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No. 141.

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and

Variations in numbers of oyster larvae in correlation with temperature and salinity. B

by

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Author

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1933.

The effects of Temperature and Salinity on the Spermatozoa, Ova and Development to the First Swimming Stage of Ostrea virginica.

By Mrs. L. R. Richardson (nee A. E. Clark)
(Part of thesis submitted to McGill University
in partial fulfilment of the requirements
for the degree of M.Sc., May, 1933.)

INTRODUCTION

The study of oyster culture and its consequent problems of the effects of environmental factors has been of great interest to the civilized world from its economic view-point. Setting aside the European and Japanese oysters and dealing only with the American oyster, Ostrea virginica, the history of oyster culture properly begins with W. K. Brooks (2), who in 1878, succeeded in artificially fertilizing the eggs of Ostrea virginica. The following summer, 1879, he arrived at the conclusion that, in the American oyster, fertilization took place outside the mantle cavity, a striking difference from the European oyster, which carries the embryos within the mantle cavity until they are quite well advanced in development, and are provided with shells of their own. This shedding of the naked spermatozoa and ova into the water makes it vital that the effects of certain environmental factors, such as temperature and salinity be known.

The study of the American oyster in Canada began in 1903 at Malpeque, on a bay of the same name in Prince Edward Island, with the establishment there of a movable Biological Station. In 1904 Dr. J. Stafford (9) started his study of the life history of Ostrea virginica, and in 1913 presented his report to the commission of Conservation, Canada.

1. Amemiya (1), a Japanese investigator, also worked on the early developmental stages of Portuguese, American and English oysters, with special reference to the effects of varying salinity.

Turning again to Prince Edward Island, Dr. Julian Nelson (7) made an investigation of oyster propagation in Richmond bay in 1915. In 1929 Dr. A. W. H. Needler (6) was put in charge of oyster investigations for the Biological Board of Canada, and a permanent laboratory known as the Prince Edward Island Marine Station was located on Bideford river, an inlet of Malpeque bay. In 1930 Mr. H. P. Sherwood, an English investigator, carried on his work there. The following series of experiments were carried out by the author in 1932 under the direction and guidance of Dr. A. W. H. Needler, to whom I am greatly indebted.

EFFECT OF TEMPERATURE

In the following experiments on the effects of different temperatures, well-insulated thermostat boxes, designed by Dr. A. W. H. Needler, were used to keep the temperature constant at whatever degree desired. Ice was put in the boxes for the temperatures lower than room temperatures, and the boxes themselves put in a cement-lined well sunk in the earth.

1. Effect of Temperature on the Spermatozoa

The temperatures used in these experiments were away beyond those that the sperms would be subjected to in nature. It has been shown by H. F. Prytherch (8) that a temperature of 20°C. is necessary before the oyster will spawn, and the water in Bideford river, having reached 20°C., rarely drops more than 5°C. and the highest temperature found by Dr. Julius Nelson

(7) was 74° F (23.6°C.). In these experiments the temperatures ranged from 5°C. to 40°C., spaced at 5° intervals.

The sperms were obtained from ripe male oysters that had not spawned or that had only partially spawned, by "stripping", i.e., a slight cut was made in the gonad and the products gently stroked towards the cut, care being taken to include as little of the tissue of the gonad as possible. The sperms thus obtained were put in beakers containing sea-water having a salinity of 27.3‰. Enough sperms were added to give the solutions a uniform cloudiness.

The object of the experiments was to determine the length of life of the sperm at different temperatures. The "life" of the sperms was defined as the period during which any motion caused by the rapid vibrations of their tails, and typical of sperm, could be observed. The sperms remained motionless until put in sea-water, then they became violently active, this activity gradually declining until "death" when they became motionless. Care had to be taken during the hourly examination of the sperm that the temperature of the test sample did not change until after the observations had been made.

The results of the experiments showed that at the highest temperature, 40°C., the life of the sperm was only 9 hours, but as the temperatures became lower the life became longer until at 5°C., the sperm lived 148 hours (Fig. 1).

These experiments show that the temperatures the sperm would encounter in nature would not have an injurious effect.

2. Effect of Temperature on the Ova.

The ova (eggs), obtained by stripping ripe female oysters,

were put in beakers containing sea-water with a salinity of 27.3‰. The life of the egg was more difficult to define as there was no visible activity, but for this work it was defined as that period during which fertilization by the sperms produced cell division and development.

