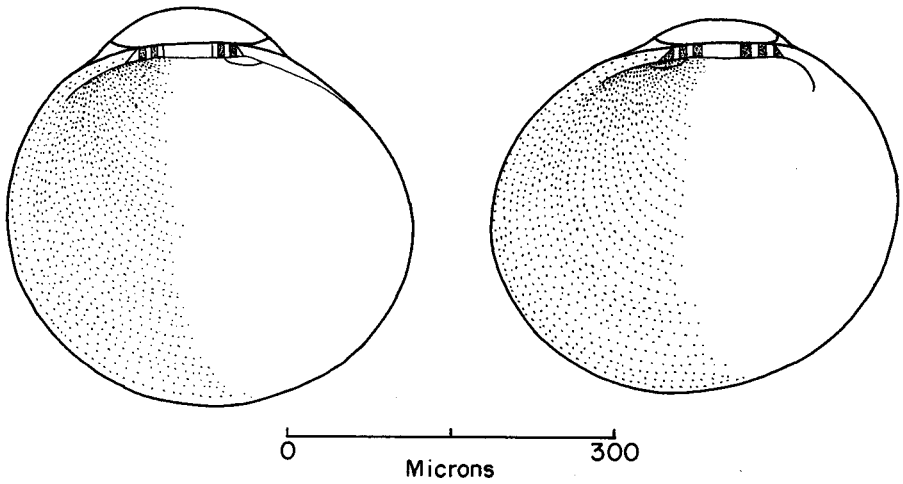

FIGURE 349.—Prodissoconch of *O. edulis* Linné. Arrangement as in figure 347. From Ranson, 1960.

stops swimming, the velum is partially withdrawn, and the larva begins to crawl on its foot. This behavior may be changed suddenly by the resumption of swimming; the foot may be withdrawn, the velum expands again, and the larva swims away. When it is ready to set, the larva crawls until it encounters suitable condition for final attachment.

Phases of setting of *C. virginica* were recorded by a motion picture camera nearly 30 years ago (Prytherch, 1934) and the photographs were recently reproduced by Medcof (1961, p. 19). To facilitate photography, the larvae were cemented with marine glue on their left valves to a glass slide which was tilted at a 45° angle. Under such conditions the larvae had no free choice in selecting the place for attachment, and the records obtained in this manner do not represent normal behavior. The attachment of fully developed larvae can be observed, however, by placing them in sea water in a petri dish and observing their behavior with a binocular microscope.

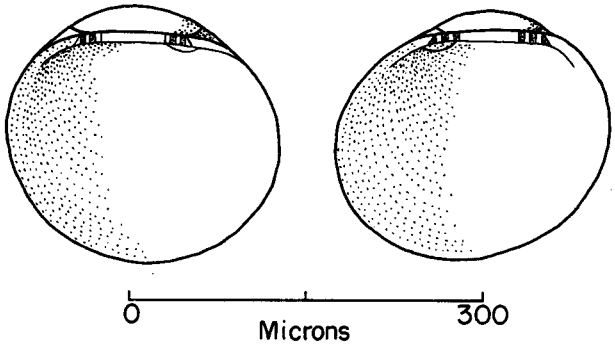

FIGURE 350.—Prodissoconch of *O. lurida* Carpenter. Arrangement as in figure 347. From Ranson, 1960.

The foot of the larva extends forward, its tip attaches temporarily to the substratum, and the whole body is pulled over by the contraction of the foot. The direction of crawling changes and occasionally reverses as the foot extends at different angles. The movement continues for some time, gradually becoming shorter and slower. Finally the foot extends far beyond the edges of the shell, the larva turns sideways with its left valve touching the substratum, and comes to a standstill. The attachment is made permanent when the byssus gland discharges a cementing fluid, which sets within a few minutes (Nelson, 1924). A similar process takes place in the setting of *O. edulis* and is probably common to other species of oysters.

The change from larva to juvenile oyster (spat) then begins immediately. The process of this metamorphosis is better known for *O. edulis* than for other species of oysters, for it has been studied by Davaine (1853), Huxley (1883), and more recently by Cole (1938b). The work of early European zoologists influenced the study of the American oyster to such an extent that in several instances the description of the metamorphosis of *C. virginica* has been repeated almost verbatim from studies on *O. edulis* with only slight changes (Ryder, 1883; Jackson, 1888, 1890). A somewhat more detailed account of the transformation of larva into spat of *C. virginica* and *O. lurida* was given by Stafford (1913).

During the metamorphosis the larval organs

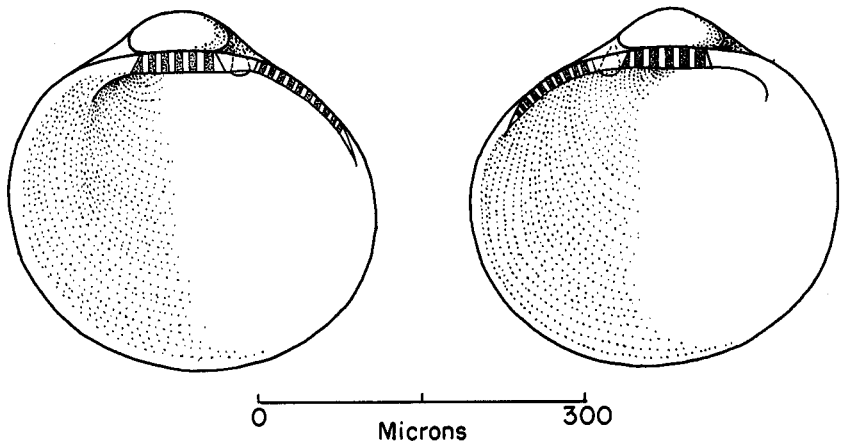

FIGURE 351.—Prodissoconch of *Pycnodonte hyotis* (L.). Arrangement as in figure 347. From Ranson, 1960.

disappear and there is an anatomical reorganization of the permanent organs. At this time the relative size of the organs and their orientation are changed. The extent of topographical changes in the relative position of organs during the transition from larva to spat can be appraised by comparing the position of some of the larval organs with that in the adult oyster. In figure 352 the principal organs of the early larval (1), fully grown larva (2), and of the juvenile oyster or spat (3) of *O. edulis* are shown diagrammatically in three drawings oriented along the dorso-ventral axis. The mouth (m.), nearly ventral in the larvae (1, 2), has shifted counterclockwise (when viewed from the right side of the oyster) about half the periphery of the larva and in the spat occupies an area in the anterio-dorsal part near the hinge. The position of the anus (a.) changes in the same direction, from the dorso-posterior part in the larva to dorso-ventral in the adult. The retractor muscles of the velum (r. v.) disappear by the end of the larval period and in the spat and adult are replaced by the radiating and marginal pallial muscles.

The most conspicuous and rapid changes take place in the velum. Davaine (1853) suggested that in *O. edulis* the velum is cast off about the end of the larval period, a conclusion not confirmed by Ryder (1883) and Stafford (1913). Illustrations by Meisenheimer (1901) of the larva

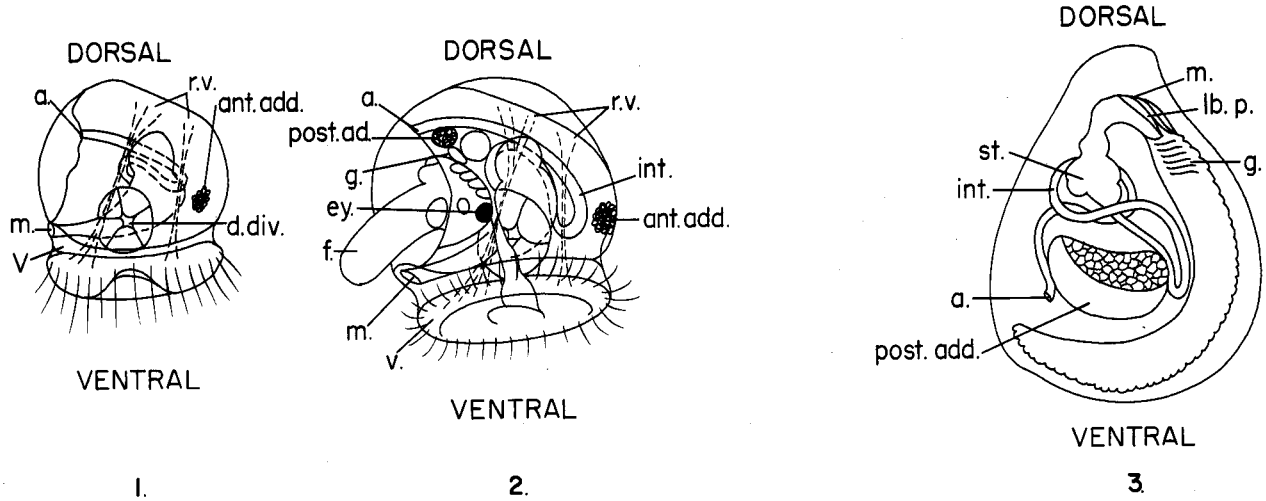

FIGURE 352.—Diagram showing the changes in the topographical relation of various organs of *O. edulis* during the transition from free-swimming larva (1) to fully developed larva ready to set (2) and juvenile oyster (3). From Erdmann (1935). a.—anus; ant. ad.—anterior adductor; ey.—eye; f.—foot; g.—gills; int.—intestine; l.p.—labial palps; post. ad.—posterior adductor; r.v.—retractors of velum; v.—velum.

