CHAPTER XIV

ORGANS OF REPRODUCTION

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ANATOMY

The ripe gonad is a massive organ located near the surface of the body within a layer of connective tissue between the digestive diverticula on the inner side and the surface epithelium on the other. In sexually mature oysters it appears as many branching tubules (follicles) which merge along the dorsal side of the body to form one continuous structure encompassing the visceral mass and extending ventrally to the tip of the pyloric process (fig. 267).

The reproductive gland is not encapsulated, and its outlines are indistinct. At sexual maturity the surface covering of the body becomes so thin that a network of fine genital canals is clearly visible through it. The diameters of the canals gradually increase as they converge into a wider gonoduct through which the germ cells are discharged. Two separate systems of genital canals, one on each side of the body, are the only sign of the paired origin of the sex gland which in an adult oyster is completely fused into a single organ.

In many bivalves sex products are discharged through the kidneys, but in the oyster the gonoduct opens into a vestibule, or atrium, which also receives the urinal duct. This relationship can be seen on a series of slightly slanted sections of the lower part of a ripe gonad. One of these sections is shown in fig. 268. The female oyster from which the tissue was taken was preserved during the act of spawning. The follicles of the lower part of the ovary (left side of the figure) are almost empty of ripe eggs; a short ciliated passage between the ovarian follicle and the pear-shaped area of the kidney vestibule are at the right; two spawned eggs are near the outside opening of the vestibule, which is lined with ciliated epithelium. A transverse section of the kidney reservoir lined with typical secretory cells is in the upper right portion of the figure. The connection between the vestibule and the reservoir is located above this section. The follicles in the inner portion of a gonad lie at an angle to the genital ducts into which they discharge their content. The structure of spermary differs from that of the ovary only in that the follicles are filled with spermatozoa.

The degree of sexual development can be estimated roughly by measuring with calipers the thickness of a transverse section of the gonad layer. Since the gonad is not uniformly thick in all its parts, the section should be made at some selected place. In conformity with the practice used by the Biological Laboratories of the Bureau of Commercial Fisheries at Milford, Conn., Oxford, Md., and Gulf Breeze, Fla., the oyster is cut with a razor blade along a line extending from the lower corner of the labial palps across the stomach to the posterior end of the body. There is considerable variability in the gonad layer of oysters of known age and similar environment. In Long Island Sound the average maximum thickness of ripe gonads of 4- to 5-year-old oysters taken from three depth levels of 10, 20, and 30 feet was, according to Loosanoff and Engle (1942), about 2.4 mm. for the shallow water oysters and only about 1.5 mm. for those found in deeper water. Much greater gonadal development was recorded in other locations. Some fully mature Cape Cod oysters used in my experimental work had a layer of gonad from 6 to 8 mm. thick, and a similar degree of development was noted in oysters from a small tidal pool near the laboratory at Beaufort, N.C.

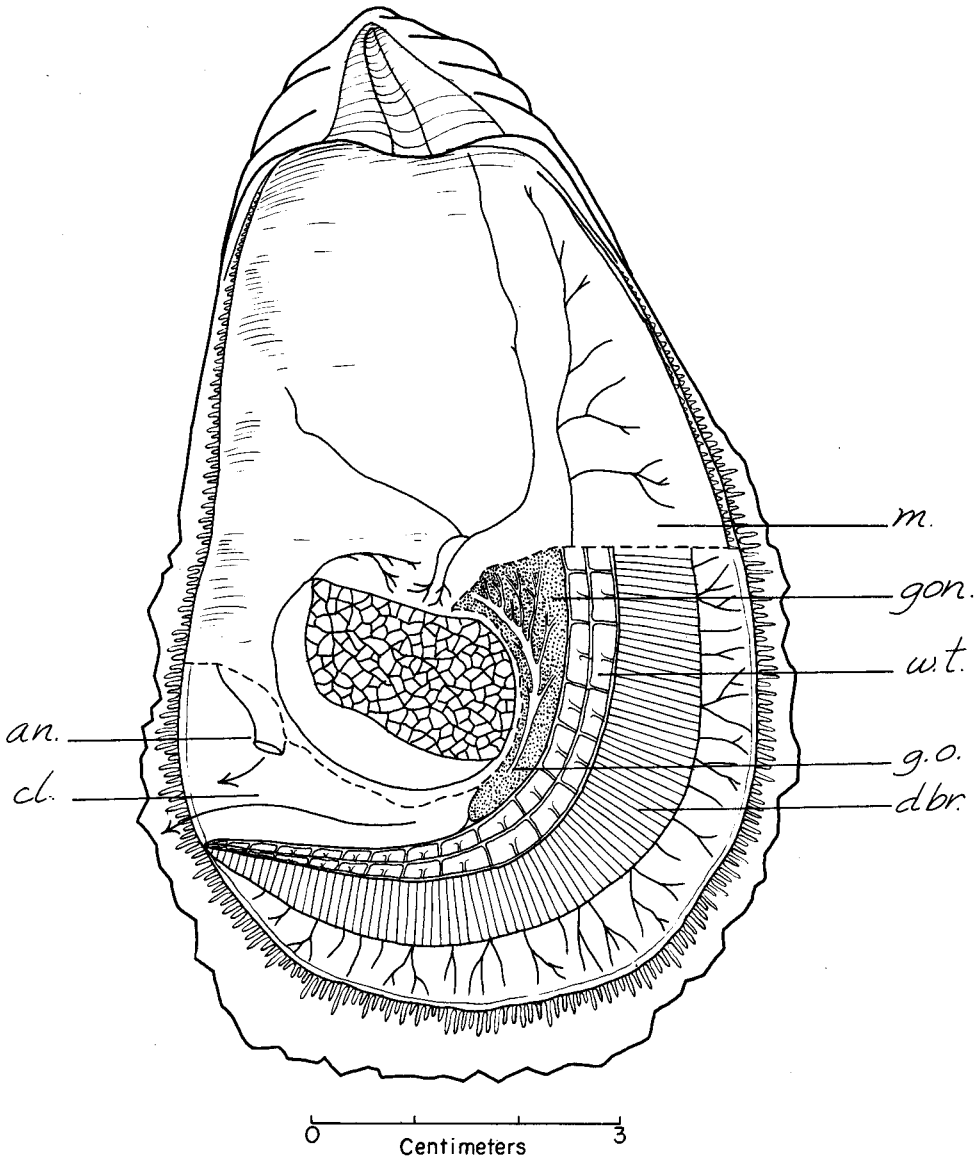

FIGURE 267.—Fully ripe *C. virginica*. The mantle of the right side was dissected above the epibranchial chamber and pushed to the right to expose the pyloric process. A network of canals leads to the single gonoduct through which the sex cells are discharged. Drawn from life. an.—anus; cl.—cloaca; d.br.—right demibranch; g.o.—genital opening; gon.—gonad; m.—mantle; w.t.—water tubes inside the gills.

Gonadal layers of about 10 mm. thickness were found in large *C. gigas* (8 inches in height) from Willapa Bay, Wash. (Galtsoff, 1930a, 1932).

In all species of *Crassostrea* the bulk of gonads varies from season to season, reaching its maximum shortly before the onset of spawning. The number of sex cells produced during one reproductive period varies, depending on the conditions of the environment. The greatest gonadal development is more likely to be found in the populations of oysters from the northern latitudes north of the Chesapeake Bay rather than in the south Atlantic and Gulf waters. This is apparently associated with the fact that the reproductive season in the northern latitudes is of short duration, 4 to 6 weeks, while in the warmer waters of the south gonadal formation and spawning, continue, with interruptions, for several months. In both groups the annual reproductive capacity (i.e., the number of eggs produced annually) maybe of the same order of magnitude or even greater in the southern oysters because of the longer reproductive period,

but the greatest bulk of a ripe gonad is more likely to occur in oysters which have only one spawning period per year.

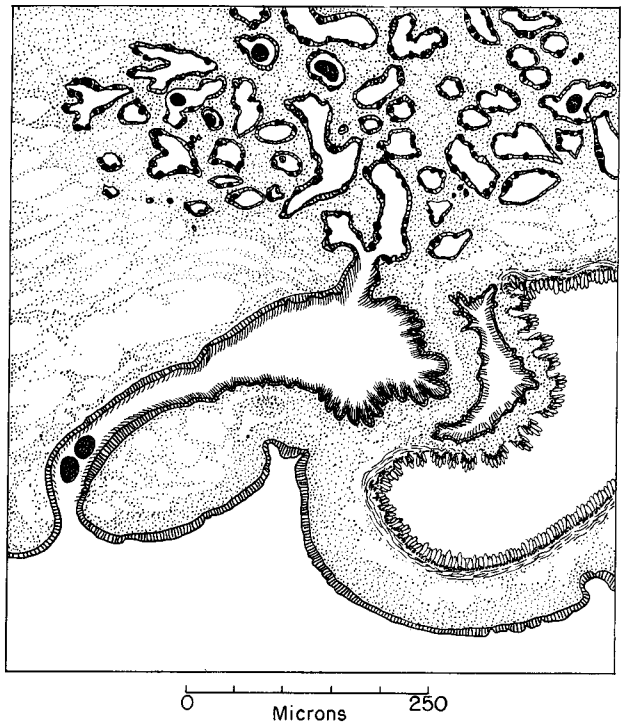

FIGURE 268.—Slightly slanted tangential section through the opening and adjacent portion of the ovary of *C. virginica*. Oviduct with two eggs at the lower right side. Kidney reservoir at upper right. Ovary follicles at left. Drawn semidiagrammatically from a photomicrograph of a preparation. Kahle, hematoxylin-eosin.

Several factors besides geography influence gonadal development. The most significant are temperature, depth (Loosanoff and Engle, 1942), salinity, available food, and pollution of oyster bottoms. Many examples of the suppression of gonadal growth by adverse conditions may be cited. For example, oysters living in waters highly polluted by various trade wastes have, as a rule, poorly developed gonads. Sometimes the development of the sex gland is suppressed to such a degree that only traces of follicles are found in the visceral mass, and the layer of the digestive diverticula is visible from the surface. These oysters have the greenish or brownish coloration typical for the digestive diverticula which in the sexually ripe oyster is not noticeable under a thick layer of gonad. Oysters with suppressed gonad development are found in the waters which receive continuous discharge of the pollutants from pulp and paper mills. Similar poor oysters are frequently encountered in waters of extremely low salinity or from areas where salinity increases to the highest limit of tolerance (34 to 40 ‰). The determination of gonadal thickness described above lacks precision because variation in the compactness of the gonadal tissue cannot be measured, but the method is, nevertheless, useful for the practical purpose of estimating expected intensity of spawning.

DETERMINATION OF VOLUME AND WEIGHT OF GONAD

The fully developed gonad is the largest organ of the oyster. The ovaries or spermaries can be separated from the underlying digestive diverticula by using small curved scissors. The excised pieces are weighed, and their volume is measured by displacement in a simple device made from a glass cylinder of appropriate dimensions (depending on the size of the sample) with a drain pipe at the bottom and a side glass tubing of about 5 mm. in diameter fused to the side at an angle of about 45° to record the water level. A convenient water level is selected and recorded, the tissue is introduced, and the water is then drained into a measuring vessel to the previous level. The body weights of adult New England oysters with fully developed gonads varied in my observations from 14.2 to 23.2 g. The gonads comprised from 31.2 to 40.7 percent of the total body weight exclusive of shell. The volume of the oysters' tissues ranged from 21 to 24 ml., with the gonads accounting for 32.8 to 33.4 percent of the total bulk. Oysters selected for these measurements were of the highest commercial quality and with maximum development of gonads. In poor oysters with light gonadal development the proportion of the weight of the gonad to body weight is only a small fraction of the figures given above.

HISTOLOGY

The gonads of the oyster originate from a group of primordial germ cells located in the mesodermal band on the ventral side of the pericardium in the vicinity of the visceral ganglion (Coe, 1943a). In embryos of bivalves primordial germ cells are identified by their relatively large size, round shape, and clear vesicular nucleus with one or two nucleoli (Okada, 1936, 1939; Woods, 1931, 1932). The primordium soon becomes separated into two groups which by continuous multiplication of the

constituent cells extend symmetrically along both sides of the body. Each group grows anteriorly, surrounded by vesicular connective tissue of the visceral mass, and forms a system of profusely branching tubular follicles. The fusion of the branches along the dorsal side obliterates any remnants of the paired origin of the gonad.

