

PHYSIOLOGY OF REPRODUCTION OF *OSTREA VIRGINICA*

I. SPAWNING REACTIONS OF THE FEMALE AND MALE

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Sexual cycles of many marine organisms are determined by specific rhythms in the development of their gonads, and by environmental conditions which to a great extent control the discharge of ripe sex cells. The simplest sexual reactions are to be found among the animals which, lacking organs of copulation, shed their eggs and sperm directly into the water. In these cases fertilization is a matter of the chance meeting of ovum and spermatozoan outside of the organism, and the propagation of the species is dependent upon the production of large numbers of sex cells and the correct timing of their emission. The latter condition is significant for it enhances the probability that the eggs will be inseminated before they become unfertilizable. Since our knowledge of the physiology of the reproduction of marine invertebrates is very inadequate, conditions which govern the discharge of their sex products are but little understood.

The present investigation of the physiological reactions involved in the reproduction of the oyster was undertaken with the view of determining the factors controlling the sexual activity of this mollusk. Because of its widespread distribution, its abundance and the anatomical simplicity of its organs of reproduction, the common oyster of commerce appears to be very suitable for such a study. The work was carried on during a number of summers at Woods Hole, Massachusetts; Milford, Connecticut; and Galveston, Texas. Supplementary observations on *O. gigas* (Japanese oyster) and *O. commercialis* (Australian oyster) were made at the Hopkins Marine Station, Pacific Grove, California, and at the Marine Laboratory of the University of Hawaii in Honolulu. The first part of this study contains a description of the sexual reactions of the female and the male oyster. The subsequent parts, which will be published separately, deal with the stimulation and specificity of sexual reactions and their significance for the survival of the species.

REPRODUCTIVE CYCLE

The annual reproductive cycle in *Ostrea virginica* begins in autumn immediately after the completion of the preceding spawning period. At

this stage the gonad follicles are lined with indifferent germinal epithelium and the sexes are often indistinguishable. A transition to the next, the sex-differentiation stage, is marked by a slight expansion of the gonad tissue and the appearance of either ovo- or spermocytes. The gonad remains in this condition until the next spring when an extensive proliferation of the follicles accompanied by rapid ovo- or spermatogenesis results in the production of large numbers of sex cells. During the following summer almost all of them are discharged and the few remaining within the follicles are cytolized and destroyed by phagocytes.

The spawning season, during which one or several emissions may occur, lasts for about six weeks in the North Atlantic States (north of the Chesapeake Bay) but in the warm waters of the South Atlantic and Gulf of Mexico may extend from March to the end of October. In every body of water a great part of the oyster population spawns more or less simultaneously. This is evidenced by the appearance in plankton collections of a large number of oyster larvæ which often occur at regular intervals or cycles corresponding to the outbursts of sexual activity (Prytherch, 1928; Hopkins, 1931).

Young oysters, excepting those living north of Cape Cod, reach sexual maturity at the end of their first year (Coe, 1936) and barring unfavorable conditions, the mollusk breeds every year until its death. The fecundity of an oyster apparently does not decrease with age for in very large specimens measuring from 6 to 10 inches in length, the gonads may be well developed and contain large numbers of fertilizable eggs.

ANATOMY OF THE ORGANS OF REPRODUCTION

A brief description of the essential anatomical features of the gonads is necessary for an understanding of the mechanism of ovulation and ejaculation. In both sexes the gonads are paired structures consisting of a large number of profusely branching tubules situated within the layer of connective tissue immediately beneath the epithelial covering of the body. In a well-developed organ the ramifying tubules conalesce along the dorsal side, forming one continuous layer investing the visceral mass. Two distinct systems of genital canals and two gonoducts, one on each side of the body (Fig. 1, *GD*) remain the only signs of the paired condition of the gonad. The thickness of the gonad layer, measured in transverse section through the anterior part of the body at the level of the stomach, varies from year to year according to the number of cells produced during the winter and spring. In cases of extraordinarily prolific development this creamy and well-defined zone may reach over 20 mm. in thickness in an adult oyster. It may be less

than 1 mm. in a poorly developed gonad. It is not clear what conditions are responsible for these differences, which may occur from year to year in the same locality and in the same stock of oysters. This problem calls for special investigation which is beyond the scope of the present work.

In a well-developed gonad of an oyster, the branching and twisted tubules are crowded with sex cells. A few follicles can be found, the

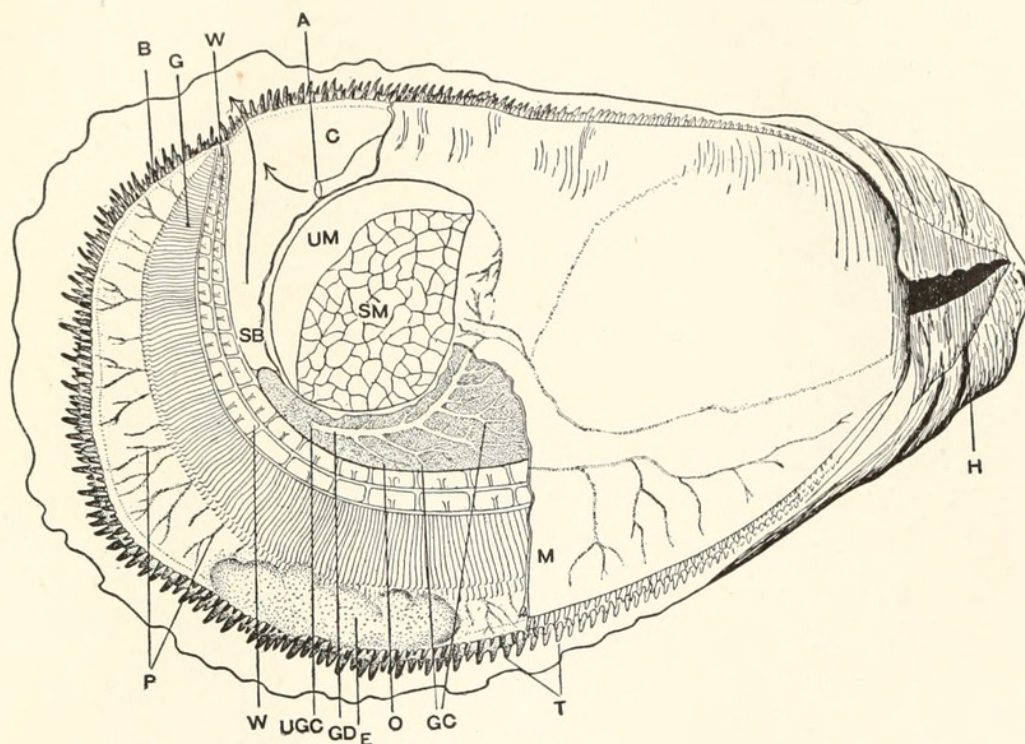


FIG. 1. Female *Ostrea virginica*, right shell and part of the mantle fold removed. The wall of the suprabranchial chamber dissected and gills pulled down to show the ovary and the water tubes of the gill lamellæ. $\times 1$.

A, anus; B, border of mantle; C, cloaca; E, eggs passed through the gills and accumulated in the pallial cavity; G, gills; GC, genital canals; GD, gonoduct (oviduct); H, hinge; M, mantle; O, ovary; P, pallial cavity; SB, suprabranchial chamber; SM, adductor muscle, portion with striated fibres; T, tentacles; UM, adductor muscle, portion with smooth fibres; W, water tubes of the gills; UGC, urinogenital cleft. Arrows indicate the direction of the cloacal current.

lining of which still consists of a single layer of undifferentiated germinal epithelium or of cells at various stages of spermatogenesis. The wall of each follicle is made of a thin layer of connective tissue fibres. The outermost portion of the gonad, adjacent to the surface epithelium, is occupied by an anastomosing system of genital canals which in a ripe specimen are easily recognizable with the naked eye. They form an arborescent structure (Fig. 1, GC) in which the branches increase in diameter toward the posterior end of the gonad.

The genital canals persist throughout the year and constitute the primary gonads, which, by the growth of their inner walls and a series of evaginations, give rise to the tubular secondary follicles. In transection the lumen of the genital canal appears as a narrow space between a thin body wall, consisting of a layer of connective tissue covered with epithelium, and a dense mass of ovarian or testicular follicles. The portion of the lumen adjacent to the body wall is lined with a ciliated epithelium while its opposite side, i.e., the inner arch of the lumen, is

