DELAYING SPAWNING OF LAMELLIBRANCHS BY LOW TEMPERATURE

By

V. L. LOOSANOFF and H. C. DAVIS

U. S. Fish and Wildlife Service, Milford, Connecticut

ABSTRACT

Spawning of oysters (*Crassostrea virginica*) and clams (*Venus mercenaria*) was delayed by transferring ripe or nearly ripe animals from Long Island Sound to the cold waters of Boothbay Harbor, Maine, where the temperature, although high enough to permit gametogenesis, was too low to induce spawning. With *C. virginica*, spawning could be postponed until six or eight weeks after the Long Island Sound population was completely spent, while *V. mercenaria* held over healthy summer spawn throughout the fall and winter and into the following spring. By similar means, laboratory workers can be provided with ripe animals during the fall when none are normally available in their own locality.

Until recently the eggs and larvae of lamellibranchs, such as oysters and clams, were available only during their normal spawning period. In our waters this period is relatively short, usually from about 2½ to 3 months. However, several years ago a method was developed to induce oysters to develop gametes and to discharge spawn in winter (Loosanoff, 1945). Later the same method, in somewhat modified form, was successfully applied to other mollusks (Loosanoff and Davis, 1950).

The method merely consists of a gradual increase in the temperature of the water from the low level prevailing in winter over the mollusk beds to that at which they spawn in summer. Using this simple method, *Crassostrea virginica, C. gigas, Ostrea lurida, O. edulis* (the European oyster), *Venus mercenaria, Mya arenaria, Mactra solidissima, Anomia aculeata, Ensis directus, Petricola pholadiformis*, and several other lamellibranchs have been conditioned at our laboratory to develop and discharge normal gametes throughout the winter and spring.

In the same way we have also succeeded in ripening the common starfish of Long Island Sound, *Asterias forbesi*. The gastropod oyster drill, *Urosalpinx cinerea*, was also conditioned to deposit egg-cases in the middle of winter. We think that the method is well suited to ripen for spawning many other invertebrates, including annelids, the eggs and larvae of which were often found when we spawned oysters in winter.

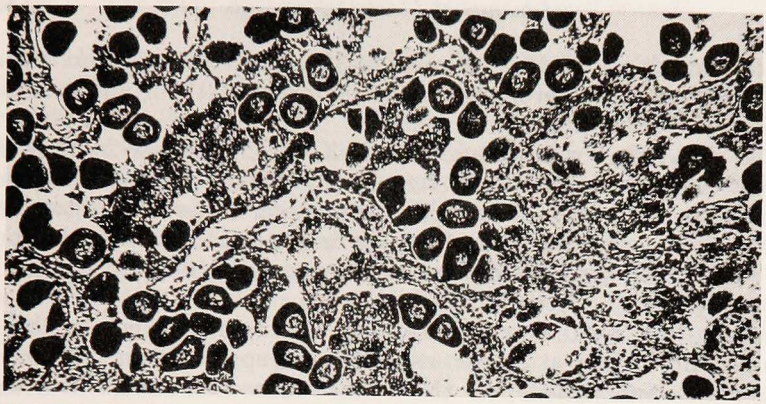
Conditioning lamellibranchs for spawning in late fall and winter is possible only after they recover from the natural summer spawning. Recovery consists of an accumulation of reserve materials, such as glycogen. In our waters that stage may not be reached by some lamellibranchs until the end of October. Thus, since these lamellibranchs become spent some time during late August or early September, and since they cannot be conditioned for fall and winter spawning until November, their ripe sex products are not available during the intervening period. To solve the problem of providing the laboratory with mollusks in spawning condition during this period, the following experiments were made.

