TEMPERATURE REQUIREMENTS FOR MATURATION OF GONADS OF NORTHERN OYSTERS

V. L. LOOSANOFF AND H. C. DAVIS

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

Gonad development and spawning of oysters (*Crassostrea virginica*) of our North Atlantic coast are well-defined seasonal phenomena. For example, in Long Island Sound, where extensive oyster beds are located, spring gonad development commences during the first part of May and spawning begins during the end of June or the first week in July (Loosanoff and Engle, 1942). There is no doubt that the rate of progress of gametogenic activities and the beginning of spawning of oysters are to a large extent influenced by the temperature of the surrounding water.

In the opinion of many students the period required for maturation of the gametes of the European oyster, *O. edulis,* is a function of time and temperature (Voisin, 1933; Orton, 1937; Korringa, 1941, and others). Few studies of a similar nature have been made, however, on the American oyster, *C. virginica.* Perhaps the only works dicussing effects of temperature on gonad development were those of Nelson (1928a), who thought that spawning of *C. virginica* should be expected approximately 160 degree-hours after the temperature of the water reaches and remains at the 20.0° C. level, and of Loosanoff (1942), who described seasonal gonadal changes of oysters of Long Island Sound and referred to the temperatures of the water prevailing during the different stages of gonad development and at spawning. In general, however, many aspects of the effect of temperature on the gonad development of *C. virginica* remained unknown.

The experiments described in this article were designed to ascertain the number of days needed for oysters of both sexes kept at different but constant temperatures to develop first mature cells; to reach the physiological state at which they can be induced to spawn by thermal or chemical means; and to reach the condition at which they will be ready to spawn normally, without any artificial stimulation. We hope that our article will contribute toward the understanding of the dependence of individuals and of populations upon ecological factors, such as temperature.

We wish to express our appreciation to our colleagues, Charles A. Nomejko, for assisting us in different phases of these studies, to David W. Calhoun for the statistical treatment of the data, and to Professor Thurlow C. Nelson of Rutgers University for reviewing the manuscript.

Method

The oysters used in these experiments ranged in age from three to five years. They were collected in winter when the temperature of the water over the beds was near 0.0° C. In the laboratory the oysters were separated into five groups and

placed in trays with running, cold sea water having a temperature of about 8.0° C., just high enough to allow the oysters to come out of hibernation. Twenty-four hours later the temperature of the water running in the trays was raised within 4 to 8 hours to 10.0, 15.0, 20.0, 25.0 or 30.0° C. and steadily maintained, within ± 1.0°C., at those levels until the end of the experiment.

In the first series of experiments small samples of oysters consisting of 5 to 15 specimens were taken at 5-day intervals for gross examination and histological studies (Loosanoff and Davis, 1949). In later experiments, however, each sample consisted of 50 oysters and when needed the oysters were examined daily. The quantitative data of this article are based on the latter samples.

The method of conditioning oysters to develop spawn in winter was, in principle, the same as that described several years ago (Loosanoff, 1945). It was modified only in the respect that instead of keeping the oysters in aquaria, the water of which was changed at certain intervals, they were kept in shallow trays through which water of the desired temperature was running continuously. The oysters were fed a rich culture of plankton which was automatically and continuously added to the running water.

Examination of the oysters was made according to the following procedure: Before the oysters were ripe enough to be induced to spawn, all 50 individuals constituting each sample were opened and the content of their gonads examined under a microscope to determine the number of males with active sperm, number of females with fertilizable eggs, and the number of immature oysters (Tables I–IV). All males containing active sperm were considered physiologically mature although not necessarily ready to spawn. Possession of fertilizable eggs was determined by adding mature sperm to the egg suspension made from each female containing large ovocytes. Formation of the polar body was taken as the criterion that the eggs were fertilizable.

After the oysters were kept long enough at the conditioning temperatures to be approaching the state in which they could be induced to spawn the procedure of the examination was changed as follows: Each of the 50 oysters constituting a sample was placed in a separate spawning dish, which was filled with water of the same temperature as that at which that group of oysters was conditioned. Then all the oysters were subjected to chemical stimulation consisting of the addition of sperm and egg suspensions to the water in the spawning dishes.

Ninety minutes after the beginning of the chemical stimulation all the oysters that failed to respond were further stimulated by an increase of the water temperature. After another period of 90 minutes the oysters that failed to spawn were opened and their gonads examined for the presence of active sperm or fertilizable eggs.

Observations

Control samples

Gonads examined at the beginning of the experiment contained small undeveloped follicles typical for hibernating oysters (Loosanoff, 1942). The male sex cells were in the early stages of spermatogenesis, predominantly spermatogonia or early spermatocytes. In the females the follicles contained mostly indifferent cells, some ovogonia and a few young ovocytes 15–18 μ in diameter. In both sexes most

of the space between the body wall and the digestive diverticula was occupied by the vesicular connective tissue which surrounded the small islands formed by the follicles (Fig. 1). The oysters were in good condition and contained large quantities of glycogen.

10.0° C. group

Even the most advanced oysters of this group, examined 35 days after the beginning of the experiment, showed only a slight development of their gonads. In the females the largest ovocytes were only 18–20 μ in diameter and contained virtually no yolk (Fig. 2). In the males, there were secondary spermatocytes and a few spermatids, but no spermatozoa. Even in these individuals, which constituted about 5 to 6 per cent of the samples, such development took place only towards the end of the experiment. In the other oysters of this temperature group the gonads even then resembled the winter condition.

It is interesting that in the 10.0° C. group the anastomosis of the follicles and gametogenesis were so slow even though these oysters, judging by the quantities of feces produced, were feeding more actively than those kept at the temperature of 25.0 or 30.0° C. Moreover, at the end of the experiment the meats of the low-temperature oysters still contained approximately as much glycogen as they had at the beginning. Considering these conditions, which indicated that the oysters were kept in a relatively favorable environment, it seems apparent that the depressed gonad development of those oysters was principally due to the low temperature.

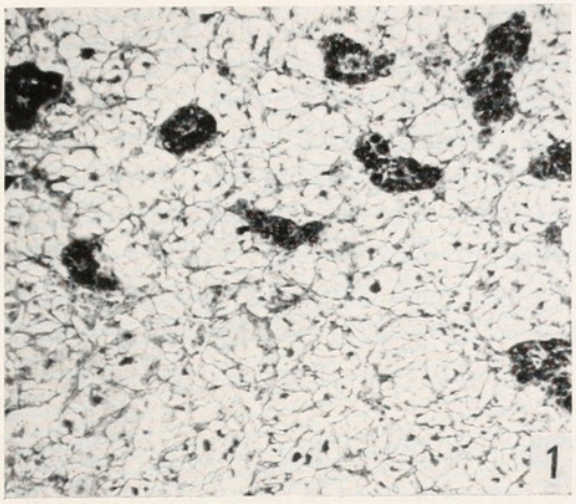
TABLE I

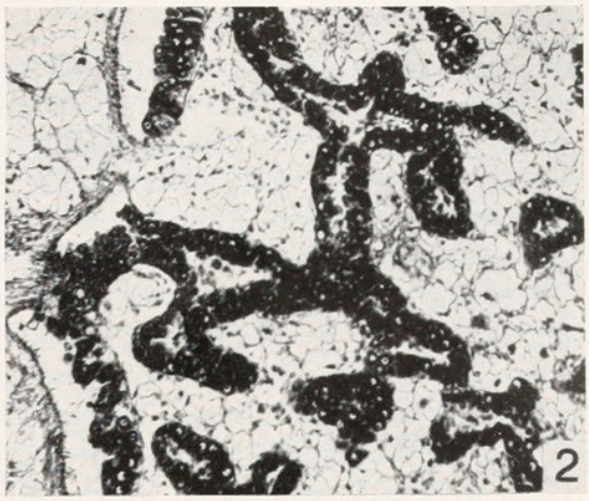
Number and per cent of oysters, kept for different periods at 15.0° C., that were induced to spawn, or showed presence of mature gametes. Number and per cent of immature oysters and those with sex undetermined are also given. Each sample composed of 50 oysters.