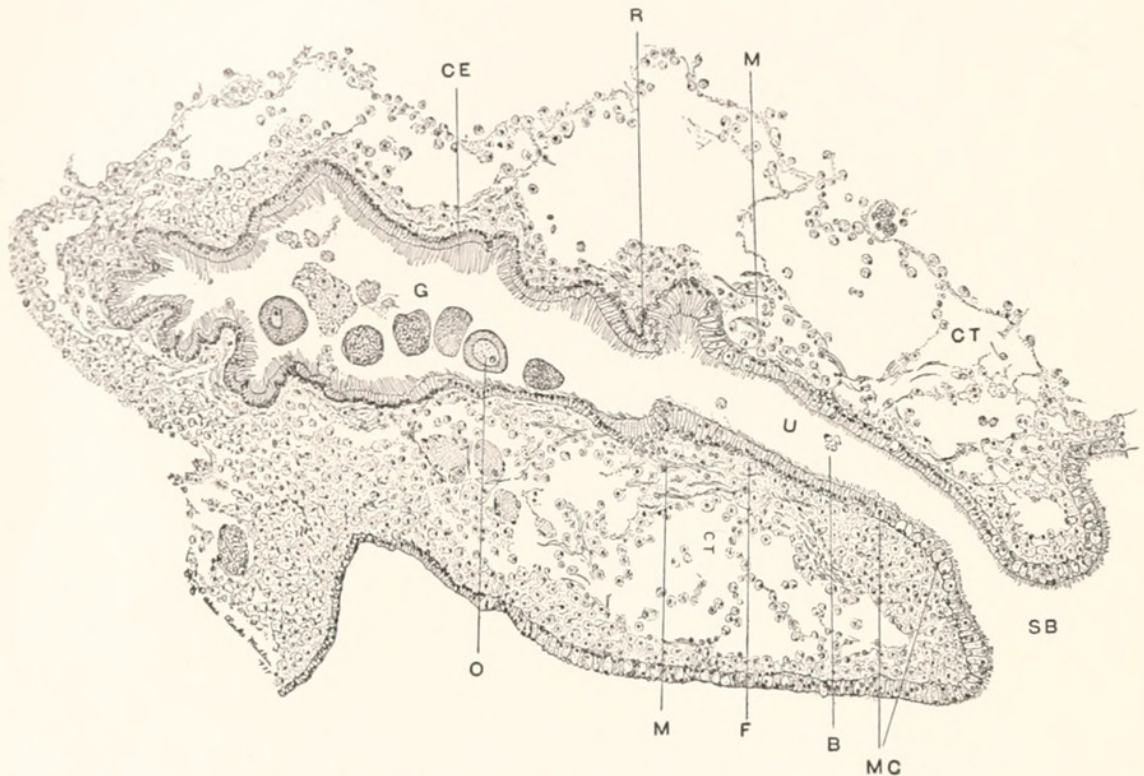


FIG. 2. *Ostrea edulis*, transverse section of the right urinogenital cleft and oviduct preserved during ovulation. Camera lucida drawing $\times 150$. Hæmatein-eosin.

B, blood cells; *CE*, ciliated epithelium; *CT*, connective tissue; *F*, connective tissue fibres; *G*, oviduct; *M*, muscle fibres; *MC*, mucous cells; *R*, ridge of ciliated cells separating the oviduct from the urinogenital cleft; *SB*, suprabranchial chamber; *O*, ripe eggs discharged from ovary; *U*, urinogenital cleft.

lined with germinal epithelium. Scattered muscle fibres are found here and there in the connective tissue of the gonad but are slightly more abundant in the body wall exterior to the genital canals. In both sexes the canals converge on each side of the gonad into a very short gonoduct (oviduct or spermiduct) through which the sperm or ova are discharged into a urinogenital cleft (*UGC*). In an adult oyster about 4 inches long, examined by the author, the oviducts did not exceed 0.15 mm. in length while the spermiducts were about 0.75 mm. long. When the

gonoduct is empty its lumen has a slit-like appearance with the ciliated lining folded in numerous ruffles (Fig. 2). In the ducts filled with eggs or sperm, the walls are greatly distended and the epithelial lining is stretched (Fig. 3).

The lumen of the gonoducts is completely lined with ciliated columnar epithelium which rests on a basal membrane surrounded by a layer of connective tissue fibres. The absence of germinal epithelium distinguishes the gonoducts from the genital canals although the transition from the canal to the duct is gradual. Circular and oblique muscles

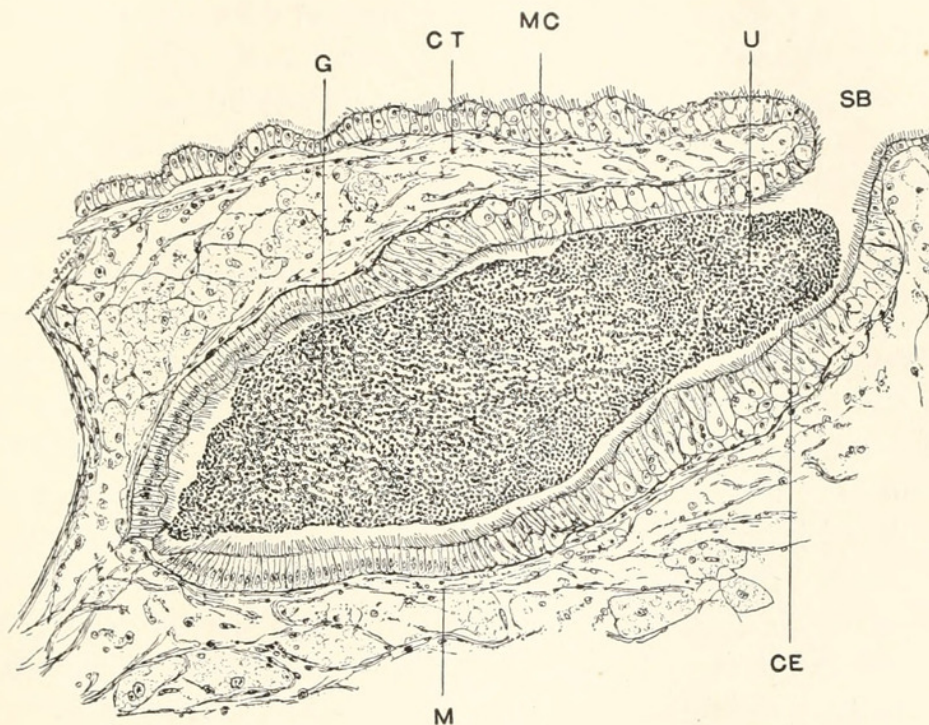


FIG. 3. *Ostrea edulis*, transverse section of left urinogenital cleft and spermiduct preserved during ejaculation. Both spermiduct (G) and the urinogenital cleft (U) are filled with ripe sperm. Camera lucida drawing $\times 150$. Hæmatein-eosin.

CE, ciliated epithelium; CT, connective tissue; G, spermiduct; MC, mucous cells; M, muscle fibres; SB, suprabranchial chamber; U, urinogenital cleft.

are scattered in the walls but do not form a continuous sheathing. In preparations stained with hematoxylin and eosin, the connective tissue fibres can easily be mistaken for muscle fibres but their true nature is clearly revealed in sections treated with the differential triple stain of Mallory which stains the connective tissue blue. Roughley (1933) states that in *Ostrea commercialis* the gonoducts are surrounded by bands of sphincter-muscle fibres immediately internal to the cleft. No such structures have been found in *O. virginica* although, as stated above, a few scattered muscles occur in the walls of the gonoducts.

Unfortunately no illustration of the sphincter is given by Roughley and a detailed comparison between the gonads of the two species of oysters is therefore impossible.

The urino-genital cleft into which the gonoducts open anteriorly and independently of the ureter is a narrow slit on the surface of the gonad (Fig. 1, *UGC*). A delicate membrane of connective tissue covered on both sides with ciliated epithelium forms the external portion of its wall. Histologically the cleft differs from the gonoduct only in the character of its epithelial lining which contains large numbers of mucous cells absent in the gonoduct (Figs. 2, 3, *MC*, *CE*). In the female the opening of the gonoduct into the cleft is marked with a well-pronounced ridge of tall ciliated cells not noticeable in the male (Fig. 2, *R*). On each side of the oyster the urinogenital cleft opens into a suprabranchial chamber in which a strong outgoing stream of water is maintained by the ciliary motion of the gill epithelium.

From an anatomical and histological study of the gonad it may be inferred that eggs and sperm are discharged primarily by the motion of the cilia of the epithelial cells lining the genital canals and gonoducts and that the contraction of the muscle fibres scattered between the follicles and in the walls of the canals is of lesser importance and probably only facilitates their release from the follicles. Judging by the distribution of the muscle and connective tissue fibres in the walls of the gonoducts, it appears possible that their contraction may to a certain extent constrict the lumen and thus impede the emission of sperm or ova. The muscle fibres appear to be better developed in the walls of the spermiducts than in the oviducts (Figs. 2, 3, *M*). Their possible rôle in ejaculation is discussed later.

SPAWNING REACTIONS OF THE FEMALE

The spawning of the female may be divided into three distinct phases: the discharge of eggs from the ovary (ovulation), the rhythmic contractions of the adductor, and the closing and opening of the mantle. Of these the first phase is obviously the principal sexual reaction of the organism, while phases two and three may be considered as accessory reactions providing a mechanism for the dispersal of eggs. Spawning may be observed in a ripe female without employing special apparatus or procedures. For observation the oyster is placed in a small glass dish filled with warm sea water (25–27° C.). When the shell opens and the mollusk begins to feed, one can easily notice that the opposite borders of the mantle are kept sufficiently apart to permit free access of water into the pallial chamber. Under ordinary circum-

stances this position of the mantle is maintained for a long time with only occasional changes (Hopkins, 1933, p. 482, Fig. 7, *A*). An approaching spawning is marked, however, by an unusual muscular activity along the edges of the mantle and by a change in the position of its inner borders and tentacles. The opposite folds which previously were flattened and kept almost parallel to the surface of the shell, now occupy positions perpendicular to it. Contractions of the mantle and the corresponding changes in the positions of its borders begin simultaneously from the posterior and anterior ends and spread toward the middle. The process continues for several minutes. During this time the borders may come in contact with one another and separate again. Finally they assume such a position that the pallial cavity is almost completely closed, only a small opening being left at a place approximately equidistant from the mouth and cloaca.