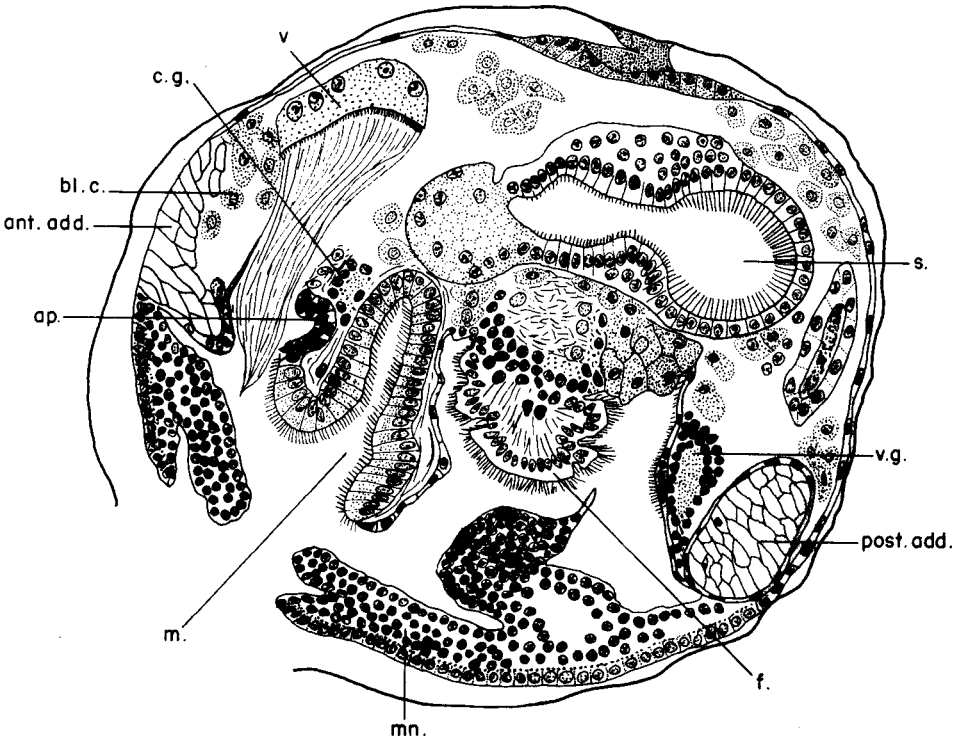

FIGURE 353.—Sagittal section of spat of *O. edulis* about 24 hours after attachment. ant. add.—disintegrating anterior adductor muscle; ap.—apical area of the velum; c.g.—cerebro-pleural ganglion; f.—foot; mn.—mantle; m.—mouth; post. add.—posterior adductor muscle; s.—stomach; v.—velum; v.g.—visceral ganglion. After Cole, 1938b.

of *Dreissensia polymorpha* and unknown to Stafford showed very clearly that the velum of this bivalve disintegrates and is absorbed, and that the apical area comes to lie outside the esophagus and later is fused with the upper lip of the mouth to form the basis of the labial palps. Cole (1938b) showed in a series of sections of *O. edulis* that as the velum collapses almost immediately after setting, its entire structure is moved upward and forward. Most of it is either cast off or disintegrates, and parts of it probably are swallowed. The apical area or apical plate of the velum becomes detached from surrounding tissues and sinks to a position dorsal to the esophagus below the surface of the body (fig. 353, ap.) where it fuses with the upper lip of the mouth. Subsequently the thickened upper lip extends laterally to form the upper labial palps. The cerebro-pleural gangial (c.g.) can be seen underlying the apical plate. In 48 hours all traces of the velum disappear.

Reabsorption of the foot begins after the discharge of the contents of the byssus gland during attachment. The foot gradually shrinks and projects behind the mouth as an irregular mass of tissue covered with ciliated cells. Phagocytes invade the interior of the foot and digest the tissue. The disentegration of the foot of *O. edulis* is completed in about 3 days.

The fully developed oyster larva has two adductor muscles. The posterior muscle, discovered by Jackson (1888, 1890), is not found in the early veliger but appears in the umbo larva. Both muscles are of approximately equal dimensions. Following attachment the anterior muscle degenerates while the posterior moves counterclockwise in the same direction as the mouth and anus.

The eyespots of *O. edulis* break down and disappear after the first 24 hours of attached life. The outlines of the epithelial cup become irregular because it is invaded by phagocytes that ingest the pigmented eye cells, thus causing the liberated pigment to lie in irregular clumps.

Many phases of larval-metamorphosis, especially of the *Crassostrea* group of oysters, are inadequately known and need to be more critically studied. With advances in the technique of artificial rearing of oyster larvae this gap in the knowledge of oyster biology may soon be filled.

DISPERSAL OF LARVAE

During the 2 or 3 weeks of free-swimming life the larvae of *C. virginica* are more or less passively carried by currents and are widely distributed in coastal waters. Biologists who have studied the distribution of planktonic bivalve larvae (Thorson, 1946) agree that their swimming is not strong enough to overcome the water movements which transport them far from the spawning grounds. To a certain extent larvae combat the currents by closing their valves and sinking to the lower level of the water column or to the bottom. However, observations of the swimming habits of artificially raised larvae of *C. virginica* kept in tall containers in the laboratory show that most of them remain swimming nearly all the time, and only those that appear to be too weak or are infected by fungi settle to the bottom.

Various methods are used in oyster research to study the distribution of larvae by taking quantitative samples, but none are satisfactory, and the results obtained by the different methods are not comparable. A pump for pumping measured volumes of water from different depths, plankton tow samplers of various designs, plankton traps, and bottle collectors of the type described by Thorson are the devices used in the study of vertical distribution of oyster larvae. The plankton tow net is most frequently employed. Larvae may be filtered out through screens, or a preserved sample of water may be placed in a glass cylinder with the bottom drawn into a funnel with a drain cock. The water may be centrifuged at high speed using the Foerst type electric centrifuge designed primarily for the collection of minute organisms that ordinarily pass through the finest mesh of the plankton net.

Many observers have found that newly attached young oysters far outnumber the free-swimming larvae, particularly of the umbo stage, found in plankton samples (Prytherch, 1924; Galtsoff, Prytherch, and McMillin, 1930; Loosanoff and Engle, 1940). Similar observations concerning the scarcity of larvae of *O. edulis* were reported by Spärck (1925) for Limfjord waters and by Gaarder (1933) for two Norwegian oyster ponds where the oyster larvae were present only in the deeper and saltier layers of water. Observations on the abundance and distribution of oyster larvae made in this country and abroad have been adequately reviewed by Korringa (1941).

The problem of adequacy of plankton sampling in relation to the physical and chemical hydrology of the James River, Va., oyster seed bed area was investigated by Pritchard (1952, 1953). His calculations show "that the concentration of late stage larvae in the overlying water sufficient to produce the large observed set needs to be, on the average, only about one larva for 100 liters." Since the basic sampling employed in these studies of distribution of oyster larvae was 100 l., the inadequacy of such a sampling technique is obvious and some better automatic sampling methods should be used to clarify these obscure points of larval behavior.

In estuaries the vertical distribution of larvae seems to depend on changes in the velocity and direction of tidal currents and the vertical salinity gradients. The oyster larvae have a more or less uniform vertical distribution in rearing tanks (Cole and Knight-Jones, 1939) and in the estuaries and bays wherever water mixing has prevented the formation of vertical gradients of temperature and salinity.

