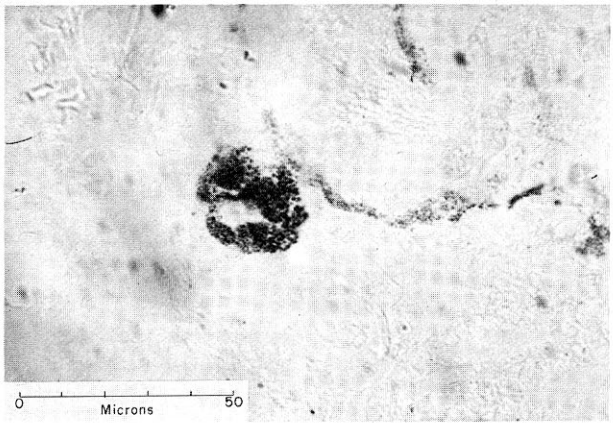

FIGURE 343.—Photomicrograph of a tangential, slightly slanted section of the larval eye of *C. virginica* preserved in osmic acid.

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

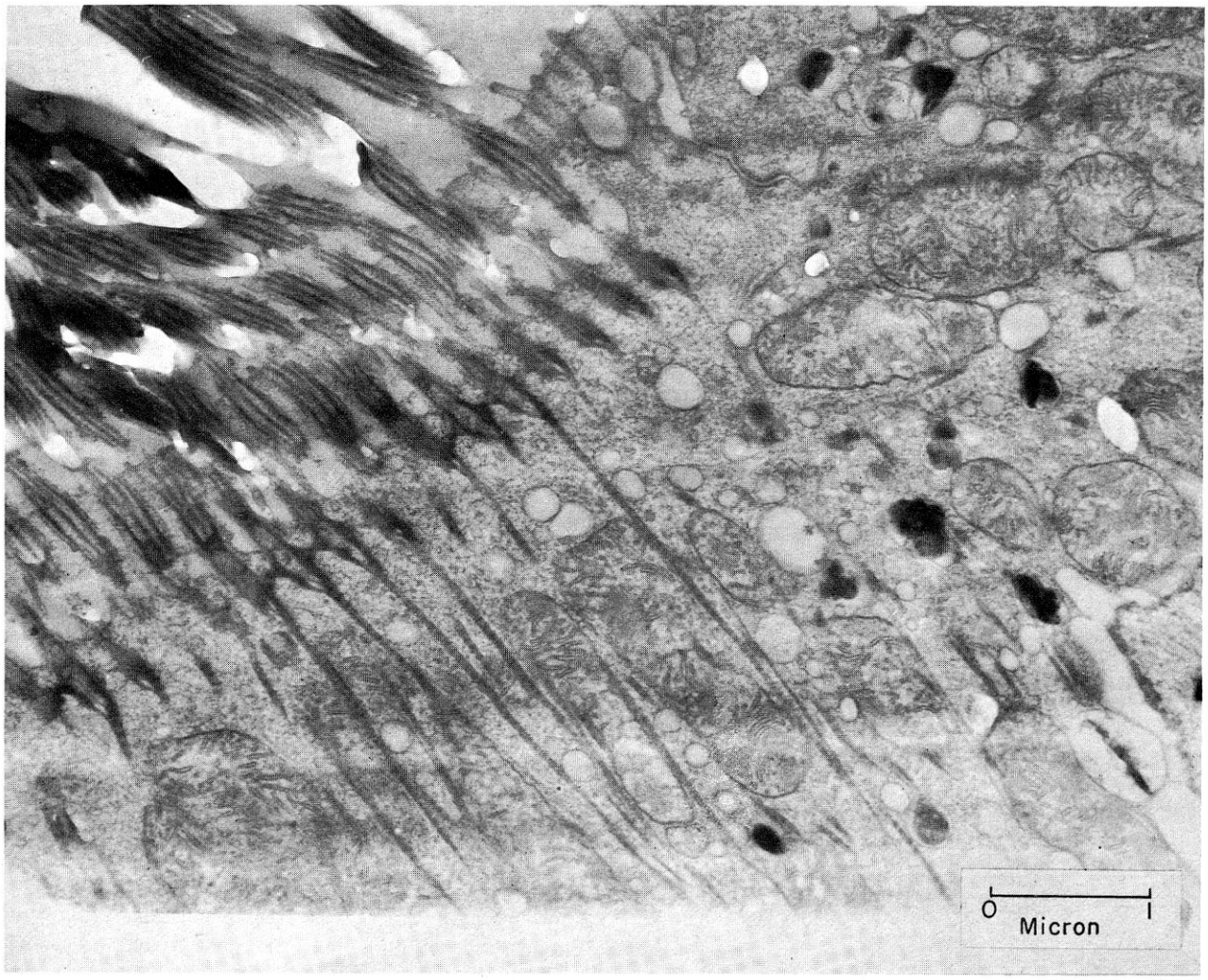

FIGURE 344.—Electron micrograph of a tangential section of a portion of a ciliated cell of the velum of the larva of *C. virginica*.

