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GROWTH AND SETTING OF LARVAE OF *VENUS MERCENARIA* IN RELATION TO TEMPERATURE

By

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ABSTRACT

Larvae of the hard shell clam, *Venus mercenaria*, were grown to metamorphosis at constant temperatures of 30.0, 27.0, 24.0, 21.0 and 18.0° C ± 1.0° C. The rate of growth of the larvae was generally, but not always, more rapid at high than at low temperatures. Within this range small differences in temperature, such as 1.0 or 2.0°, were not extremely important in affecting the rate of growth. The average time needed by larvae to reach a certain size or to grow to metamorphosis at different temperatures is given. Larvae obtained from the same sources and grown under identical conditions often showed considerable individual variations in the rate of growth and in the time needed to reach the stage of metamorphosis. Fertilized eggs placed in water of 15.0 or 33.0° C ± 1.0° C showed abnormal development and heavy mortality, few ever reaching veliger stage. Some food requirements of the larvae are discussed.

INTRODUCTION

At some time or other almost every student of ecology or physiology of larvae of lamellibranch mollusks has commented that temperature is the factor influencing the duration of their pelagic life. Unfortunately, most of these comments were usually too brief and incomplete. Others, even if lengthy, lacked supporting data to be of much value in analyzing the relationship between temperature and growth of larvae.

Since a review of the literature devoted to the effects of temperature upon larvae of lamellibranchs has recently been offered by Thorson (1946) and Baughman (1947), there is no need to repeat it here. We shall merely mention a few articles that have a close relation to our studies. Nelson (1908) stated that within the temperature range from 24.0 to 27.0° C the American oyster, *Ostrea virginica*, has a free-swimming period of only one week. At 23.0° C, however, this period is extended to 13 days, and at 20.0° C to 17 days. Medcof (1939) concluded that oyster larvae require approximately 24, 26 and 30 days to reach maturity at a constant temperature of 21.0, 20.0 and 19.0° C respectively. According to Korringa (1941), *Ostrea edulis* of Holland has a pelagic life of six days at a temperature of 22.0–23.0° C, 9 to 10 days at 18.0–21.0° C, and 13 to 14 days at 16.0–17.0° C. Belding (1912) thought that the free-swimming period of larvae of *Venus*

mercenaria is 10 to 12 days at a temperature of approximately 22.0° C, but somewhat longer at lower temperatures.

Thus, the general opinion is that temperature may hasten or prolong the larval period. Furthermore, it is believed that a difference of only one degree, between 19.0 and 20.0° C, may prolong the swimming period of larvae by as much as four days (Medcof, 1939). However, the accuracy of the day-degree relationships offered may be questioned, since the number of days needed by larvae of different lamellibranchs to reach the setting stage at certain temperatures was decided largely upon the basis of field observations, where it is always difficult and usually impossible to evaluate the relative importance of other factors such as salinity, pH, food, etc. It is thought, therefore, that such relationships can be best determined by laboratory experiments where most of the factors can be rigidly controlled. This paper offers a description of studies devised to determine the rate of growth, length of free-swimming period, and other aspects of the behavior of larvae of the hard shell clam, *Venus mercenaria* L., grown at different but constant temperatures.

We wish to express our appreciation to our colleague, John H. Peterson, for tabulating some of the data on which this article is based.

METHODS

The larvae were grown at constant temperatures of 33.0, 30.0, 27.0, 24.0, 21.0, 18.0 and 15.0° C ± 1.0° C. They were kept in earthenware crocks of 20-liter capacity. These crocks, which were covered with black painted glass to exclude the light, were immersed in large containers through which water of a constant temperature was running continuously, thus maintaining a constant temperature within the crocks. Duplicate crocks were used for each temperature. The crocks were filled with sea water filtered through a thick layer of cotton.

The largest part of this work was done during the winter and early spring, the time which we found most convenient to control the temperature of the water (Loosanoff, 1949). Sperm and eggs were taken from clams which were conditioned to spawn in winter (Loosanoff and Davis, 1950). To avoid the shock of temperature changes, the fertilized eggs were gradually brought up or lowered to the temperatures in which they were to be cultured. Usually one million eggs were introduced in each crock, making the initial concentration of 50,000 eggs per liter of water.