The microscopical structure of the gonad varies, depending on the age of the oyster, degree of maturity, season, and environmental condition. In Ostreidae and in some other pelecypods (*Pecten*, *Mytilus*, *Volsella*) the follicles of a fully developed gonad consist almost entirely of primitive sex cells (gonia) at various stages of development with only a few minute follicular cells between them. Because of the absence of a capsule or membrane around the gonad, the sex cells are in direct contact with the surrounding tissues (fig. 269). It may be assumed that the role of follicular cells in the growth of the gonad of oysters is insignificant and that the gametogenic cells obtain their nourishment directly from the connective tissue which surrounds them. In *Mya*, *Teredo*, *Bankia*, and other Adesmacea the follicular cells are large and function as accessory nutritive cells.

In immature oysters and in the adults, that had completed the spawning period, the germinal epithelium consists of undifferentiated sex cells, some at the early stages of gametogenesis. Their sex can be recognized only by careful cytological examination. As the follicles grow and ramify they spread throughout the surrounding layer of connective tissue. The maturing sex cells inside

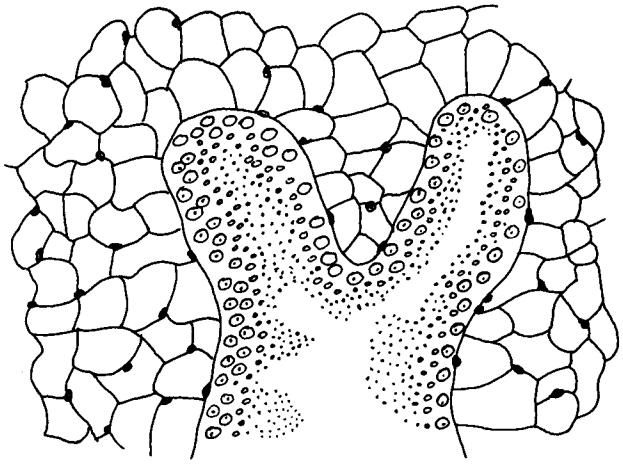

FIGURE 269.—Terminal portion of the follicle of a gonad of *C. virginica* at early stage of development (male phase). The follicle is surrounded by a large mass of connective tissue. Redrawn from Coe, 1936a.

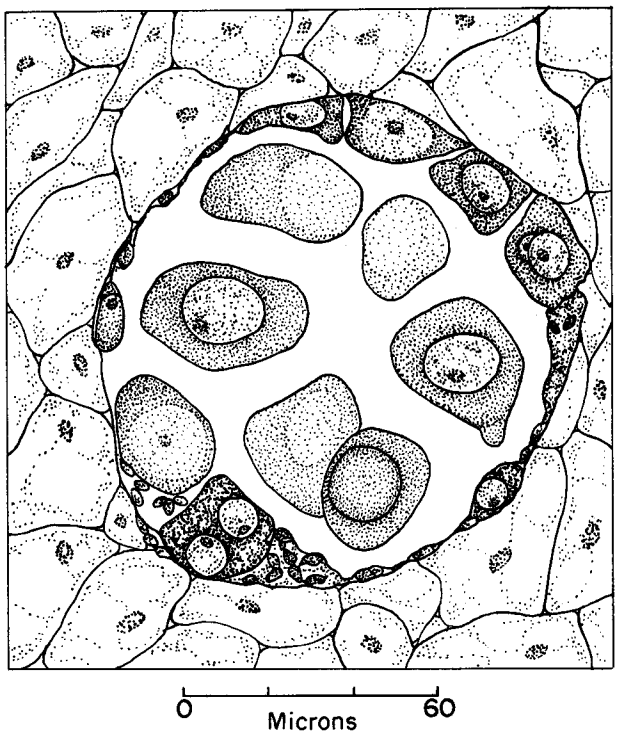

FIGURE 270.—Cross section of one surface follicle in a fully developed ovary of *C. virginica*. Bouin, hematoxylin-eosin.

them multiply, grow, and fill up the lumen (fig. 270). The follicles near the surface of the gonad are distinctly different. Their outer walls facing the body surface are lined with ciliated epithelium, and only the inner sides of the follicles retain the germinal cells (fig. 271). This differentiation of the germinal epithelium into two distinct types is probably common to all species of oysters. The follicles lined with ciliated cells, function as the genital canals through which mature sex cells are moved by ciliary action. They were first described by Hoek (1883) for *O. edulis* and subsequently were found in *O. lurida* and in several species of *Crassostrea*.

Since the transformation of germinal cells into ova and sperm is a gradual process which does not involve all the cells of the germinal epithelium at the same time, numerous undifferentiated or so-called residual cells are usually found along the inner periphery of a follicle. Some of them can be found even in a fully developed gonad (fig. 270).

The bulk of a functional ovary is made up of fully developed ova which fill up the lumina of

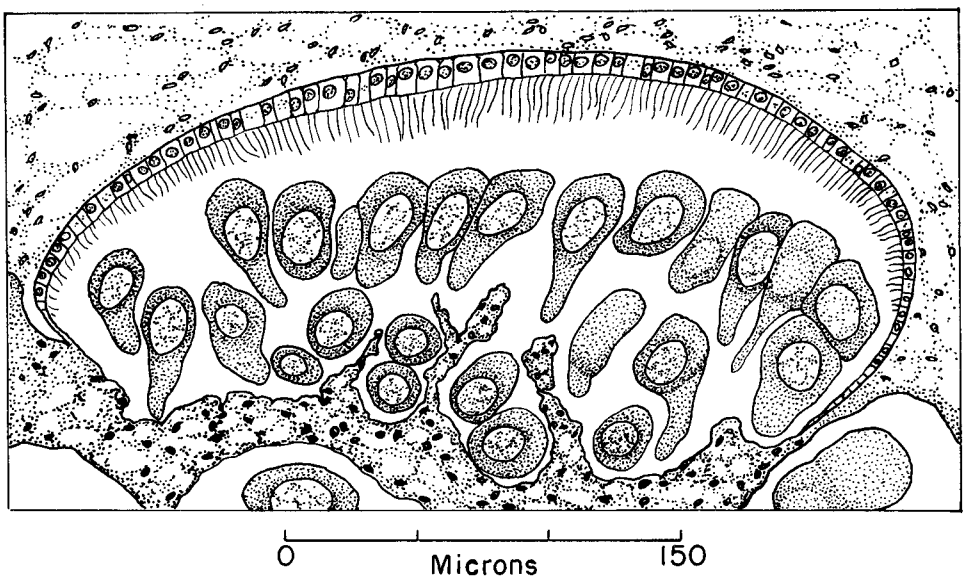

FIGURE 271.—Cross section of a surface follicle from a fully developed ovary of *C. virginica*. Germinal epithelium is formed by cells at different stages of ovogenesis and by small undifferentiated cells. Bouin, hematoxylin-eosin.

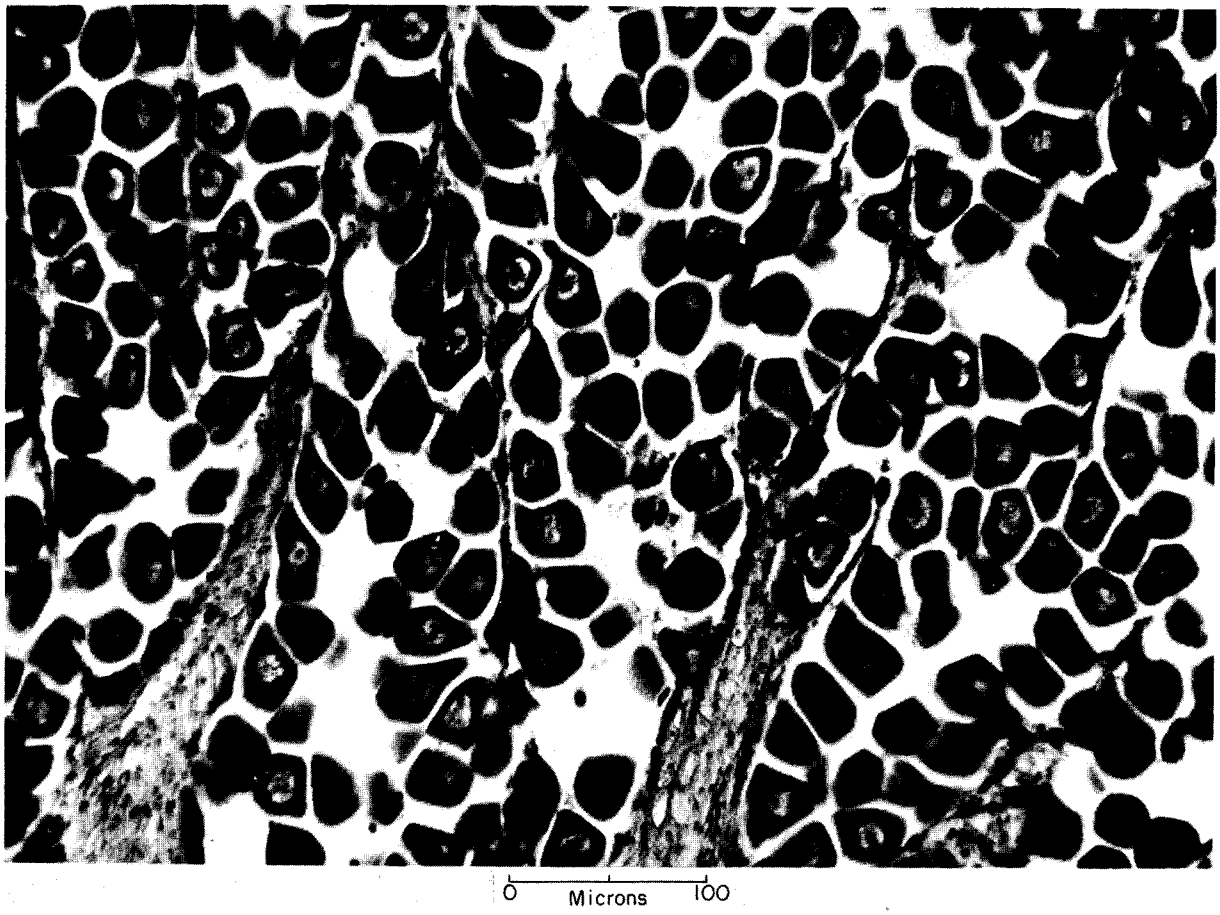

FIGURE 272.—Photomicrograph of an oblique section of a ripe ovary of *C. virginica* shortly before spawning. Bouin, hematoxylin-eosin.

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FIGURE 273.—Photomicrograph of a cross section of a ripe spermary of *C. virginica*. Bouin, hematoxylin-eosin.

the follicles and of a number of cells at different stages of the development nearer to the walls. The eggs are attached to the walls of the follicles by elongated peduncles, giving them a pear-shaped appearance. At the height of sexual development the layer of undifferentiated germinal epithelium is reduced to a very narrow band of small cells hardly visible at low magnification (fig. 272). The connective tissue between the follicles also very nearly disappears.

The arrangement of cells in a ripe spermary (fig. 273) is similar to that in the ovary. Fully formed spermatozoa are massed together inside the follicle with their tails inward. They become separated as the sperm moves through the genital canals, which are frequently distended by the accumulation of spermatozoa ready to be ejected. In *O. edulis* and *O. lurida* the sperm in the lumen of a follicle form distinct balls which retain their shape until they are discharged by the oyster. A number of sex cells remain in the follicles at the completion of spawning. Consequently, at the end of the reproductive season the gonads contain mature ova and sperm as well as undifferentiated cells; many of them are pycnotic and detached from the germinal lining of the wall. Phagocytosis becomes pronounced as large numbers of leucocytes invade the follicles to digest and cytolize the remaining sex cells (fig. 274). The connective tissue between the follicles becomes disorganized. After the reabsorption of the gonad is completed only a narrow band of germinal epithelium remains in a few follicles and the entire layer has shrunk to such a thin band that it is not visible to the naked eye. The oyster is now at an indifferent stage. Its sex can be recognized in some individuals in which young ovocytes or spermatocytes are present, but in many others

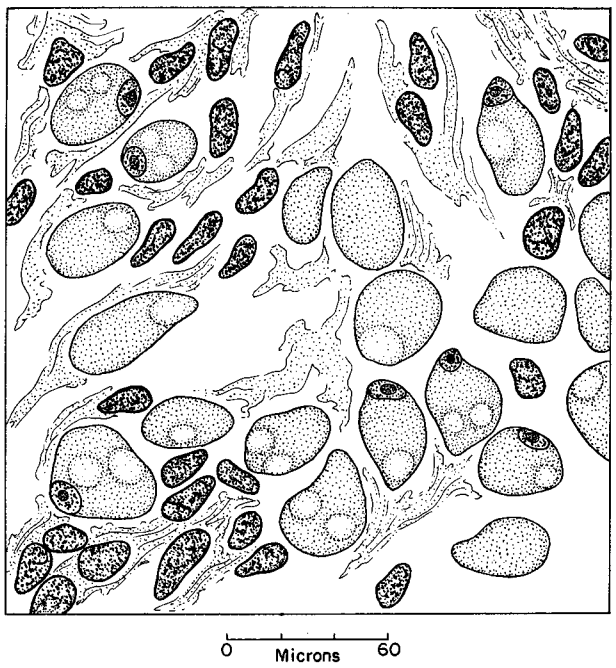

FIGURE 274.—Ovary of *C. virginica* shortly after completion of spawning. Unspawned ova are cytolized. Note the invading phagocytes and the disorganization of connective tissue. Bouin, hematoxylin-eosin.

the young sex cells are not sufficiently differentiated and their sexes can not be identified. Ovocytes, when sufficiently developed, can be distinguished from spermatocytes by their large nuclei and granular cytoplasm.