Several observers have attempted to correlate the distribution of larvae with different stages of tides. Julius Nelson, one of the pioneer students of the biology of the larva of *C. virginica* in New Jersey waters, believed that the larvae could migrate toward land by rising at the beginning of flood tide and settling to the bottom before the turn to the ebb. By this reaction to tidal changes their dispersion in tidal estuaries is avoided. This idea influenced the research of his son, Thurlow Nelson, and his students, who modified and elaborated the original concept (Nelson, 1917, 1921; Nelson and Perkins, 1931; Carriker, 1951).

According to these observations, which were made in New Jersey estuaries, the swarms of larvae are distributed along definite lanes up and down stream from the spawning grounds. If the salinity of water is uniform from bottom to surface, the greatest number of larvae is found at the level of the highest current velocity. In bodies of water with distinct salinity stratification the larvae congregate just above the zone of greatest salinity change. Nelson believed that the advanced larval stages drop to the bottom and remain near it during slack water, and that the increased salinity of early flood tide stimulates their swimming upward. This performance repeated at each change of tide enables the larvae to move upstream by progressive stages. This

mechanism, if true, would explain the location of many setting areas in tidal rivers above the principal oyster grounds. The theory has stimulated a great deal of field observation, but, unfortunately, the experimental evidence upon which it rests has not been fully documented. Only a few laboratory observations have been made on the effects of changes in current velocities and salinity on the behavior of oyster larvae, and the experiments reported by Nelson and Perkins were performed under the most primitive conditions. Great experimental difficulties were involved in conducting this type of study, and the elaborate equipment necessary for recording larval behavior was not available to the investigators.

Observations on larval distribution in waters other than New Jersey differ from those described by Nelson and his associates. Prytherch (1929) states that in Milford Harbor, Conn., "the oyster larvae were found to be most abundant at the time of low slack water and gradually disappeared as the tide began to run flood." He further states that no larvae could be found swimming in the water when the flood current had reached a velocity of 0.6 foot per second, and supports this statement by observations on oyster larvae kept in a tank. Oyster larvae remained swimming in the tank while the water was at a standstill, but dropped to the bottom when the current velocity produced by artificial circulation was from 0.3 to 0.5 foot per second. The experimental technique was very primitive, and the results cannot be considered convincing. Observations made by Loosanoff (1949) in Long Island Sound do not confirm Prytherch's interpretations. No evidence was found that early and late umbo larvae were common near the bottom. On the contrary, in several instances "their number was greatest midway between the high and low water when the tidal current was near the maximum velocity." A similar conclusion that larvae do not descend during periods of rapid tidal flow was reached by Carriker (1959) from studies of conditions in a salt-water pond on Gardiners Island at the eastern end of Long Island, N.Y.

Current views of the movement of oyster larvae up estuaries were summarized by Carriker (1961). The consensus of opinions of those who studied the problem in typical estuaries indicates that fully developed larvae (pediveligers) have a tendency to remain in lower, more saline strata and are passively conveyed toward the upper reaches of an estuary by the net, nontidal flow of deeper and denser layers of water (circulation in the estuaries is discussed in Chapter XVIII, p. 402). The discrepancy between the observations made in New Jersey waters and in Long Island Sound may be explained by differences in hydrography. Long Island Sound is not an estuary in the strict meaning of the term, but can be regarded as an embayment with several true estuaries, as for instance, the mouth of the Housatonic River, Milford Harbor, New Haven Harbor, and many others. The distribution of the larvae in the Sound is not, therefore, comparable to that observed in New Jersey waters. Further, salinity change from surface to bottom is small, rarely exceeding 2‰, and there is considerable exchange of sea water between the Sound and the outside waters. Under these conditions one may expect substantial losses of larvae during a tidal cycle. It is known that abundance of fully grown larvae in the Sound area is so low that quantitative sampling is not reliable.

The evidence that oyster larvae are actually conveyed by tidal current to the upper part of a tidal river is provided by the investigations of Dimick, Egland, and Long (1941) on *O. lurida* in Yaquina Bay, Oreg. Yaquina Bay and River is a short estuary, about 12 miles, on the coast of Oregon. The natural oyster beds cover only 101.9 acres. Plankton samples taken systematically at known distances from the mouth of the bay showed that "up-river limit of the free-swimming larvae was . . . approximately 4 miles above the upper limits of the natural oyster beds." No larvae were found in this area at near low tide. There is no doubt that these larvae were carried upstream by flood tide.

Lack of agreement on the results of field observations on the relation of larvae to tidal stages is the result of inadequacy of sampling techniques and a lack of understanding the responses of the larva to environmental changes. Changes in temperature, salinity, current velocities, oxygen, and food content of water vary in each estuary, so the occurrence or absence of larvae cannot be related to a given tidal phase unless the major conditions during this stage of tide are fully understood and their effects on larvae are known.

The volume of water transported by ebb flow in estuaries usually exceeds the volume of water re-entering at flood, the difference being equal to the volume of river discharge at the head. If

the larvae are uniformly distributed in the water and swim most of the time, a certain percentage of them will be carried away and lost in the sea. Many more are lost as prey to enemies, disease, and other causes.

In the light of present knowledge only two general assumptions regarding the larval behavior can be made: oyster larvae are able to move by their own power within only a very limited area, and they are dispersed by tidal currents beyond the immediate vicinity of spawning grounds.

A survival relationship exists between the age of the larvae and tidal cycles. After analyzing daily counts of larvae of *O. edulis* in plankton samples taken in Oostersheld, Holland, Korringa (1941) concluded that the longer the duration of the pelagic period the greater is the loss of larvae and the lower is the percentage reaching maturity: In 6 to 7 days, equal to 13 tides, 10 percent reach maturity; in 10 days, equal to 19 tides, 5 percent reach maturity; and in 12 days, equal to 23 tides, 2.5 percent reach maturity. If the original number of larvae is A and the rate of dispersal and other losses of larvae are equal during their free-swimming period, the number of larvae at the completion of pelagic life is $A(1-1/p)^n$ where $1/p$ is the decrease during one tidal cycle and n is the number of tides. The loss during one tidal cycle is estimated by Korringa at between 13 to 15 percent. About 10 percent of the losses he attributed to predators and only about 4 percent to tides. Because of the greater duration of the pelagic life of the oviparous *C. virginica*, it is reasonable to expect that losses of larval populations of this species probably exceed those determined by Korringa for the larviparous *O. edulis*.

It is generally known that mortality among the planktotrophic larvae during their pelagic life is tremendous and that only an insignificant percentage of them reach metamorphosis. Korringa made an interesting computation which shows that out of one million *O. edulis* larvae produced in Oostershelde only about 250 attach themselves and metamorphose, and of this newly set spat 95 percent die before the onset of winter.

The rate of survival of larvae of *C. virginica* and the percentage reaching attachment are not known, but the principles of Korringa's method can be applied to the American species. His studies show that the success of oyster setting depends on prolific and simultaneous spawning of oysters in an estuary. By determining the abundance of larval population and the rate of exchange of water during a tidal cycle, an estimate can be made of the intensity of the forthcoming setting, barring, of course, unforeseen circumstances which may destroy the larvae.

REACTION OF LARVAE TO EXTERNAL ENVIRONMENT

Little is known about the reactions of larvae to changes in temperature and salinity of water. Temperature fluctuations during the reproductive season apparently have no direct effect on the behavior of larvae of *C. virginica*, *O. edulis*, and *C. gigas*. Davis (1958) has demonstrated in a series of laboratory tests that the reduction of salinity from the normal (for Long Island Sound oysters) level of 26‰ to 27‰ to 15‰ has no effect on the growth of larvae and that inhibition of growth became noticeable in salinities of 12.5‰ and lower (Davis and Ansell, 1962). In water of 10‰ salinity 90 to 95 percent of the larvae died by the 14th day, and at a salinity of 5‰ they appeared to be moribund within 48 hours. In these experiments the behavior of larvae was not recorded. It would be interesting to repeat these studies and determine the reactions of larvae to sudden and to gradual changes of salinities.

Vertical distribution of larvae of *O. edulis* apparently is not affected by light (Korringa, 1941). This is probably true also for the larvae of *C. virginica*, but because no experiments have been made under controlled laboratory conditions, it is premature to assume that larvae of the American oyster are not sensitive to light. The phototactic responses of larvae to light intensity and color have not been explored, but the presence of the eye in the fully developed larva suggests that this organ is somehow used during the last days of larval life. Before attachment the larva crawls over the surface exploring the substratum with its foot, which acts as a tactile organ. It has not been established that the eye participates in this exploration. Nelson (1926) believes, however, that the "eyed" larvae of *C. virginica* are stimulated by light and continue to move until they reach a shaded place where they become quiescent. Hopkins (1937) expresses the opposite view and states that in setting of *O. lurida* light is not an orienting factor. He inclines toward Prytherch's (1934) view that the larval eye has an entirely different function. Since neither of the quoted authors can corroborate their impressions by ex-

perimental evidence, the whole question of the factors influencing the behavior of oyster larvae at the time of setting needs to be examined.