In the majority of cases the tonus level of the adductor muscle changes during this time and the valves open more widely. Then the adductor begins a series of contractions which continue until the end of spawning. Coincident with the relaxations of the adductor and gaping of the valves, a white cloud of ova appears at the anterior part of the pallial chamber (Fig. 1, *E*) and, descending posteriorly, fills it. The ova accumulate in front of the small opening left between the two opposite borders of the mantle and by sharp contraction of the adductor are expelled into the water (Fig. 6). Almost immediately the adductor relaxes, the valves open again, the borders of the mantle come together leaving the opening at approximately the same place it occupied before, and a new batch of eggs, which in the meantime has entered the pallial chamber, is expelled. The process repeats itself with great regularity and may continue for more than one hour, its duration depending on the amount of ripe ova in the gonad. Gradually the discharge of eggs ceases; the contractions of the adductor become weaker and irregular, and the reaction reaches an end.

The activity of the mantle during spawning is less important than the contraction of the adductor for in many instances, especially when only a small number of eggs are discharged, the mantle borders remain flattened and the pallial cavity is open along its entire length. It is evident that the formation of a small aperture, although helpful in producing better dispersal of eggs, is not indispensable to the success of spawning.

For a more detailed study of the behavior of the adductor muscle during spawning the following technique was used. An oyster immobilized on its left side with a mixture of plaster of Paris and cement was placed on a suitable support in a glass tank containing 27.5 liters

of sea water. The right valve was attached to a lever which recorded the shell movement on a slow-motion kymograph. Temperature was maintained constant within $\pm 0.1^\circ \text{C}$. by means of a heater, stirrer, and mercury temperature controller, operated through a relay. The entire set was mounted on an adjustable base supported by a tripod. Water was continually aerated or, if necessary, was kept running. The lever was arranged in such a way that the closed positions of the shell corresponded to the peaks of the kymograph curve. By this method several hundred records of shell movement were obtained. Those reproduced in this paper are the photographic prints of the originals.

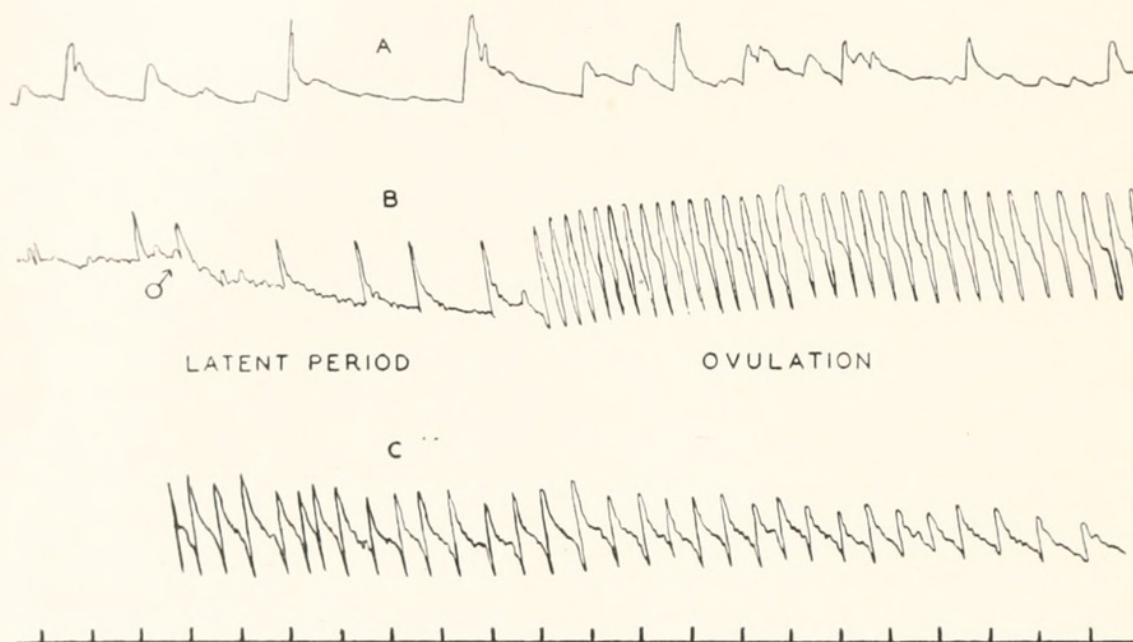


FIG. 4. *Ostrea virginica*, spawning reaction of a female. *A*, normal muscular behavior during feeding; *B* and *C*, spawning reaction. Time interval, 1 minute (bottom line). Note the change in the tonus level during the latent period. ♂ indicates time when live sperm was added to the water.

The closing of the shell of an oyster is affected by the contraction of the adductor muscle; its opening is due to the elasticity of the hinge ligament which acts as a spring forcing the valves apart when the adductor relaxes. Spontaneous shell movements are therefore entirely controlled by the contractions and relaxations of the adductor, whereas the purely mechanical action of the hinge depends exclusively on the expansion of the compressed elastic material of which it is composed. Under ordinary conditions the shell movements of an oyster are rather irregular (Fig. 4, *A*). Occasional strong contractions of the adductor resulting in a complete closure of the valve are followed by periods of relaxation interrupted by one or several light contractions causing par-

tial closing of the shell. Strong contractions are either ejection reactions by which the oyster gets rid of the material accumulated in the branchial chamber, or are responses to a great variety of chemical and physical stimuli; as for instance—mechanical disturbances, sudden change in the intensity of illumination, chemical irritation by acids, salts, and various other organic and inorganic compounds. The magnitude of the reaction in these cases is usually directly proportional to the intensity of the stimulus.

Partial closure of the valves is caused by the contraction of the striated part of the adductor muscle, while the maintenance of the valves in a given position against the pull of the hinge is attributed to the smooth component or so-called "catch" muscle. Under normal environmental conditions shell movements are characterized by long relaxation periods which vary from two to several minutes and are often interrupted by secondary contractions. A comparison of the records obtained during feeding with those performed at the time of spawning, reveals a great difference in the muscular behavior. The approach of ovulation is very often accompanied by a change in the tonus level (Fig. 4, *B*) and greater relaxation of the adductor. Then begins a series of rapid contractions and relaxations following one after another with remarkable regularity and continuing for a considerable time. Constancy in the amplitude of the relaxation curve, especially during the first half of the reaction, and the remarkable rhythmicity of the contractions are the most distinctive features of the sexual reaction of the female. These phenomena do not occur under any other circumstances. Attempts to reproduce this type of reaction in non-spawning females by physical stimulation (pricking, electric current) or by applying various drugs and chemicals, have been unsuccessful. Rhythmical contractions of the striated component of the adductor in *O. gigas* were described by Hopkins (1936, p. 500, Fig. 1), who noticed their relations to the discharge of water from the cloaca and suggested that the impulses of the activity originate within the digestive system. That the rhythmicity of muscular behavior can be due also to other causes is obvious from the observations on oysters left for several hours without aeration or changing water (Fig. 5). In these cases a peculiar behavior of the adductor is probably caused by the accumulation of CO_2 and other products of metabolism for the normal activity is restored as soon as water is renewed. Injection of 1:10,000 solution of adrenalin into the pallial cavity also results in the rhythmical contraction which, however, lasts only for a short time (Fig. 5, *C*). All these contractions have longer relaxation periods and are less regular than those associated

with the sexual activity of the oyster. This can easily be noticed by comparing the records reproduced in Figs. 4 and 5.

The question may be raised whether the behavior of the adductor during spawning represents ejection reactions in response to irritation caused by the presence of ova in the pallial chamber. That this is not the case is indicated by the continuation of typical spawning reactions after the discharge of ova has ceased. Furthermore, the quick relaxation, which always precedes a sharp contraction of the adductor during spawning, begins before the eggs appear in the pallial chamber and the amplitude of the contraction is entirely independent of the amount of the discharged material. It is typical of the spawning reactions that

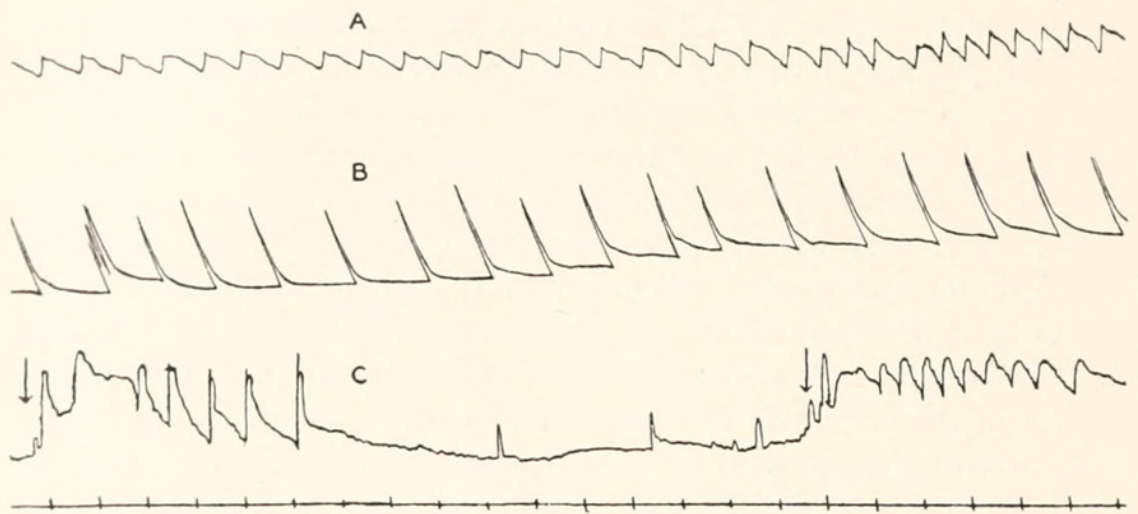


FIG. 5. Non-spawning rhythmical contractions in female oysters. *O. virginica* (A) and *O. gigas* (B) kept for several hours without aeration. C, effect on adductor muscle produced by 1:10,000 adrenalin solution injected into pallial cavity of *O. virginica*. Time of two treatments indicated by arrows. Time interval, 1 minute (bottom line).

the contractions begin with the maximum amplitude of the up and down strokes (Fig. 4), although at the very beginning only a few ova are discharged. If the rhythmical contractions were only ejection reactions comparable to the oyster's response to irritation caused by the accumulation of foreign material in its pallial chamber, one would expect that the amplitudes of the curves would have varied with the amount of material expelled. All the records obtained during the present investigation show, however, that the strokes of the greatest amplitude occur at the beginning of the reaction and remain constant for a considerable period of time. The supposition that the contractions of the adductor during spawning are ejection reactions is therefore untenable. It may be inferred that the adductor of the ovulating female receives specific

internal stimuli which control its action, and which are present only during ovulation.