During spawning the sex cells are moved inside the genital canals by ciliary motion of the epithelium. How they are released from the follicles and reach the genital canals located near the surface of the sex gland is not known. Histological examination discloses no contractile elements in the tissues surrounding the follicles, and no contraction of the gonad or part of it could be detected during spawning. Upon reaching the gonoduct the released cells continue to be moved by the powerful cilia of the duct and vestibule (fig. 275). Longitudinal and circular muscle fibers are found under the basal membrane of the vestibule and appear to be better developed in the male gonad than in the female. Roughley (1933) refers to the presence of sphincter muscles in the gonoducts of *Ostrea* (*Crassostrea*) *commercialis*, but no structure resembling a sphincter is found in *C. virginica* (Galtsoff, 1938a). It is conceivable that a contraction of circular muscle fibers of the spermiduct to a certain degree controls the passage of sperm through the genital opening, but no such action has been detected under experimental conditions.

The epithelium of the distal end of the gonoduct and of the urinogenital vestibule contains, besides the ciliated cells, a large number of mucous cells (MC) not present in the lining of the canals. In the ovary the opening of the oviduct into the vestibule is marked by a small ridge of ciliated cells (Galtsoff, 1938a, 1938b).

SPAWNING

The sexual apparatus of oysters is of the simplest type for it lacks the accessory sex organs which in some mollusks are used for mutual excitation, storage of sex cells, copulation, and secretion of egg capsules. In spite of the structural simplicity of the reproductive organs, the spawning of the female oyster is a rather complex action which involves coordination of the gills, nervous system, mantle, and the adductor muscle, while the sexual act of the male is much simpler. It is, therefore, more convenient to describe separately the phenomena involved in the spawning of the two sexes. Under natural conditions simultaneous release of sperm and eggs, essential for successful reproduction of the species, is attained through mutual stimulation.

SPAWNING REACTIONS OF THE FEMALE

Spawning of the female proceeds in several consecutive steps which, in the order of their participation, involve the ovary, the gills, the mantle, and the adductor muscle. The behavior of these organs follows a distinct pattern, one action succeeding the other in a precise sequence which finally terminates in the dispersal of eggs in the surrounding water.

The first step is ovulation, i.e., the discharge of eggs from the ovary into the epibranchial chamber. The moment the eggs appear in the epibranchial chamber the two edges of the mantle come together and effectively seal the pallial cavity and cloaca. This peculiar behavior of the mantle may be observed in a spawning female oyster placed close to the wall of a rectangular tank. In an actively feeding animal the space between the two mantle edges is wide open; the pallium and the tentacles are extended outward parallel to the surface of the valves and the gills, exposing the side of the adductor muscle, the rectum, and the inner part of the cloaca (fig. 276). A different picture is seen in a spawning female. A few minutes before ovulation the edges of the

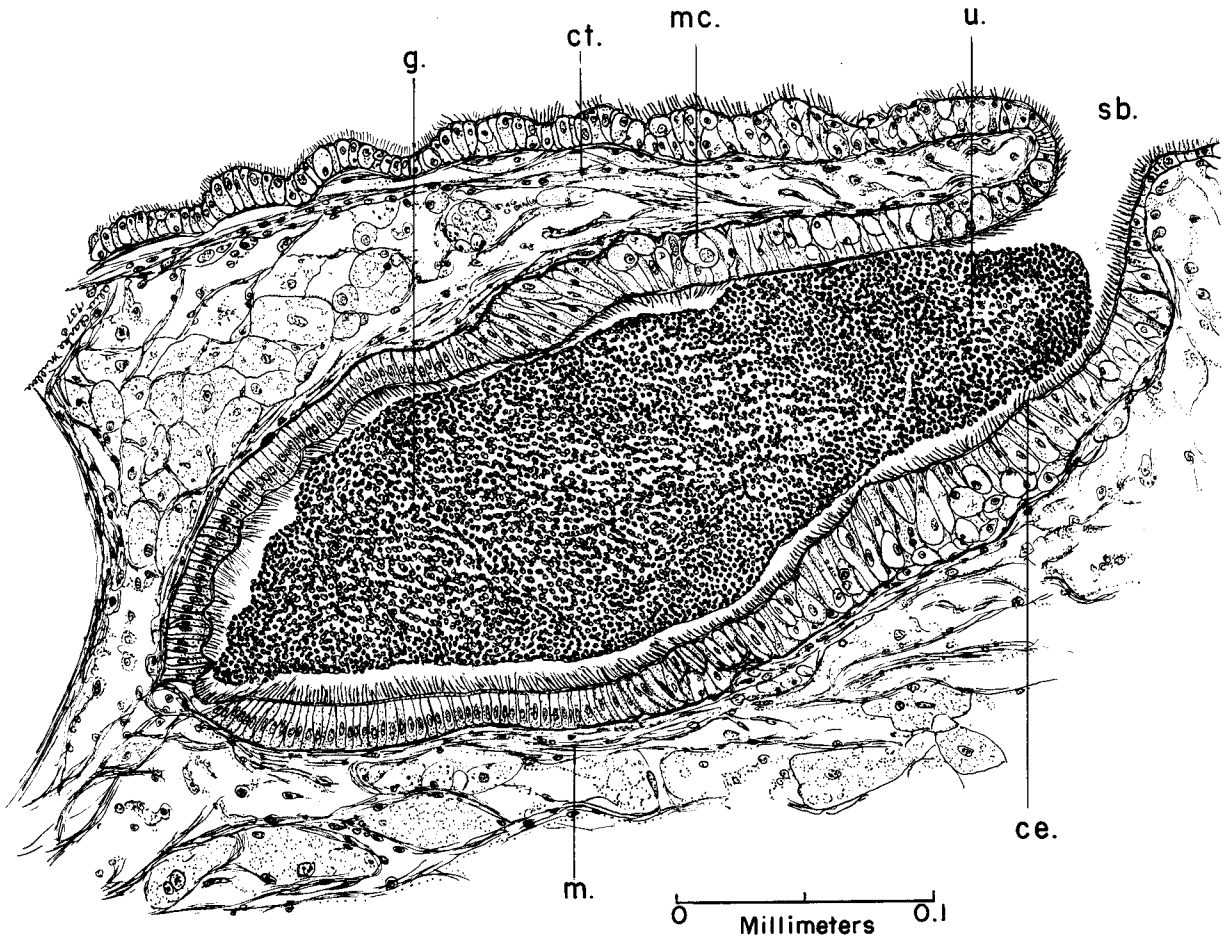

FIGURE 275.—Section through the lowest part of spermiduct and the adjacent portion of the vestibule. Both organs are grossly distended by the discharged sperm. Tissue of *C. virginica* was preserved during the act of spawning. G—approximate position of the junction of spermiduct and the vestibule; CE—ciliated epithelium; CT—connective tissue; M—longitudinal muscle fibers; MC—mucous cell; SB—opening of the vestibule into the epibranchial chamber; U—lower part of the urinogenital vestibule. Bouin, hematoxylin-eosin. From Galtsoff, 1932.

mantle display unusual activity; they come together and temporarily close the access to the gills; then for a few seconds they open again. Both the rate and range of shell movements at this time gradually increase. Finally, the entrance to the gill cavity closes completely except for one small opening or "window" as shown in fig. 277. Soon a white cloud of unfertilized eggs appears at the window, the adductor muscle contracts sharply, and the eggs are discharged and dispersed several inches away from the oyster (fig. 278). The opening between the edges of the mantles may be formed at any place along their periphery but once formed its position remains unchanged throughout the duration of spawning. Spawning may last from a few minutes to nearly 1 hour, depending on the amount of mature eggs in the ovary.

Eggs trapped inside the epibranchial chamber have to pass through the water tubes in order to accumulate in the space between the gills and the mantle since there is no other way by which they can reach this area (Galtsoff, 1938a). This conclusion was confirmed by microscopic examination of a section of the gills of a female preserved during the act of spawning (fig. 279). Any other minute particles suspended in water pass through the ostia and water tubes into the exhalant chambers and are swept by the outgoing current. The eggs released through the genital pore, however, take the opposite course when they enter the water tubes of the gills.

While the eggs pass through the gills the ostia are wide open and the ciliary currents along the filaments are neither inhibited nor reversed. The eggs, therefore, flow against the current produced

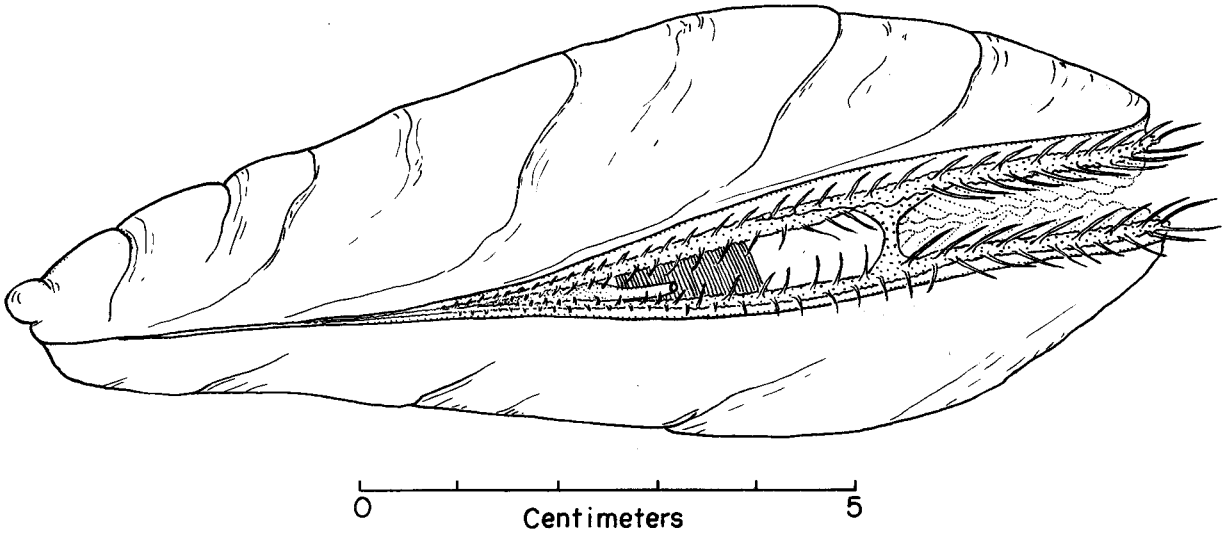

FIGURE 276.—Actively feeding *C. virginica*. The valves are wide open, the pallium and the tentacles are extended outward and the cloaca, the adductor muscle, and rectum are clearly visible. Drawn from life. The opening between the valves is slightly exaggerated.