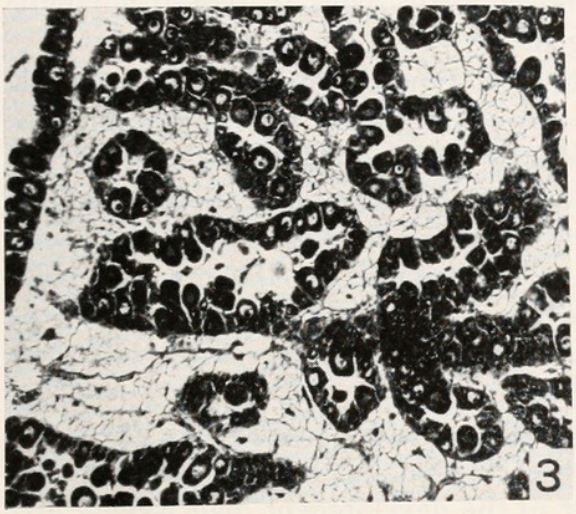
Days of conditioning	Induced to spawn				Unspawned				Per cent		
	By chemical stimulation		By chemical and thermal stimulation		Mature gametes		Immature gametes		Spawned	Unspawned but with mature gametes	Immature
	Male	Female	Male	Female	Active sperm	Fertilizable eggs	Sex recognizable but immature	Sex undetermined			
10	—	—	—	—	5	0	11	34	—	10	90
15	—	—	—	—	6	0	14	30	—	12	88
20	—	—	—	—	6	3	16	25	—	18	82
25	—	—	—	—	16	6	10	18	—	44	56
30	0	0	0	0	26	9	11	4	0	70	30
35	0	0	2	1	17	17	7	6	6	68	26
40	0	0	3	0	19	19	7	2	6	76	18
45	0	0	4	2	16	20	7	1	12	72	16
55	1	0	14	5	3	27	0	0	40	60	0

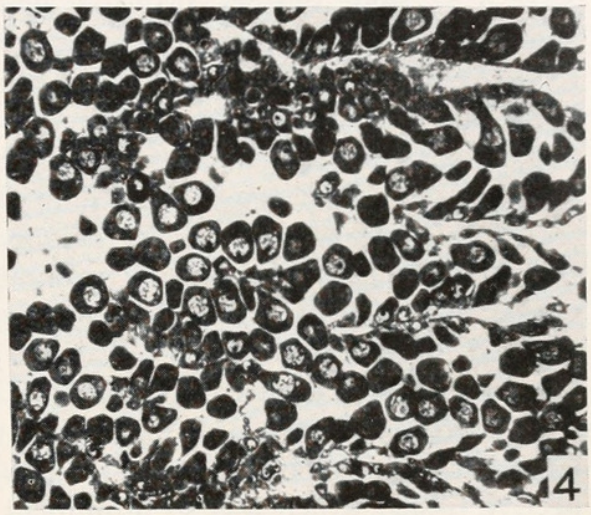
15.0° C. group

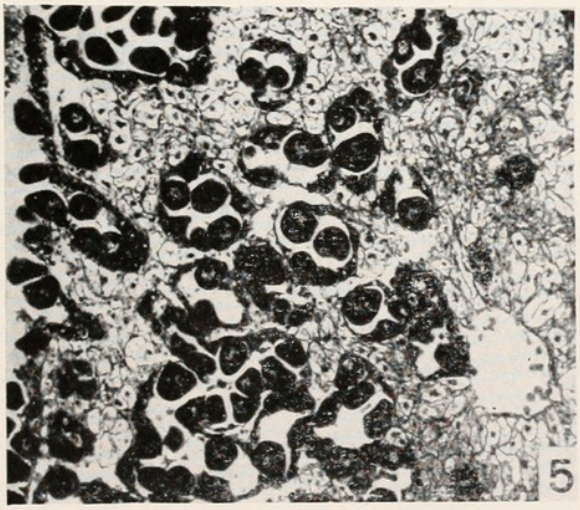
During the first five days of exposure the oysters showed little gametogenic activity and their gonads still remained in winter condition. The advanced males











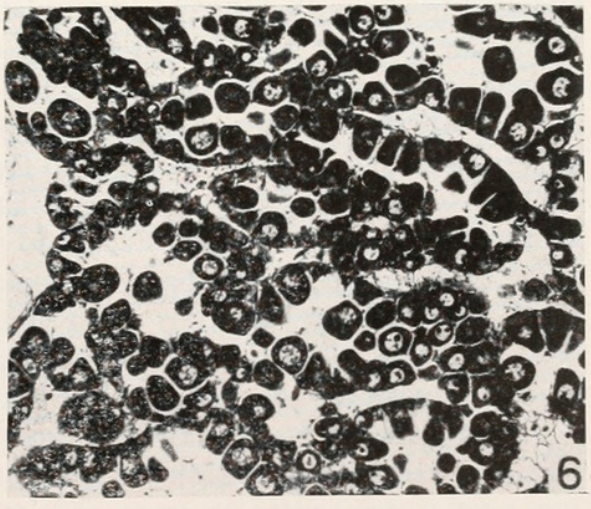


FIGURE 1. Winter gonad of oyster showing undeveloped follicles surrounded by connective tissue. × 112.

FIGURE 2. Gonad of most advanced female oyster of the group kept 35 days at 10.0° C. × 112.

FIGURE 3. Gonad of female oyster showing proliferating follicles containing growing ovocytes; 15 days at 15.0° C. × 112.

FIGURE 4. Gonad of female oysters; 40 days at 15.0° C. × 112.

FIGURE 5. Partly discharged gonad of female oyster; 55 days at 15.0° C. × 112.

FIGURE 6. Gonad of female oyster approaching ripeness; 13 days at 20.0° C. × 112.

showed a slight proliferation of follicles but gametogenesis proceeded only as far as the formation of spermatids. After 10 days the female oysters still showed little progress, while in the males a slight anastomosis of the follicles was noticed and in five of them a few spermatozoa were found (Table I). Towards the 15th day the largest ovocytes were already about 35 μ in diameter, but the vesicular connective tissue still occupied a large part of the interfollicular spaces (Fig. 3).