At the beginning of spawning the adductor muscle may contract as many as three times a minute but gradually the rhythm slows down, the contractions become less frequent and less regular; the duration of the relaxation periods increases, and the muscle resumes its normal activity. Sometimes the change takes place so gradually that the end of the reaction can not accurately be determined.

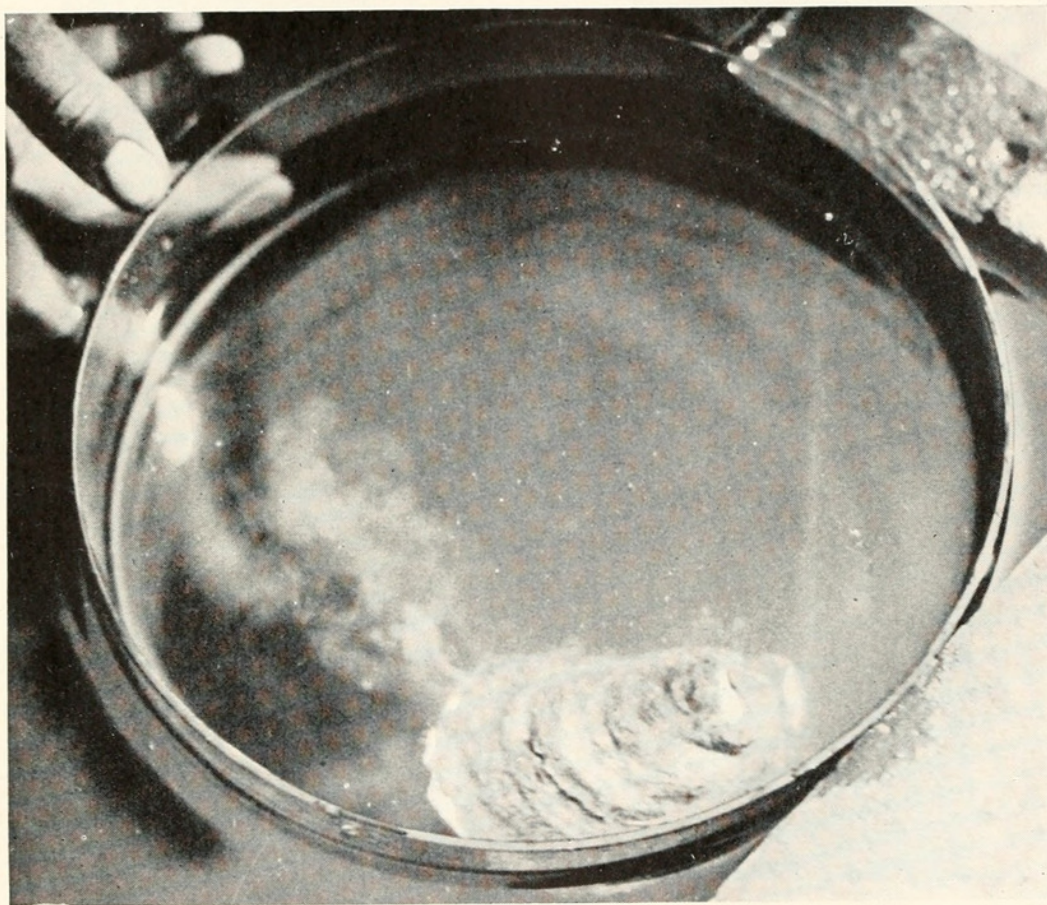


FIG. 6. Female *O. virginica* in the act of spawning. The photograph was taken at the moment the eggs were discharged by snapping of the valves. Note near the edges of the shell, a narrow jet of eggs expelled through a small opening between the borders of the mantle.

Kymograph records show that during spawning the relaxation periods are interrupted by brief pauses which give the curve a ladder-like appearance usually more pronounced toward the end of the spawning (Fig. 4, C). As will be shown later, this second half of each relaxation period coincides with the appearance of ova in the pallial chamber.

Contractions of the adductor, plus the action of the mantle, provide an efficient mechanism for the dispersal of ova in the surrounding water. The small hole between the two borders of the mantle through which

the ova are ejected, produces the same effect as a narrow nozzle at the end of a garden hose, considerably increasing the zone of their dispersal. Spawning females taken out of the water were observed to shoot their spawn to a distance of about four feet. Since fertilization takes place outside the organism and since the ova, being heavier than water, quickly settle on the bottom, the biological significance of this arrangement and the advantages it provides for the propagation of the species are obvious.

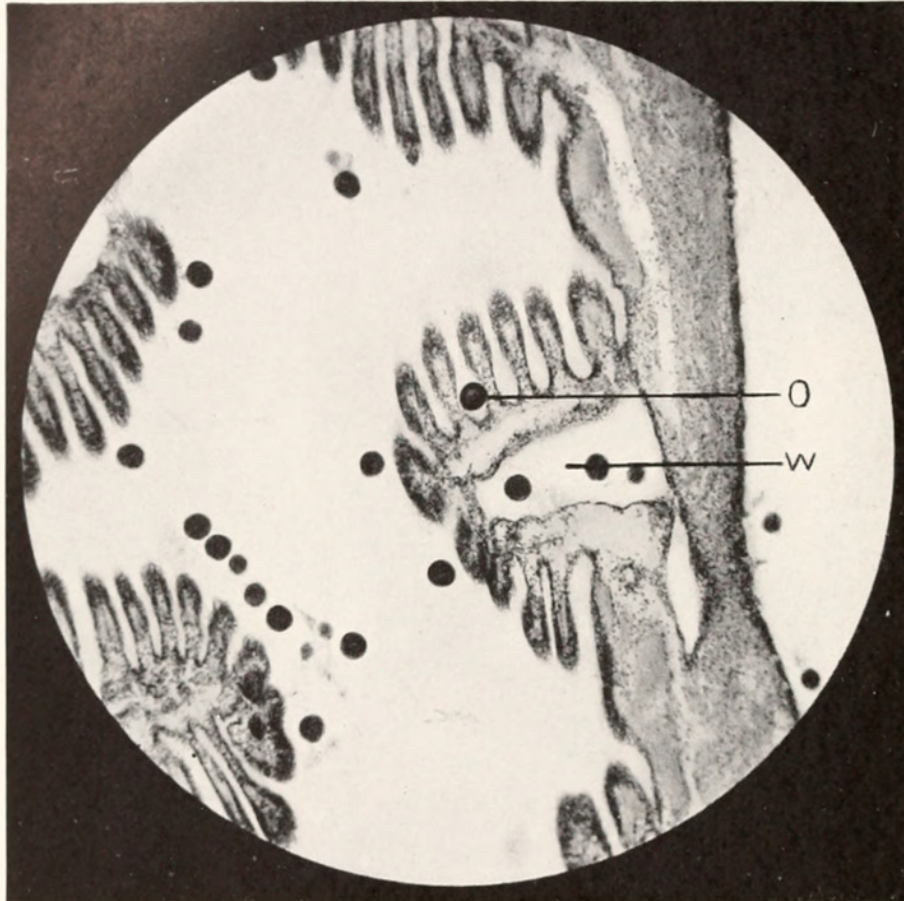


FIG. 7. Transverse section of the gills of the female *O. virginica* killed during spawning. Photomicrograph $\times 88$. Hæmatein-eosin. Note the presence of eggs in the water tube, *W*, and in the ostium, *O*.

During ovulation ripe eggs are discharged through the oviduct and urinogenital cleft into the suprabranchial chamber and cloaca. Their passage from the ovary and through the ducts can easily be observed in a living organism. For this purpose a spawning female is taken out of the water, one of its valves is removed, and the urinogenital cleft exposed by making an incision in the wall of the cloaca and suprabranchial chamber. The oyster is immediately returned to the water. The passage of eggs along the genital canals, oviduct and through the cleft can now easily be observed with a magnifying lens. Ova expelled by the

ciliary action of the epithelial lining of the ducts form a continuous stream and settle in the exposed part of the cloaca. Some of them are carried away by the cloacal current. Ovulation, once started, continues without interruption for a long time and is not stopped even by severe injury to the organism. The contractions of the severed adductor muscle persist but are not correlated with the delivery of eggs from the ovary. There are no noticeable contractions in the walls of the genital ducts or in the urinogenital cleft.