TO THE ANGLE OF SURFACE

French oyster growers take advantage of the preference of oyster larvae for the under surfaces of submerged objects and use special spat collectors made of tiers of tiles set one upon the other with their concave surfaces underneath. New spat is always found in larger numbers on the lower surfaces. According to Cole and Knight-Jones (1939) the larvae of *O. edulis* reared in large tanks in Conway, Wales, set more intensely on the under surfaces of test shells. A study of the effect of the angle of a flat surface on the attachment of larvae was made by Hopkins (1935) in his work on *O. lurida* of the Pacific Coast. He used glass plates, each 2,400 sq. in., placed at different angles over the oyster grounds. The under horizontal surface was designated as 0° and the upper horizontal as 180°. Other plates were set at 45° intervals between the two extremes. The average number of larvae attached to each surface were:

0° (under horizontal)	1,195
45°	181
90°	11
135°	3
180° (upper horizontal)	1

Similar observations were made by Schaefer (1937) with the larvae of *C. gigas*. He set 150 glass plates in positions varying by 45°; some of the plates were parallel to the direction of the tidal current while others were transverse to it. The plates were left for 5 days before the spat were counted. There is a functional relationship between the intensity of setting and the angle of the surface on which the larvae set, the number of larvae being greatest on the under horizontal surface (0° angle) and lowest as the angle approaches 180°. The curve shown in figure 354 is drawn between the points taken as the average numbers of larvae attaching during 5 days on a glass surface held at different angles. The curve is hyperbolic. Schaefer attributes the setting behavior of *C. gigas* to the upward position of the foot of the swimming larvae and possibly to negative geotaxis. No experimental evidence is given to substantiate either point.

The behavior of oyster larvae does not differ from that of many other fouling organisms which

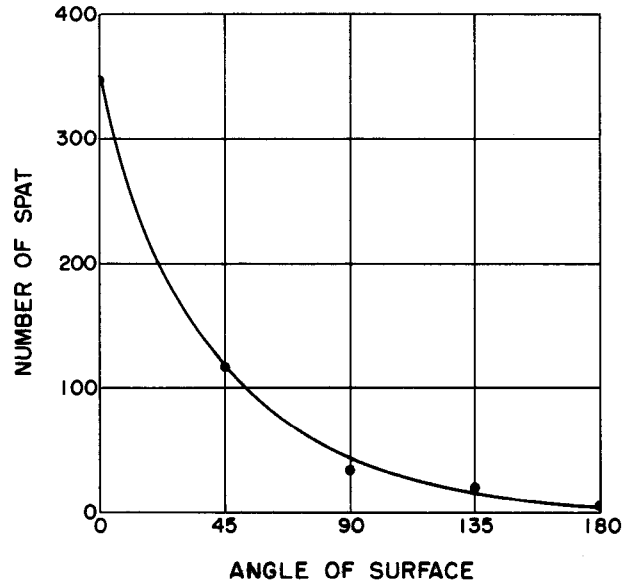

FIGURE 354.—Effect of angle of the surface of plate glass on the number of spat of *C. virginica* which attach to it within 5 days. Glass area 2,400 sq. in.; 0° is under horizontal surface. (Figure 1 from Schaefer, 1937).

were found by Pomerat and Reiner (1942) to attach in greatest abundance to the under surfaces of plates held in a horizontal position.

Contradictory results were obtained, however, in experiments with cement covered boards held at several angles either suspended in water or placed near the bottom. These tests made by Butler (1955) on oyster bottoms near Pensacola, Fla., showed a preponderence of spat on the upper surfaces of the boards. Setting on upper surfaces (135° and 180°) comprised 78 percent of the total number set, and only 18 percent were counted on the lower surfaces. The remaining 4 percent were found on vertical boards. Butler was not able to confirm the results of Pomerat's and Riener's tests made earlier at Pensacola in which frosted glass plates suspended in water attracted the greater percentage of oysters (and barnacles) to the lower surfaces. Bonnot (1937, 1940) built a spat collector from plywood strips dipped in a mixture of cement and sand. He fastened five or six strips of plywood one above the other so that the horizontal surfaces were separated by three-fourths of an inch. Setting of *O. lurida* averaged 98 larva per square inch on the upper surfaces and 67 per square inch on the lower ones. The turbulent current caused by narrow spaces between the strips possibly was

responsible for the greater number of larvae setting on the upper surfaces.

A lack of consistency in observations of various investigators in different environments indicates that it is impossible to ascertain the effects of a single factor of the environment while testing under complex and variable natural conditions. Real progress in the study of the reaction of oyster larvae may be achieved if further observations are made under controlled conditions.

Larvae of *C. virginica* that are grown artificially in culture jars and not disturbed by stirring or aeration are more or less uniformly distributed. Eyed larvae frequently congregate on the surface, swimming with their vela uppermost and touching one onother with the tips of the cilia. They form groups or "rafts" visible to the naked eye. Some of them close their valves, fall rapidly to the bottom, and after a short time resume swimming. Falling to the bottom should not be confused with negative geotaxis, which has not been demonstrated for oyster larva. In the laboratory, larvae often attach themselves to the sides of plastic or glass containers and apparently do not discriminate between light and dark surfaces.

TO THE PROPERTIES OF SURFACE

Oyster larvae attach themselves to many kinds of hard and semihard surfaces. They are found on rocks, gravel, cement, wood, shells of other mollusks, on stems and leaves of marsh grass, and on a great variety of miscellaneous objects such as tin cans, rubber boots and tires, glass, tar paper, and pieces of plastic that may be accidentally thrown on the bottom or deliberately used as spat collectors. There is no evidence that the larvae are selective in finding a suitable place to set, provided the surface is not covered with a slimy film, detritus, or soft mud. Under natural conditions they are never found on shifting sand or on a bottom covered with loose sediment. Success of setting always depends primarily on the availability of clean surfaces rather than on other factors. Shells covered with oil and greasy substances in polluted areas are not suitable for the attachment of larvae. Cole and Knight-Jones (1939) found that it is difficult to induce *O. edulis* to set on smooth glass, but the larvae of *C. virginica* raised in the laboratory readily attach to polished glass. In fact live preparations of spat may be obtained for microscopic examination of small oysters by suspending glass slides in a tank with fully grown larvae.

GREGARIOUSNESS

An interesting gregarious tendency has been observed by Cole and Knight-Jones (1949) among the larvae of *O. edulis*. During experiments in large rearing tanks they found that larvae set more readily on shells already bearing 50 to 100 spat than on shells bearing fewer spat. They suggest that a substance secreted into the surrounding water by the spat, and possibly by the fully developed larvae, encourages the setting. No attempts were made to isolate the substance and test its effect. The authors make another observation which may throw some doubt on the validity of their interpretation. They state (p. 36) that "Larvae set more readily on shells which had remained uncleaned in the tanks for 2 or more weeks, and which bore a visible film of bacteria or diatoms, than on similar shells which were cleaned daily." In their study of gregariousness they placed shells of uniform size and shape in pairs in a tank containing fully developed larvae, and the number of spat attached to them was counted daily. One shell of the pair was considered a control and was cleaned every day, and the other (experimental) remained uncleaned. By the end of the setting period the total spat settled on the experimental shells significantly exceeded the total spat settled on the controls by a ratio of 2.5 to 1. The figures suggest that the observed differences may be due to the attraction of larvae by those which had already settled on the shell, but the conclusion cannot be accepted without further verification. The possibility is not excluded that some other unknown factor, such as the position of the controls in relation to the experimentals, affected the results or that handling and removal of spat from the control shells caused changes to the surface which made them less attractive to the larvae. It would be profitable to conduct a series of tests designed to eliminate bias by placing experimental and control shells at random and making a statistical analysis of the significance of the differences.

Yonge (1960) expresses no doubt "that larvae (of *O. edulis*) settle more readily on surfaces to which others are already attached," and points out that this tendency aids in reproductive efficiency and is, therefore, a major benefit to attached animals. In view of the fact that a single oyster

shell has sufficient space for only a few spat to grow to maturity, heavy concentrations of spat are of doubtful value for reproduction and may even be harmful by creating overcrowded conditions.