By the 20th day 9 oysters, representing 18 per cent of the sample, contained active sperm or fertilizable eggs, while 16 other oysters possessed recognizable sex cells (Table I). Thus, on this date the sex of 50 per cent of the oysters composing the sample was already recognizable.

On the 25th and 30th days attempts were made to induce the oysters to spawn. They were first stimulated by the addition to the water of a suspension of ripe spermatozoa or eggs, and later by a rapid increase of the temperature to about 32.0° C. Some oysters, probably females, moved their shells in typical rhythmic spawning motions, but no eggs were discharged.

Spawning was successfully induced for the first time on the 35th day (Table I). However, of 50 oysters composing the sample only three, two males and one female, spawned and then only after the combined effects of chemical and thermal stimulations. The eggs obtained in this spawning developed into normal larvae.

By this time a difference in the gonad development of the individuals composing the samples became especially pronounced. The thickness of the gonadal layer varied from almost 0.0 to 3.0 mm. and while the most advanced oysters could be induced to spawn, the least advanced possessed gonads so undeveloped that their sex could not be easily determined (Table I).

Between the 35th and 45th days the oysters continued to develop, their enlarged gonadal follicles coming in contact with each other, while the vesicular connective tissue, which was so prominent earlier in the development, had largely disappeared (Figure 4). Yet, even on the 45th day none of the oysters could be induced to spawn by chemical stimulation alone.

After 55 days all the oysters had either fertilizable eggs or active sperm, and 20 of them, constituting 40 per cent of the sample, were induced to spawn (Table I). Of these, however, only one responded to chemical stimulation alone, while all others required the rapid increase in temperature. It is possible that this happened because several oysters in the sample had already discharged some spawn, and were, therefore, comparatively indifferent to the stimulating effect of sex products. That some of the oysters may have spawned was shown by histological examination of the gonad tissue taken from the oysters of an auxiliary experimental group, some of which showed partially discharged gonads, characterized by contracting follicles and the invasion of connective cells into the interfollicular spaces (Fig. 5).

At the end of the 90-day period an egg and sperm suspension was added to the trays to determine if the oysters would respond to this stimulation and begin spawning, even if there were no increase in temperature. Within a few minutes after the addition of the suspension almost all the oysters in the tray commenced spawning and continued to do so for about two hours. During the spawning the temperature steadily remained at 15.8° C. The eggs collected from this spawning developed into normal larvae.

20.0° C. group

The stimulating effect of this temperature was clearly seen early in the experiment. At the end of the first five days 28 per cent of the oysters already contained either active sperm or fertilizable eggs (Table II). After eight days the oysters were first induced to spawn by a combination of chemical and thermal stimuli, and two days later some oysters responded to chemical stimulation alone (Table II). Although some ripe eggs were found in the females after only five to ten days of exposure, nevertheless, such eggs still represented a minority of the follicular cells.

TABLE II

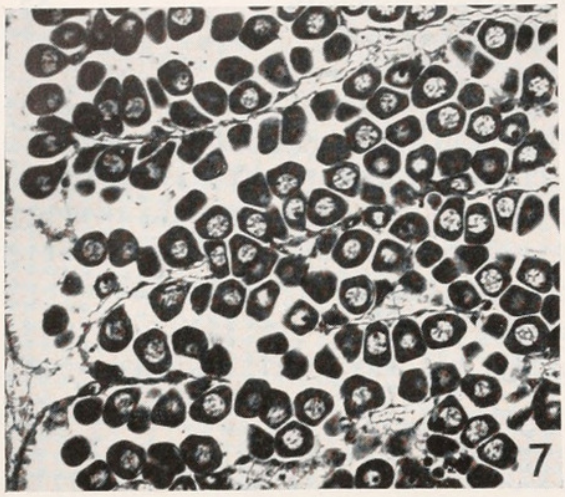
Number and per cent of oysters, kept for different periods at 20.0° C., that were induced to spawn, or showed presence of mature gametes. Number and per cent of immature oysters and those with sex undetermined are also given. Each sample composed of 50 oysters.

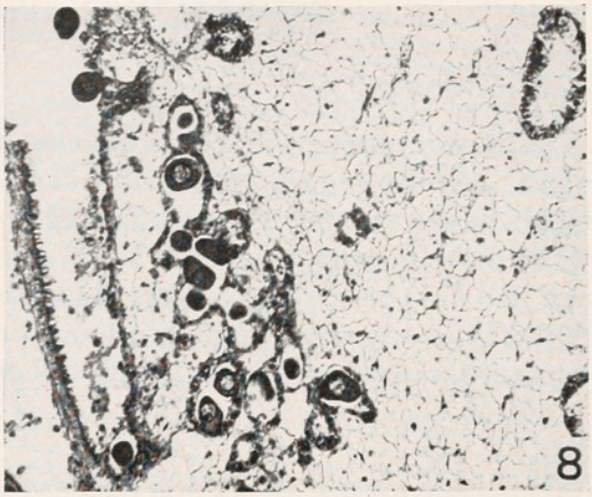
Days of conditioning	Induced to spawn				Unspawned				Per cent		
	By chemical stimulation		By chemical and thermal stimulation		Mature gametes		Immature gametes		Spawned	Unspawned but with mature gametes	Immature
	Male	Female	Male	Female	Active sperm	Fertilizable eggs	Sex recognizable but immature	Sex undetermined			
5	0	0	0	0	9	5	7	29	0	28	72
8	0	0	2	0	14	4	18	12	4	36	60
10	2	0	11	0	9	7	14	7	26	32	42
13	1	0	7	8	8	16	9	1	32	48	20
15	3	3	9	6	4	14	10	1	42	36	22
18	6	6	3	11	8	12	2	2	52	40	8
20	1	1	16	12	1	18	1	0	60	38	2
25	0	0	19	6	4	21	0	0	50	50	0

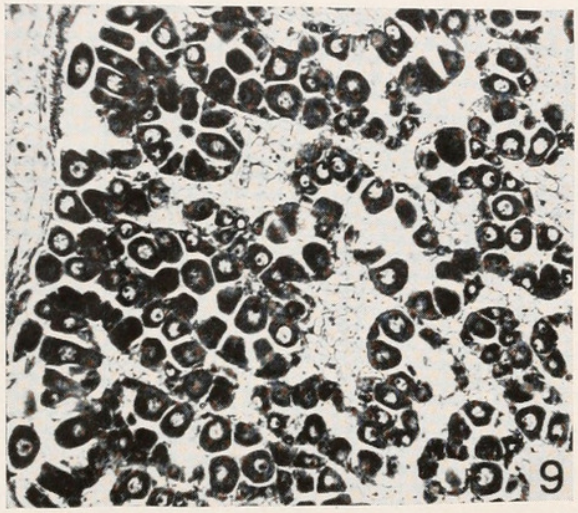
Again, as in the preceding temperature classes, the individual differences among the oysters of the same group were striking. While 26 per cent of the oysters kept at 20.0° C. for ten days could be induced to spawn, 7 others still possessed gonads resembling the winter condition (Table II). This condition of slow gonad development persisted even though the oysters were feeding vigorously and showed rapid growth of shell. Similar individual differences in gonad development were also found in oysters growing under natural conditions in Long Island Sound (Loosanoff, 1942).