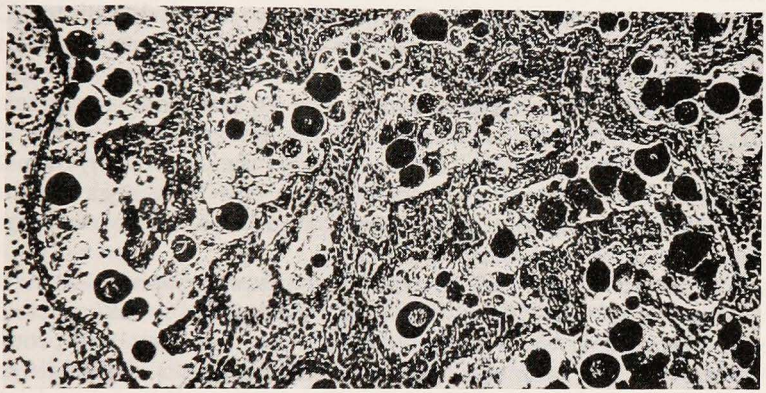
At the end of May 1950, several bushels of adult oysters (*C. virginica*) and clams (*V. mercenaria*) from Long Island Sound were planted in the waters of Boothbay Harbor, Maine. Since the water of Boothbay Harbor in summer is about 5–8° C. cooler than that of Long Island Sound, we reasoned that the temperature of the Boothbay Harbor water, while not arresting the development of the gametes of our mollusks, would, nevertheless, remain low enough to prevent the mollusks from spawning. Thus, by the time the oyster and clam populations of Long Island Sound were completely spawned out, or nearly so, those kept in the Maine water would still retain sperm or eggs and could, therefore, be induced to spawn when needed.

Oysters and clams of the first group, brought from Maine on September 7, 1950, were ripe with extremely thick gonads and were easily induced to spawn with a discharge of large quantities of eggs and sperm.

The second group was brought to Milford on October 3 nearly a month later. These oysters were ripe also, but unlike the first ones, only some of them could be induced to spawn while others already showed evidence of resorption of the unspawned ova (Fig. 1). The clams, on the other hand, showed no evidence of spawning or of resorption of gonadal material. They responded quickly to spawning stimulation, which consisted of a rapid increase in the water temperature to about 32.0° C. and the addition of egg or sperm suspension; large quantities of normal spawn were discharged.

The third group was brought from Maine on October 24, about three weeks later than the second batch. At that time the temperature of the water at Boothbay Harbor was approximately 9.0° C. After being brought to Milford, the mollusks were placed in the large tide-filled outdoor concrete tank where the temperature was about 10.0° C. An attempt to spawn the oysters of this group on October 26 was unsuccessful. Of the 40 oysters used, only one male responded, and even this was only a feeble spawning, consisting of a discharge of sperm with considerable quantities of amorphous matter and blood





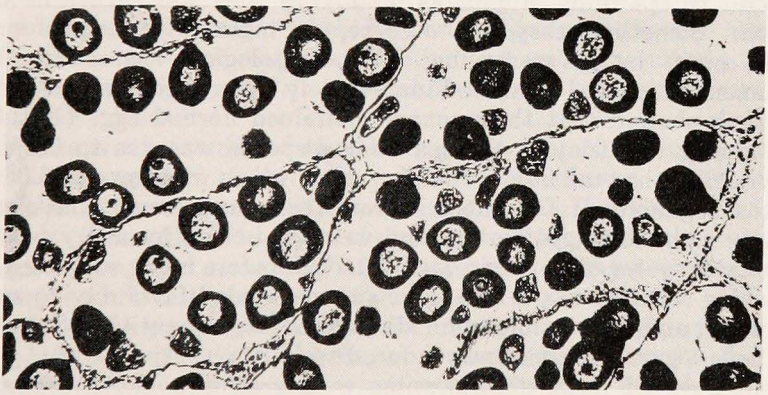


Figure 1. Gonad of female oyster in early stages of resorption. X112 Figure 2. Gonad of female oyster in advanced stages of resorption. Notice many cytolized eggs. X112 Figure 3. Section of gonad of experimental clam, *Venus mercenaria,* preserved in December. X112

cells. Examination of the remaining 39 oysters showed that resorption was already in progress in the majority of them and was well advanced in some (Fig. 2).

The clams that were brought to Milford on October 24 were distinctly different from the oysters in both condition and behavior. There was no evidence of resorption of gonadal material, and two days after arrival every one of the 15 clams responded to spawning stimuli. The discharged eggs developed into larvae which were cultured to metamorphosis.

Because the oysters were resorbing their gonads by the end of October, no further attempts were made to spawn them. However, systematic observations on the clams were continued, since their condition and their physiological behavior were different. The source of supply for these observations was the large group of clams that was brought from Maine on October 24, 1950; these were kept in our outdoor tank. A sample consisting of 10 individuals was taken from the tank on November 7 and again on November 15. All of the clams spawned in response to stimulation. In the latter case the clams came directly from water of only 8.1° C. The eggs obtained from both spawnings developed normally and the larvae were again cultured to the setting stage.