The cultures were changed every two days by using the method already described (Loosanoff and Davis, 1950). After the water had been changed, the larvae were fed a mixture of *Chlorella* sp. and a

purple sulfur bacteria, *Chromatium perty*, creating in the culture crocks a concentration of approximately 300,000 cells of *Chlorella* and 400,000 cells of sulfur bacteria per cc of water. It is interesting that the sulfur bacteria, which were rejected as food by the oysters (Loosanoff, 1949a), were readily taken in and apparently assimilated by the larvae of *V. mercenaria*.

Altogether four major experiments were conducted. In the first, no larvae were measured until the fourth day. In the subsequent experiments, however, larvae from all the cultures were measured at the end of the second day after fertilization. In one 30.0° C culture, measurements were made as soon as the larvae reached the straight hinge stage, which occurred in less than 24 hours.

From each crock 50 larvae were taken for measurement on each occasion. However, because the cultures were run in duplicate, the number of larvae taken as a sample for each temperature group was actually 100. As a rule, in all experiments the duplicate samples showed extremely close agreement.

Measurements of the larvae were made in the usual manner, using a Sedgwick-Rafter cell to hold the larvae. Since each of the small divisions of our ocular micrometer was equal to seven microns, most of our figures were based on these intervals. In some cases, when the necessity arose to determine the measurements more precisely, the larvae were measured under high-dry power where each division of the ocular micrometer was equal to only 1.7 microns. In general, however, it was found impractical to be confined to such accurate measurements, because the variation in the measurements of the larvae depended to some extent upon the position in which the larva was lying on the slide. This was especially true of the older larvae, the shapes of which were more rounded than those of the relatively flat young ones.

RESULTS

We would like to mention here that this discussion is based on the results of experiments in which the clams of only one geographic area—Long Island Sound—were used to furnish the spawn. This point is emphasized because it is considered possible that within the general population of *Venus mercenaria*, which extends from the Gulf of St. Lawrence to Texas, there are local races of clams whose physiological requirements may differ from those of Long Island Sound clams. That such physiologically-different races of the same genus may exist among lamellibranchs has been shown recently for the common American oyster, *Ostrea virginica* Gmelin (Loosanoff, unpublished manuscript).

First our discussion will be confined to the temperatures within the range of 18.0 to 30.0° C. The results observed at 15.0 and 33.0° C will be discussed later, because the development of the eggs and larvae at these temperatures was usually abnormal.

Within the range from 18.0 to 30.0° C the mortality of the eggs and then the larvae was comparatively low. It is estimated that often not less than 85% of these were carried to the stage of metamorphosis. The only exceptions were several cultures grown at 18.0° C, where somewhat fewer eggs developed into larvae of straight hinge stage. However, those that reached that stage usually lived through metamorphosis.

The larvae in all cultures behaved normally. They were usually vigorous and rapid swimmers, this condition necessitating that they be killed with formalin before measurements were taken. Their color was usually pinkish-yellow-green, characteristic of the type of food they were fed. Incidentally, our experience has shown conclusively that the color of larvae of *V. mercenaria*, as well as that of larvae of some other lamellibranchs, such as *Mya arenaria* and *Mactra solidissima*, with which we worked at different times, should not be considered as a reliable specific characteristic that may be helpful in identifying larvae. We formed this conclusion because we found that the color of the larvae may be changed at will, within an hour or so, by feeding them micro-organisms of different colors.

The data on growth of larvae at different temperatures in Experiments 1 through 4 are given in Figs. 1, 2, 3 and 4. The curves of these figures are based upon the mean length of the larval populations as determined by measurements of representative samples at two-day intervals. As already mentioned, the exception was Experiment 1, where the larvae were not measured until the end of the fourth day. In Experiment 4, which was conducted in the early summer when the water temperature was already higher than 18.0° C, no cultures were grown at that temperature. In Figs. 1, 2, 3 and 4 the curves terminate at 185 microns. This arbitrary size was taken as the terminal point of the curves because in some instances it was found that, when the mean length of the larvae in the culture was approaching 185 μ, many larvae were already metamorphosing, thus dropping out of the free-swimming population.