The question naturally arises as to how, in the intact organism, the ova discharged into the suprabranchial chamber reach the pallial chamber from which they are completely separated by the gills and walls of the branchial chambers (Fig. 1). Investigations of Kellogg (1892); Yonge (1926); Galtsoff (1928); Hopkins (1933) and others show that water is pumped from the pallial chamber through the ostia and water tubes of the gills into the suprabranchial chamber from whence it is expelled into the cloaca. In *O. virginica* a portion of it goes into an asymmetrical promyal chamber. In an actively feeding oyster a stream of water can easily be noticed at the dorso-posterior and dorso-anterior sides of the body. One would naturally expect that every particle introduced into the suprabranchial chamber would be carried away with one of these streams (Fig. 1). Yet the ova of a spawning female find their way into the pallial chamber against the current produced by the gills and are expelled from the posterior side of the body (Fig. 6).

Observations on ovulation in females in which the valve has been removed and the cloacal wall has been dissected, show that the only way the eggs discharged into the suprabranchial chamber can reach the pallial cavity is through the water tubes and ostia of the gills. This conclusion is fully corroborated by examination of sections of female oysters killed during the act of spawning in which unfertilized ova were found inside the water tubes and near the ostia (Fig. 7, *W*, *O*). Since the beating of the lateral cilia throws the suspended particles toward the outer surface of the gill lamellæ (Galtsoff, 1928) where they are caught by the frontal cilia and eventually conveyed toward the mouth, the presence of an egg just underneath the lateral cilia is good evidence that it has reached this place from inside through the water tubes and suprabranchial chamber. This conclusion is fully supported by experiments in which the passage of eggs through the gills was observed in vivo. The following technique has been developed for making such observations. A piece of shell of a ripe female was sawed off and carefully removed avoiding any injury to the tissues. After the operation, the underlying piece of mantle usually rolled up exposing the gills (Fig. 8, *A*). The oyster was then placed in a finger-bowl filled with sea water

(25° C.) and ovulation was stimulated by the addition of sperm (Galtsoff, 1930*b*). The shell movement was recorded on a kymograph by means of a lever attached to the remaining portion of the valve. The exposed gills were illuminated by a strong light and examined under a binocular microscope mounted on a suitable stand. Under these conditions the passage of eggs through the gills could easily be observed.

It has been noticed that they emerge from the ostia in the area which

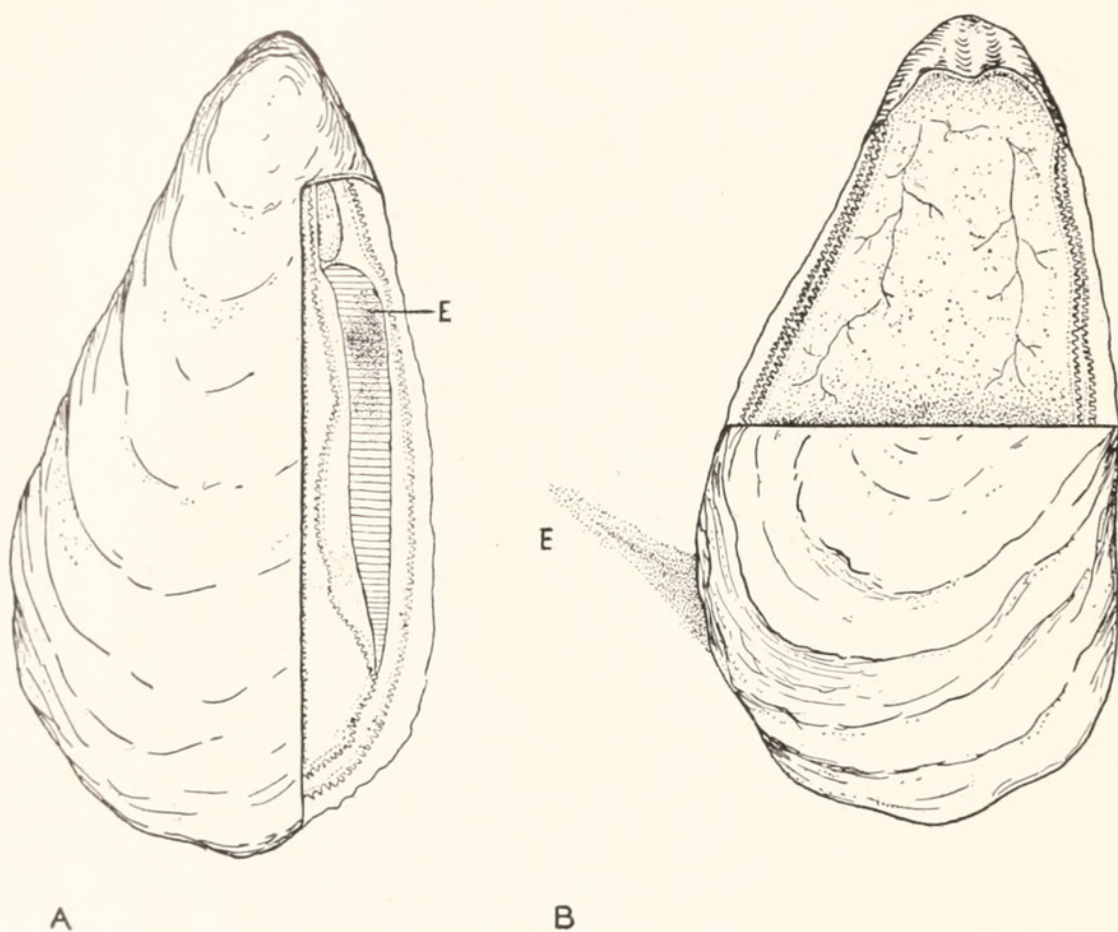


FIG. 8. *Ostrea virginica*, passage of eggs through the gills. *A*, portion of shell is sawed off and the mantle rolled up. Eggs (*E*) pass through the anterior part of the gills. *B*, anterior half of the shell is removed but the adductor is intact. Eggs are discharged through the cloaca.

extends from the anterior part of the gills for about two-thirds of their length. The greater number of eggs pass along the proximal half of the gill lamellæ, although some of them escape through the distal portion of the gill. This experiment has been repeated several times and each time the moment of the appearance of eggs on the surface of the gills was marked on the kymograph curve. The emergence of eggs through the ostia coincided with the maximal gaping of the valves and was therefore associated with the rhythmical contractions of the adductor.

What forces the eggs to take the course through the narrow passages of the water tubes against the current produced by the gill epithelium? Several possible factors have been considered: a reversal of ciliary motion in the gills, suction caused by the rhythmical movements of the valves, and difference in pressure between the suprabranchial and pallial chambers. While the evidence is not sufficiently conclusive to permit a definite answer to the question, certain of these possibilities may be ruled out.

The possibility of the reversal of ciliary motion cannot be verified by observation because the action of the lateral cilia cannot be observed in the intact gill lamella. The beating of the frontal cilia may, however, be observed by noting the movement of particles of carmine powder. During the passage of eggs it appears to be normal. If there is a reversal of ciliary motion it is therefore limited to the lateral cilia. The possibility of a reversal of the lateral cilia may not be completely excluded, but confirmatory evidence is lacking. Nelson (1936) states that the rate of pumping of water greatly decreases during the spawning of the female oyster. This may be indicative of a disturbance in the functioning of the lateral cilia. Another explanation is possible, however, for we know that the mantles of the spawning female reduce the opening, or even almost close the pallial aperture. This action obviously will decrease or completely stop the exchange of water. The reduced rate of pumping during spawning does not, therefore, mean that the beating of the lateral cilia ceased or was reversed. Other observations cast further doubt on the reversal of ciliary motion in the gills. Experiments were performed in which the anterior portion of the shell was cut away, preventing shell movement but leaving the muscle with adjacent part of the shell intact (Fig. 8, *B*). Because the hinge was severed, the oyster was unable to open its valves, but the adductor muscle and its nerve ganglia were in no way affected by the operation. Under these conditions eggs discharged from the ovary do not pass through the gills but are washed away from the cloaca by the respiratory current. If there were a reversal of the ciliary beating during ovulation a continuous oozing of eggs through the gills would be expected. Additional negative evidence is supplied by the fact that in several instances spawning oysters have been observed to discharge eggs both through the gills and through the cloaca.

We have already seen that the emergence of eggs through the ostia coincides with the period of maximal gaping of the valves. These facts led the author to consider whether rhythmical shell movement might cause a suction which would draw the eggs from the suprabranchial chamber into the water tubes and pallial cavity (see reference to this

in Hopkins' paper, 1937, p. 457). Observations on oysters in which the gills were exposed by cutting a piece of shell, thus forcing the mantle to roll up and leave the pallial chamber wide open, make this explanation untenable.

Observations made during the summer of 1937 with sex-inverted oysters (Galtsoff, 1937) are of particular interest because they show that something more than rhythmical shell movement is necessary for the passage of eggs through the gills. In these experiments one adult female, which during the preceding summer was a male, discharged the eggs through the cloaca in male fashion in spite of the well-developed contractions of the adductor which are typical for a spawning female.