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

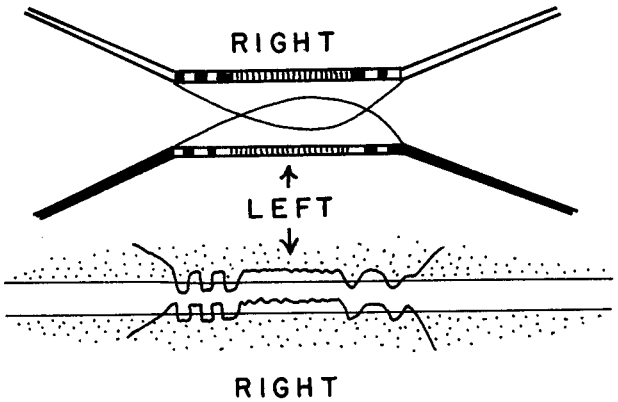

FIGURE 345.—Type of hinge of the families Ostreacea and Pteriacea. Redrawn from Rees, 1950.

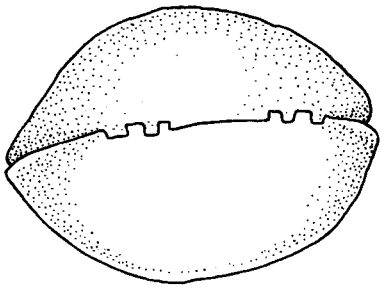

FIGURE 346.—Drawing of a 5- to 6-day-old prodissoconch of *C. virginica*, 70 μ long, examined from the dorsal side.

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

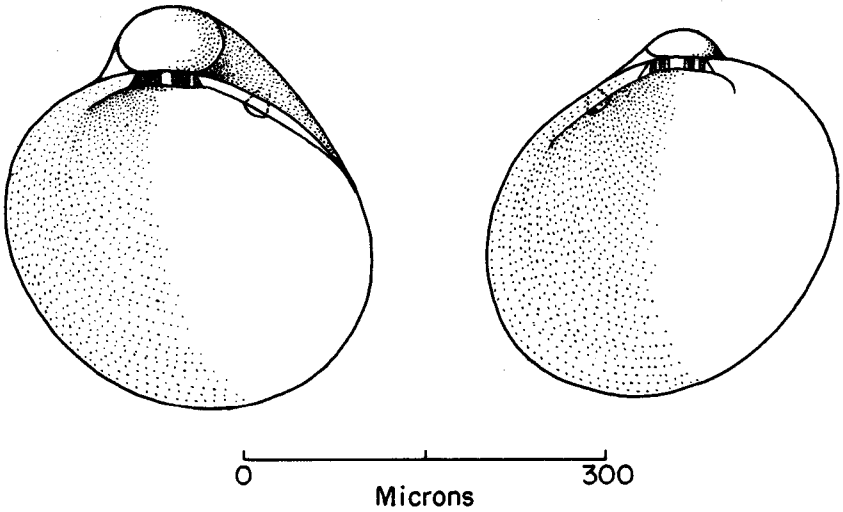

FIGURE 347.—Prodissoconch of *C. virginica* (Gmelin). Inner view of the valves. Left valve on the left and right valve on the right. Knoblike structure indicates the location of the ligament. From Ranson, 1960.

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

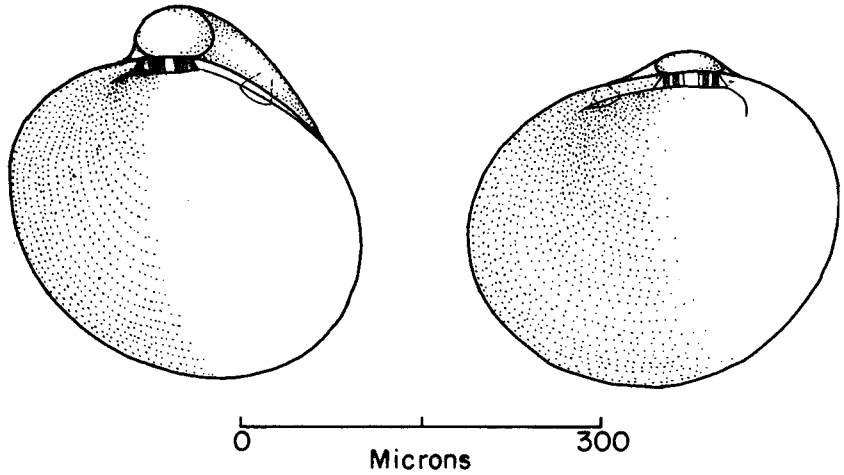

FIGURE 348.—Prodissoconch of *C. rhizophorae* (Guilding). Arrangement as in figure 347. From Ranson, 1960.