ARTIFICIAL REARING OF OYSTER LARVAE

Early attempts to rear oyster larvae under artificial conditions produced uncertain results. Sometimes a small number of spat were obtained, but the experiments could not be repeated under similar conditions. At that time oyster larvae were placed in 5-gallon carboys, and the water was aerated and circulated. At 2-day intervals the larvae were concentrated by centrifuging and transferred into fresh sea water (Wells, 1920). In another method, tried with only partial success, the larvae were reared in slowly running sea water which was filtered through a 2-inch layer of white sand or porous stone (filtrose) placed on the bottom of a container. The rates of filtration and of addition of new water were regulated by a valve placed below the filtering layer (Prytherch, 1924). In both types of experiments no food was added to the containers under the assumption that enough was present in the water. Then Gaarder and Spärck (1933) and Gaarder (1933) studied the food of the larva of *O. edulis* in Norwegian oyster ponds and made what may be considered the first significant step toward solving the problem of rearing larvae under artificial conditions. Spärck observed that the water of the ponds contained considerable numbers of a small green unicellular alga which later on was isolated and cultured in the laboratory. It appeared to be a species of *Chlorella* which was consumed by the larvae. Studies by these investigators revealed also that nannoplankton of the ponds consisted principally of small green algae and flagellates measuring from 2μ to 3μ. Fertilization of experimental tanks by the addition of liquid manure greatly increased the production not only of *Chlorella* but also of various diatoms, chiefly *Nitzschia*, flagellates, various large unicellular green algae, and bacteria. In this enriched water a few larvae grew to a size of 300μ but failed to attach (Spärck, 1927). After it was found that *Chlorella* is present in the Norwegian oyster ponds, in experimental tanks in Conway, Wales, and in certain experimental basins in Denmark, Kändler (1933) attempted to grow oyster larvae on a diet of this alga alone but had little success. This led him to conclude that oyster larvae are unable to digest *Chlorella*, which left the intestine apparently unchanged. Feeding experiments with *Carteria* and *Chlamydomonas* were also unsuccessful. More critical experiments conducted at Conway, Wales, showed that the larvae are unable to utilize nonmotile green algae such as *Chlorella* and *Collomyxa* but that yellow-brown chrysomonads (not identified but designated as flagellate C) gave satisfactory results (Cole, 1937).

The Conway experiments demonstrated that organic enrichment of the water of the large tanks was consistently successful in giving rise to a good crop of flagellates with the resulting good growth and setting of larvae (Cole, 1939). The most satisfactory fertilizer was the meat of the shore crab *Carcinus* ground with sand and heated to the boiling point. The suspension of meat was added to a 90,000-gallon tank at the average rate corresponding to 12.5 medium-sized crabs per day for a period of 3 to 4 weeks. Production of nannoplankton was judged by pH readings, and as soon as the readings reached 8.3 to 8.4 and the tank had a distinct slight cloudiness, no more crab meat was added.

Evidence presented by Cole showed that growth and attachment of *O. edulis* larvae in tanks were significantly increased by organic enrichment which stimulated the development of the nannoplankton. Under laboratory conditions the oyster larvae grew and set satisfactorily in the water containing cultures of *Platymonas tetrahele*. The larvae of oysters and other bivalves apparently are not able to swallow microorganisms which exceed 8μ, but according to Thorson (1950) the size of nannoplankton normally devoured by larval forms is smaller (2μ to 3μ).

Difficulties in obtaining reproducible results from using organic enrichment for rearing larvae suggested that variations in the composition and quantity of nannoplankton may be responsible. To determine the food requirements of *O. edulis* larvae, Bruce, Knight, and Parke (1940) isolated from sea water six flagellate organisms ranging in size from 1.5μ to 7μ in diameter. A known number of oyster larvae were introduced into glass vessels filled with 16 l. of uncontaminated, sterile sea water which was stirred and aerated. The water was changed continuously by a drop feed; the loss of larvae was prevented by covering the outflow tubes with bolting silk. The larvae were

fed pure cultures of flagellates grown in the so-called "Erdschreiber" medium of the following composition (Gross, 1937):

Sodium nitrate ($NaNO_3$)	0.1 g.
Sodium orthophosphate (Na_2HPO_4)	0.02 g.
Soil extract	50 ml.
Sea water	1,000 ml.

Soil extract is made by boiling 1 kg. of good potting or garden soil with 1 l. of distilled water in an autoclave for 1 hour. The flask is set aside for 2 or 3 days, and the muddy dark fluid is decanted and sterilized by heating to the boiling point. After standing 3 to 4 weeks the suspended particles settle on the bottom, and the transparent brown or red fluid is poured into another container and boiled for a short time. Boiling of the medium should be avoided once the required quantities of nitrates and phosphate have been added.

Since the six flagellates used in these experiments (Bruce, Knight, and Parke, 1940) were not identified and were labeled only by letters, inconsistencies in the results reported may be attributed to the appearance in the culture of other species, or, as the authors state, "to the supervention of factors outside experimental control." The authors suggest that one of these conditions may be the fact that larvae from different oysters are not equally viable.

The feeding of oyster larvae (*O. edulis*) with pure cultures of nannoplankton was repeated by Walne (1956). In this case the larvae were kept in vessels of 1 l. capacity without change of sea water, and the species of flagellates grown in cultures were identified. Among the Chlorophyceae, only *Pyramimonas grossii* Parke gave consistently good results. Tests made with *Chlorella stigmatophora* Butcher seemed to indicate that those chlorococcales which have a thick cell wall are poor food for oyster larvae. The best results were obtained with *Isochrysis galbana* Parke, a chrysophycean of about 5μ to 6μ in length. *Prymnesium parvum* Carter was found to be toxic to larvae. So far there is no proof that the species of flagellates used in these experiments form a significant component of the natural population of nannoplankton and that their presence in estuaries is necessary for larvae living under natural conditions.

Imai and Hatanaka (1949, 1950) reported that the larvae of *C. gigas* can be reared on a culture of colorless flagellate, *Monas* sp., which abounds in brackish waters of Japan. The authors believe that the flagellate of the *Monas* type plays an important role in the production of oysters in Japan. The possibility remains, however, that in their experiments other flagellates were present in the culture of *Monas* enriched with glucose, cane sugar, nitrates, and phosphates.

The pelagic life of *C. virginica* and *C. gigas*, and probably of all oviparous oysters, is longer than that of larviparous *O. edulis* and *O. lurida*. Consequently, the rearing of these oviparous larvae under artificial conditions presents additional difficulties. Considerable advances in the rearing of larvae of various bivalve species were made by Loosanoff, Davis, and their collaborators at the Bureau of Commercial Fisheries Biological Laboratory, Milford, Conn. Phases of the work are summarized by Loosanoff (1954) and Loosanoff and Davis (1963a, 1963b). Oysters were induced to spawn by increasing the temperature and by adding sperm suspension (see p. 308, Chapter XIV). The fertilized eggs were freed from debris by passing the water through a series of fine screens and placed in 5-gallon earthenware jars until free-swimming larvae emerged. Then the water was changed every 24 to 48 hours by straining it through fine sieves which retained the larvae. The sea water in which the larvae lived was filtered through cotton to remove detritus and zooplankton. Aeration and mechanical agitation were considered unnecessary if the water was changed every other day. The larvae were given measured amounts of cultures of various micro-organisms. In general the results obtained in Milford corroborate the findings of British investigators. Davis (1953) established that oyster larvae can utilize as food the following species of flagellates: *Dicrateria inornata*, *Chromulina pleiades*, *Isochrysis galbana*, *Hemiselmis rufescens*, and *Pyramimonas grossii*. *Chlorella* sp. was used only by advanced larval stages and not by young veligers.

The utilizable flagellates were added to the rearing tanks at the rates of 15,000 and 25,000 cells per ml. per day but no toxic effects were noticed in these heavy concentrations, and the larval oyster population of approximately 5,000 per 1. showed satisfactory growth. The actual number of flagellates ingested by the larvae was not determined, but the inference was made that "the rate of growth of oyster larvae had an inverse relation to the number of larvae per unit volume" (Davis, 1953). Cole (1939) states that a population of 20,000 to 30,000 small flagellates per 1 ml.

is adequate to promote growth of larvae of *O. edulis* but is insufficient for the spat.

With the exception of the toxic *Prymnesium parvum*, the naked flagellates provided better food for young oyster larvae than the organisms with heavy cell walls, which can be utilized only by older larvae. The best single foods were found to be *Isochrysis galbana*, *Monochrysis lutheri*, *Chromulina pleiades*, *Dicrateria inornata*, and some other unidentified species of *Dicrateria*. Since the cultures used in these experiments were not free of bacteria, the question naturally arises whether the marine bacteria are utilized as food. Davis (1953) states that none of the 13 species of marine bacteria tested by him were used by the larvae. The species of bacteria have not been identified. However, the probability that larvae may derive a certain amount of food from some bacteria is strengthened by the observation reported by Davis (1953) and Loosanoff (1954) that larvae kept in cotton-filtered sea water without algal food continued to grow for as long as 14 to 18 days. The role of marine bacteria in the feeding of oyster larvae needs further experimental study.