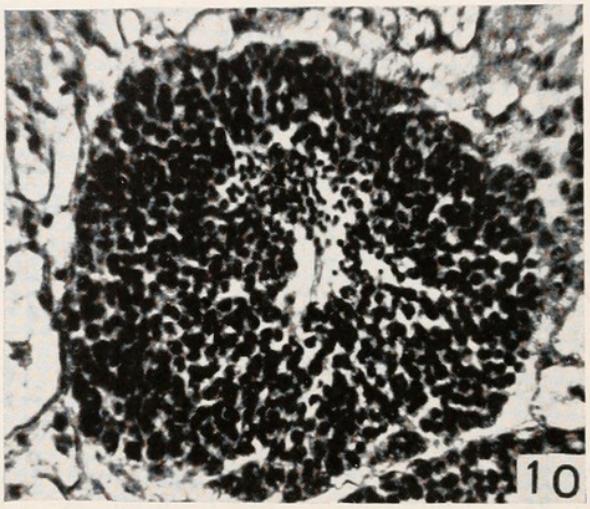
The spawning behavior of the females within the samples also showed that they were in physiologically-different states of ripeness. Some of them did not respond at all to stimulation. These were usually immature individuals, the sex of which could not be determined. Others moved their shells as in spawning but released no eggs. Still others discharged a few apparently immature eggs measuring only 45 μ, *i.e.*, about 5 μ smaller than normal eggs. Finally, there were females that discharged normal eggs which developed into healthy larvae. The ratio between these groups changed continuously, as can be judged from the data in Table II.

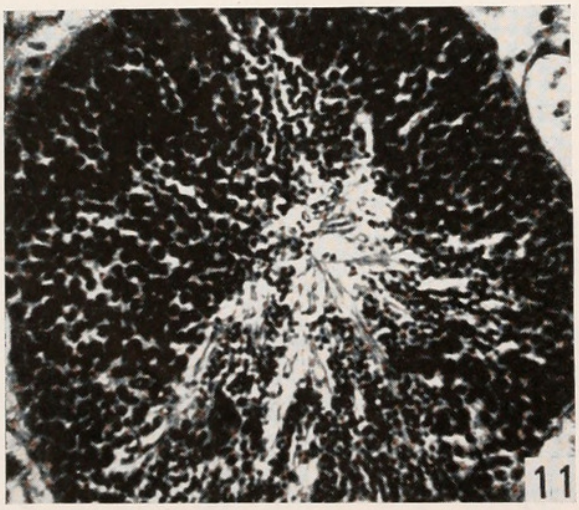
Between the 13th and 18th days many oysters were either approaching ripeness (Fig. 6) or were ripe (Fig. 7). At the latter date 52 per cent of the oysters











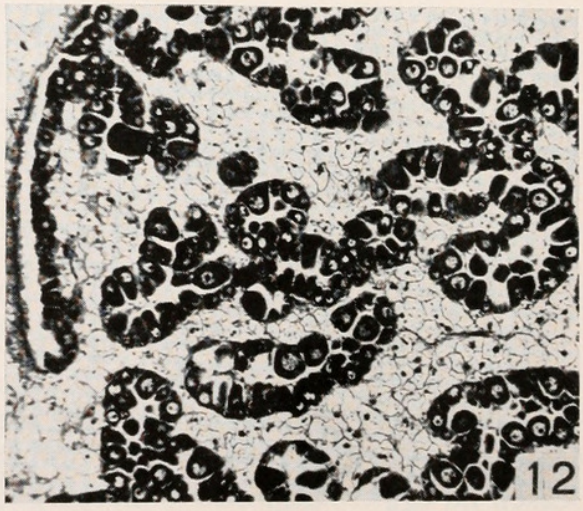


FIGURE 7. Gonad of ripe female oyster; 18 days at 20.0° C. × 112.

FIGURE 8. Gonad of female oyster in advanced stage of spawning; 25 days at 20.0° C. × 112.

FIGURE 9. Gonad of female oyster containing some ripe eggs but still not quite mature; 8 to 10 days at 25.0° C. × 112.

FIGURE 10. Gonadal follicle of male oyster showing few ripe spermatozoa in center of lumen; 3 days at 30.0° C. × 475.

FIGURE 11. Gonadal follicle of male oyster; 5 days at 30.0° C. × 475.

FIGURE 12. Gonad of female oyster containing some fertilizable eggs; 5 days at 30.0° C. × 112.

spawned, when stimulated, and the number of individuals with sex undetermined was only one or two per sample of 50. After 20 days 98 per cent either spawned or contained ripe gametes.

On the 20th day of exposure the number of oysters that could be induced to spawn showed a considerable decrease, as compared with the record of the 18th day (Table II). Between these two dates some of the oysters had apparently spawned in the natural, unprovoked way, and the presence of their sex products in the surrounding water may have made them unresponsive to this type of stimulation when it was applied experimentally a day or so later. This contention is supported by the observation that on the 25th day of the experiment none of the oysters could be induced to spawn by chemical stimulation alone. Histological studies of their gonads also showed that some were already in the typical post-spawning condition characterized by contracted follicles still retaining some ripe eggs, a large number of phagocytes, and a rapid invasion of connective tissue throughout the space between the body wall and the digestive diverticula (Fig. 8). Such oysters, obviously, could not be easily stimulated and, therefore, were not included in the number of oysters induced to spawn on that date. Instead, they were classified together with those that were unspawned but contained active sperm or fertilizable eggs. This explains why on the 25th day of exposure the number of oysters that could be induced to spawn showed a decrease, while the number of individuals which were unspawned but contained ripe gametes showed an increase (Table II).

25.0° C. group

In this group rapid gametogenesis and proliferation of gonadal follicles began within the first few days. After five days 17 oysters, constituting 34 per cent of the sample, already contained mature gametes (Table III). Of these 13 were males containing active sperm and the rest were females with fertilizable eggs. In both sexes, however, there were only a few ripe cells, indicating that the oysters were just entering the state of ripeness.

TABLE III

Number and per cent of oysters, kept for different periods at 25.0° C., that were induced to spawn, or showed presence of mature gametes. Number and per cent of immature oysters and those with sex undetermined are also given. Each sample composed of 50 oysters.

Days of conditioning	Induced to spawn				Unspawned				Per cent		
	By chemical stimulation		By chemical and thermal stimulation		Mature gametes		Immature gametes		Spawned	Unspawned but with mature gametes	Immature
	Male	Female	Male	Female	Active sperm	Fertilizable eggs	Sex recognizable but immature	Sex undetermined			
5	—	—	—	—	13	4	10	23	—	34	66
6	0	0	0	0	19	8	11	12	—	54	46
7	0	0	8	4	7	13	10	8	24	40	36
8	5	1	3	1	13	17	10	0	20	60	20
9	1	3	7	5	8	13	9	4	32	42	26
10	9	13	3	5	5	10	3	2	60	30	10

By the end of the seventh day some of the oysters were induced to spawn by the combined effects of chemical and thermal stimuli, and the next day five males and one female responded to chemical stimulation alone.