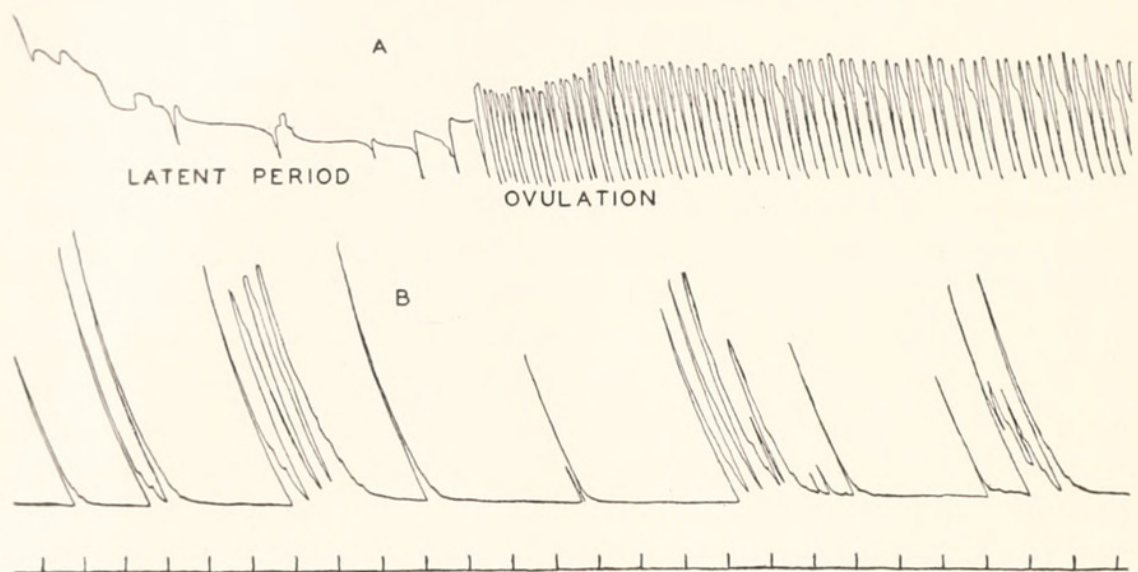


FIG. 9. *Ostrea gigas*, spawning reaction of two females. *A*, beginning of the reaction; note change in tonus level during the latent period and rhythmical contractions during ovulation. *B*, spawning reaction about one hour after its beginning; during the brief periods of contractions eggs are discharged through the gills; during the relaxation periods (horizontal parts of the curve) they are discharged through the cloaca. Time interval, 1 minute (bottom line).

Obviously some other conditions necessary to drive the eggs through the gills were still wanting.

The fact that the eggs are forced through the gills from the inside indicates that pressure in the suprabranchial chamber is higher than on the other side of the gill lamellæ. The increase in pressure can easily be produced by the discharge of a large amount of material from the gonad and closure of the cloacal opening. Although direct evidence is lacking, this supposition is in accord with other observed facts. Since it is known that the discharge of eggs from the ovary once started continues without interruption for a long time, it is logical to expect that pressure inside the suprabranchial chamber increases when the contrac-

tion of the adductor closes the valves and compresses the gill lamellæ. During the following relaxation and gaping of the valves the gill lamellæ spread apart and eggs which had accumulated inside are forced through the water tubes and ostia. This may be possible only if the cloacal opening remains closed for otherwise they would be carried away with the outgoing current of water. Whether the soft and contractile edges of the cloaca come together, closing the opening during ovulation, has not been ascertained by observation but the spreading and contraction of the gill lamellæ has often been noticed.

The rather complex and circuitous method of discharging eggs described for *O. virginica* occurs also in other bivalves. It is known that in many incubatory forms the ova are retained in the interlamellar branchial spaces. In *Ostrea edulis*, *O. angasi*, *O. lurida*, and *Entovalva* sp. (Pelseneer, 1906, p. 244) eggs develop in the pallial chamber outside the gills, but in the *Unionidæ* they are retained in the interlamellar branchial spaces in which special pouches or marsupia are developed (Ortmann, 1911; Lefevre and Curtis, 1910). Ovulation in the latter forms was observed in but a few cases (Latter, 1891; Ortmann, 1911, p. 298; Howard, 1914, p. 35). Latter, describing the process in *Anodonata* and *Unio*, states that "In order that the ova may reach their final resting place there must be some reversal of the respiratory currents." He could not, however, detect any reversal of the ciliary motion and attributed the passage of eggs from the cloaca into the gills to a suction caused by the gaping of the valves. This conclusion, however, was not verified by observation.

Stafford (1915) states that in *O. lurida* eggs liberated from gonoducts into the suprabranchial chambers, being too heavy to be carried in the respiratory current, flow naturally into the water tubes and are forced through them into the pallial chamber by the pressure of their mass. This explanation, apparently not based on observation, requires further verification for it is very doubtful that the ova of this species are much heavier than those of *O. virginica*. In the latter species, as has been shown above, they are easily carried away by the current. Furthermore, it seems improbable that the weight of eggs will be sufficient to counteract the current maintained in the water tubes by the ciliated epithelium.

Spawning in all three groups of bivalves mentioned above may be considered as representing three consecutive stages of one process. In *Unionidæ*, the ova penetrate into the water tubes which develop into special broodchambers, to remain there until the emergence of the glochidia; in the incubatory species of oysters, *O. edulis* and *O. lurida*, the ova completely pass through the gill lamellæ but are retained in the

pallial chamber between the demi-branches; in *O. virginica*, *O. gigas* and *O. commercialis*, they are discharged from the pallial chamber immediately after being forced through the gills. Here the process has reached its final development. All three different types of spawning may be attributed to a greater or lesser efficiency of the combined activity of the adductor muscle, gills, and cloaca during ovulation. A detailed physiological study of the spawning reactions of various species of *Unionidae* and of the incubatory oysters will therefore be interesting.

SEXUAL REACTIONS OF *O. gigas* AND *O. commercialis*

Sexual reactions in *O. gigas* and *O. commercialis* are similar to those described for *O. virginica*. Kymograph tracings of shell movements of spawning females of these oysters show the same typical characteristics, namely, change in tonus level during the latent period, rhyth-

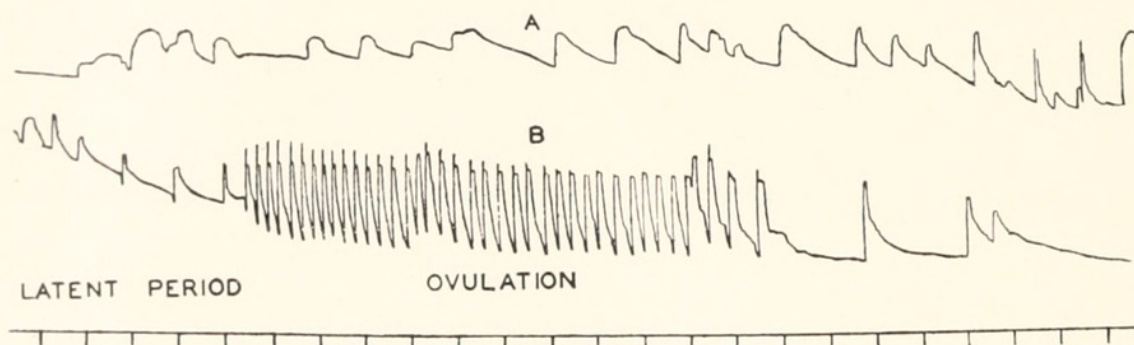


FIG. 10. *Ostrea commercialis*, spawning reaction of a female. A, muscular behavior during feeding. B, spawning reaction. Time interval, 1 minute (bottom line).

micity in the contractions of the adductor muscle and gradual fading of the reaction (Figs. 9, 10). The reaction in *O. gigas* differs slightly, however, from that of the other two species. As a rule, the Japanese oyster discharges eggs both through the gills and cloaca. In one of the experiments, for instance, muscular contractions and discharge through the gills continued for 13 minutes while ovulation and emission of eggs through the cloaca lasted 21 minutes longer. It is impossible to state which of the two methods of discharge is the principal one in this species. Eggs discharged with the respiratory current through the cloaca often come in large lumps, several millimeters long, which immediately settle on the bottom. Large numbers of eggs discharged in this manner are smothered and perish while those discharged through the gills are more widely distributed throughout the water and have a better chance to survive. No discharge of eggs through the cloaca was observed in *O. commercialis*.

DURATION AND NUMBER OF SPAWNINGS

Spawning once started may continue for a long time and cannot be stopped by taking the oyster out of the water or even by inflicting