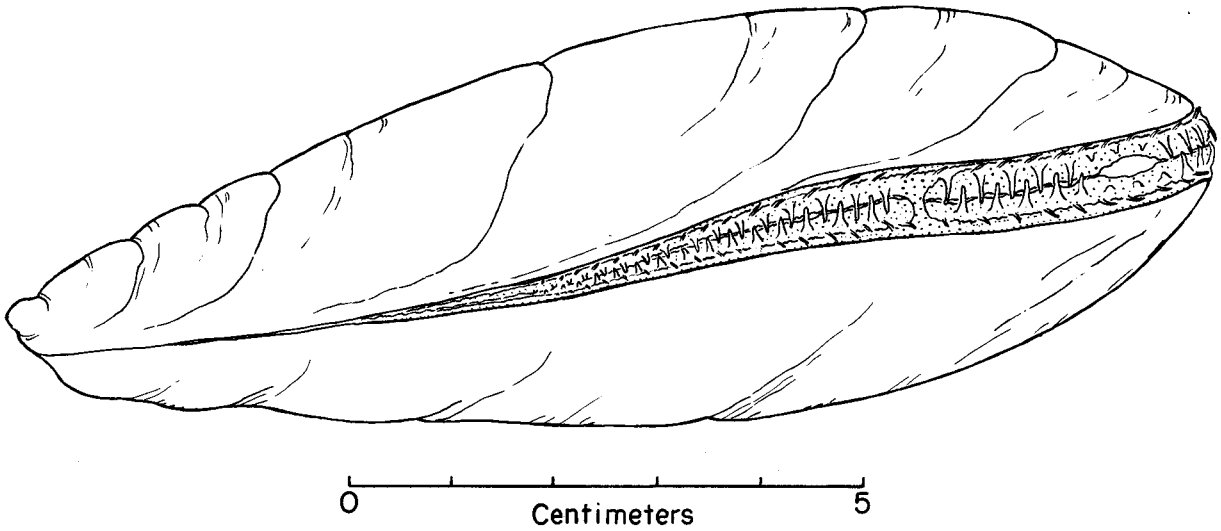

FIGURE 277.—Position of the mantle and tentacles of *C. virginica* shortly before the discharge of eggs through a small window left open (right side of figure). Drawn from life.

by the lateral cilia. Laboratory observations show that when the valves open the gill lamellae also expand and the water tubes dilate. At the same time the closing of the cloaca and of the mantle cavity cuts off the access of water from the outside. As a result, a suction is produced inside the water tubes by the expansion of gill lamellae, forcing the eggs into the water tubes and through the ostia to the surface of the gills.

Eggs of larviparous oysters (*O. lurida* and *O. edulis*) also pass through the gills but are retained in the pallial cavity until fully developed larvae are formed and escape from the mother's body.

Stafford (1913) thought that the eggs of *O. lurida* are too heavy to be carried by the respiratory current and so fall into the water tubes and are forced through the ostia by the pressure of their own mass. The correctness of such an explanation seems doubtful. The act of spawning in the larviparous species has not been studied adequately, probably because the ovulation and passage of eggs into the pallial chamber proceed without any outward indication (Yonge, 1960). Eggs of these species are fertilized inside the body

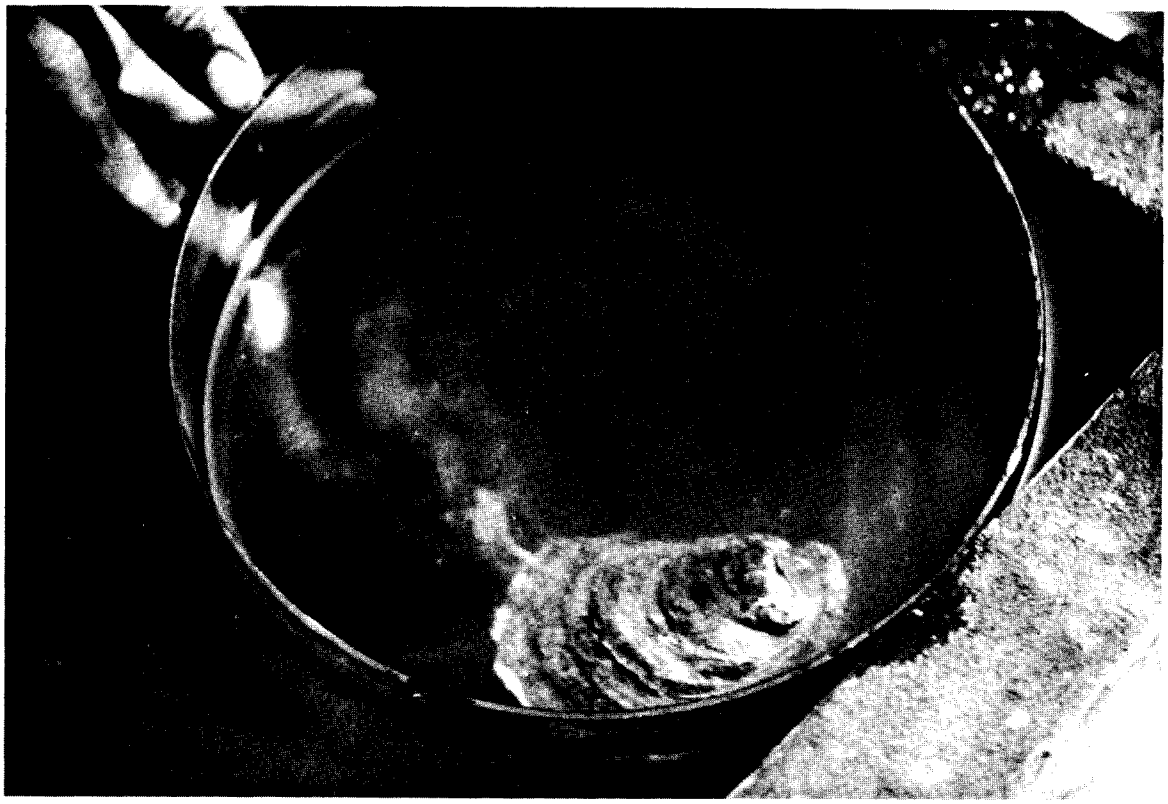

FIGURE 278.—Spawning of large female *C. virginica*, photographed in the laboratory.

by sperm drawn in from the outside with the respiratory current and are extruded as well-developed larvae. The process is called "swarming" (Korringa, 1941). Careful studies of shell movements of *O. lurida* or *O. edulis* during the reproductive period may uncover some peculiarities of the behavior of their adductor muscles associated with swarming.

If the shell movements of a spawning female are prevented by cutting off a piece of valve between the adductor muscle and the hinge, the eggs cannot pass through the gills and are discharged through the cloaca. This has been demonstrated in the experiments illustrated in fig. 280, A and B. In both cases fully mature Cape Cod oysters were placed in finger bowls under a low-power binocular microscope. In oyster A the gills were exposed by cutting off a piece of the right valve without injuring the adductor muscle. Its shell movement remained normal. In oyster B the entire dorsal half of the right valve above the muscle attachment was removed, and in this way shell movements were prevented. During the spawning of oyster A the released eggs (e) passed through the gills, while in oyster B they were discharged through the cloaca.

Female spawning of *C. gigas* and *O. cucullata* follows the same pattern as the American oyster (Galtsoff, 1932). It is apparent that the mantle, gills, and adductor muscle of *Crassostrea* species temporarily assume the role of accessory sex organs and through coordination and adjustment of their activities perform a specific role which is distinct from their primary functions.

The release of sex cells from sexually mature oysters often requires a stimulus which causes a triggerlike effect and initiates spawning. Very often this effect is associated with a sudden rise in the temperature of the water. Numerous ecological observations show that under natural conditions oysters spawn at rising temperature. This led to the concept of "critical temperature," and for many years the temperature of 20° C. was considered the lowest at which spawning takes place. It was postulated that "once this critical temperature' of 20° C. is reached a trigger mechanism is released which requires some hours for its consummation" (Nelson, 1928a). Further obser-

vations disproved this concept. Nelson reported that *C. virginica* transplanted from the United States to England could be induced to spawn at 19.1° C. (Nelson, 1931). Some of the oysters of Long Island Sound spawn at 16.4° C. (Loosanoff, 1939).

Ecological evidence shows that spawning of an oyster population often coincides with a rapid rise of temperature but it is not determined by a specific "critical" temperature. Physiological studies at the Woods Hole laboratory indicate that temperature and chemical stimulation, acting singly or jointly, may induce spawning in sexually ripe oysters. On the other hand it is apparent that certain internal and external conditions inhibit spawning.

The effect of temperature on spawning can be observed by placing a sexually mature oyster in a tank of water, connecting its right valve to the writing lever of a kymograph, rapidly warming the water and then maintaining the temperature at a desired level. Shell movements of the

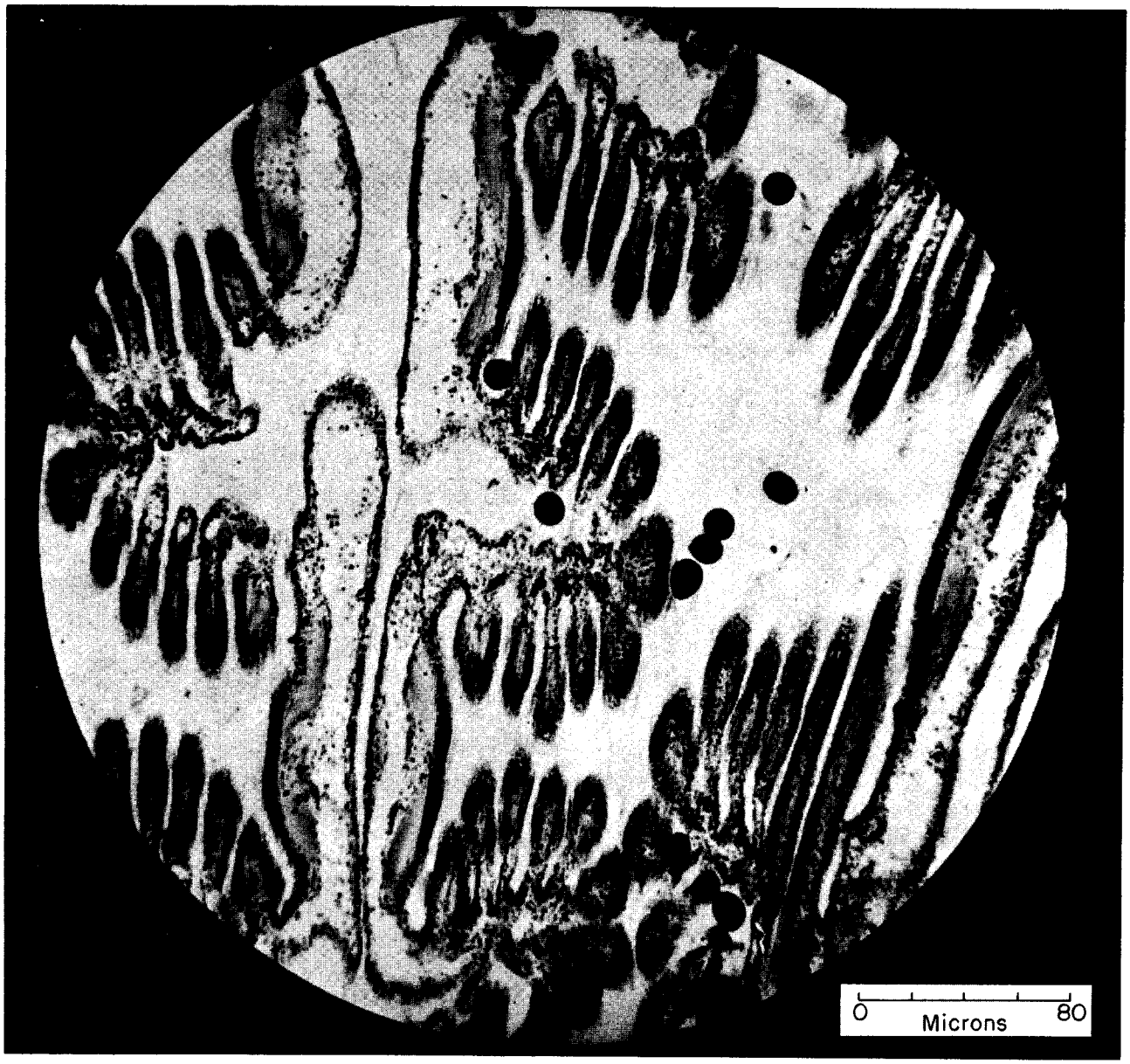

FIGURE 279.—Transverse section of the gills of female *C. virginica* preserved during spawning. Hematin-eosin. Note the eggs inside the water tube (center) and in the ostium.

