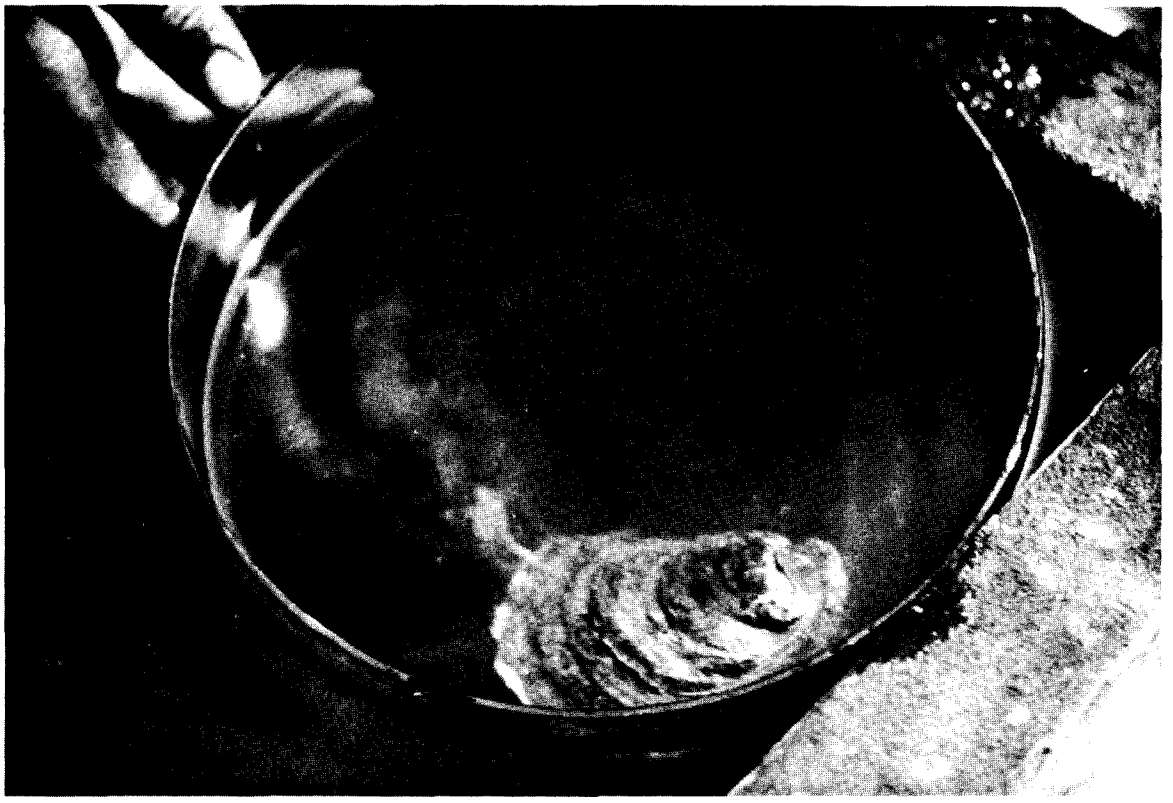

FIGURE 278.—Spawning of large female *C. virginica*, photographed in the laboratory.