Apparently the best results in rearing larvae under artificial conditions are obtained with a mixed food of *Isochrysis galbana*, *Monochrysis lutheri*, *Chromulina pleiades*, and *Dicrateria* sp. With such a diet and at 30° C., the larvae of *C. virginica* begin setting between the 10th and 12th days after fertilization; at 24° C., the sibling larvae are ready to set on the 24th to 26th day; at 20° C., only a few of the larvae set by the 38th day. Setting of larvae of *O. lurida* at a temperature of 22° C. takes place on the 7th day after release of larvae from the brood chamber (Loosanoff and Davis, 1963a).

Under laboratory conditions in Woods Hole the young larvae of *C. virginica* are often found on the bottom of vessels entangled in lumps of several individuals. These larvae never recover and usually die within the next 24 hours. Sometimes the larvae of oysters and clams are attacked and killed by a fungus which has been tentatively identified by the workers at the Bureau of Commercial Fisheries Biological Laboratory at Milford, Conn., as belonging to the genus *Sirolpidium zoophtorum* Vishniac (Davis, Loosanoff, Weston, and Martin, 1954; Johnson and Sparrow, 1961). There are undoubtedly other bacteria and possibly viruses which inflict epizootic mortality on larval populations in the laboratory and in natural waters.

The technique of rearing oyster larvae has progressed sufficiently to be applicable to practical purposes of oyster culture. Details of techniques, organization, and operation of a mollusk hatchery are summarized by Loosanoff and Davis (1963b).

BIBLIOGRAPHY

ANDREWS, JAY D.
1951. Seasonal patterns of oyster setting in the James River and Chesapeake Bay. Ecology, vol. 32, No. 4, pp. 752–758.

BERNARD, FÉLIX.
1898. Recherches ontogéniques et morphologiques sur la coquille des lamellibranches. Partie I. Taxodontes et anisomyaires. Annales des Sciences Naturelles, Zoologie, série 8, tome 8, pp. 1–208.

BONNOT, PAUL.
1937. Setting and survival of spat of the Olympia oyster, *Ostrea lurida*, on upper and lower horizontal surfaces. California Fish and Game, vol. 23, No. 3, pp. 224–228.
1940. Methods of collecting oyster spat. Transactions of the American Fisheries Society, vol. 69, for the year 1939, pp. 263–267.

BORISIAK, A.
1909. Pelecypoda du plankton de la Mer Noire. Bulletin Scientifique de la France et de la Belgique, tome 42, pp. 149–184.

BRUCE, J. R., MARGERY KNIGHT, and MARY W. PARKE.
1940. The rearing of oyster larvae on an algal diet. Journal of the Marine Biological Association of the United Kingdom, vol. 24, No. 1, pp. 337–374.

BUTLER, PHILIP A.
1955. Selective setting of oyster larvae on artificial cultch. Proceedings of the National Shellfisheries Association, vol. 45, August 1954, pp. 95–105.

CARRIKER, MELBOURNE ROMAINE.
1951. Ecological observations on the distribution of oyster larvae in New Jersey estuaries. Ecological Monographs, vol. 21, No. 1, pp. 19–38.
1959. The role of physical and biological factors in the culture of *Crassostrea* and *Mercenaria* in a saltwater pond. Ecological Monographs, vol. 29, No. 3, pp. 219–266.
1961. Interrelation of functional morphology, behavior, and autoecology in early stages of the bivalve *Mercenaria mercenaria*. Journal of the Elisha Mitchell Scientific Society, vol. 77, No. 2, pp. 168–241.

CHESTNUT, A. F., and WILLIAM E. FAHY.
1953. Studies on the vertical distribution of setting of oysters in North Carolina. Proceedings of the Gulf and Caribbean Fisheries Institute, Fifth Annual Session, November 1952, pp. 106–112.

COLE H. A.
1937. Experiments in the breeding of oysters (*Ostrea edulis*) in tanks, with special reference to the food of the larva and spat. Fishery Investigations, series II, vol. 15, No. 4, 1936, 25 pp.

1938. A system of oyster culture. Journal du Conseil, vol. 13, No. 2, pp. 221–235.
1938b. The fate of the larval organs in the metamorphosis of *Ostrea edulis*. Journal of the Marine Biological Association of the United Kingdom, vol. 22, No. 2, pp. 469–484.
1939. Further experiments in the breeding of oysters, (*Ostrea edulis*) in tanks. Fishery Investigations, series II, vol. 16, No. 4, 47 pp.

COLE, H. A., and E. W. KNIGHT-JONES.
1939. Some observations and experiments on the setting behaviour of larvae of *Ostrea edulis*. Journal du Conseil, vol. 14, No. 1, pp. 86–105.
1949. The setting behaviour of larvae of the European flat oyster *Ostrea edulis* L., and its influence on methods of cultivation and spat collection. Fishery Investigations, series II, vol. 17, No. 3, 39 pp.

DANTAN, J. L.
1917. La larve de l'*Ostrea edulis* (L.). Annales de l'Institut Océanographique, tome 7, fascicule 6, pp. 1–20.

DAVAINE, C.
1853. Recherches sur la génération des huîtres. Comptes Rendus des Séances et Mémoires de la Société de Biologie, tome 4, série 1, année 1852, pp. 297–339.

DAVIS, HARRY C.
1953. On food and feeding of larvae of the American oyster, *C. virginica*. Biological Bulletin, vol. 104, No. 3, pp. 334–350.
1958. Survival and growth of clam and oyster larvae at different salinities. Biological Bulletin, vol. 114, No. 3, pp. 296–307.

DAVIS, HARRY C., and ALAN D. ANSELL.
1962. Survival and growth of larvae of the European oyster, *O. edulis*, at lowered salinities. Biological Bulletin, vol. 122, No. 1, pp. 33–39.

DAVIS, H. C., V. L. LOOSANOFF, W. H. WESTON, and C. MARTIN.
1954. A fungus disease in clam and oyster larvae. Science, vol. 120, No. 3105, pp. 36–38.

DAWSON, C. E.
1955. A study of the oyster biology and hydrography at Crystal River, Florida. Publications of the Institute of Marine Science, University of Texas, vol. 4, No. 1, pp. 279–302.

DIMICK, R. E., GEORGE EGLAND, and J. B. LONG.
1941. Native oyster investigations of Yaquina Bay, Oregon. Progress Report II covering the period July 4, 1939 to September 30, 1941. Oregon Agricultural Experiment Station, Corvallis, Oreg. Cooperating with the Fish Commission of the State of Oregon and the Lincoln County Court, 153 pp.

ERDMANN, WILHELM.
1935. Untersuchungen über die Lebensgeschichte der Auster. Nr. 5. Über die Entwicklung und die Anatomie der "ansatzreifen" Larve von *Ostrea edulis* mit Bermerkungen über die Lebensgeschichte der Auster. Wissenschaftliche Meeresuntersuchungen herausgegeben von der Kommission zur wissenschaftlichen Untersuchung der deutschen Meere in Kiel und der Biologischen Anstalt auf Helgoland, Neue Folge, Band 19, Abteilung Helgoland, Heft 3, Abhandlung Nr. 6, pp. 1–25.

FASTEN, NATHAN.
1931. The Yaquina oyster beds of Oregon. American Naturalist, vol. 65, No. 700, pp. 434–468.

FOX, DENIS L., JOHN D. ISAACS, and EUGENE F. CORCORAN.
1952. Marine leptopel, its recovery, measurement and distribution. Journal of Marine Research, vol. 11, No. 1, pp. 29–46.

FUJITA, TSUNENOBU.
1934. Note on the Japanese oyster larva. Proceedings of the Fifth Pacific Science Congress, vol. 5, pp. 4111–4117.

GAARDER, TORBJØRN.
1933. Untersuchungen über Produktions- und Lebensbedingungen in norwegischen Austern-Pollen. Bergens Museums Årbok, 1932, Naturvidenskapelig Rekke, nr. 3, pp. 1–64.

GAARDER, TORBJØRN, and R. SPÄRCK.
1933. Hydrographisch-biochemische Untersuchungen in norweigischen Austern-Pollen. Bergens Museums Årbok, 1932, Naturvidenskapelig Rekke, nr. 1, pp. 1–144.