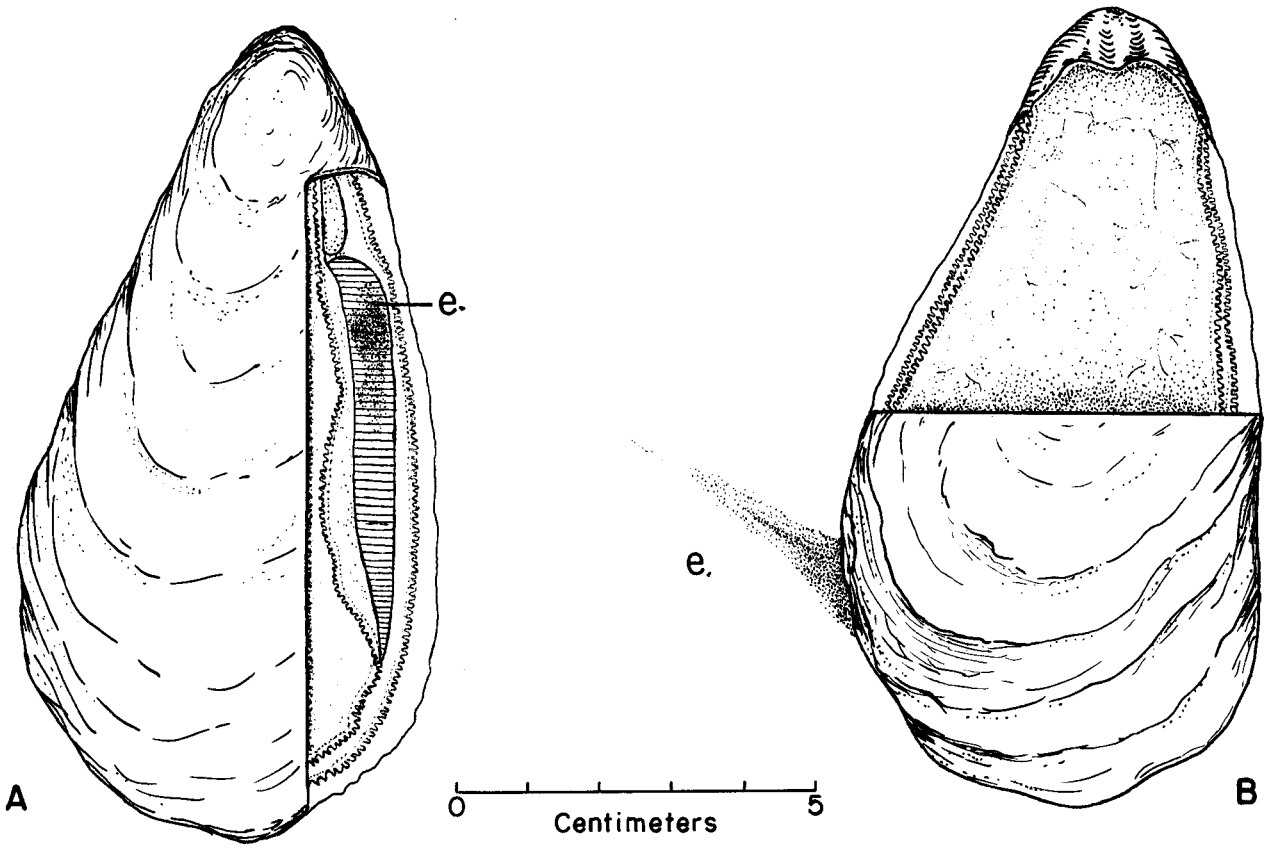

FIGURE 280.—Experiment showing the role of shell movements in the discharge of spawned eggs of *C. virginica* through the gills. A.—Portion of the right valve was removed to expose the gills; the adductor muscle was not injured and shell movements during spawning were normal. Eggs (e) pass through the gills. B.—Portion of the right valve between the adductor muscle and the hinge was cut off to prevent shell movements. Eggs (e) pass through the cloaca. Drawn from life.

spawning oyster are recorded on a kymograph, and the presence of discharged eggs or sperm in the water is ascertained by microscopic examination of samples taken at frequent intervals. In the case of heavy spawning so many sex cells may be shed that the water becomes milky and opaque; when there is light spawning the presence of eggs should be checked by collecting material which settles on the bottom of the tanks.

Spawning of sexually ripe females of *C. virginica* may be induced by warming the water from 18° to 20° C. to 22° to 23° C. and maintaining this temperature for several hours. Relatively few oysters respond to this mild stimulation. A more effective method, which in my experience gave positive results in about 40 percent of the oysters tested, consisted in rapidly raising the temperature of the water from about 20° C. to 33° to 34° C. The remaining 60 percent of the oysters which did not respond to thermic stimulus required additional stimulation by live sperm. Using this technique I found that the population of oysters from a single small bed in Onset, tested within a few days, consisted of individuals which greatly varied in the degree of their response to spawning stimuli. The tests were made at intervals of 2° C. The females that failed to spawn at 22° to 23° C. spawned at this temperature when sperm was added to the water. Some of the oysters spawned at 25° to 27° C., but still others required the addition of sperm to induce ovulation at this temperature level. Similar results were obtained at 29° to 31° C. and 32° to 33° C. In each of the groups tested there were specimens which did not respond to the rise of temperature and required additional stimulation by live sperm. All the oysters used in these experiments were mature; they had fully developed gonads, the eggs were fertilizable, and spawning, when induced, was copious.

The threshold temperature of spawning is not a "critical" temperature in the sense that it automatically induces the discharge of eggs in all physiologically ripe oysters. The success or failure of thermic stimulation depends on the responsiveness of the organism. It would be more appropriate to speak of the "critical condition" of the organism which makes it responsive to stimulation rather than of critical temperature of spawning. Within broad limits between 15° and 32° to 34° C., spawning of *C. virginica* may occur at any temperature; mass spawning of an oyster population is more likely to take place in warm water above the 22° to 23° C. level.

Stimulation by live sperm is of great importance in the reproduction of *Crassostrea* oysters. In the Woods Hole experiments the time elapsed between the addition of sperm suspension and the beginning of shedding of eggs varied between 6 and 38 minutes. At about 20° C. the sperm added to the pallial cavity passed through the gills and was expelled from the cloaca within 7 to 8 seconds. The latent period of spawning reaction lasts several minutes. This suggests that possibly the sperm acts upon the female organism after it has been absorbed by the cells of the water transport system or by the digestive tract. Direct evidence, however, is absent since attempts to prevent the penetration of sperm into the digestive tract by plugging the mouth and esophagus were not successful.

Rhythmic contractions of the adductor muscle are associated with the release of eggs from the ovary and are not directly stimulated by temperature or by any known chemical agent. This becomes clear from the observations which show that spawning contractions proceed in the same manner whether the spawning was induced by temperature or by sperm. Two kymograph tracings of shell movements of the two females shown in fig. 281 are similar in spite of the fact that in one of them (upper line) spawning was induced by the addition of sperm, while in the other by rapidly warming the water from 21.6° to 30.2° C.

Experiments were made to determine whether some substances causing the contraction of the adductor muscle are released into the blood stream during spawning. A female was induced to spawn by thermic stimulation, and a sample of its blood withdrawn from the pericardium was immediately injected into the visceral mass and into the circulatory system of a sexually mature but nonspawning female. Six experiments of this type were made with negative results.

Shell movements during female spawning are so typical that they cannot be mistaken from any other type of muscular activity. Spawning reaction is recognized by the duration of the latent period of not less than several minutes; the uniformity of the tonus level at the points of relaxation; regular rhythm of the contractions, particularly at the beginning of the reaction; and the presence of a small "plateau" about half-way on the relaxation curve (see fig. 281). The plateau is indicative of the slowing down of the relaxation phase; its significance is due to the fact that it coincides with the oozing out of eggs through the ostia of gill filaments (Galtsoff, 1938b). This type of

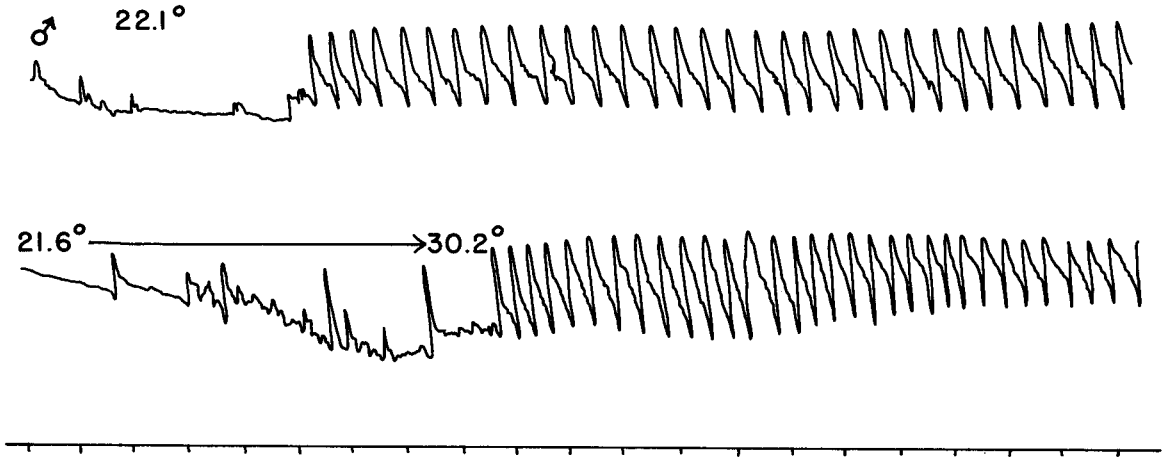

FIGURE 281.—Kymograph records of shell movements during spawning of two female *C. virginica*. Upper line—spawning induced by the addition of sperm at 22.1° C.; lower line—spawning induced by rapid rise of water temperature from 26.1° to 30.2° C. Time interval, 1 minute.

spawning curve appears in hundreds of records obtained in the laboratory in the course of several years of studies. It does not occur after the spawning season is over and cannot be provoked by temperature or chemical stimulation of the oysters devoid of mature eggs. Injections of low concentrations of adrenalin cause rhythmical contractions of the adductor muscle but of an entirely different type. The spawning reaction is always followed by a refractory period of two to several days during which the female is not responsive to stimulation.

SPAWNING REACTION OF THE MALE

Spawning of the male does not involve the participation of the mantle and adductor muscle. Sperm is discharged from the spermary into the epibranchial chamber by ciliary motion inside the genital ducts and is swept away by the respiratory current (figs. 282 and 283). The pallium remains wide open and quiescent. Muscular contractions of the adductor play no role in the release and discharge of sperm, and there is no visible change in the velocity of the cloacal current during ejaculation (Galtsoff, 1938a). Shedding of sperm occurs sometimes in sudden outbursts of brief duration which may be repeated at frequent intervals. Toward the end of the reproductive season the discharge of sperm may continue for several hours without interruption until the male is completely spent. Ejaculation proceeds either from one or from both spermiducts simultaneously. In the latter case the flow of milky water containing suspended spermatozoa can be seen emerging from the cloaca and from the promyal chamber simultaneously. The males of *C. gigas and C. commercialis* behave in a manner similar to the males of *C. virginica.*

Males of *C. virginica* are more responsive to spawning stimuli than the females of the species. They are more readily stimulated by rising temperature, and shedding of sperm is easily induced by various substances; a suspension of eggs or filtered egg water (sea water in which eggs were kept for some time); eggs of various bivalves (*Pecten irradians, Mercenaria mercenaria, Mytilus edulis*); and eggs of starfish, *Asterias forbesi.*

The latent period of stimulation varies depending on the substance used and its concentration, but in general it is much shorter than in female spawning. Suspension of eggs or egg water of *C. virginica* induces spawning of the male within 5 to 6 seconds at 24° to 25° C.; eggs of *Pecten irradians* are more effective, provoking a response in a male oyster in 4.6 to 4.8 seconds; the latent period in the case of clam eggs (*Mya arenaria, Mercenaria mercenaria*) is from 8 to 9 seconds at

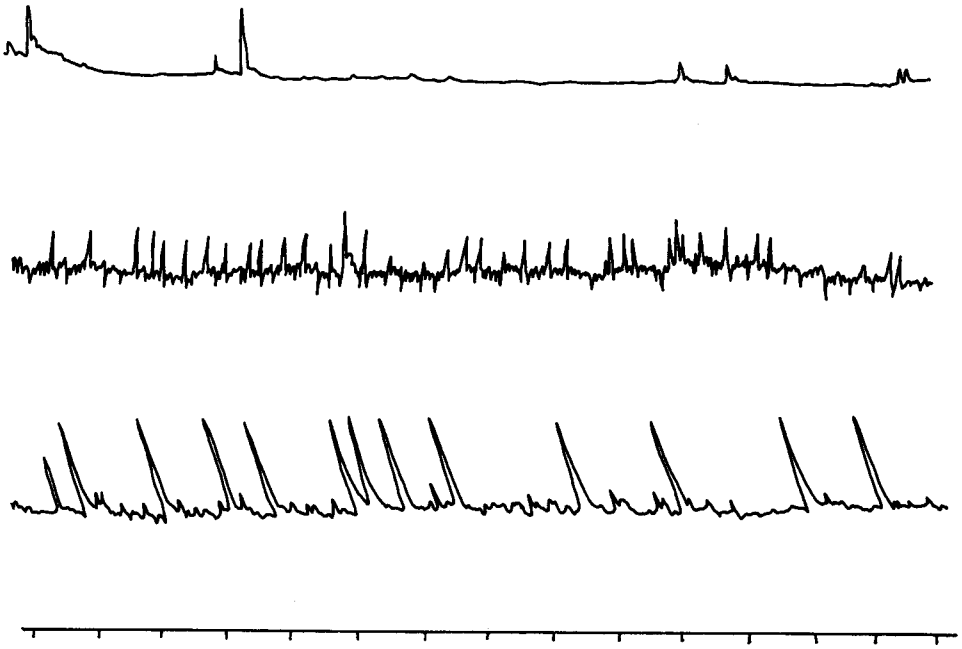

FIGURE 282.—Shell movements of three males of *C. virginica* recorded during the shedding of sperm. There was no change in muscular contraction before, during, or after spawning. Temperature 23.5° C. Time interval, 1 minute.