After 9 and 10 days of conditioning the samples gave 32 and 60 per cent of spawners respectively, and at the latter date the number of immature oysters was only 10 per cent (Table III). However, some of those that were capable of spawning still possessed gonads containing many immature cells. This condition is well illustrated in Figure 9, where together with mature ovocytes there are many unripe cells. Furthermore, the large quantity of connective tissue in the interfollicular spaces indicated that further growth of the follicles was still to occur.

Mass spawning of the oysters of this temperature group took place during the eleventh day. In some earlier experiments, however, mass spawning of one group kept at 25.0° C. occurred on the ninth day (Loosanoff and Davis, 1949). In both cases the spawning was unprovoked since the temperature remained steadily at 25.0° C. and no sex products capable of stimulating the experimental oysters could be present in the water flowing into the trays, because the experiments were conducted in February when the oysters in the Harbor, from which our water supply is obtained, were hibernating and, obviously, could not release any spawn.

30.0° C. group

This group, as a whole, responded extremely rapidly to the effect of high temperature and after three days of exposure 18 per cent of the oysters already possessed physiologically-ripe cells (Table IV). Although the most advanced females showed expanding follicles already containing a few fertilizable eggs, their gonads, nevertheless, still had a large quantity of connective tissue separating the follicles. In the males rapid spermatogenesis was in progress, already resulting in a few ripe spermatozoa in the centers of the lumens of the follicles (Fig. 10).

TABLE IV

Number and per cent of oysters, kept for different periods at 30.0° C., that were induced to spawn, or showed presence of mature gametes. Number and per cent of immature oysters and those with sex undetermined are also given. Each sample composed of 50 oysters.

Days of conditioning	Induced to spawn				Unspawned				Per cent		
	By chemical stimulation		By chemical and thermal stimulation		Mature gametes		Immature gametes		Spawned	Unspawned but with mature gametes	Immature
	Male	Female	Male	Female	Active sperm	Fertilizable eggs	Sex recognizable but immature	Sex undetermined			
3	—	—			7	2	3	38	—	18	82
4	—	—			26	3	6	15	—	58	42
5	16	3	Not		7	3	8	13	38	20	42
6	15	12	Employed		10	1	8	4	54	22	24
7	22	13			1	2	6	6	70	6	24

After five days the oysters were successfully induced to spawn. Because they were conditioned at the temperature of 30.0° C. it was impractical to use a much higher temperature to induce spawning and, therefore, chemical stimulation

alone was used. Nineteen oysters of the total number of 50 spawned but the quantities of spawn released were still small. Histological examination of the gonads showed that although the males conditioned at 30.0° C. for five days (Fig. 11) had considerably more mature sperm than after three days (Fig. 10), the unripe cells, nevertheless, still predominated. The same could be said for the females in which ripe ovocytes were not too common (Fig. 12).

After six days 54 per cent of the oysters were induced to spawn. Yet, 24 per cent were still immature and in four per cent the sex could not be determined (Table IV). After seven days 70 per cent could be spawned, and in one of the experiments an unprovoked mass spawning took place at the end of the seventh day. Both sexes participated in the spawning, the males discharging large quantities of sperm, but the number of eggs released still remained comparatively small.

By the tenth day the gonads of the oysters were already in advanced post-spawning stages, characterized by contracted follicles similar to those shown in Figures 5 and 8.

Discussion

As has already been mentioned, in studying the maturation of gonads of oysters kept at different but constant temperatures we were primarily interested in the number of days needed for formation of the first physiologically-mature gametes in each sex. We were also interested in finding how soon oysters can be induced to spawn by artificial means, *i.e.*, by the addition of sex products or by a rapid increase in temperature and, finally, how soon they would begin to spawn normally, without the help of any artificial stimulation.

Our experiments showed that the temperature of 10.0° C. is not high enough to induce gametogenic activities in most of the oysters, and in those few in which such activities were initiated, the development proceeded at an extremely slow rate. Apparently this temperature was too low to permit the conversion of glycogen and other materials stored in the oyster bodies into sex products. While the exact mechanism of this complex process is still not understood, it is thought that it involves the action of a certain enzyme or chain of enzymes, which is retarded, or completely inhibited, at low temperatures.

There are ample indications that some processes in animals, including oysters, are slowed down by low temperature more than others, thus showing that the effect of such low temperature is, obviously, differential. For example, while the growth of the shell of the oysters of our waters may proceed even at a temperature of one or two degrees below 10.0° C., the gonad development at such a temperature is either entirely arrested or proceeds at such a slow rate that a successful completion of that process cannot be expected. Perhaps this is a good example of the so-called developmental or biological zero of Bĕlehrádek (1935) who considers such a zero as the highest temperature threshold at which a certain protoplasmic activity is still arrested by cold.

Our observations, that at a temperature of 10.0° C. even the most advanced oysters did not form spermatozoa after 35 days of exposure, differ from those of Nelson (1928b) who, in his studies of oysters in Barnegat Bay, found that "active spermatozoa may be found in a few oysters at temperatures as low as 9°–10° C., Table I." Nelson's table shows, however, that the temperature data which he

offers were the averages for the week preceding the date of collection of the gonad samples. It is possible, therefore, that the maximum temperature during, or prior to, this period exceeded 10.0° C., causing spermatogenic activities which resulted in the formation of a few spermatozoa. Nevertheless, the results of our controlled experiments are in full agreement with the field observations of Nelson (1928a) who emphasized the wide individual differences often found in the condition of the gonads of oysters collected at the same time and from the same bed.

The experiments also demonstrated that under certain conditions ripening of gonads and spawning of oysters of both sexes may be accomplished at a temperature of only about 15.0° C. These experiments prove rather conclusively that the old conception that 20.0° C. is the minimum temperature at which spawning of *C. virginica* is possible does not hold for the oysters of Long Island Sound.

Laboratory observations on ripening of gonads at such a relatively low temperature corroborate the studies in Long Island Sound, which showed that spawning of oysters may take place at a temperature as low as 16.4° C. (Loosanoff, 1939). Recently we have found that in nature oysters may possess ripe gonads in early summer when the temperature of the surrounding water is only 14.6 to 15.4° C. On July 1, 1948 samples of oysters were taken from two of our collecting stations located in Long Island Sound at a depth of 30 feet. At the time of collection the temperature of the water at the first station was 14.3° and at the second, 15.4° C. The highest temperature recorded that summer at the first station, prior to collection of the sample, was 14.6° C., while at the second station it was 15.4° C. These oysters were placed in the laboratory in separate trays of slowly running water at about 22.0° C. A few hours later the oysters began to spawn copiously, both sexes participating in the act. The fertilized eggs were cultured and became normal larvae.

A similar experiment was repeated in early June, 1949. The oysters were collected from a depth of 35 feet, where the highest temperature recorded for the season was only 14.5° C. Again the oysters spawned a few hours after they were placed in warm water. Thus, the oysters were near the spawning condition at the time of collection even though the highest temperature the water had reached that season was only about 15.0° C.