To determine the length of the life of the eggs under natural conditions six tubes were partially filled with sea-water from Bideford River and eggs were added to give uniform solutions. To the first tube sperms, freshly removed from a male, were added immediately. To the second tube sperms were added after an interval of 2 hours, to the third after an interval of 4 hours, to the fourth after 6 hours, to the fifth after 8 hours, and to the sixth after 10 hours. In order to keep the temperatures constant and the same as that of the river, the tubes were suspended in the river at a depth of about 3 feet. Later examination showed that in tubes 1, 2, and 3, i.e., in the tubes to which the sperms had been added immediately, and after intervals of 2 and 4 hours, respectively the eggs developed normally. But in tube 4, where the eggs had been allowed to stand 6 hours before sperms were added, although polar cells were formed, no further development took place. Also the eggs in tubes 5 and 6 showed no signs of development and were apparently dead.

The conclusion from these results was that the life of the unfertilized egg under normal conditions of temperature and salinity was from 4 to 6 hours. (Fig. 2). These conditions might be called ideal in that the eggs were protected from salt, currents,

enemies, etc. Julius Nelson (7) found that in Richmond Bay, P.E.I., the eggs tend to sink to the bottom in about ten minutes if the water is still, and to become covered over with silt or to be smothered by one another and be killed, so it is necessary that the eggs be reached by the sperms before they sink to the bottom. Nature seems to have provided most admirably for this, as, according to Dr. Paul Galtsoff (4), the males respond to temperature changes more readily and seem to spawn first. The presence of the sperms in the water causes the females to spawn, so when the eggs are freed in the water, the sperms are already present and active due to their long life in comparison with that of the ova.

Next the effect of high temperature on the ova was determined. The first temperature used was 40°C. Solutions of ova were heated to this temperature for 5, 15, 30, and 60 minutes, then they were allowed to cool to room temperature and sperms were added to test their vitality. Later examination showed no signs of development, so it was concluded that this temperature killed the eggs in all cases.

Then a temperature between 38°C. and 39°C. was tried for the same time periods. After the addition of sperms the eggs developed to a certain stage, but never reached the swimming larvae stage (Fig. 3).

In a third series a temperature between 36°C. and 37°C. did not prevent the eggs from producing swimming larvae after being heated for 5, 15, and 30 minutes, but did when the eggs were heated for 60 minutes (Fig. 4).

Eggs heated to 35°C. produced swimming larvae even when heated for 60 minutes (Fig. 5).

3. Effect of Temperature on Early Development

For this work the same range of temperatures was used as in the sperm experiments i. e., from 5°C. to 40°C. Sperms and eggs were mixed in beakers containing salt-water having a salinity of 27.3‰, and the beakers were placed in the pneumatic troughs of the thermostat boxes. The results are shown in Fig. 6.

At 40°C. the eggs did not show any signs of development, as was to be expected from the previous set of experiments.

A temperature of 35°C. also proved too high for the development of swimming larvae, although polar cells were formed and some segmentation took place. This seemed to be a contradiction of one experiment in the previous series, i.e., when the eggs were heated to 35°C., and, after being allowed to cool to room temperature, on being fertilized with fresh sperms produced swimming larvae (Fig. 5). The conditions of the two experiments, however, are entirely different. In the former experiment on the effect of temperature on the eggs, fertilization took place at room temperature, by sperms that had not been subjected to the high temperature; while in the experiments on the effect of temperature on the development, fertilization had to take place at 35°C., by sperms subjected to that abnormally high temperature. Although fertilization did take place at 35°C., normal development did not follow.

The next four temperatures used proved suitable for the

development of the swimming larvae, i.e., 30°C., 25°C., 20°C. and 15°C., the only difference being the time involved. At 30°C. only 5 hours were needed from the time the ova and sperm were mixed until swimming larvae were produced. At 15°C. the process was much slower, taking 25 hours for the production of swimming larvae.

At 10°C. only a few polar cells were formed and no further development was seen.

Finally at 5°C., the lowest temperature used, not even polar cells were formed.

The deduction from the above experiments was that that the most suitable temperatures for the early development of Ostrea virginica are between 20°C. and 30°C. This work is also in agreement with that done by H. C. Dannevig (3), who found that in the development of fish eggs high temperatures increased the rate of development and low temperatures prolonged it.

Effect of Salinity

The salinities of the following experiments were determined by using a Knudsen's hydrometer. The salinities higher than normal, i.e., higher than 27‰ - 28‰, were obtained by concentrating sea-water, then dilution with normal sea-water until the desired salinity was reached. The lower salinities were obtained by diluting normal sea-water with rain water caught in glass jars, so no factor other than salinity would be changed. Sperms and ova for this work were obtained in the same manner as for the temperature experiments, and the solutions were kept at room temperature.