The metamorphosis of a larva of *V. mercenaria* into a young clam is not as sharply defined as is the metamorphosis or, as it is commonly called, setting of an oyster, at which time the larva ceases crawling entirely and cements itself to a shell or other clean object. In *V. mercenaria*, as well as in some other clams, metamorphosis is an extended process beginning with the gradual replacement of a ciliated

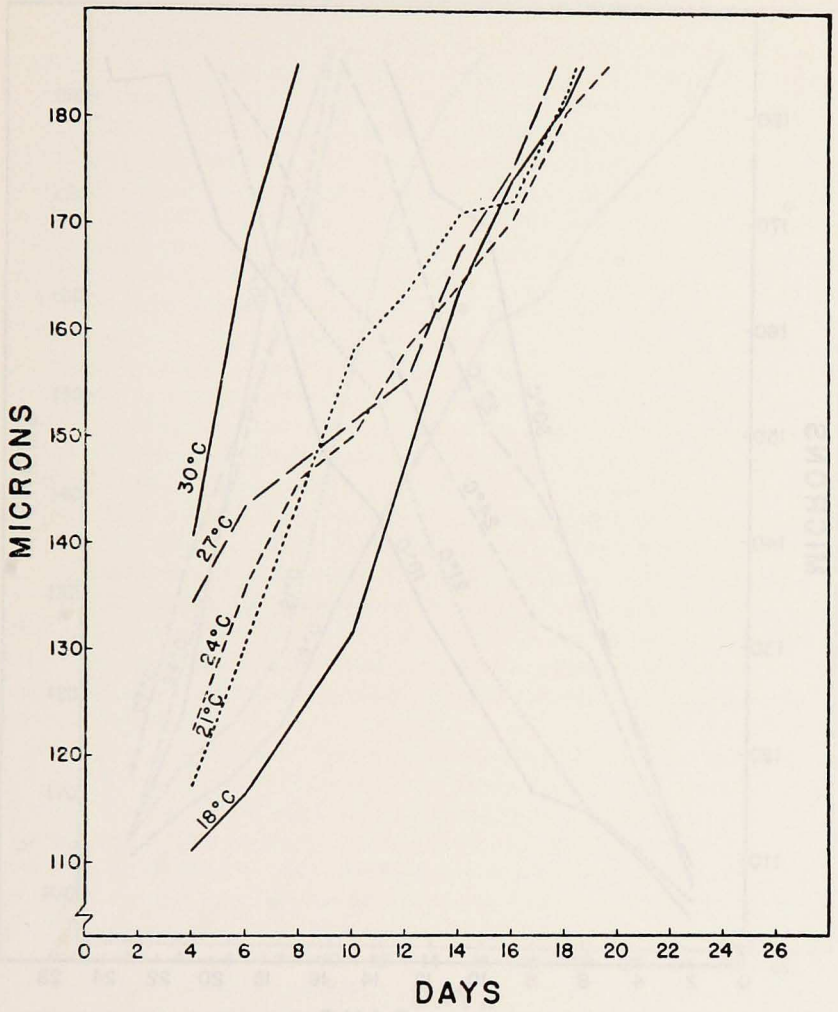

Figure 1. Mean length of larvae of different ages grown at five constant temperatures. Experiment 1.

velum with a large muscular foot and ending with the development in the foot of a functional byssal gland. This point is often difficult to establish, because many young clams, although possessing a byssus gland, do not always attach. Therefore, it may be difficult at times to distinguish a very young clam from an old ready-to-metamorphose larva.