Since spawning is possible at temperatures lower than 20.0° C., Nelson's (1921) conclusion that if the temperature of the water fails to reach 70.0° F., (21.2° C.) and remain at that level for some time, the oysters will not spawn at all, should be regarded as not applicable to the oysters of Long Island Sound. For the same reason Nelson's (1928a) suggestion that spawning of *C. virginica* may be expected to begin approximately 160 degree-hours after the temperature of the water has reached 20.0° C. is not true for Long Island Sound oysters. We think that the discrepancies between our observations and Nelson's may be explained on the basis that the two studies were made in geographical areas where ecological conditions are different, and where the local populations of oysters may possess significantly different physiological traits. That such physiologically-different groups of oysters may exist was recently shown by Stauber (1950) who by reviewing published and some unpublished records concluded that there appear to be three physiological races of oysters, *C. virginica,* which require different minimum temperatures at which they are capable of spawning. Stauber showed that the race with the lowest critical temperature is not found in Canada, at the northern limit of the

geographical range of the species, but in Long Island Sound, some 600 miles airline farther south. Loosanoff and Nomejko (1951), by conducting experiments in Milford Harbor, Connecticut, with oysters brought from different geographical areas along the Atlantic coast, found that the breeding temperature requirements of the northern oysters were somewhat lower than those of the southern groups, thus corroborating Stauber's conclusions and suggesting once more that some physiological requirements of oysters of different geographical districts may be decidedly different. We would also like to mention that our colleague, Professor Thurlow C. Nelson, called our attention to the most interesting fact that many years ago Lamarck from shell characteristics alone designated the Long Island Sound oyster as *Ostrea borealis,* which he believed to be a species distinctly different from *O. virginica* (now *Crassostrea virginica*).

TABLE V

Per cent of oysters of both sexes with mature gametes and estimated number of females in samples of 50 oysters kept at temperature of 15.0, 20.0, 25.0 or 30.0° C. for different numbers of days. Calculated days' exposure compensated for inequalities in sex ratio are also shown.

Days exposed	Temperatures in °C.											
	15.0			20.0			25.0			30.0		
	Compensated days exposed	% Oysters with mature gametes	Estimated number of females	Compensated days exposed	% Oysters with mature gametes	Estimated number of females	Compensated days exposed	% Oysters with mature gametes	Estimated number of females	Compensated days exposed	% Oysters with mature gametes	Estimated number of females
3										3.0	18	27
4										5.2	58	18
5				4.9	28	28	4.9	34	28	5.7	58	23
6							6.1	52	27	6.7	76	24
7							6.1	64	33	7.6	76	25
8				7.8	40	29	7.8	80	29			
9							7.9	74	33			
10	9.8	10	29	10.6	58	26	8.8	90	33			
13				11.2	80	34						
15	14.5	12	30	12.9	78	34						
18				16.1	92	33						
20	17.4	18	34	18.4	98	32						
25	25.9	44	28	26.6	100	27						
30	35.9	70	23									
35	35.6	74	29									
40	43.2	82	27									
45	44.9	84	30									
55	52.3	100	32									

As is well known, the rate of metabolic processes of an animal increases almost to the extreme upper limit of the temperature at which this animal carries on its activities. This is especially well noticed in poikilothermous animals, the body temperature of which is usually at or near that of their environment. To express more or less accurately the influence of temperature on biological phenomena and to demonstrate the relation existing between temperature and the speed of physiological

processes, a number of formulae have been proposed by many students including the most often quoted Krogh (1914), Arrhenius (1915) and Bělehrádek (1935). It is not the purpose of our article to evaluate the relative merits of the different formulae, except to mention that although there is not a universally acceped mathematical expression for the effect of temperature upon the rate of biological processes, it is widely recognized, nevertheless, that some of the formulae, including those mentioned above, offer biologists a tool for an approximate solution of the problems and, at times, for prediction of the results of the studies concerning relationships between temperature and physiological processes, such as gonad development or spawning.

Choice of indices of the gonadal development of a group of oysters is somewhat arbitrary. The two most satisfactory for examination were the number of individuals with active sperm and the number with fertilizable eggs. Accordingly, an analysis was made of the sums of these two indices, as a percentage in each sample of 50 oysters (Table V). This percentage is hereafter called the degree of maturity of the sample. It was then transformed to the probit scale, and compared with the exposure time on a logarithmic scale. The four groups of samples at the different temperatures gave four series of points. By the method of maximum likelihood four parallel straight lines with the slope 4.343 were fitted, one to each series. Deviations did not appear to come from systematic curvature or non-parallelness of the fitted lines.

This analysis indicated that (1) since straight lines follow use of the log-time scale, individual differences may be explained by variability in the rates of development of different individuals. (2) Parallelism of the four lines is evidence that a given temperature increase affects all individuals in the same way, accelerating their rates of development by the same factor. (3) The slope of the fitted lines measures the variation of the oyster population with respect to differences in time required to reach maturity. For example, at a given temperature, it will take the slowest developing 20 per cent of the population over 2.5 times as long to reach maturity as it does the fastest 20 per cent (Fig. 13).

Our experiments have shown that males mature earlier than females (Tables I–V). Therefore, we would expect that samples largely female would be slow and those predominantly male would be farther advanced than the average. Since the sex of oysters with poorly developed gonads could not be recognized, the actual number of each sex in some samples was unknown. Hence, the sexes could not be analyzed separately, and random fluctuations in sex ratio have presumably disturbed the temperature averages, making comparison uncertain. A refinement of the analysis was obtained by allowing for the estimated proportion of females in each sample. An appropriate allowance was then subtracted from the actual time for predominantly female samples and added to the time of the samples with more than the average number of males.

Estimated sex ratios in each sample were based on the preliminary fitted lines, by dividing the number of sexually indeterminate individuals roughly in proportion to the expected unripe fractions for each sex. The resulting estimate, expressed as number of females, is shown in Table V. Based on these estimates an allowance was calculated, using partial regression, leading to compensated exposure times: $x = 1.03035x_1 - .01212x_2 + .31226$, where x_1 is the original log-time, x_2 is the

estimated number of females in the sample and x is the log of the compensated time. The latter is the time that would presumably be required for a sample of 50 oysters containing 28.37 females, which is the weighted average for the experiment, to reach the same degree of maturity as the given sample. This is presented in Figure 13, showing degree of maturity plotted against compensated exposure time, with