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

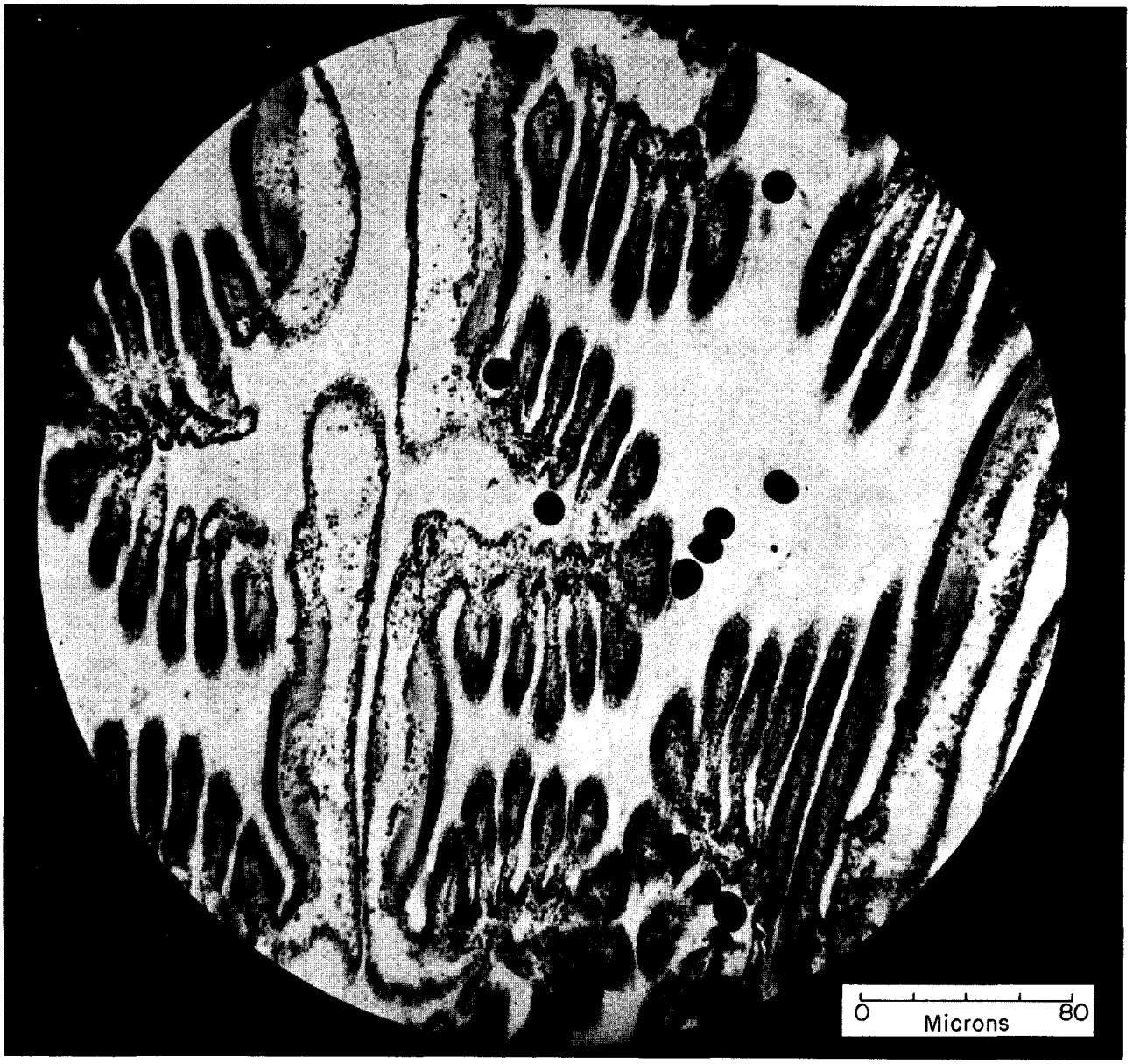

FIGURE 279.—Transverse section of the gills of female *C. virginica* preserved during spawning. Hematin-eosin. Note the eggs inside the water tube (center) and in the ostium.