2. Effect of Salinity on Sperms.

Nineteen solutions of sperms were made up in sea-water varying in salinity from 0°/°° to 40.68°/°°. The results were interesting in that the salinities giving the longest life periods were several per mille lower than normal for Bideford river. In the solution with salinity of 23.2°/°° the sperms lived, i.e., they showed activity, for 42 hours, and at a salinity of 20.3°/°° the life period was 41 hours. At 0°/°° no movement of the sperms was seen, and at 3°/°° the movement was very slight, so slight that this salinity is not included on the graph (Fig. 7).

The salinities included on the graph range from 4.6°/°° to 40.68°/°°, with fifteen intermediate between the minimum and maximum. As was to be expected, at the two extremes the life periods were shortest, being 21 hours at 4.6°/°° and 22 hours at 40.68°/°°.

In Bideford river the salinity seldom drops below 27°/°°, except at the very head after a heavy rank when it may reach 23°/°°, nor does it often exceed 29.5°/°°, the greatest variation being between 27°/°° and 29°/°°.

The results of the above experiments show that the natural variation in salinity as found in the river would have little or no effect on the life of the sperms.

2. Effect of Salinity on Early Development

Ova and sperms were mixed in beakers of sea-water having the same salinity range as that used in the experiments on the effects of salinity on the sperms, i.e., between 4.6°/°° and 40.68°/°°. The results showed that at all salinities between 14.5°/°° and 38.8°/°° the development progressed as far as

The first swimming larvae stage. This agrees with the results obtained by I. Amemiya (1) in his work on the early developmental stages of the American oyster. At salinities lower than 14.5‰, although polar cells were formed and some segmentation took place, the development was irregular and did not result in swimming larvae.

The results of the experiments on the effects of salinity bear out the statement made by Julius Nelson (7) in 1915 in his report of his work on oyster propagation in Richmond Bay, viz., that too much emphasis was placed on density.

Owing to difficulties in keeping larvae alive during the stages of development between the first swimming stage and the time of settling as spat, the experiments were not carried further. Possibly the variations in salinity would have more effect during this period, as was suggested by A. E. Hopkins (5) in his discussion of the factors influencing the spawning and settling of oyster larvae in Galveston Bay, Texas. He suggests that the larvae depend either directly or secondarily on a salinity greater than 20‰ in order to develop to the setting stage.

CONCLUSION

1. The life of the sperm was prolonged by lower temperatures.
2. The life of the unfertilized egg, under normal conditions, was from 4 to 6 hours; 40°C. killed the eggs; 35°C. apparently did not harm them; and 38°C. - 39°C. and 36°C. to 37°C. had intermediate effects.
3. Swimming larvae were developed at temperatures between

15°C. and 30°C. At 15°C. 25 hours were required, and at 30°C. only 3 hours. Above and below these temperatures the larvae failed to reach the swimming stage.

4. The sperms had the longest life at a salinity between 20.3‰ and 23.2‰; at these salinities the sperms were active for 41 and 42 hours respectively.

5. Swimming larvae developed at all salinities between 14.5‰ and 38.8‰.

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Variations in Numbers of Oyster Larvae in Correlation
with Temperature and Salinity

By Mrs. L. R. Richardson (nee A. E. Clark)
(Part of thesis submitted to McGill University
in partial fulfilment of the requirements
for the degree of M.Sc., May 1933).

INTRODUCTION

The following paper outlines the results so far obtained from a systematic study of the numbers of oyster larvae present in plankton collections, taken at the Prince Edward Island Marine Station, during the summers of 1930 and 1931. Although at present certain conclusions appear to be obvious, it will be necessary to continue further along these lines before a definite statement as to results may be made.

The spawning of the American oyster, Ostrea virginica, does not take place until the water reaches a temperature of 20°C. The ova and sperm are shed into the water, and fertilization is external. The subsequent segmentation is irregular, the rate depending on the temperature and salinity. The embryo, after a few hours, develops into a swimming larva, the trochophore, which, in laboratory experiments at least, tends to rise to the surface.

With the formation of a larval shell, the prodissoconch (Stafford), the larva becomes a veliger, typical of mollusca. This veliger increases in size, the early stages being known as the "straight-hinge larve," having the two valves of the shell symmetrical. Later the symmetry is lost, the left valve becoming larger and more convex than the right, the larva changing

to the "umbo-larva". Throughout the trochophore and veliger stages, the oyster larva is free-swimming, and an increase in size and in complexity of the organs of locomotion and digestion taken place. (Stafford and Brooks).