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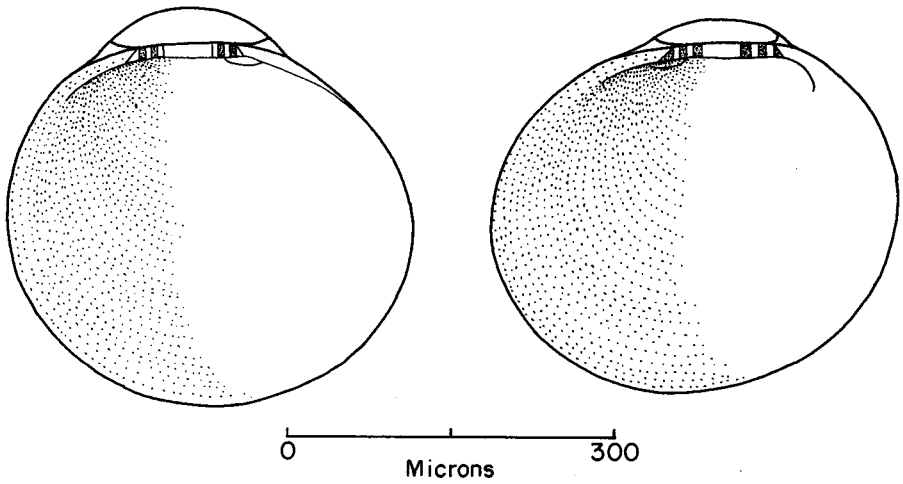

FIGURE 349.—Prodissoconch of *O. edulis* Linné. Arrangement as in figure 347. From Ranson, 1960.

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.

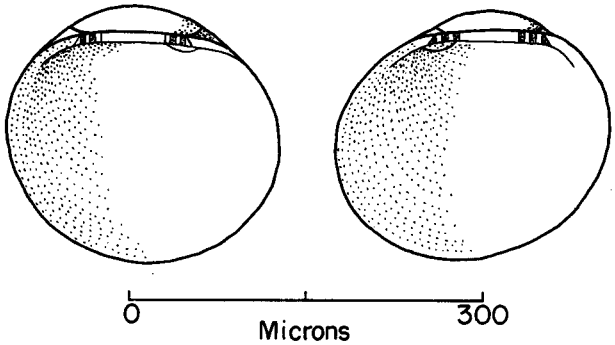

FIGURE 350.—Prodissoconch of *O. lurida* Carpenter. Arrangement as in figure 347. From Ranson, 1960.

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

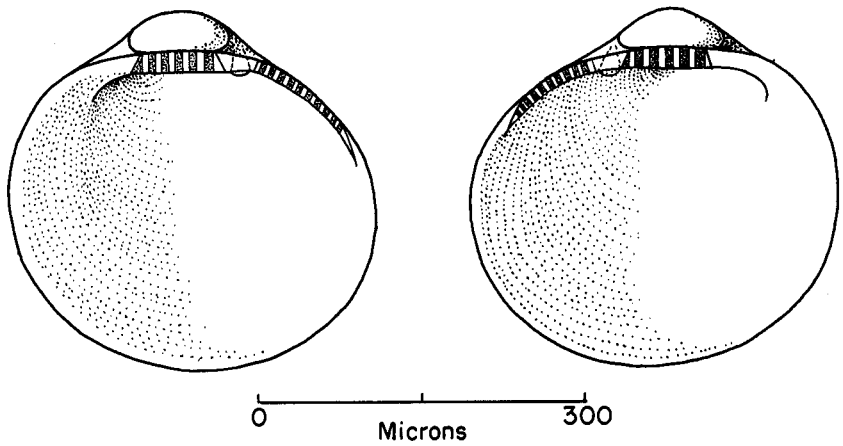

FIGURE 351.—Prodissoconch of *Pycnodonte hyotis* (L.). Arrangement as in figure 347. From Ranson, 1960.