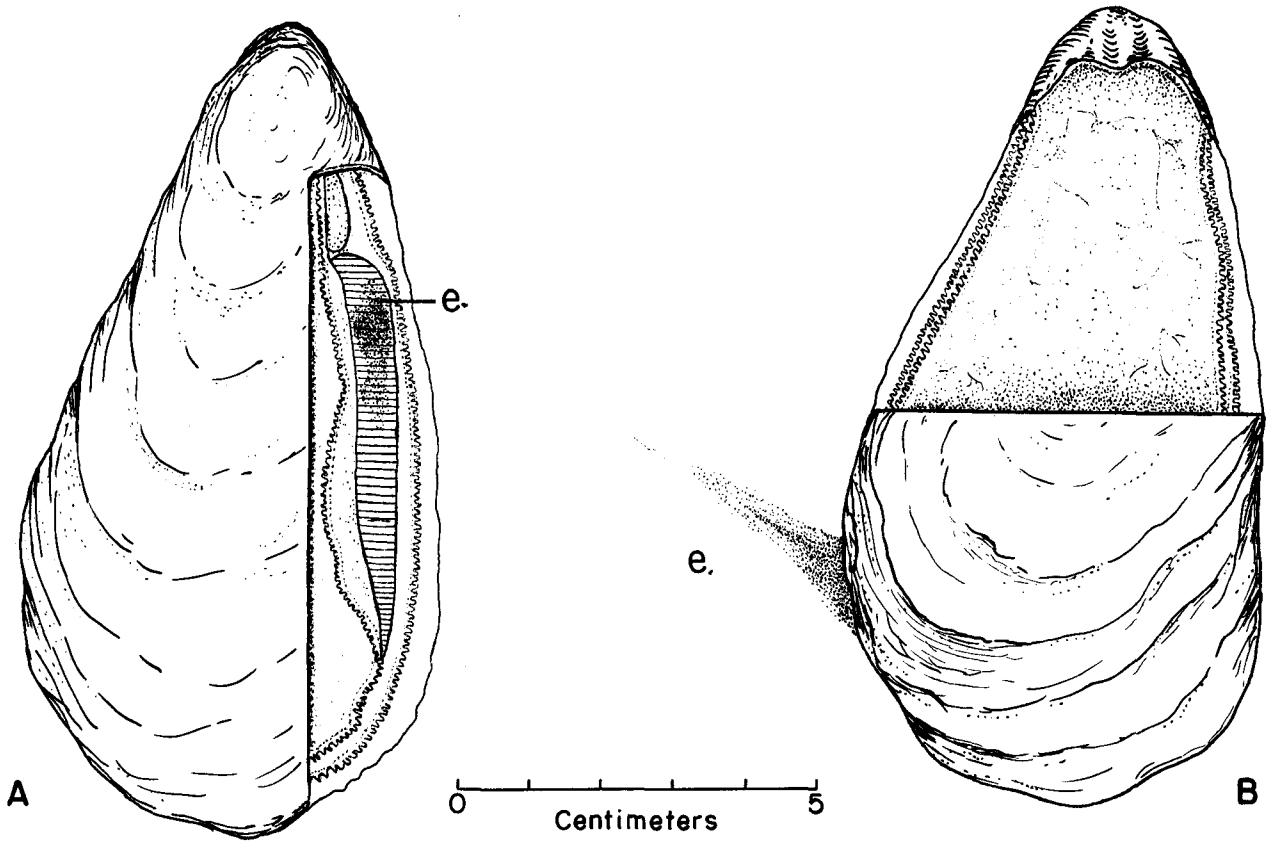

FIGURE 280.—Experiment showing the role of shell movements in the discharge of spawned eggs of *C. virginica* through the gills. A.—Portion of the right valve was removed to expose the gills; the adductor muscle was not injured and shell movements during spawning were normal. Eggs (e) pass through the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

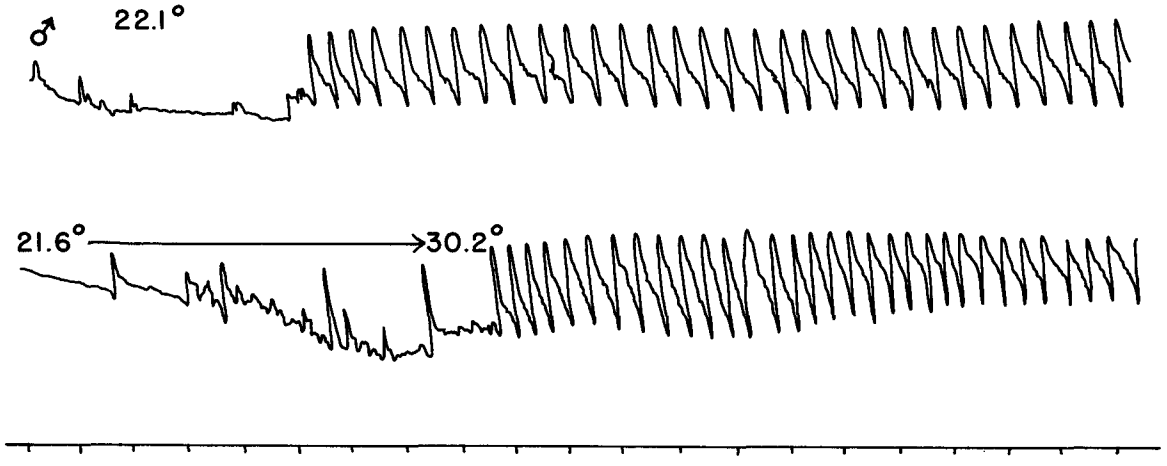

FIGURE 281.—Kymograph records of shell movements during spawning of two female *C. virginica*. Upper line—spawning induced by the addition of sperm at 22.1° C.; lower line—spawning induced by rapid rise of water temperature from 26.1° to 30.2° C. Time interval, 1 minute.