FIGURE 283.—Photograph of the spawning male, *C. virginica*. The V cut in the left valve was made several weeks before the experiment. Temperature 23.0° C. Sperm ejected through the opening of the promyal chamber is seen as a white jet opposite the V cut.

24° to 25° C. Many other substances including various hormones (thyroxin, adrenalin, estrogen), desiccated anterior and posterior pituitary, thymus, thyroidin, cysteine, glutathione, peptone, egg albumen, urea, different sugars (dextrose, maltose, d-arabinose), starch, and yeast stimulate ejaculation in various degrees of effectiveness. Suspension of desiccated thyroid gland (thyroidin) in sea water was found to be the most effective stimulant, and it has been used in the Woods Hole laboratory in preference to egg suspension or egg water.

Other substances may also provoke sexual response. Miyazaki (1938) found that the extracts from several algae, *Ulva pertusa*, *Enteromorpha* sp. and *Monostroma* sp. induce spawning in the males of *C. gigas*.

Mature males (*C. virginica*) respond also to live

sperm of the species. In this case the latent period of spawning reaction is much longer, varying from 6 to 27 minutes at 20° to 21° C. The interesting fact is that in the case of sperm stimulating male spawning the latent period is of the same order of magnitude as that of the female spawning reaction. Possibly the sperm acts as a stimulant only after it has been absorbed by the oyster, while eggs and egg water act upon the receptors located on the body surface.

The active principle of sperm of *C. virginica* can be extracted with ethyl alcohol and benzene. The residual powder of the extract can be mixed with sea water and added to the gills of the female to induce a typical spawning reaction (Galtsoff, 1940). Spermatozoa of *C. virginica* carry another hormonelike substance which may be recovered after alcohol extraction. The substance was named "diantlin" by Nelson and Allison (1940), who found that it dilates the ostia and stimulates the increase of water flow through the gills.

FREQUENCY OF SPAWNING

Under laboratory conditions male oysters may be induced to spawn many times at very brief intervals. It is, therefore, reasonable to assume that they behave in a similar way in their natural environment. The females spawn only a limited number of times within one breeding period. Out of several hundreds of oysters tested in the laboratory, the majority of the females were induced to spawn two or three times within a 6-week period (July–August), and only one spawned seven times. Similar conditions exist with *C. gigas*. Frequently a substantial percentage of females of these two species fail to shed eggs in spite of a ripeness of their ovaries and favorable environmental conditions. The spawn may be retained in the gonads until late fall when it is reabsorbed.

The number of times the adult female oysters spawn under natural conditions can only be surmised from examination of gonads and the occurrence of larvae in plankton. It is very difficult to decide from plankton observations whether the entire oyster population spawned several times or if different groups of oysters produced the larvae appearing at intervals in plankton. In Long Island Sound, for instance, four or more "waves" in the occurrence of straight hinge larvae were recorded (Loosanoff and Nomejko, 1951a) in 1943, but the described periodicity may have been due to spawning of different populations living in shallow and deep water. Inasmuch as the laboratory experiments show that repeated spawning may be induced in the same female, it is reasonable to infer that in their natural habitat oysters spawn more than once during every breeding season.

Laboratory observations show that spawning of sexually mature *C. virginica* is sometimes inhibited and that the oysters fail to respond to all known methods of stimulation. Similar conditions exist with *C. gigas*, which sometimes fail to spawn in spite of full gonad development. Artificial stimulation by suspension of sex cells may facilitate spawning but is not always successful. The reason for this inhibition of spawning in sexually ripe oysters has not been established, but the work of Lubet (1955) on *Chlamys* and *Mytilus* throws some light on the problem. Lubet discovered that the excision of cerebral ganglia in these mollusks provokes precocious spawning and that the mutilated animals spawn much earlier than the controls. Excision of the visceral ganglia seems to retard spawning. These experiments suggest that spawning is under the control of the nervous system. It also appears significant that neurosecretion in the ganglia cells precedes gametogenesis and that maximum accumulation of the neurosecretory products occurs at the time of the maturation of sex cells. In the species studied by Lubet the neurosecretory granules were absent in the ganglia of the recently spawned out animals. Whether Lubet's findings on neurosecretion apply to sexually mature oysters is not known, but his work seems to indicate that in the bivalves he studied, the release of sex cells was facilitated by the removal of internal inhibition (excision of cerebral ganglia) and that the disappearance of the neurosecretory products from the cerebral ganglia was necessary for the mollusk to become receptive to spawning stimuli. The latter inference is based on the observation that partial disappearance of neurosecretory granules always occurs a few days before spawning. After the completion of spawning all neurosecretory cells are empty. These findings are not in accord with the results of studies conducted by Antheunisse (1963) on zebra mussels (*Dreissena polymorpha* Pallas) from the Amstel River near Amsterdam. The mussels were collected once a month, from November 1957 to November 1958, for histological examination. For extirpation experiments only adult females were used during the spring and

summer of 1959. Antheunisse states that in spite of the parallelism between the neurosecretory and reproductive cycles in zebra mussels spawning and neurosecretion are not interrelated. Extirpation experiments were difficult to perform, and most of the mussels died some days after the operation. It is therefore apparent that further studies are needed to determine the role of the neurosecretion in reproduction of mussels, oysters, and other bivalves.

FECUNDITY OF THE OYSTER

The intensity of spawning as judged by the number of eggs or spermatozoa discharged in each instance is variable. In both sexes the number of sex cells produced by a ripe female or male depends on the size of the oyster and the degree of development of the gonad. The range of variation is enormous. If the female gonad is poorly developed, only a few thousands of eggs may be released. On the other hand, the number of eggs produced and discharged by a well-developed gonad may reach many millions. Potential capacity of the ovary, i.e., the total number of eggs produced by a female during the breeding season, is not indicative of its actual reproductive ability which is expressed by the number of eggs actually spawned. The following procedure is used for estimating the number of eggs released by the female. The oyster is placed in a 20 l. tank and spawning is stimulated by warming the water and adding sperm suspension. After the completion of spawning five samples of 100 ml. each are taken while the water is agitated by an electric stirrer. Eggs in the sample are killed by adding two to three drops of 1 percent osmic acid, allowed to settle, and are counted in a Sedgwick-Rafter chamber. The oysters used in four separate tests varied from 9.2 to 13.3 cm. in height. The number of eggs (in millions) discharged in one spawning were 15, 30.3, 70.3, and 114.8 (Galtsoff, 1930b). After discharging over 100 million eggs the last oyster had a gonad about 5.5 mm. thick containing vast numbers of eggs.

The results of these counts were questioned on the basis that the computed volume of the discharged eggs exceeds the total volume of the body (Burkenroad, 1947). Rechecking the data confirmed my estimate. The counts are correct within ± 10 percent, the principal source of error being the difficulty in obtaining uniform distribution of eggs in the tank.

In the ovary the eggs are tightly packed and compressed; upon their release the diameter of their rounded part is increased. The spawned eggs in the above tests averaged 40 μ in diameter. The volume of a given number of eggs can be computed by using the conversion table from diameters to volumes of spheres given in Perry (1941). Since the volume of one egg of 40 μ diameter is 33,510.3 μ^3, the volume of 115 millions of eggs, solidly packed would correspond to 3.8 cm.^{3} With an allowance of 25 percent for interspaces the volume of spawned eggs in the ovary would be about 4.8 cm.^{3} The latter figure is within the range of magnitude of the volume of the gonad obtained by the displacement method.

Not all the ovocytes become mature at the same time. During the intervals between spawning some of them grow and replace those discharged by the preceding ovulation.

The fecundity of *C. gigas* is even greater. The five large oysters of this species forced to spawn in the laboratory averaged 55.8 million eggs per oyster; post mortem examination showed that after ovulation they retained the major part of the gonadial material. In comparison to *C. virginica* and *C. gigas* the fecundity of the larviparous European oyster is rather low. Estimates of the mean number of larvae per oyster were made by Dantan (1913), Cole (1941), Cerruti (1941), and Millar (1961). In British waters the mean number of larvae vary from 90,000 for a 1-year-old oyster to over a million for a 4-year-old oyster. French oysters relaid in West Loch Tarbert, Scotland, after 1 year produced as many larvae as the native oysters on English beds. The number of larvae is dependent, of course, on the size of the oyster, as can be seen from the table given by Millar.

Diameter in cm.	Mean number of larvae estimated from five samples	Diameter in cm.	Mean number of larvae estimated from five samples
6.2–7.3	733,400	7.4–7.6	1,155,000
7.3–7.4	1,543,000	8.3–8.5	616,000

The fully grown *O. lurida* bear broods of 250,000 to 300,000 larvae, the number depending generally upon the size of the maternal oyster (Hopkins, 1936, 1937).

SEX RATIO, HERMAPHRODITISM, AND SEX CHANGE

The oviparous species of oysters of the genus *Crassostrea* usually are not hermaphroditic; specimens in which functional eggs and sperm are found together are relatively rare. This condition exists in *C. virginica, C. gigas, C. angulata, C. rhizophorae,* and is probably common to all members of the genus. The frequency of hermaphroditism in *Crassostrea* oysters varies with age and environment. The earliest record was made by Kellogg (1892), who found one hermaphrodite among the many adult *C. virginica* he kept in breeding tanks. Burkenroad (1931) reported that about 1 percent of the oyster population on the coast of Louisiana were hermaphrodites. Needler (1932a, 1932b) found only four hermaphrodites (less than 0.4 percent) among the 1,044 oysters of various ages growing on beds in the waters off Prince Edward Island. The hermaphrodites were found only among the 2-, 3-, and 4-year-old oysters; none were encountered in oysters from 5 to 8 years old. In the course of my studies I found only two hermaphrodites among several thousand sexually ripe oysters from 5 to 7 years old.

Amemiya (1929) reported only one hermaphroditic specimen among 120 sexually mature *C. gigas* (0.8 percent). The percentage is apparently higher in the Bombay oyster, *O. cucullata,* for Awati and Rai (1931) reported 23 hermaphroditic specimens (2.9 percent) among the 794 oysters they examined

The larviparous oysters of the genus *Ostrea* (*O. edulis, O. lurida, O. equestris,* and others) are as a rule ambisexual, i.e., they undergo rhythmical changes in sexuality. The initial phase in these species is usually male, followed by alternating female and male phases.

Orton (1927) distinguishes several arbitrary categories of sexual changes in *O. edulis* from pure male or female to hermaphrodites which contain an equal abundance of ripe spermatozoa and ova. Different transitional phases of sex changes which take place during the life of the European oyster are discussed later in this chapter.

Oysters have no secondary sexual characters, and their sex can be recognized only during the reproductive periods by microscopic examination of gonads. Sperm suspension, which can be forced out by gentle pressure on the surface of the gonad, is viscous and white; the suspension of eggs is creamy and has a granular appearance. Sex determination made with the naked eye should be verified by microscopic examination of smears.