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

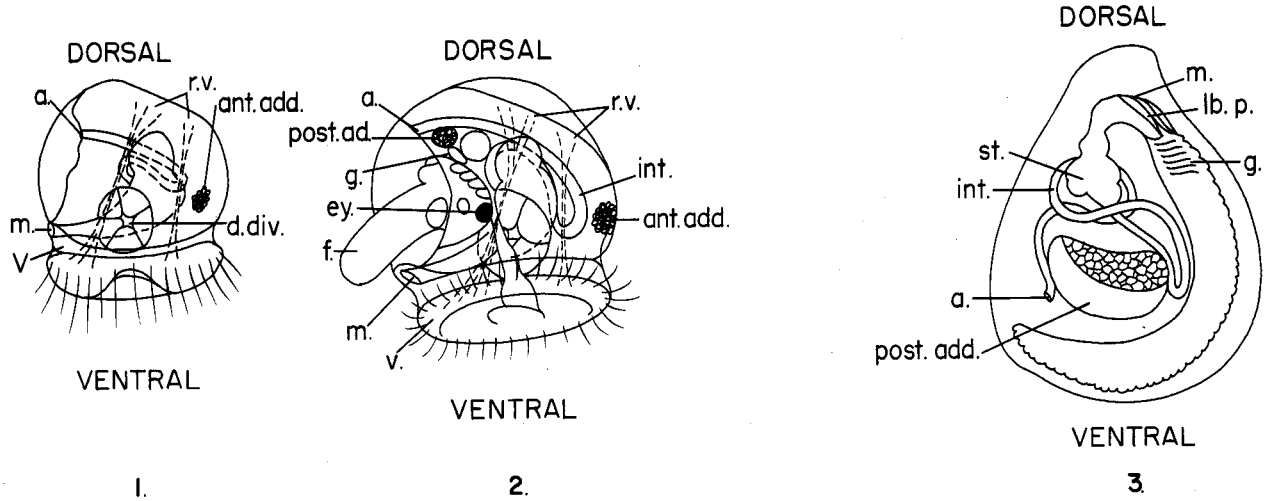

FIGURE 352.—Diagram showing the changes in the topographical relation of various organs of *O. edulis* during the transition from free-swimming larva (1) to fully developed larva ready to set (2) and juvenile oyster (3). From Erdmann (1935). a.—anus; ant. ad.—anterior adductor; ey.—eye; f.—foot; g.—gills;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

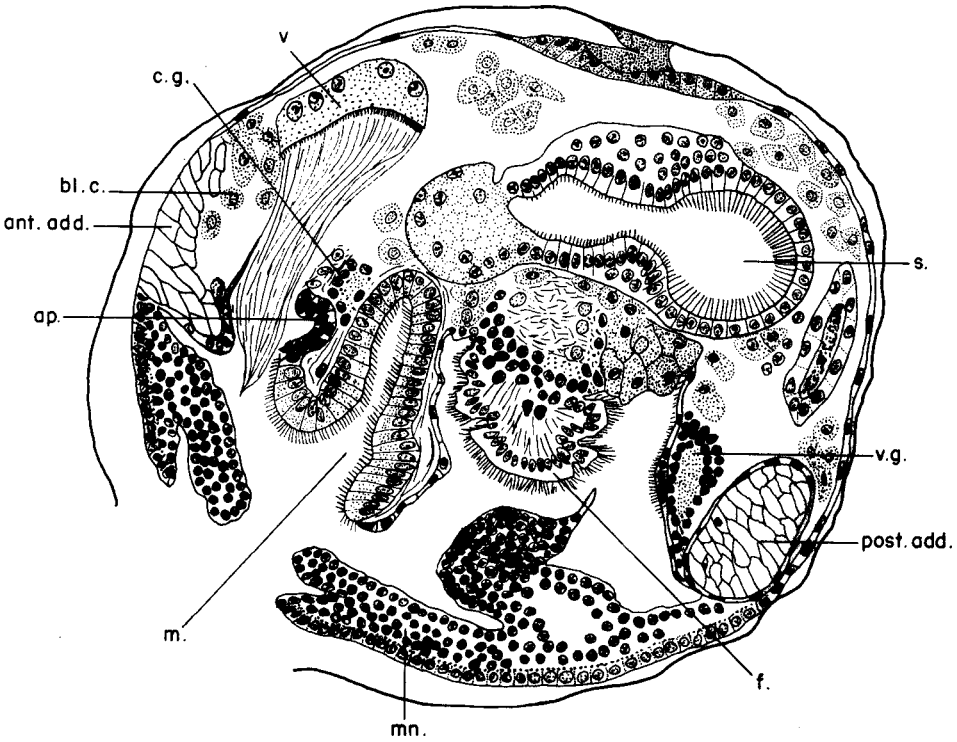

FIGURE 353.—Sagittal section of spat of *O. edulis* about 24 hours after attachment. ant. add.—disintegrating anterior adductor muscle; ap.—apical area of the velum; c.g.—cerebro-pleural ganglion; f.—foot; mn.—mantle; m.—mouth; post. add.—posterior adductor muscle; s.—stomach; v.—velum; v.g.—visceral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