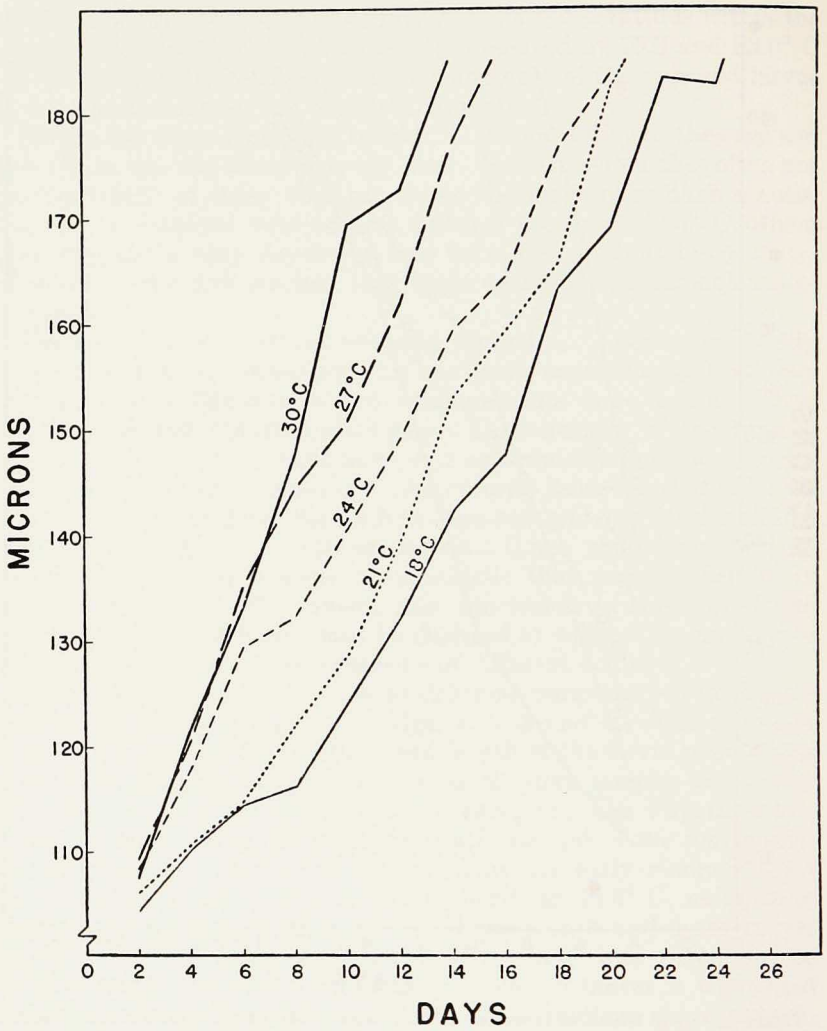

Figure 2. Mean length of larvae of different ages grown at five constant temperatures. Experiment 2.

The length at which metamorphosis took place in our cultures ranged from approximately 175 to 236 μ, occurring most commonly between 200 and 210 μ. The largest larvae did not always metamorphose first. In several cultures some comparatively small individuals of only approximately 180 μ did metamorphose, while larger

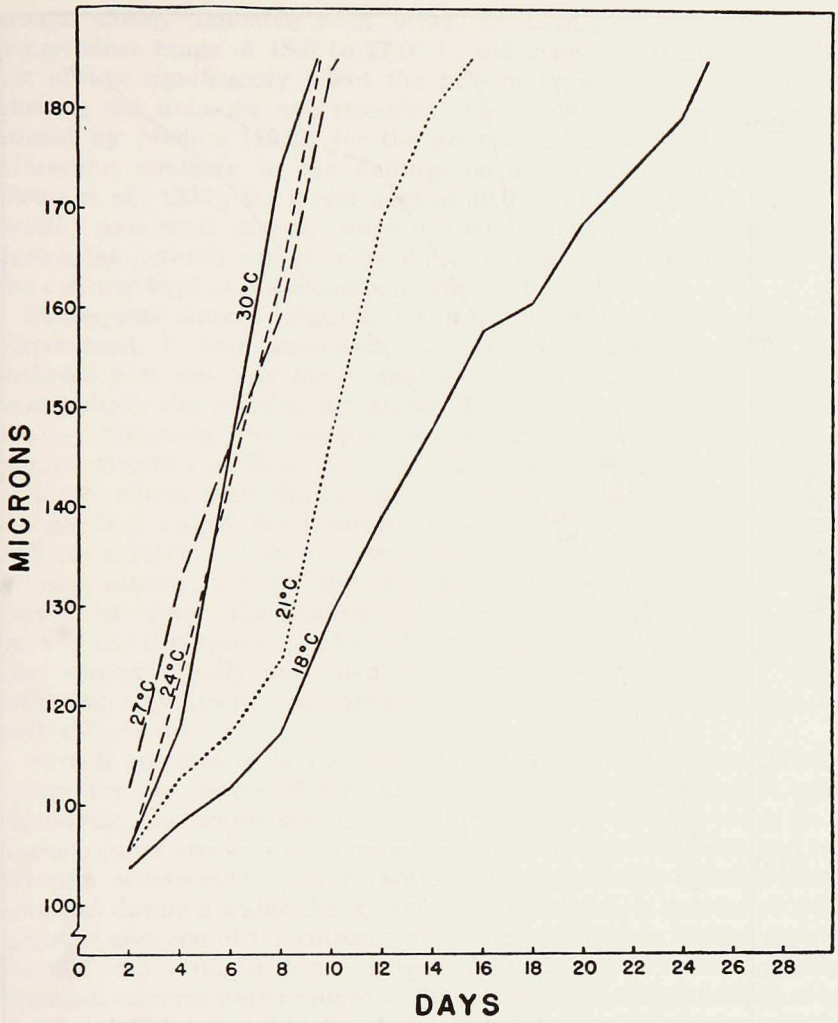

Figure 3. Mean length of larvae of different ages grown at five constant temperatures. Experiment 3.

larvae of more than 200 μ still continued swimming, at the same time displaying a powerful velum and a comparatively small foot.