In many species of bivalves sex is unstable, and hermaphroditism and alternation of sex are common. With respect to sex change oysters fall into two groups: oysters in which sexual phases change regularly in a definite rhythm, as in *O. edulis, O. lurida;* and those belonging to the *Crassostrea* type, in which the sexes of the adults are separate, as in *C. virginica, C. gigas, C. angulata,* and *O. cucullata.* The gonads of the oysters of the first group contain functional ova and spermatozoa simultaneously. These oysters are hermaphrodites. In the second group hermaphroditic individuals are relatively rare.

The difference between the two groups is not as explicit as it appears since the primary gonad of *Crassostrea* is bisexual, i.e., it contains the germinal cells of both sexes.

As early as 1882 the outstanding Dutch naturalist, Hoek (1883), in his studies of *O. edulis* made the important observation that "at the time when an oyster is sexually mature, it always functionates as a male as well as a female; it is, therefore, physiologically dioecious." The significance of this important discovery was appreciated nearly half a century later after Orton (1927, 1933) showed experimentally that maleness developed in 97.3 percent of young or adult females which carried eggs, embryos, or larvae. He further established the fact that the earlier state of maleness was always found in the more recently spawned females. Great advances in the understanding of sex changes in *O. edulis* and other species were made by the works of Stafford (1913) on *O. lurida,* by experimental research conducted by Spärck (1925), and particularly by observations on the American species made by Coe (1932–41). It was clearly established by these investigations that the young oysters of the larviparous species (*O. edulis, O. lurida*) become sexually mature first as males then gradually change into functional females; later they become males again, and such alternation with some modification continues throughout life. Comparable phases of sex changes occur in the *Crassostrea* species although the rhythm of sex alternation is different. At the age of 12 to 16 weeks the primary gonad of *C. virginica* is bisexual (ambisexual, according to Coe's terminology) since both ovogonia and spermatogonia are found in the same follicles

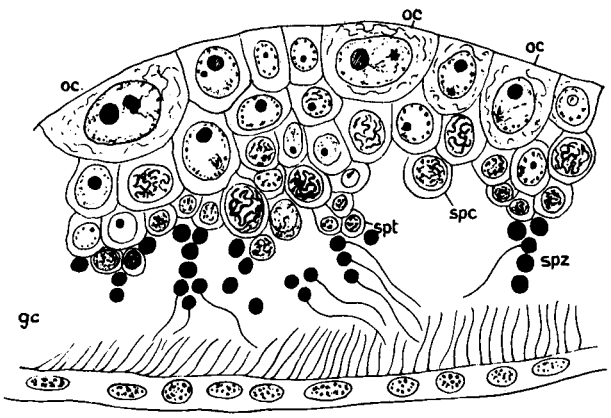

FIGURE 284.—Portion of bisexual gonad in young male *C. virginica*. gc—genital canal; oc—ovocytes; spt—spermatid; spc—primary spermatocyte; spz—spermatozoa. Photographically reproduced from Coe, 1934, fig. 5.

(fig. 284). At this stage the *C. virginica* gonad resembles that of *O. lurida* at the completion of the male phase and transition to female (fig. 285). In *C. virginica* the spermatogonia proliferate more rapidly than do the ovogonia and soon the young gonad attains a predominantly male appearance. Variation in the rhythm of gonad development in the oysters from different localities and even among those occupying the same bed results in different "categories" or "phases" of maleness or femaleness.

Development of the primary bisexual gonad in young *C. virginica* in New England waters is checked by the approach of winter when the growth of ovocytes is inhibited while the number of spermatogenic cells increases. A small number of spermatids may be formed early in November when the oysters are about 4 months old. The spermary of these secondary males contain scattered ovocytes, many of which degenerate, but some of them continue to develop into ova capable of fertilization. Even at the stage designated by Coe (1934) as "true male" the spermary at sexual maturity still retains a small number of ovocytes on the walls of the follicles.

At the close of the first breeding season many undifferentiated cells remain in the gonad to form the germinal cells of the following year.

Transformation of a bisexual gonad of *C. virginica* into an ovary begins before the formation of spermatozoa. At this stage the spermatogenesis is inhibited by the growth of ovocytes and the female phase is attained in a certain percentage of young oysters. The protandry, i.e., the development of maleness before the female phase, is well pronounced in *C. virginica*.

At their first breeding season young oysters form several sex classes: immature individuals in which the sex cells have not differentiated; males; hermaphrodites in which functional spermatozoa and ova are found in the gonad; and females. Hermaphroditic oysters are capable of self-fertilization and produce apparently normal larvae. The relative abundance of different sex phases of young oysters varies greatly, as can be seen from table 34,

TABLE 34.—*Frequency of different sex phases among* C. virginica *at first breeding season*

[According to Coe, 1934]

Locality	Immature	Hermaphrodite	Males	Females	Total examined
W. Sayville, N.Y.	77	4	154	48	283
Great South Bay, N.Y.	21	0	197	7	225
New Haven, Conn. (1932)	17	4	389	13	423
New Haven, Conn. (1933)	3	0	129	7	139
Woods Hole, Mass.	373	3	9	4	389

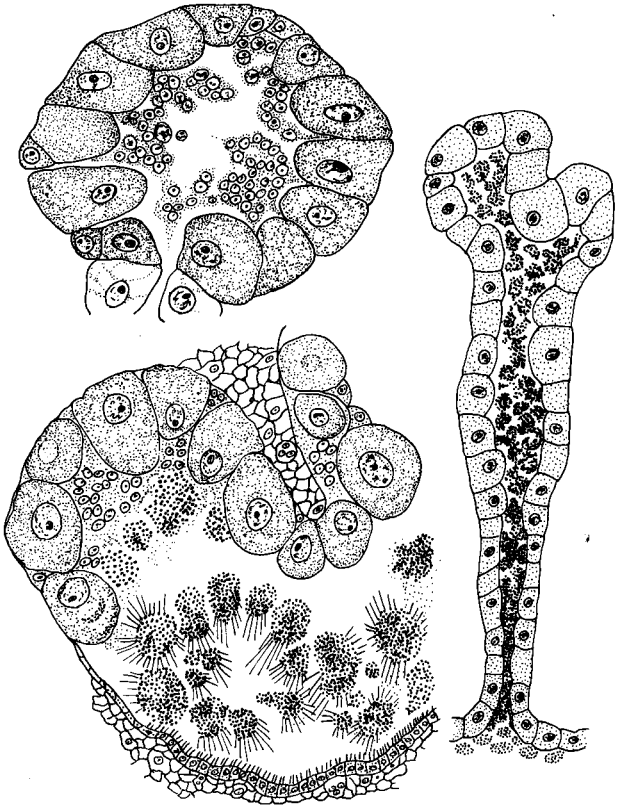

FIGURE 285.—Transition from male to female phase in *O. lurida*. Lower left—genital canal filled with sperm balls ready to be discharged; spermatogonia on the surface of large ovocytes. Right—advanced male phase in an older oyster; ovarian follicle is packed with cells in the later stages of spermatogenesis. Upper left—female phase preceding ovulation; many spermatogonia in the lumen of the follicle. Redrawn from Coe, 1932c.

which summarizes Coe's observations (1934) made on oysters from four different places along the coast of Massachusetts, Connecticut, and New York.

During the second breeding season the number of males may still exceed that of the females, but generally the sex ratio approaches equality. Great differences in the degree of protandry among the 1-year-old oysters is associated with the differences in the growth rate. Coe's observations suggest that there is a correlation between the development of ovocytes in the bisexual gonad and the rate of body growth. At the first breeding season the average size of the females is much larger than that of males (Needler, 1932a, 1932b). Coe (1934) found that the mean height of 389 yearling males from the New Haven area was 31.28 mm. (Std. dev. ±6.33) and that of the 13 females of the same age 38.54 mm. (Std. dev. ±8.12). The difference does not seem to be statistically significant, and several interpretations were advanced by Coe. He suggested that the females require more favorable conditions in order to mature, that they are metabolically more active, and that at the critical period of sex differentiation the metabolic factor determines the predominance of the male or female cells in the primary bisexual gonad. These proposals require corroboration. Since it is known that the growth rate of young oysters is accelerated by keeping them suspended above the bottom, there apparently would be no difficulties in conducting a comparative test using slow and fast growing oysters selected from a single population.

After spawning the gonad of *C. virginica* retains its bisexual potencies and its sex may alternate in either direction. Needler (1932a, 1932b) was the first to demonstrate that such a change actually occurs among adult *C. virginica*. She found that out of 24 surviving oysters which were known to be males during one summer, 5 became functional females the following year, and out of 12 females 5 changed to males. Among the 57 *C. virginica* studied by Needler (1942) for a period of 4 years there was a high proportion of males which remained unchanged while other oysters changed sex at least once. Some of the individuals changed sex every year. Needler suggested, without providing corroborative evidence, that the sex of the males which remained unchanged was genetically determined and that the other oysters in which sex alternation occurred at random were hermaphrodites.

Adult Japanese oysters, *C. gigas*, may change their sex during the interval between the two breeding seasons. Amemiya (1929), who established this fact, found that the rate of change was higher in the males (60 percent) than in the females (25 percent). Sex change occurs also in *C. commercialis;* 95 percent of the very young oysters of this species were found by Roughley (1933) to be males, but among the adult specimens of large size the females predominated at the rate of 270 to 100 males.

The sex of the oysters used in the investigations by Needler and Amemiya was determined by drilling a hole in the shell and pinching off a piece of gonad for microscopy. The injury caused by the operation constitutes a factor which may affect the unstable gonad and influence its sex change. By removing about one-third of all gill lamellae in adult *C. gigas* at the time when the gonad was at the indifferent phase after spawning, Amemiya (1936) demonstrated that the percentage of males in the mutilated group in all cases was larger than those in the control. Removal of the gill tissue may have indirectly influenced the development of male sex by reducing the rate of feeding and growth. This assumption also needs further corroboration.

Injury to the oyster used for observation on sex change can be avoided by inducing spawning in each individual oyster, obtaining kymograph tracings of muscular contractions, and examining the discharged sex cells. This technique was employed in the Woods Hole laboratory. In a test which continued for 5 consecutive years, 4-year-old oysters were obtained from one of the private oyster beds near Onset, Mass. During the first summer 202 oysters were induced to spawn, their sex was recorded, and an identifying number was engraved on the right (upper) valve. Upon completion of the tests the oysters were returned to outdoor tanks or were placed in the harbor and remained there until the next reproductive season. The testing was repeated every summer (Galtsoff, 1961).

Because the males respond to spawning stimuli more readily than the females, their number at the beginning of the experiment was greater than that of the females. The disparity does not represent an actual sex ratio of the population of 4-year-old oysters which was found to be about 1 to 1. The mortality, especially among the 7-year-old oysters

used in the test, was high, and the number of nonspawning oysters gradually increased toward the end of the experiment (table 35).

When oysters failed to respond to spawning stimulation, the testing was repeated at 4- to 5-day intervals for 5 consecutive weeks. Negative results were assumed to indicate the oysters were nonfunctional sexually, and they were returned to the holding tanks for another year. In several instances the oysters that failed to spawn became sexually active the following breeding season. It is not known at present whether the increased number of failures to spawn and increased mortality (table 35) should be attributed to aging or to unfavorable conditions in the winters. On several occasions the holding tanks and live cars in which the oysters were kept were swept by stormy waters and everything inside was covered with a deposit of mud.

TABLE 35.—*Changes in the percentage of sexes in a selected group of* C. virginica *tested consecutively for 4 years*

[Initial number of males and females 4 years old does not represent the normal sex ratio which was about 1 to 1]

Age in years	Males	Females	Non-spawning	Total survivors
Number	*Percent*	*Percent*	*Percent*	*Number*
4	64.4	35.6	0	202
5	65.2	32.6	2.2	181
6	61.2	31.6	7.2	139
7	50.1	40.3	9.6	104
8	44.1	41.2	14.7	68

During the 5-year period of observations the number of spawning males decreased from 119 to 15 in 9-year-old mollusks. The number of females decreased from 63 to 18. Consequently the sex ratios of males to females of the experimental oysters changed from 1.9:1 at the beginning to 0.8:1 at the end of the observations. The predominance of oysters of female sex in the surviving oysters can not be attributed to more frequent sex changes from male to female. In table 36 no significant differences were recorded in the rates of sex alternation in the two groups. The predominance of females at the end of the test could be explained, therefore, by greater survival rate of oysters at the female phase. This interesting point requires further corroboration.