According to Stafford the free-swimming period of the American oyster lasts for about two weeks after the shell-bearing stage is reached. The larva then settles, becoming cemented by its left valve to some solid surface, such as a rock or an oyster shell, and is known as the "spat". This develops the spat shell or dissoconch, and remains fixed, unless dislodged by outside forces, during the rest of its life.

Some knowledge of the geography and hydrography of the area under consideration is necessary for the interpretation of the observations. Bideford river is an inlet of Malpeque bay, and not a "river" in the true sense of the word, the water being salt, not fresh. The area of Malpeque bay and its inlets is about 40,000 acres; across the mouth of the bay is a string of islands preventing, to a great extent, the mixing of the water of the bay, and of the Gulf of St. Lawrence. The bay is shallow, the greater area having a depth of about 12 feet only, and the greatest depth 50 feet. For these reasons the temperature of the bay and its inlets is comparatively high for this latitude. Since only a few streams flow into Malpeque bay, the salinity is quite high even at the heads of the inlets.

Experimental work on oyster culture has been carried on at the Prince Edward Island Marine Station, established on Bideford river, by the Biological Board of Canada. Several oyster beds were reserved for this work, the two chosen for the following

study being Paugh's bed (Station 2001) at the head of the river, and Pulpit bed (Station 2002) $1\frac{1}{2}$ miles nearer the bay.

Collection of Samples

A rotary pump was employed in taking 50 gallon samples of water from the surface and from the bottom, the water being strained through a tow-net of No. 18 bolting silk, and the plankton collected in a wide-mouthed jar. The catch was preserved in formalin and later concentrated to between 10 c.c. and 15 c.c. The samples were received in this condition.

At the same time as the pump samples were taken, temperature readings for the surface and the bottom waters were made. Samples of the water were also taken, from which the salinities were later calculated by means of a Knudsen's hydrometer.

Examination of samples

The samples were brought to a uniform volume, 15 c.c., by the addition of 5% formalin. The bottle containing the sample was shaken until the contents were evenly dispersed, then two one-half c.c. samples were taken with a pipette, and placed in watch-glasses. By a circular movement of the watch-glass the material was collected at the centre, and was then examined under a binocular microscope.

Numerous forms were found in the samples, including gastropod larvae, diatoms, bivalve larvae, etc., as well as much debris, the latter especially in the bottom samples. Special attention was directed to the study of the many bivalve larvae present. Employing the method used by Stafford (11), the larvae were separated and measured by an ocular micrometer, until the identification of the oyster larvae was determined.

The older umbo-larvae were easily recognizable on account of the symmetry, previously mentioned, of the valves. No attempt was made to distinguish the straight-hinge larvae of the oyster from those of the other bivalves, because the very great similarity was confusing.

The number of umbo-larvae in each one-half c.c. was counted; the two results added together; and the sum multiplied by 15 to give the approximate number of larvae of the later stages present in the original 50 gallon sample.

Using the oyster larva, the salinity, and the temperature data, graphs were made for each of the stations for the oyster larva seasons of 1930 and 1931.

Observations

In the interpretation of these graphs, it must be borne in mind that the results are only approximate, and give a very generalized picture.

Pulpit Bed (Station 2002), 1930 - (Figure 1).

The first oyster larvae were found in the bottom sample taken June 26th, and the last larvae were observed in the surface sample of August 15th, giving a larva season of about seven weeks. For this time the greatest number of larvae were found in bottom samples of July 10th and July 22nd and in the surface samples of July 22nd, 24th and August 6th.

There was little temperature variation during the seven week period, the maximum being 23.1°C. at the surface on June 27th, and the minimum 19.7°C. at the bottom, August 12th. The temperatures hovered between these two extremes with no exceptional rise or fall.

The salinity was also relatively constant, varying between 29.7‰ and 28.5‰. Since the water is comparatively deep at this station, changes in salinity due to heavy rains are not strongly marked.

Paugh's bed (Station 2001), 1930 - Figure 2).

Oyster larvae were first found in the sample taken July 15th, and the last in the sample of August 25th, indicating a larva season of only 41 days, about a week shorter than for Station 2002 for the same year. The two main peaks of the larva curve occurred on July 24th and 30th.

The temperature variations at this station were greater than at station 2002, as was to be expected on account of the shallowness of the water. The maximum temperature was 24.4°C. at the surface, July 25th, and the minimum 19.0°C. at the bottom, August 12th and 18th.

The salinity also showed ^{greater} variations than at Station 2002. At the surface a drop on July 6th, and on July 15th, was due to heavy rain diluting the water during low tide.