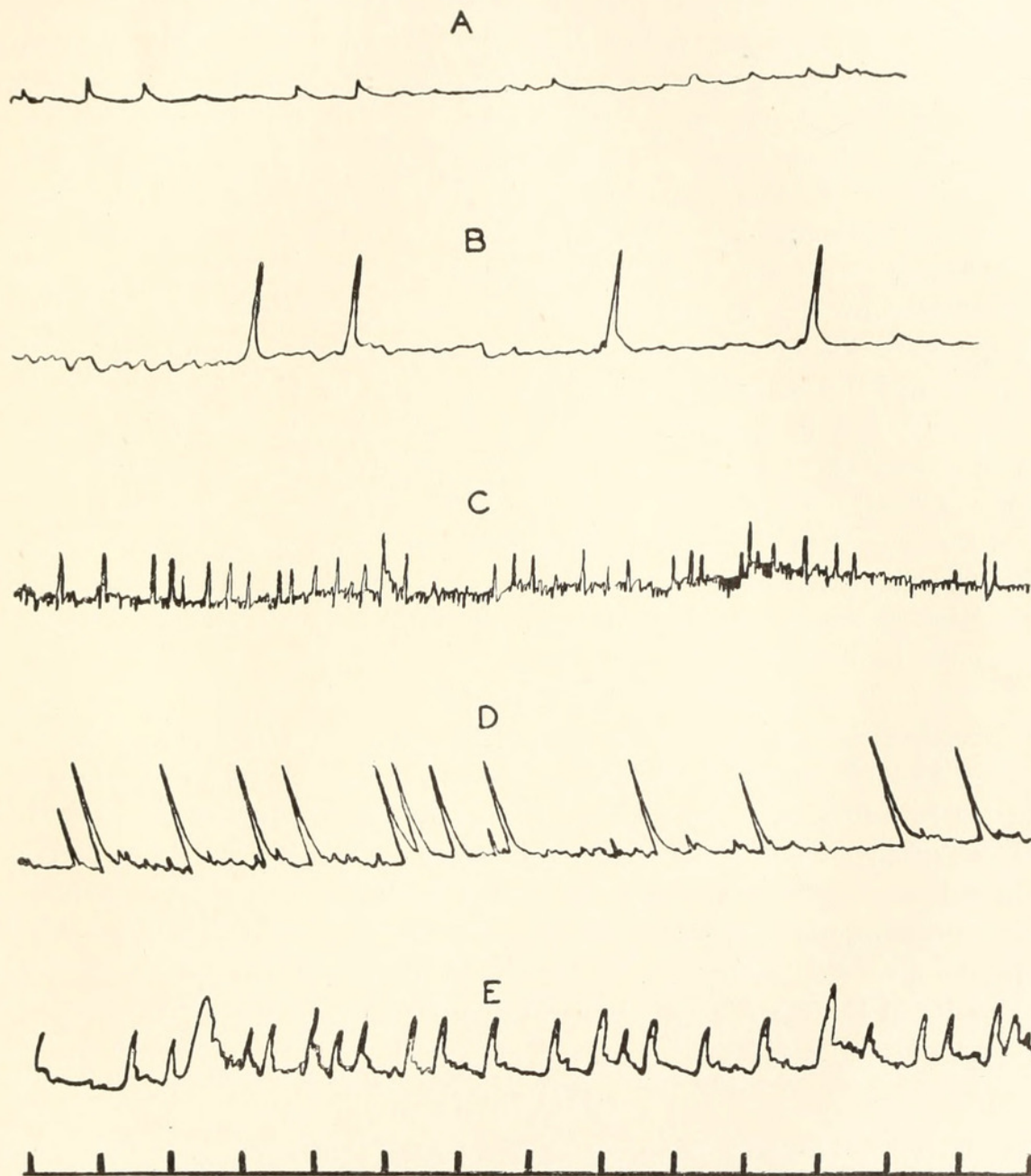


FIG. 11. *Ostrea virginica*, muscular behavior of five males (A-E) during ejaculation. Time interval, 1 minute (bottom line).

severe injury to its body. Duration of ovulation, i.e., of the discharge of ova from the gonad, cannot be measured with accuracy since the release of small numbers of eggs can easily be overlooked. Duration of the typical spawning reaction of the adductor, on the other hand,

can be determined from the kymograph records, except in the cases where the fading of the curves is so gradual that the cessation of the reaction is indistinct. In many instances, however, the curves of muscular activity show changes sufficiently sharp to indicate the end of spawning. In 101 records of *O. virginica* available for analysis, the duration of spawning varied from 4 to 118 minutes. The shortest periods were recorded either at the beginning or toward the end of the reproductive season. In *O. gigas* (13 records), the duration of the discharge of eggs either through the gills or through the cloaca varied from 15 to 130 minutes. In the two records obtained with *O. commercialis* spawning lasted 12 and 29 minutes.

Copious ovulation was observed always to be accompanied by a large amplitude of the up and down excursions of the adductor and the greater frequency of its contractions. The greatest number of eggs discharged during one spawning was found to be 114.8 millions in *O. virginica* and 55.8 millions in *O. gigas* (Galtsoff, 1930a). In both cases histological examination of the spawned females revealed that only a small portion of the sex cells had been released. Judging by the thickness of the gonad layer, the fertility of *O. gigas* appears to be much greater than that of *O. virginica*. There is no doubt that the number of eggs discharged during one spawning, given above, does not represent the maximal figure.

That *O. virginica* may spawn several times during one season was ascertained by experiments conducted during several summers between June 15 and August 7, at Woods Hole. Under laboratory conditions a single female was induced to spawn seven times during this period. How often an individual oyster spawns in nature has not been determined but it may be assumed that under favorable conditions it behaves in its natural environment in the same manner as in the laboratory. Repeated spawning was also observed in *O. gigas*. In the laboratory the females of this species were induced to spawn two or three times during one month.

EJACULATION

During ejaculation sperm discharged from the testis passes through the spermiduct and urinogenital cleft into the suprabranchial chamber from whence it is carried by the current of water into the cloaca and washed away. Muscular contractions of the adductor play no rôle in the release of sperm which is effected primarily by the ciliary motion of the epithelial lining of the gonad and respiratory current produced by the gills. The behavior of the adductor during ejaculation shows a great variety of conditions from almost a complete quiescence (Fig.

11, *A*) to greatly increased activity characterized by small and irregular contractions (Fig. 11, *C* and *E*). These contractions are not, however, concerned with the release of sperm.

The sudden appearance of a jet of sperm at the beginning of ejaculation gives an impression of increased velocity of the cloacal current. The question whether there is an increase in ciliary activity during ejaculation was carefully studied in a number of experiments. Two methods were employed for measuring the strength of the cloacal cur-

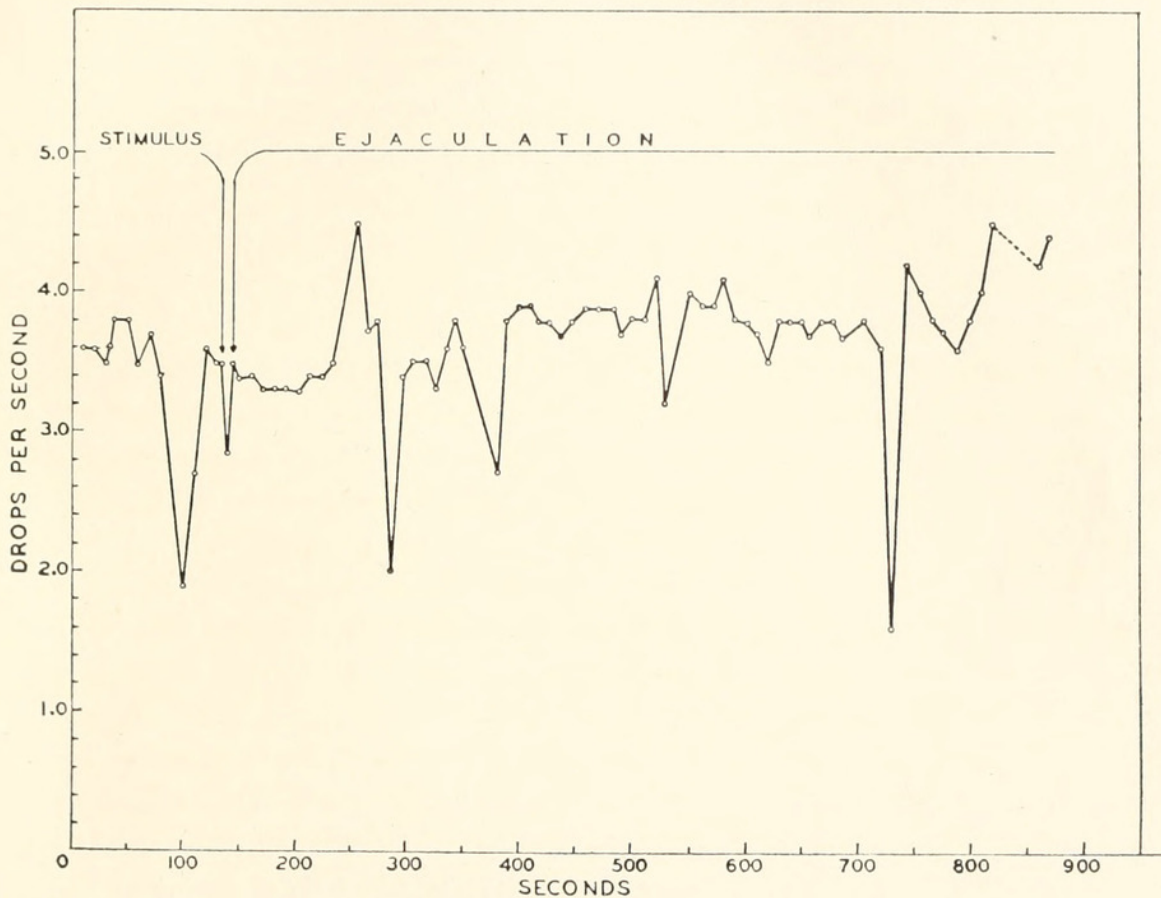


FIG. 12. Rate of pumping of water of the male *O. virginica* during continuous ejaculation. Electric drop-counting method. Each point represents 10-second average of the number of drops of water discharged by the gills.

rent. In the first series of experiments an electric drop-counting technique, fully described in a previous publication (Galtsoff et al, 1935, p. 172) was used. The method consists in recording on a kymograph each drop of water pumped by the gills through a rubber tubing inserted into the cloaca. The method completely eliminates the effects of the adductor muscle and of the mantle which may constrict the pallial aperture, making it possible to obtain measurements of the efficiency of the ciliary epithelium alone. The results of the two experiments in which continuous records were obtained before, during and after ejaculation

are presented in Figs. 12 and 13. The experiments lasted 14 and 32 minutes respectively. Each point plotted on the curves represents an average number of drops per second for the preceding 10-second interval. The exact time of stimulation and of the release of sperm is indicated by the arrows. In the first experiment (Fig. 12) ejaculation lasted continuously for several minutes. There was no increase in the ciliary activity at the beginning of ejaculation and, with the exception of one high peak and four depressions, the rate of pumping remained more or less constant. Stimulation, which in this case consisted in adding