Out of the 68 survivors at the end of the breeding season of the fourth year, 31 had alternated their sex at least once during the period of testing (Galtsoff, 1961). The frequency of changes were as follows:

One change	18 instances.
Two changes	10 instances.
Three changes	2 instances.
Four changes	1 instance.

The oyster which changed sex every year was a male at the beginning and returned to the male phase at the last test. No distinct pattern is apparent in the rhythm of changes except the greater persistence of the female phase. The sex ratio within the sex-reversed oysters changed from 23 males and 8 females at the beginning to 11 males and 20 females at the end of the observations.

TABLE 36.—*Frequency of sex alternation in adult* C. virginica *from 5 to 9 years old*

[The figures in the table indicate the number of males (column 2) and females (column 5) of each age and the number and percentage of changes to females (columns 3 and 4) and to males (columns 6 and 7) that occurred during each year]

Age in years	Males	Changes to females		Females	Changes to males	
Number	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
5	119	10	9.2	63	11	17.4
6	88	10	11.3	38	9	23.7
7	65	15	23.1	33	3	9.1
8	25	3	12.0	24	6	25.0
9	15	1	6.7	18	1	5.6

The cause of sex instability and the factors which may influence the shift of a gonad from one sex to another are not known. Coe believed that the physiological state of the organism in each breeding season is the key to the determination of the sexual phase of the oyster. No concrete proof to substantiate this idea can be found in his experiments. Egami (1952) attempted to transplant pieces of gonad of *C. gigas* to another oyster of the same or of the opposite sex. He found no evidence that the sex of the host affected the sex differentiation of the graft and concluded that the sex of the grafted pieces has been determined at the operating season (December) at their morphologically undifferentiated state. In another work (Egami, 1953), he corroborated the results of Amemiya's observation on the decrease of growth rate of *C. gigas* by the removal of gill tissue and the increase in the percentage of males among the mutilated oysters. He concluded that maleness could not be attributed directly to mutilation but was associated with the decreased growth rate of oysters without gills. Egami found that among normal individuals of *C. gigas* those growing more rapidly during the autumn tended

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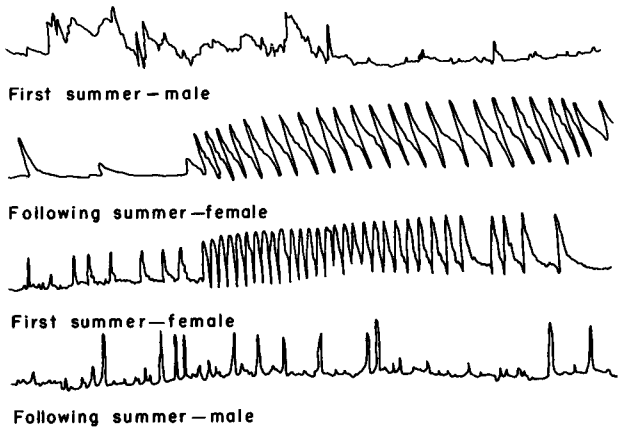
to develop into females the following reproductive season.

The conclusions may be considered only tentative since they are based on a small number of experiments which need to be repeated on a larger and more comprehensive scale. Sex alternation in oysters offers fascinating possibilities for further research on this fundamental biological problem.

The spawning reactions of sex-reversed oysters, as reflected in the type of shell movements and in the manner of dispersal of sex cells, are in every respect identical to those of the reactions of true males and females (fig. 286). In some sex-reversed oysters the change from male to female behavior is however delayed. Examination of records shows that in several instances females which were males during the preceding year spawned at the beginning of the reproductive season in a male fashion, discharging the eggs through the cloaca. Full female reaction involving rhythmical contractions of the adductor muscle was fully developed toward the end of the spawning season (fig. 287). It can be inferred from these observations that the mechanism of female sexual behavior develops at a slower rate than the morphological changes in the gonads. There was no retention of female behavior in oysters which returned to the male phase. The male reaction was apparent in them at the beginning of the season.



FIGURES 286.—Kymograph records of sex-reversed male (two upper lines) and sex-reversed female (two lower lines) *C. virginica* recorded at two consecutive breeding seasons. Both oysters were 5 years old at the "first" summer. In both instances eggs were dispersed through the gills, the sperm through the cloaca. Time interval, 1 minute.

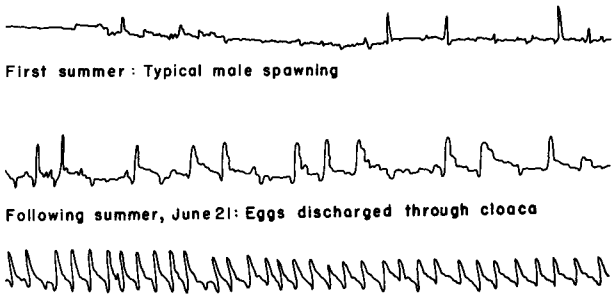


FIGURE 287.—Spawning of the sex-reversed male *C. virginica*. First line—spawning at the male phase. Second line—sex of the gonad changed to female; spawning proceeds in the male fashion. Third line—typical female spawning. Oyster was 5 years old during the "first" summer. Time interval, 1 minute.

In one instance the spawning of an hermaphroditic oyster was recorded (Galtsoff, 1961). Both eggs and sperm were discharged simultaneously through the cloaca, and the rhythmical contractions of the adductor muscle were not fully developed (fig. 288). A small portion of the gonad of this oyster is shown in fig. 289. Microscopic examination of the tissue revealed the presence of relatively few mature eggs in the follicles occupied by spermatozoa. Spawning of this oyster was induced by raising the water temperature. Eggs removed from the spawning tank were found to be fertilized and their development traced to trochophore stage was normal.

LUNAR PERIODICITY

The modern zoologist may disregard the early popular beliefs and superstitions which endowed the moon with mysterious effects on human affairs, on animals, and plants; nevertheless, he is confronted with several undeniable instances of lunar periodicity in the reproduction of marine invertebrates. Probably the most famous and generally known examples are the swarming and breeding of the Palolo worm (*Eunice viridis* Gray) of the South Pacific at the moon's last quarter of

FIGURE 288.—Shell movements of a spawning hermaphroditic *C. virginica*. Temperature 24.5° C. Time interval, 1 minute.

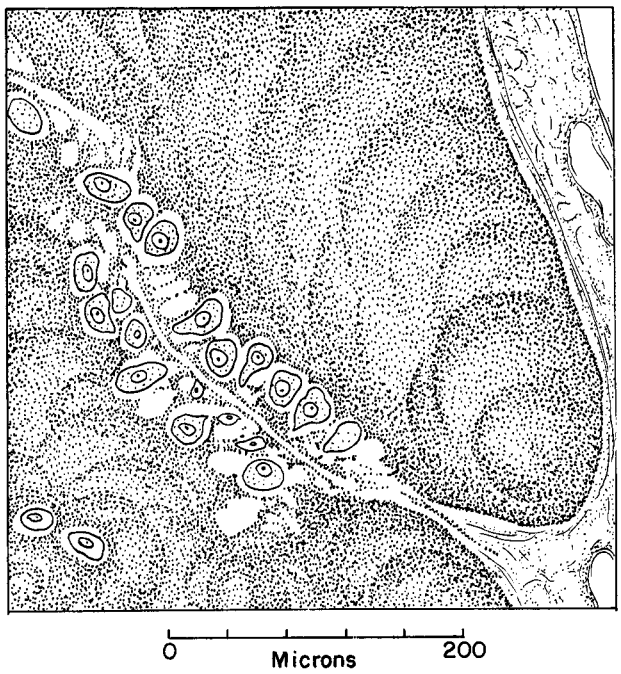


FIGURE 289.—Section of a small portion of an hermaphroditic gonad of *C. virginica*. Bouin, hematoxylin-eosin.

October and November; the swarming of the Atlantic Palolo (*Odontosyllis enopla* Verrill) at Bermuda and *Eunice fucata* Ehlers, at Tortugas, Florida (Mayer, 1908); and the breeding habits of *Heteronereis* form of *Nereis limbata* at Woods Hole (Lillie and Just, 1913). Legendre (1925) gives an interesting historical account of the effect of the moon on marine organisms. A comprehensive review of the instances of lunar periodicity of breeding among many marine invertebrates, including several species of pelecypods, is given by Korringa (1947).

Evidence of a relationship of breeding of *O. edulis* to moon phases was first presented by Orton (1926), who examined weekly samples of adult oysters from Fal estuary and found two important maximums in spawning at the full moon spring tides in the year 1925. He further concluded that the population as a whole gave maximal percentage of spawn (based on presence of embryos in the oysters) in the weeks after the July and September full moons. Later observations by Korringa (1941, 1947) in the commercial oyster district of Oosterschelde, Holland, confirmed the existence of a relationship between breeding of *O. edulis* and moon phases. He found that the full moon exercises the same influence on the breeding of oysters as does the new moon. Korringa based his studies on determinations of the time and abundance of oyster larvae in plankton and found a marked periodicity in the maximums of oyster larvae occurring about 10 days after full and new moon. Fluctuations in water temperatures, according to Korringa's view, are apparently of little or no importance in causing the periodicity in swarming which appears to be correlated with the spring tides. Unfortunately no experimental evidence is available to substantiate this inference which is based entirely on the concurrence of the two phenomena.

Spawning of *C. virginica* has no relationship to lunar phases. The existence of such a relationship was postulated by Prytherch (1929), who stated that Long Island Sound oysters spawn "at the end of full moon tidal period, or eight days after the time of full moon," but the correlation could not be corroborated by careful studies of Loosanoff and Nomejko (1951a), who continued the observations in Long Island Sound over a period of 13 years after the termination of Prytherch's work. Negative results were also reported by Hopkins (1931) in Galveston Bay, Tex., and by R. O. Smith in South Carolina waters (unpublished reports on file in the Bureau of Commercial Fisheries).

BIOLOGICAL SIGNIFICANCE OF SPAWNING REACTION

The most outstanding single factor in oyster reproduction is the difference in spawning behavior of the two sexes. The males are more responsive to sexual stimulation than the females and are easily stimulated to spawn by rising temperatures and by a great variety of organic substances, some of them not found in natural sea water. The spawning response of the male is nonspecific.

The less responsive, sexually mature females require stronger stimuli and are highly specific to chemical stimulation; they respond only to suspensions of sperm of the same or related species and are indifferent to the sperm of other bivalves and various chemical substances tested. The specificity of females is an insurance that eggs cannot be discharged when there is no sperm in the water.

Males are usually the first to initiate spawning; the discharge of sperm by even one individual induces spawning by those next to it, and the process spreads over the entire oyster community. This sequence has been observed among oysters

FIGURE 290.—Spawn of *C. gigas* carried down by the tidal river near Vancouver, British Columbia. Courtesy of D. Quayle. Black and white enlargement of Kodachrome slide.

kept on floats and among specimens living under natural conditions on oysters beds near low tide level. The spawning of an entire oyster population can be artificially initiated by mincing the meats of several sexually mature oysters and spreading them into the waters of the oyster bed. This method, based on my laboratory experiments, has been applied on a commercial scale by oyster growers in British Columbia (Elsey, 1936).

Simultaneous spawning of oyster populations is essential for the production of a large brood of oyster larvae and for obtaining setting of commercial value. In the estuaries where the majority of oyster beds are located, the tides carry the released spawn for some distance before the eggs sink to the bottom. A transport of oyster spawn by a strong current can be seen in the photograph (fig. 290) taken by Quayle near Vancouver, British Columbia, and kindly given to me for reproduction. The white streak in the foreground of the clear river water was formed by billions of eggs and sperm discharged by a population of *C. gigas* several miles up river.

The method of discharging sperm and eggs is also of considerable significance. Spermatozoa carried away by the respiratory current remain in suspension for several hours. When eggs are discharged in the same manner by some sex-reversed oysters, they rapidly sink to the bottom only a few inches away from the female. Laboratory observations indicate that under such conditions only a very small percent of them are fertilized or have even a slim chance of developing. On the other hand, eggs discharged in the usual manner through the gills and forcibly ejected from the mantle cavity, have a much better chance to be fertilized and survive. Furthermore, because of the specificity of female response to sperm, eggs are ejected only when the water contains free spermatozoa of the same species. The female spawning reaction is an adaptation of an oviparous organism to the conditions of its existence and assures the survival of the species.

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