The 1930 material had been previously worked over by H. P. Sherwood (10). His results differed from the above in certain aspects, although some of his major peaks are coincident with those in Fig. 1 and Fig. 2. This difference may be explained by the condition of the samples when studied for the second time, or else by different methods of procedure.

Pulpit Bed (Station 2002), 1931 - (Figure 3).

The charts for 1931 give a much better picture of the correlation between temperature and the numbers of oyster larvae.

At Station 2002 the oyster larvae were first observed in the surface sample taken July 11th; and were last seen on August 22nd, a larva season of about six weeks. The curve has two main peaks, one on July 22nd, the other on August 5th. In the bottom sample taken July 22nd, approximately 375 oyster larvae were found, about seven for each gallon of the original 50 gallons. This is a greater number than was found for this station in 1930, when less than five per gallon were present.

The temperature curve also exhibits two main peaks, the one on July 11th, and the other, continued over a longer period, from July 23rd to 31st.

It is to be pointed out, that, in studying these graphs, the temperature and salinity at the time of spawning is more important, than the temperature and salinity during the peak time of oyster larvae, since this is only the result of spawning.

In Fig. 3, the oyster larva peaks lag several days behind those of the temperature curve. On July 5th the temperature reached 20°C. causing some oysters to spawn and giving the larva peaks of July 14th, 15th and 16th. On July 11th the temperature reached 23.6°C. causing the greatest spawning burst of the season, resulting, on July 22nd, in the maximum number of oyster larvae. The temperature again rose on July 23rd and continued high until July 31st, as mentioned before, resulting in the second burst of spawning, giving the second larva peak on August 5th.

The lag in days between the temperature peak and the larva peak is probably due to the time that the oyster taken in reaching the size at which it is readily recognizable. The fact that there is a greater lag between the high temperature of

July 11th and the larva peak of July 22nd may be explained by the sudden drop in temperature on July 18th, which, since the rate of development depends on the temperature, resulted in a longer lapse of time before the larvae were recognizable.

Paugh's Bed (Station 2001), 1931 - (Figure 4).

The first larvae were found in samples taken on July 9th, and the last in samples taken September 1st, giving a larva season of nearly eight weeks. Two high peaks occurred during this season, one on July 16th yielding about ten oyster larvae per gallon (9520 for 50 gallons), and the other, on August 3rd yielding an average of slightly more than 16 larvae per gallon (or 810 for 50 gallons). These constitute record high figures for this area.

The temperature peaks for Station 2001 were slightly in advance of those for Station 2002, as was to be expected on account of the shallowness of the water. On July 11th the temperature reached 27.1°C., resulting in the spawning of a large number of oysters, the subsequent larva peak extending from July 16th to July 21st. The fact that the larva peak is broad may be explained by the continued high temperature after July 3rd, which did not fall below 20°C. This would also explain the short lag of the larva peak behind the maximum temperature. The temperature on July 18th dropped to 17.3°C., which was followed by a rise to 24.6°C. on July 24th, and remained high until July 31st. This second temperature rise resulted in a burst of spawning, followed by a correlated peak in the number of larvae.

After the second temperature and larva peaks, both curves smooth out gradually, the number of oyster larvae dropping until none were found after September 5th.

Although not shown in Fig. 4, oyster larvae, at the ratio of about one in three gallons, were found in the sample taken on September 21st. This was unusual, because the temperature after August 29th was below 20°C.; however, on September 14th, there was a rise in temperature to 18.2°C., and this rise must have been sufficient to cause some ripe oysters, which were caught by the earlier falling temperature, to spawn.

A similar case, not indicated in Fig. 4, was found at Station 2002. Here the same number of larvae, i.e., one in about three gallons, was found in the sample taken September 19th. This was probably due to a similar rise in temperature to 19.4°C. on September 14th.

CONCLUSION

The conclusions arrived at from the study of the graphs are only tentative, more extensive work being necessary before any definite statements may be made, but, at the present, the results so far obtained appear to indicate that:-

1. For the year 1930, at both stations, there was very little correlation between the temperature and the number of oyster larvae, due to the slight range of temperature variation throughout the larva season.

2. For the year 1931, at both stations, there was a fairly close correlation between the temperature and the number of oyster larvae, due to more pronounced temperature changes during the season.

3. The lag between the temperature peak and the larva peak is due to the length of time required for the oyster larvae to reach a size easily recognizable.

4. The length of the lag between the temperature and larva peaks varies with the temperature.

5. No correlation could be found between the salinity and the number of oyster larvae.

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