As the season progressed, groups of clams were brought into the laboratory from the outdoor tank at approximately biweekly intervals to ascertain whether they still could be induced to spawn and produce viable zygotes. It was noticed, however, that the clams did not respond to spawning stimuli as readily as they did earlier in the season. Sometimes they had to be kept at high temperatures for 10 or 12 hours before responding. Yet, histological examination of specimens killed and preserved immediately upon their removal from the tank showed that their gonads contained normal eggs (Fig. 3). Apparently the delay in the spawning response was due to keeping the clams in the tank at a low temperature, which was down to 6.0° C. by the beginning of December. From then on, however, the clams were brought to the laboratory and were kept there for a day or two in running water of approximately 20.0° C. before being subjected to spawning stimuli. As a rule the clams responded favorably to such treatment and, upon stimulation, discharged large quantities of spawn.

The last group of clams was induced to spawn on April 9, 1951; the larvae received from this spawning were grown to metamorphosis. Unfortunately, the sample of April 9 exhausted our supply and observations had to be discontinued.

In general, the experiments showed that it is possible to delay spawning of ripe or nearly ripe mollusks by keeping them at a com-

paratively low temperature. In oysters it can be delayed as late as mid-October. In other words, under natural conditions most of the oysters in our waters would be spent in August, but by using this method we can delay spawning for 6 or 8 weeks, i. e., until the time of year when the oysters living in Long Island Sound will be ready to be conditioned for winter spawning. Spawning of *V. mercenaria* can be delayed even longer than that of oysters. Thus, using a combination of our two methods, one of conditioning for winter spawning (Loosanoff and Davis, 1950) and the other of delaying spawning in late summer and fall by low temperature, it is possible now to have ripe lamellibranchs available throughout the year.

Somewhat similar results in the delay of spawning were achieved by taking ripe mollusks from Long Island Sound in midsummer and by keeping them in the laboratory in an insulated container through which mechanically-cooled sea water was flowing. Basically the principle is the same as keeping them in the cool water of Maine. However, only a small number could be conveniently kept in the laboratory under semirefrigerated conditions. Furthermore, a rapid increase in temperature due to failure of the mechanical devices always caused major and complete spawning, thus rendering the whole group useless.

Although observations on the fate of the unspawned ova and sperm of clams and oysters are not the subject of this article, it should be mentioned here that the ability of the clams to carry undischarged spawn far into the winter, without undergoing such cytological changes that would make the gametes unviable, is of extreme biological importance and interest. It is even more significant that the retention of healthy spawn through late winter and spring occurred although the clams were exposed for several months to temperatures ranging between 0 and 6° C.

Perhaps this ability of the clam, unlike that of the oyster, which resorbs its gonads early in the fall, can be explained by the general pattern of its seasonal gonadal changes (Loosanoff, 1937). According to this pattern the production of a new crop of eggs in *Venus mercenaria* begins soon after spawning, and the chief growing period of young ovocytes is in autumn during October and November. By the onset of winter the gonads of female clams usually present a virtually mature appearance. The cells developed in autumn remain in the clam gonads throughout the winter without undergoing any degenerative processes. Nevertheless, regardless of this consideration, we should not underestimate the biological significance of the observation that normal eggs and spermatozoa which were discharged by the clams on April 9, 1951 were those which were formed some time in October or November of 1949, i. e., almost two years ago.

We are deeply indebted to our colleagues, John B. Glud and Walter R. Welsch of the U. S. Fish and Wildlife Service biological laboratory at Boothbay Harbor, Maine, whose help and friendly cooperation enabled us to carry on this study, to Charles A. Nomejko for preparing the photographs for this article, and to William S. Miller for his help in taking the mollusks to Boothbay Harbor.

SUMMARY

1. It was possible to delay spawning of clams and oysters for considerable periods by keeping them in water, the temperature of which was high enough to permit gametogenesis but too low to induce spawning.

2. While the oyster, *C. virginica*, resorbs unspawned gonads in late fall, the clam, *V. mercenaria*, carries undischarged eggs or spermatozoa through the winter and even into spring; the latter may be induced to spawn during this time, discharging viable gametes which develop into normal larvae.

REFERENCES

Loosanoff, V. L.

1937. Seasonal gonadal changes of adult clams, *Venus mercenaria* (L.). Biol. Bull. Woods Hole, *72:* 406–416.

1945. Precocious gonad development in oysters induced in midwinter by high temperature. Science, *102:* 124–125.

Loosanoff, V. L. and H. C. Davis

1950. Conditioning *V. mercenaria* for spawning in winter and breeding its larvae in the laboratory. Biol. Bull. Woods Hole, *98:* 60–65.