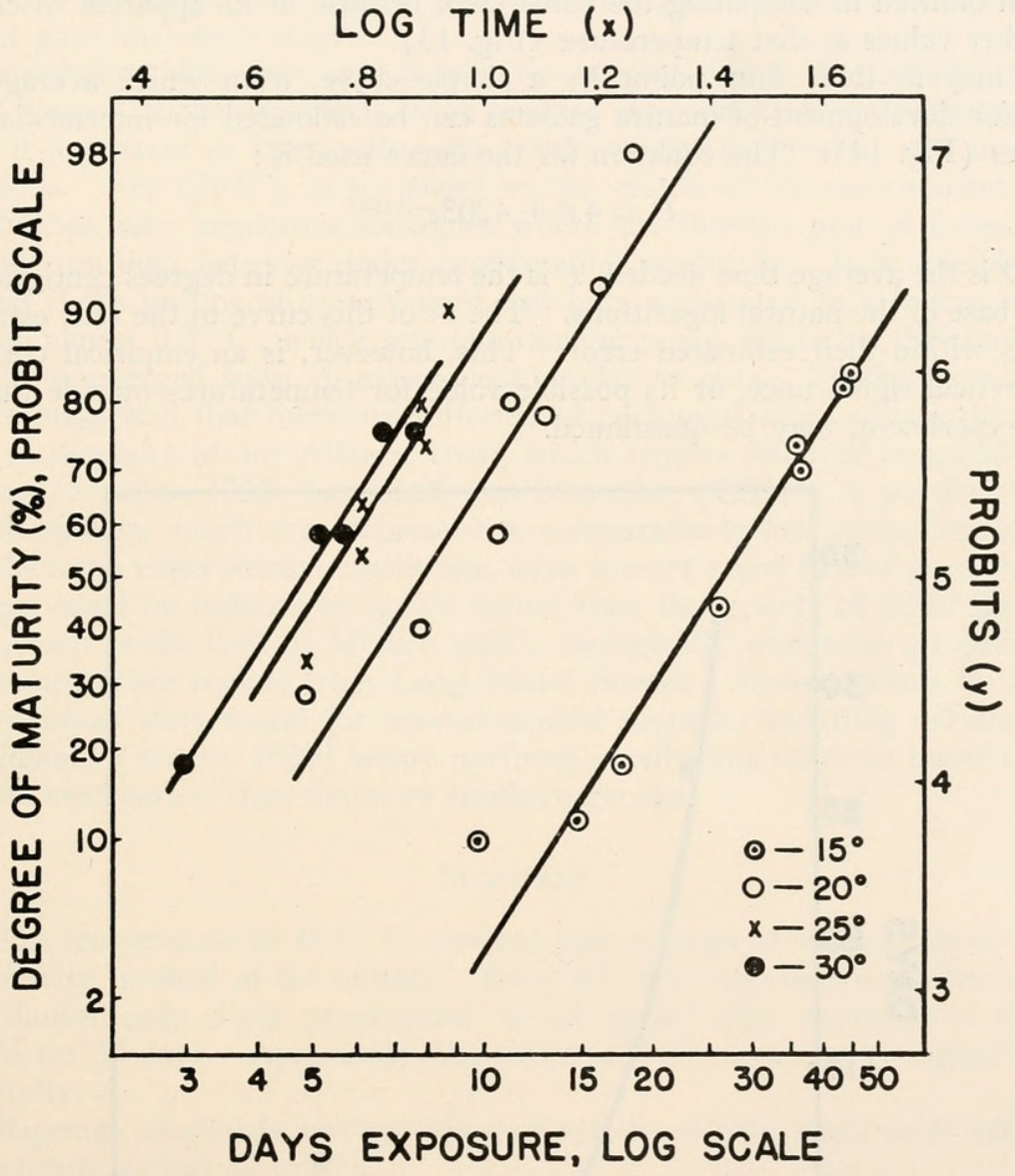


FIGURE 13. Degree of maturity (%) in samples of 50 oysters plotted against period of exposure to four experimental temperatures. Each period of exposure is compensated for the estimated sex ratio of the sample.

a straight line of slope 4.343 fitted for each of the four temperatures. This fit is satisfactory after compensation for sex ratio: chi-square equals 30.8, with 22 degrees of freedom, which is just at the 10 per cent level of significance.

From the equation for each line, $y - \bar{y} = 4.343\ (x - \bar{x})$, where y is the probit of degree of maturity and $\bar{x}$ and $\bar{y}$ are the average values of x and y for a given temperature, the average time required for 50 per cent of oysters to develop mature

gametes can be calculated for each temperature. This corresponds to the value of x when $y = 5$. For the four temperatures this average time was:

15.0° C. — 26.5 days	25.0° C. — 5.4 days
20.0° C. — 7.9 days	30.0° C. — 4.9 days

Their error is estimated as about 10 per cent. The lowest observation at 15.0° C. has been omitted in computing the value 26.5, because of its apparent discrepancy from other values at that temperature (Fig. 13).

We may fit these four points by a simple curve, from which average times needed for development of mature gametes can be estimated for intermediate temperatures (Fig. 14). The equation for the curve used is:

$$D = 4.8 + 4205e^{-.3554T}$$

where D is the average time needed, T is the temperature in degrees centigrade, and e is the base of the natural logarithms. The fit of this curve to the four established points is within their estimated error. This, however, is an empirical curve, and its theoretical significance, or its possible value for temperatures outside the range of the experiment, may be questioned.

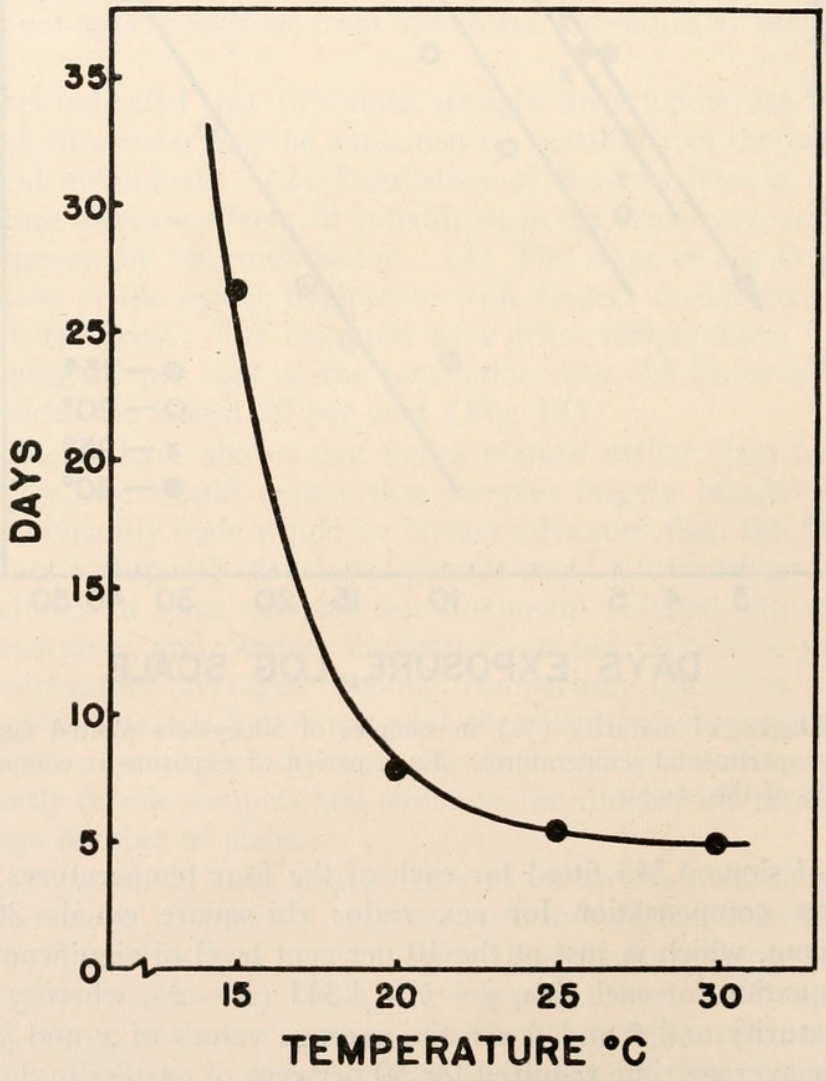


FIGURE 14. Average time needed for 50 per cent of an oyster population to develop mature gametes at different temperatures.