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

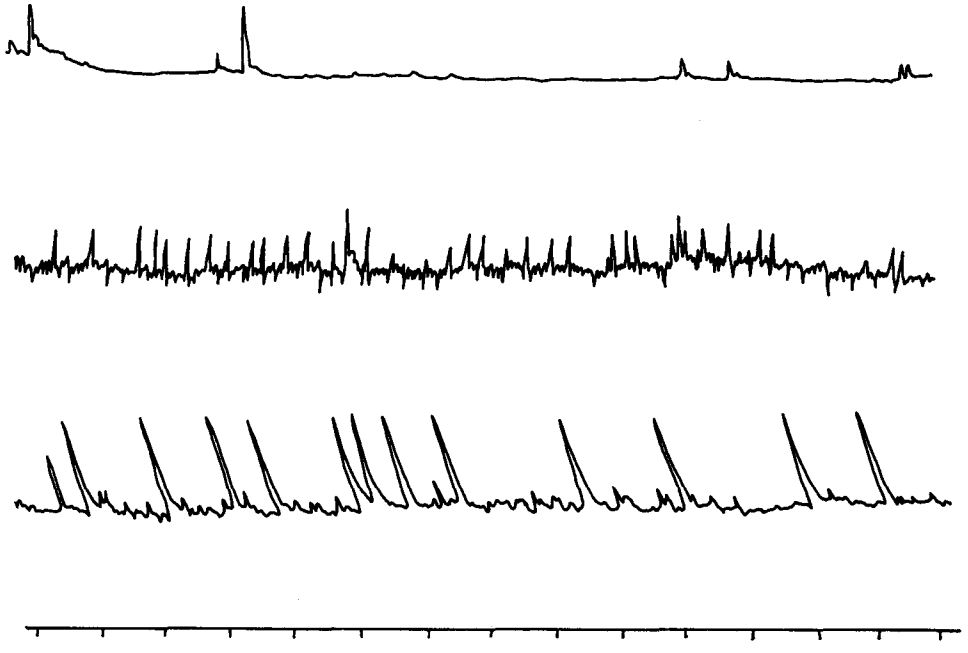

FIGURE 282.—Shell movements of three males of *C. virginica* recorded during the shedding of sperm. There was no change in muscular contraction before, during, or after spawning. Temperature 23.5° C. Time interval, 1 minute.

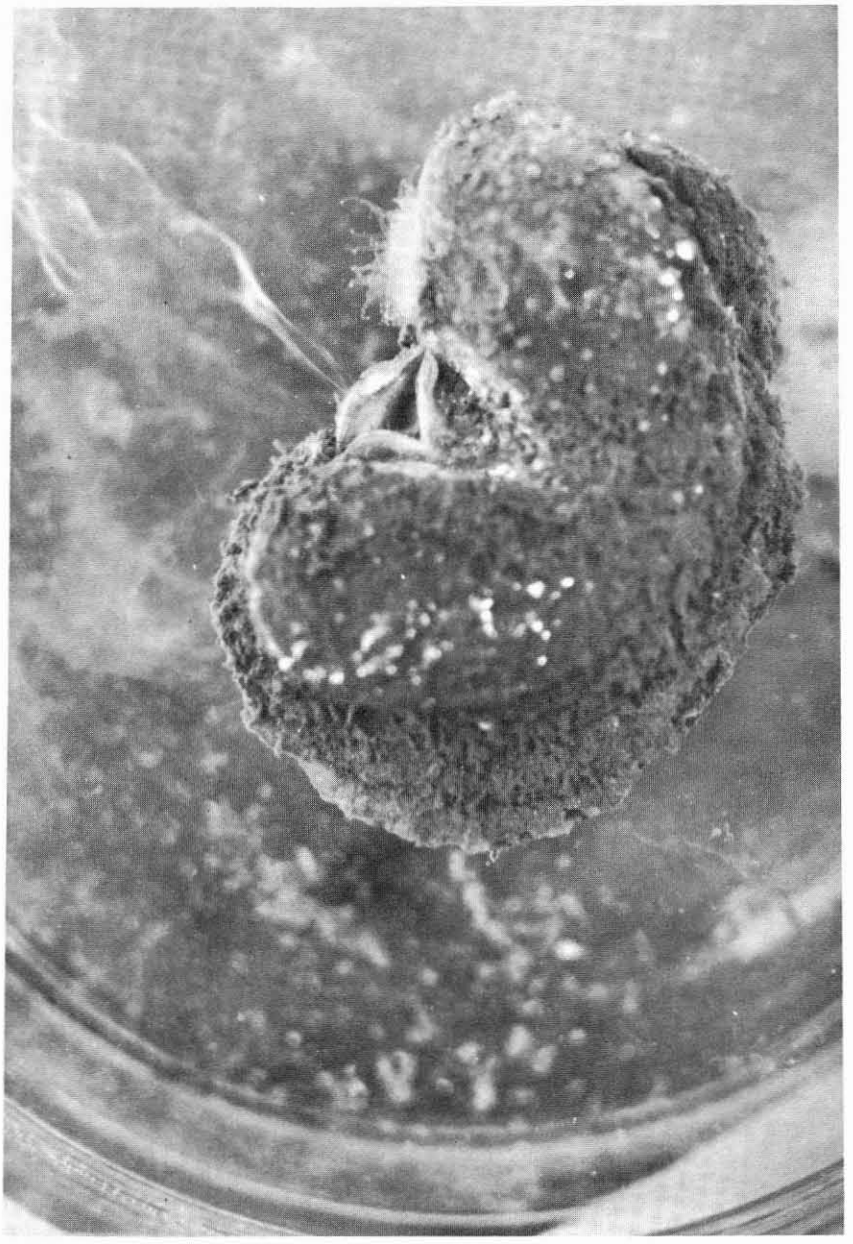

FIGURE 283.—Photograph of the spawning male, *C. virginica*. The V cut in the left valve was made several weeks before the experiment. Temperature 23.0° C. Sperm ejected through the opening of the promyal chamber is seen as a white jet opposite the V cut.

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