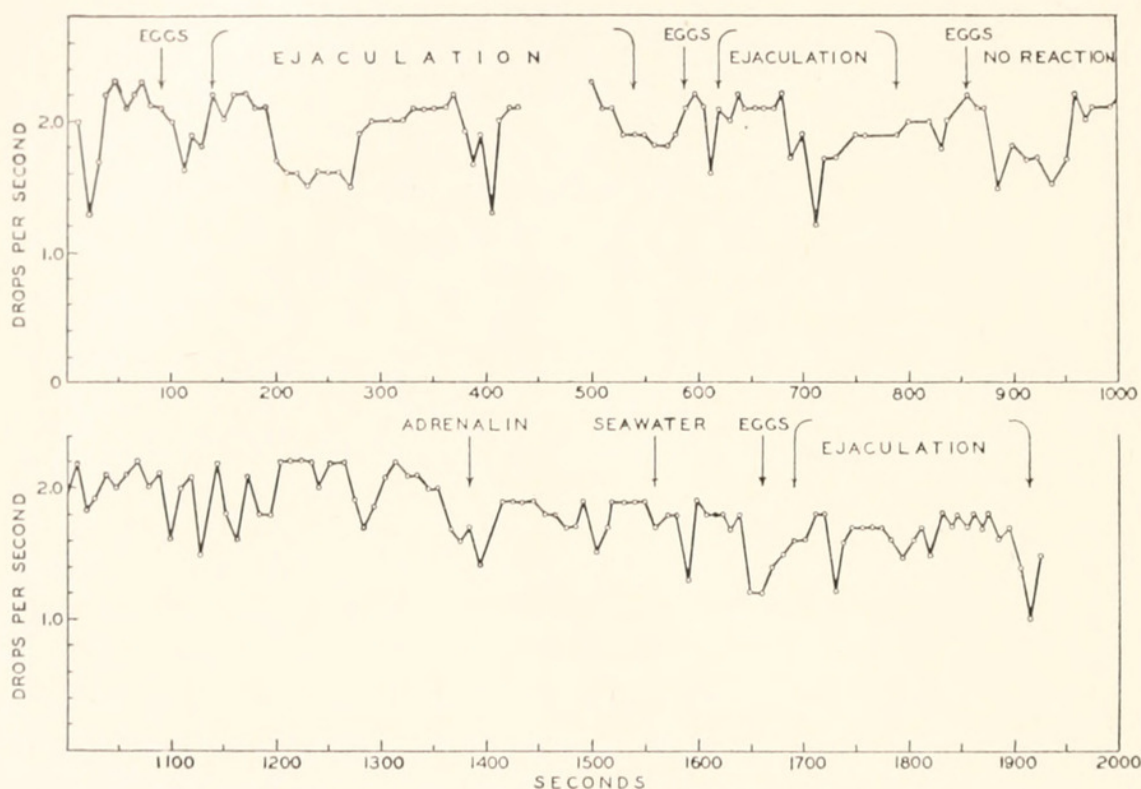


FIG. 13. Rate of pumping of water by the male *O. virginica* during and between ejaculations. Electric drop-counting method. Each point represents 10-second average of number of drops of water discharged by the gills.

thyroidin to the pallial cavity of the oyster, was followed by a temporary inhibition of the rate of pumping. This was probably due to mechanical stimulation, as a similar effect was noticeable in other cases when sea water of the same temperature was injected between the valves (Fig. 13). In the second experiment ejaculation was induced three times by the addition of oyster eggs. Not only was there no increase in the rate of pumping of water, but in two cases (Fig. 13, upper line), the rate materially decreased during ejaculation.

Similar results were obtained by using the technique of Hopkins (1933) for recording the relative strength of the cloacal current of an

intact oyster. In this method the velocity of the current is measured by the deflection of a light lever, one arm of which supports a small paper or celluloid cone placed in front of the cloacal opening. The deflections of the lever are proportional to the velocity of the current striking the cone. The method permits the study of the operations of the entire complex mechanism which controls the pumping of water by the gills. A photographic reproduction of a portion of one of the records is given in Fig. 14. The exact time of stimulation and the beginning and end of ejaculations are indicated by vertical marks (third line). The upper line represents the shell movement of the spawning male and the changes in the strength of current are shown on the second line. The uppermost points of both curves correspond to the closure

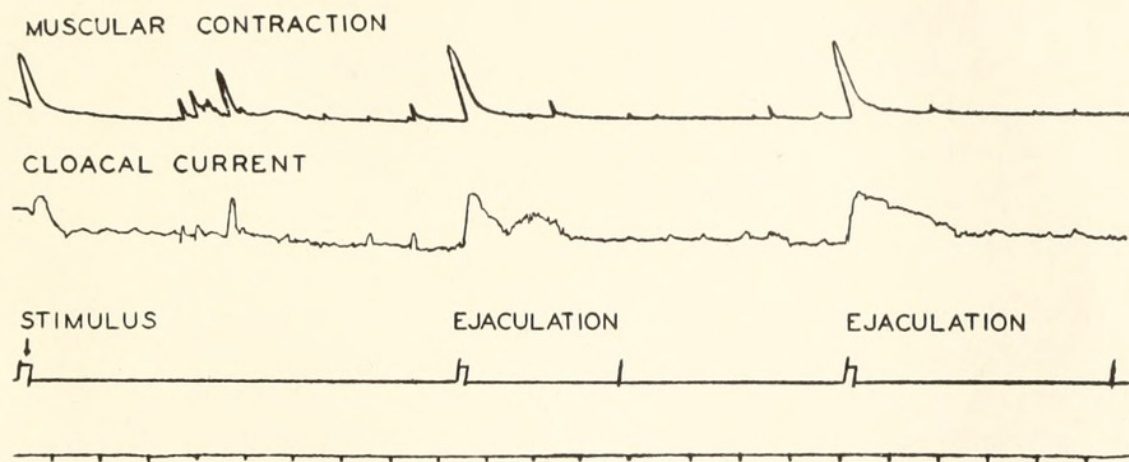


FIG. 14. Muscular contraction and relative strength of cloacal current during ejaculation of *O. virginica*. Double vertical bars (third line from top) mark the beginning of ejaculation. Single bars indicate its cessation. Time interval, 1 second (bottom line).

of shells and cessation of current. The record clearly shows a correlation between the strength of the current and shell movement and lack of significant change in the activity of the ciliated epithelium during ejaculation. The experiments repeated several times under various conditions always gave the same results indicating that sudden discharge of sperm during ejaculation is not due to the outburst of increased ciliary activity. Although no distinct sphincter is present in the spermiducts or in the urinogenital cleft, the ability of the male repeatedly to release small amounts of sperm under the influence of a proper stimulus suggests a sphincter-like action. Histological examination provides certain basis for this assumption for it shows (Fig. 3, *M*) the existence in the walls of the spermiducts of muscle fibres which are stronger and more abundant than in the oviducts. It is therefore probable that while the ciliated lining of the genital canals and spermiducts provides a motive

power which conveys the ripe sperm from the testis, the release of sperm is regulated by the contractions of muscle fibres scattered in the walls of the ducts.

Ejaculation may proceed either from one or simultaneously from both spermiducts. At the beginning of the spawning season only a small amount of sperm is discharged at each ejaculation, which lasts only a few seconds, but repeated emissions can be induced immediately one after another. In several experiments a single male was stimulated to shed sperm more than a hundred times during a ten-hour period, each time releasing a small amount of spermatozoa. As the season proceeds and the amount of ripe sperm in the testis increases, each ejaculation lasts longer. Finally a stage is reached when ejaculation, once started, continues for hours and cannot be stopped until the male is completely spent.

Sperm discharged with the cloacal current remains in suspension for a considerable time and is widely distributed in the water.

The males of *O. gigas* and *O. commercialis* behave in the same manner as the males of *O. virginica*.

SUMMARY

The gonad of an oyster consists of a large number of branching tubules containing sex cells and emptying into genital canals, the lumen of which is lined on one side with the ciliated epithelium. There are two gonoducts, one on each side of the gonad, which open into supra-branchial chambers. Spawning of the female consists in the discharge of eggs from the ovary (ovulation); rhythmical contractions of the adductor which cause opening and closing of the shell valves; and in the contraction and change in the position of mantle borders which almost completely close the pallial cavity. The last two phases of spawning are accessory sexual reactions which provide mechanism for better dispersal of eggs in the water. Muscular behavior during spawning is characterized by its remarkable rhythmicity and maintenance of a constant tonus level. It cannot be reproduced by artificial stimulation. Eggs discharged into the suprabranchial chamber are not carried away by the cloacal current but penetrate through the gills into the pallial cavity and are expelled by vigorous movements of the shell valves. Their passage through the gills coincides with the periods of greatest relaxation of the adductor and widest gaping of shell. If the shell movement is prevented by severing the hinge, eggs fail to pass through the gills and are discharged by the cloacal current. The possibility of the reversal of the ciliary motion and of the suction produced by shell

movement as the forces responsible for the passage of eggs through the gills are discussed and rejected as untenable. The following tentative explanation is offered. The discharge from the ovary and the closure of the cloaca produce an increased pressure in the suprabranchial chamber which forces the eggs through the water tubes and gill ostia into the pallial cavity. The difference in pressure on both sides of the gill reaches its maximum when the valves open and the gill lamellæ spread apart. This accounts for the penetration of eggs through the gill during the periods of greatest relaxation of the adductor.

Ejaculation is not accompanied by the rhythmical contractions of the adductor and the sperm discharged through the spermiduct is carried away by the cloacal current. There is no increase in the rate of pumping of water during spawning.

The discharge of sperm is probably controlled by the contractions of numerous muscle fibres in the walls of the spermiduct. Definite sphincter-like structure is absent.

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