GALTSOFF, PAUL S., and R. H. LUCE.
1930. Oyster investigations in Georgia. [U.S.] Bureau of Fisheries, Report of the Commissioner of Fisheries for the fiscal year 1930, appendix 5 (Document 1077), pp. 61–100.

GALTSOFF, P. S., H. F. PRYTHERCH, and H. C. MCMILLIN.
1930. An experimental study in production and collection of seed oysters. Bulletin of the U.S. Bureau of Fisheries, vol. 46, for 1930, pp. 197–263. (Document 1088.)

GARSTANG, WALTER.
1929. The origin and evolution of larval forms. Sectional Presidents' Addresses, Section D, Zoology. British Association for the Advancement of Science, Report of the ninety-sixth meeting (ninety-eighth year), Glasgow, 1928, September 5 to 12. Office of the British Association, Burlington House, London, pp. 77–98.
1951. Larval forms and other zoological verses. Basil Blackwell, Oxford, England, 85 pp.

GROSS, F.
1937. Notes on the culture of some marine plankton organisms. Journal of the Marine Biological Association of the United Kingdom, vol. 21, No. 2, pp. 753–768.

HAGMEIER, A., and A. SCHUBERT.
1930. Untersuchungen über die Biologie der Auster. Nr. 4. Die Austernbrut im Wattenmeer. Wissenschaftliche Meeresuntersuchungen herausgegeben von der Kommission zur wissenschaftlichen Untersuchung der deutschen Meere in Kiel und der Biologischen Anstalt auf Helgoland, Neue Folge, Band 18, Abteilung Helgoland, Heft 1, Abhandlung Nr. 1, pp. 1–26.

HAVINGA, B.
1939. Prediction of the time of setting of oyster spat and a method of control. Journal du Conseil, vol. 14, No. 3, pp. 394–400.

HESS, OSWALD.
1962. Entwicklungsphysiologie der Mollusken. Fortschritte der Zoologie, Band 14, pp. 130–163.

HOPKINS, A. E.
1935. Attachment of larvae of the Olympia oyster, *Ostrea lurida*, to plane surfaces. Ecology, vol. 16, No. 1, pp. 82–87.
1936. Ecological observations on spawning and early larval development in the Olympia oyster (*Ostrea lurida*). Ecology, vol. 17, No. 4, pp. 551–566.
1937. Experimental observations on spawning, larval development, and setting in the Olympia oyster, (*Ostrea lurida*). [U.S.] Bureau of Fisheries, Bulletin No. 23, vol. 48, pp. 439–503.

HORI, J.
1926. Notes on the full-grown larva and the Japanese common oyster, *Ostrea gigas* Thunberg. Journal of the Imperial Fisheries Institute, vol. 22, No. 1, pp. 1–7.

HORST, R.
1882. On the development of the European oyster (*Ostrea edulis* L.). Quarterly Journal of Microscopical Science, vol. 22, pp. 341–346.
1883. A contribution to our knowledge of the development of the oyster (*Ostrea edulis* L.). Bulletin of the U.S. Fish Commission, vol. 2, for 1882, pp. 159–167.

HUXLEY, T. H.
1883. Oysters and the oyster question. The English Illustrated Magazine, vol. 1, No. 1, pp. 47–55; vol. 1, No. 2, pp. 112–121. Macmillan and Company, London.

IMAI, TAKEO, and MASAYOSHI HATANAKA.
1949. On the artificial propagation of Japanese common oyster, *Ostrea gigas* Thunb., by non-colored naked flagellates. Bulletin of the Institute of Agricultural Research, Tôhoku University, Sendai, Japan, vol. 1, No. 1, pp. 39–46. [In Japanese with English summary.]
1950. Studies on marine non-colored flagellates, *Monas* sp., favorite food of larvae of various marine animals. I. Preliminary research on cultural requirements. Science Reports of the Tôhoku University, series 4, Biology, vol. 18, No. 3, pp. 304–315.

INGLE, ROBERT M.
1952. Spawning and setting of oysters in relation to seasonal environmental changes. Bulletin of Marine Science of the Gulf and Caribbean, vol. 1, No. 2, pp. 111–135.

JACKSON, ROBERT TRACY.
1888. The development of the oyster with remarks on allied genera. Proceedings of the Boston Society of Natural History, vol. 23, pp. 531–556.
1890. Phylogeny of the Pelecypoda. The Aviculidae and their allies. Memoirs of the Boston Society of Natural History, vol. 4, No. 8, pp. 277–400.

JOHNSON, T. W., JR., and F. K. SPARROW, JR.
1961. Fungi in oceans and estuaries. J. Cramer, Weinheim, Germany; Hafner Publishing Company, New York, 668 pp.

JØRGENSEN, C. BARKER.
1946. Lamellibranchia. *In* Gunnar Thorson's Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund), ch. 9, pp. 277–311. Meddelelser Fra Kommissionen for Danmarks Fiskeri-og Havundersøgelser, serie: Plankton, bind 4, nr. 1.

KÄNDLER, RUDOLPH.
1927. Muschellarven aus dem Helgoländer Plankton. Bestimmung ihrer Artzugehörigkeit durch Aufzucht. Wissenschaftliche Meeresuntersuchungen herausgegeben von der Kommission zur wissenschaftlichen Untersuchung der deutschen Meere in Kiel und der Biologischen Anstalt auf Helgoland, Neue Folge, Band 16, Abteilung Helgoland, Heft 2, Abhandlung Nr. 5, pp. 1–8.
1928. Untersuchungen über die Biologie der Auster. Nr. 3, Verbreitung und Wachstum der Austernbrut im Wattenmeer. Planktonuntersuchungen im Sommer 1926 und 1927 nach der Aussetzung holländischer Saataustern. Wissenschaftliche Meeresuntersuchungen herausgegeben von der Kommission zur wissenschaftlichen Untersuchung der deutschen Meere in Kiel und der Biologischen Anstalt auf Helgoland, Neue Folge, Band 17, Abteilung Helgoland, Heft 1, Abhandlung Nr. 3, pp. 1–35.
1933. Die Kultur der Auster. Emil Abderhalden's Handbuch der biologischen Arbeitsmethoden, Abteilung 9, Methoden der Erforschung der Leistungen des tierischen Organismus, Teil 5, Band 1, Methoden der Meerwasserbiologie, pp. 599–716. Urban and Schwarzenberg, Berlin.

KNIGHT-JONES, E. W.
1952. Gregariousness and some other aspects of the setting behaviour of *Spirorbis*. Journal of the Marine Biological Association of the United Kingdom, vol. 30, No. 2, pp. 201–222.

KORRINGA, P.
1941. Experiments and observations on swarming, pelagic life and setting in the European flat oyster, *Ostrea edulis* L. Archives Néerlandaises de Zoologie, tome 5, pp. 1–249.

LEES, H.
1930. Paper and glass collectors for oyster spat. Journal du Conseil, vol. 5, No. 1, pp. 383–384.

LOOSANOFF, VICTOR L.
1932. Observations on propagation of oysters in James and Corrotoman Rivers and the seaside of Virginia. The Virginia Commission of Fisheries, Newport News, Va., 46 pp.
1949. Vertical distribution of oyster larvae of different ages during the tidal cycle. [Abstract.] Anatomical Record, vol. 105, No. 3, pp. 591–592.
1954. New advances in the study of bivalve larvae. American Scientist, vol. 42, No. 4, pp. 607–624.

LOOSANOFF, VICTOR L., and HARRY C. DAVIS.
1963a. Rearing of bivalve mollusks. *In* F. S. Russell (editor), Advances in marine biology, vol. 1, pp. 1–136. Academic Press, Inc., London.
1963b. Shellfish hatcheries and their future. U.S.

Fish and Wildlife Service, Commercial Fisheries Review, vol. 25, No. 1, pp. 1–11.

LOOSANOFF, VICTOR L., and JAMES B. ENGLE.
1940. Spawning and setting of oysters in Long Island Sound in 1937, and discussion of the method for predicting the intensity and time of oyster setting. [U.S.] Bureau of Fisheries, Bulletin No. 33, vol. 49, pp. 217–255.

MACBRIDE, ERNEST WILLIAM.
1914. Invertebrata. *In* Walter Heape (editor), Text-book of embryology, vol. 1, 692 pp. Macmillan and Company, Ltd., London.

MACKIN, JOHN G.
1946. A study of oyster strike on the seaside of Virginia. Virginia Fisheries Laboratory of the College of William and Mary and Commission of Fisheries of Virginia, Contribution No. 25, 18 pp. Commonwealth of Virginia, Division of Purchase and Printing, Richmond, Va.