We have noticed that the quantity of glycogen contained by the oysters at the beginning of gonad development may control the quantity of spawn that will be produced. If the oysters are "poor," *i.e.*, containing little glycogen, they usually do not develop good gonads. For example, our attempts to condition for spawning in winter several groups of oysters shipped to us from the Florida waters of the Gulf of Mexico failed because those oysters were thin and watery, containing virtually no glycogen. Similar experiments with poor oysters from other bodies of water gave the same results.

In concluding this discussion it may be mentioned that the knowledge of the thermal history of the organisms prior to their exposure to the experimental conditions is important in interpreting the results of the experiment and in forming conclusions. Fry (1947), in his paper on the effects of the environment on animal activities, cites numerous examples where the thermal past of fishes reflects profoundly on their behavior under experimental conditions. It is possible, therefore, that if the groups of oysters used had been acclimated to a higher temperature than almost 0.0° C., which was our starting point, somewhat different results would have emerged from an experiment similar to ours. Furthermore, since it has been suggested that there are different physiological races among the population of *C. virginica* of our Atlantic coast, which require different temperatures for spawning (Stauber, 1950; Loosanoff and Nomejko, 1951), it is possible that our northern oysters, which are acclimated to comparatively low temperature, would, under the same experimental conditions, show a more rapid rate of gonad development and could be induced to spawn earlier than the oysters of other geographic regions, such as the Gulf of Mexico which, ecologically, maintains an entirely different temperature regime from Long Island Sound. Such relations to temperature conditions were found for several aquatic animals, including certain species of amphibians (Moore, 1939) where northern populations survived lower temperatures and bred earlier than the more southern groups.

Summary

1. The temperature of 10.0° C. was not high enough to induce normal gametogenic activity in most of the oysters. Even the most advanced individuals of this group showed only slight development of the gonad after exposure to this temperature for 35 days. Apparently this temperature is near the biological zero for this activity.

2. Ripening of gonads and spawning of oysters of both sexes were achieved at a temperature as low as 15.8° C.

3. Wide individual differences in the extent of gonad development among the individuals constituting the same groups were commonly found.

4. In all temperature groups, ranging from 15.0 to 30.0° C., the physiologically ripe gametes were generally formed earlier in the males than in the females.

5. At 15.0° C. the most advanced males contained a few ripe spermatozoa on the 10th day. Fertilizable eggs were found on the 20th day and spawning was induced by the 35th day. At 20.0° C. oysters with ripe spermatozoa and fertilizable eggs were found on the fifth day. Spawning was induced on the 10th and 13th days in males and females respectively. At 25.0° C. ripe spermatozoa and fertilizable eggs were found by the fifth day. Spawning was induced on the seventh day. At 30.0° C. ripe spermatozoa and a few fertilizable eggs were found three

days after the hibernating oysters were taken from their winter environment and placed at this temperature. Spawning was induced on the fifth day.

6. The average time at each of the experimental temperatures required for 50 per cent of the oysters to develop mature gametes was calculated to be:

15.0° C. – 26.5 days	25.0° C. – 5.4 days
20.0° C. – 7.9 days	30.0° C. – 4.9 days

To estimate the average time needed for development of mature gametes at temperatures intermediate to those given above, a simple curve is offered based on the equation:

$$D = 4.8 + 4205e^{-.3554T}$$

where D is the average time needed, T is the temperature, and e is the base of the natural logarithms.

7. The quantity of glycogen in the oysters at the beginning of gonad development may control the quantity of spawn produced.

LITERATURE CITED

ARRHENIUS, S., 1915. Quantitative laws in biological chemistry. Bell, London, pp. 1–164.

BĚLEHRÁDEK, J., 1935. Temperature and living matter. Borntraeger, Berlin, pp. 1–277.

FRY, F. E. J., 1947. Effects of the environment on animal activity. Publications of the Ontario Fisheries Research Laboratory, No. 68. The University of Toronto Press, Toronto, pp. 1–62.

KORRINGA, P., 1941. Experiments and observations on swarming, pelagic life and setting in the European flat oyster, *Ostrea edulis* L. *Contr. from Governmental Inst. for Biol. Fish. Research. Extrait des Archives Neerlandaises de Zool.*, **5**: 1–249.

KROGH, A., 1914. The quantitative relation between temperature and standard metabolism in animals. *Int. Zeits, physik.-chem. Biol.*, **1**: 491–508.

LOOSANOFF, V. L., 1939. Spawning of *Ostrea virginica* at low temperatures. *Science,* **89**: 177–178.

LOOSANOFF, V. L., 1942. Seasonal gonadal changes in adult oysters, *O. virginica,* of Long Island Sound. *Biol. Bull.,* **82**: 195–206.

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

LOOSANOFF, V. L., AND H. C. DAVIS, 1949. Gonad development and spawning of oysters at several constant temperatures. *Anat. Rec.,* **105**: 112.

LOOSANOFF, V. L., AND JAMES B. ENGLE, 1942. Accumulation and discharge of spawn by oysters living at different depths. *Biol. Bull.,* **82**: 413–422.

LOOSANOFF, V. L., AND CHARLES A. NOMEJKO, 1951. Existence of physiologically-different races of oysters, *Crassostrea virginica. Biol. Bull.,* **101**: 151–156.

MOORE, J. A., 1939. Temperature tolerance and rates of development in the eggs of Amphibia. *Ecology,* **20**: 459–478.

NELSON, T. C., 1921. Aids to successful oyster culture. I. Procuring the seed. N. J. Agric. Exp. Stat. Bull. No. 351, pp. 1–59.

NELSON, T. C., 1928a. Report of the Dept. of Biol. of the N. J. Agric. Coll. Exp. Stat. for the year ending June 30, 1927, pp. 77–83.

NELSON, T. C., 1928b. Relation of spawning of the oyster to temperature. *Ecology,* **9**: 145–154.

ORTON, J. H., 1937. Oyster biology and oyster culture. The Buckland Lectures for 1935, pp. 1–211, Edward Arnold and Co., London.

STAUBER, L. A., 1950. The problem of physiological species with special reference to oysters and oyster drills. *Ecology,* **31**: 109–118.

VOISIN, P., 1933. La reproduction de l'huitre. *La Peche Marit., la Peche fluv. et la Pisciculture,* **16**: 61–63.

Loosanoff, V L and Davis, Harry C. 1952. "TEMPERATURE REQUIREMENTS FOR MATURATION OF GONADS OF NORTHERN OYSTERS." *The Biological bulletin* 103, 80–96. https://doi.org/10.2307/1538408.

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