MARSHALL, S. M., and A. P. ORR.
1955. The biology of a marine copepod, *Calanus finmarchicus* (Gunnerus). Oliver and Boyd, London, 188 pp.

MAZZERELLI, GIUSEPPE.
1923. Note sulla biologia dell 'Ostrica (*Ostrea edulis* L.). I. Nascita delle larve e durata del periodo larvale. Bollettino della Societa dei Naturalisti in Napoli, vol. 34 (serie 2, vol. 14), pp. 151–159.

MEDCOF, J. C.
1961. Oyster farming in the Maritimes. Fisheries Research Board of Canada, Bulletin No. 131, 158 pp.

MEISENHEIMER, JOHANNES.
1901. Entwicklungsgeschichte von *Dreissensia polymorpha* Pall. Zeitschrift für wissenschaftliche Zoologie, Band 69, pp. 1–137.

NELSON, THURLOW C.
1917. Report of the Department of Biology of the New Jersey Agricultural College Experiment Station, New Brunswick, N.J., for the year ending October 31, 1961, pp. 399–430. MacCrellish and Quigley Company, State Printers, Trenton, N.J.
1921. Aids to successful oyster culture. I. Procuring the seed. New Jersey Agricultural Experiment Stations, Bulletin No. 351, New Brunswick, N.J., 59 pp.
1924. The attachment of oyster larvae. Biological Bulletin, vol. 46, No. 3, pp. 143–151.
1926. Report of the Department of Biology of the New Jersey State Agricultural Experiment Station for the year ending June 30, 1925, pp. 281–288. Published by the State, Trenton, N.J.
1928. Relation of spawning of the oyster to temperature. Ecology, vol. 9, No. 2, pp. 145–154.

NELSON, THURLOW C., and EARLE B. PERKINS.
1931. Annual report of the Department of Biology, July 1, 1929–June 30, 1930. New Jersey Agricultural Experiment Station, New Brunswick, N.J., Bulletin 522, 47 pp.

POMERAT, C. M., and E. R. REINER.
1942. The influence of surface angle and of light on the attachment of barnacles and other sedentary organisms. Biological Bulletin, vol. 82, No. 1, pp. 14–25.

PRITCHARD, D. W.
1952. A review of our present knowledge of the dynamics and flushing of estuaries. Chesapeake Bay Institute of the Johns Hopkins University, Technical Report 4, Reference 52–7, 45 pp.
1953. Distribution of oyster larvae in relation to hydrographic conditions. Proceedings of the Gulf and Caribbean Fisheries Institute, Fifth Annual Session, November 1952, pp. 123–132.
1954. A study of flushing in the Delaware model. Chesapeake Bay Institute of the John Hopkins University, Technical Report 7, Reference 54–4, 143 pp.

PRITCHARD, D. W., and RICHARD E. KENT.
1953. The reduction and analysis of data from the James River operation oyster spat. Chesapeake Bay Institute of the Johns Hopkins University, Technical Report 6, Reference 53–12, 92 pp.

PRYTHERCH, HERBERT F.
1924. Experiments in the artificial propagation of oysters. [U.S.] Bureau of Fisheries, Report of the Commissioner of Fisheries for the fiscal year 1923, appendix 11 (Document 961), pp. 1–14.
1929. Investigation of the physical conditions controlling spawning of oysters and the occurrence, distribution, and setting of oyster larvae in Milford Harbor, Connecticut. Bulletin of the U.S. Bureau of Fisheries, vol. 44, for 1928, pp. 429–503. (Document 1054).
1930. Improved methods for the collection of seed oysters. [U.S.] Bureau of Fisheries. Report of the Commissioner of Fisheries for the fiscal year 1930, appendix 4 (Document 1076), pp. 47–59.
1931. The role of copper in the setting and metamorphosis of the oyster. Science, vol. 73, No. 1894, pp. 429–431.
1934. The role of copper in the setting, metamorphosis, and distribution of the American oyster, *Ostrea virginica*. Ecological Monographs, vol. 4, No. 1, pp. 47–107.

RANSON GILBERT
1943. Les prodissoconques des Ostréidés actuels et fossiles. Classification et évolution des Ostréidés. Titres et Travaux Scientifiques de M. Gilbert Ranson, pp. 52–58. Masson et Cie, Paris.
1960. Les prodissoconques (coquilles larvaires) des Ostréidés vivants. Bulletin de l'Institut Oceanographique No. 1183, 41 pp.

REES, C. B.
1950. The identification and classification of lamellibranch larvae. Hull Bulletins of Marine Ecology, vol. 3, No. 19, pp. 73–104.

RYDER, JOHN A.
1883. On the mode of fixation of the fry of the oyster. Bulletin of the U.S. Fish Commission, vol. 2, for 1882, pp. 383–387.

SCHAEFER, MILNER B.
1937. Attachment of the larvae of *Ostrea gigas*, the Japanese oyster, to plane surfaces. Ecology, vol. 18, No. 4, pp. 523–527.
1938. The rate of attachment of the larvae of the

Japanese oyster, *Ostrea gigas*, as related to the tidal periodicity. Ecology, vol. 19, No. 4, pp. 543–547.

SPÄRCK, R.
1925. Studies on the biology of the oyster (*Ostrea edulis*) in the Limfjord, with special reference to the influence of temperature on the sex change. Report of the Danish Biological Station to the Board of Agriculture, 30, 1924, 84 pp.
1927. Studies on the biology of the oyster (*Ostrea edulis*). Report of the Danish Biological Station to the Board of Agriculture, 33, 1927, pp. 43–65.

STAFFORD, J.
1912. On the recognition of bivalve larvae in plankton collections. Contributions to Canadian Biology being studies from the marine biological stations of Canada, 1906–1910, No. 14, pp. 221–242.
1913. The Canadian oyster, its development, environment and culture. Committee on Fisheries, Game and Fur-bearing Animals, Commission of Conservation, Canada. The Mortimer Company, Ltd., Ottawa, Canada, 159 pp.

SULLIVAN, CHARLOTTE M.
1948. Bivalve larvae of Malpeque Bay, P. E. I. Fisheries Research Board of Canada, Bulletin No. 77, 36 pp.

THORSON, GUNNAR.
1946. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund). Meddelelser Fra Kommissionen for Danmarks Fiskeri-Og Havundersøgelser, Serie: Plankton, bind 4, nr. 1, 523 pp. C. A. Reitzels Forlag, Copenhagen.
1950. Reproductive and larval ecology of marine bottom invertebrates. Biological Reviews of the Cambridge Philosophical Society, vol. 25, No. 1, pp. 1–45.

WALNE, P. R.
1956. Experimental rearing of the larvae of *Ostrea edulis* L. in the laboratory. Fishery Investigations, series 2, vol. 20, No. 9, 23 pp.

WELLS, WILLIAM FIRTH.
1920. Artificial propagation of oysters. Transactions of the American Fisheries Society, vol. 50, pp. 301–306.

WERNER, BERNHARD.
1940. Über die Entwicklung und Artunterscheidung von Muschellarven des Nordseeplanktons, unter besonderer Berücksichtigung der Schalen entwicklung. Mit einem Beitrag zur Kenntnis der Laichzeiten der Nordseemuscheln. Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere, Band 66, Heft 1, pp. 1–54.

WOODS HOLE OCEANOGRAPHIC INSTITUTION.
1952. Marine fouling and its prevention. Prepared for the Bureau of Ships, Navy Department, United States Naval Institute, Annapolis, Maryland, 1952, 388 pp. (Chapter 4, pp. 42–47, Temporal sequences and biotic successions.) Woods Hole Oceanographic Institution, Contribution No. 580.

YONGE, C. M.
1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. Journal of the Marine Biological Association of the United Kingdom, vol. 14, No. 2, pp. 295–386.
1959. Evolution within the bivalve mollusca. Proceedings of the XVth International Congress of Zoology, sec. 4, Invertebrate zoology, pp. 367–370.
1960. Oysters. Collins Clear-Type Press, London, 209 pp.

ZOBELL, CLAUDE E.
1938. The sequence of events in the fouling of submerged surfaces. Official Digest, Federation of Paint and Varnish Production Clubs, September 1938, Scripps Institution of Oceanography, University of California, Contributions, 1938, new series, No. 35, 8 pp. La Jolla, Calif.

ZOBELL, CLAUDE E., and ESTHER C. ALLEN.
1935. The significance of marine bacteria in the fouling of submerged surfaces. Journal of Bacteriology, vol. 29, No. 3, pp. 239–251.