In the course of these experiments we tried to determine whether larvae grown at low temperatures, such as 18.0° C, would reach a larger size before setting than larvae grown at higher temperatures. Some of the preliminary experiments did indicate a tendency of this

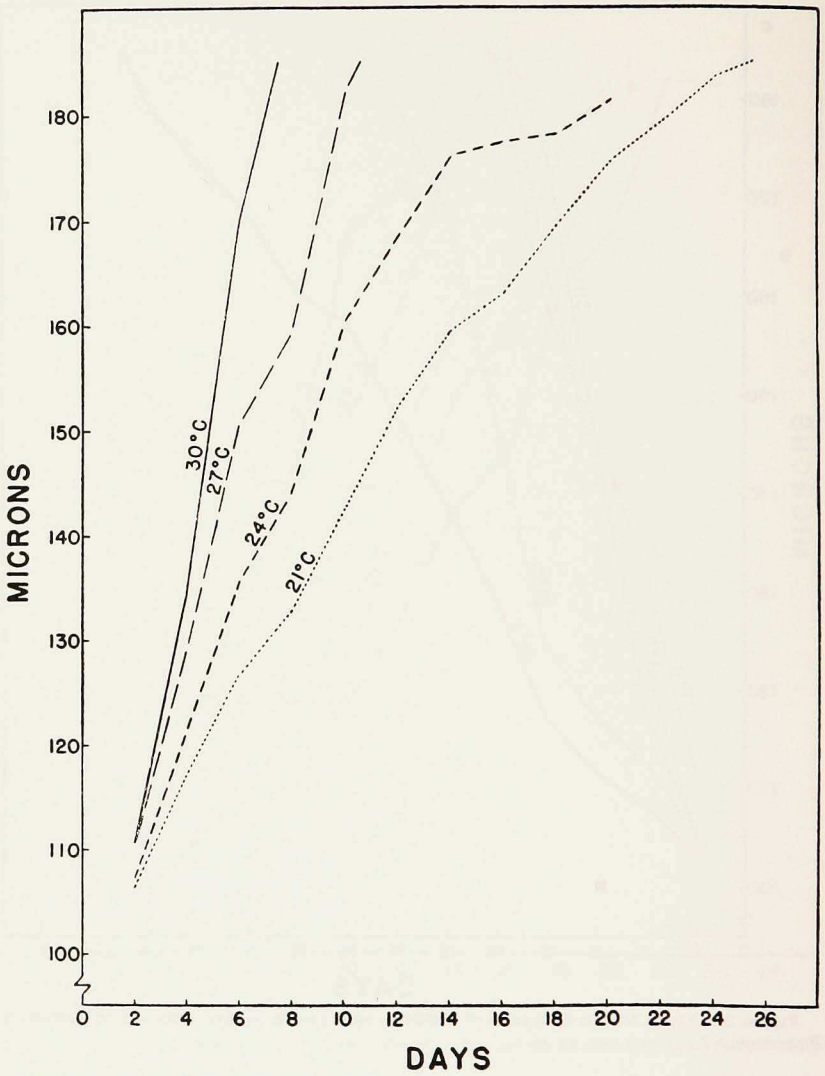

Figure 4. Mean length of larvae of different ages grown at four constant temperatures. Experiment 4.

type, but further and more extensive experiments did not support this contention.

With exception of the 30.0° C group, the results of the first experiment showed that the rates of growth of the other four temperature

groups closely resemble each other, indicating that, within the temperature range of 18.0 to 27.0° C, differences in temperature do not always significantly affect the rate of growth (Fig. 1). When plotted, the data do not resemble the exponential growth curves offered by Medcof (1939) for the growth of larvae of *O. virginica.* Moreover, contrary to the findings on some other lamellibranchs (Seno, et al., 1926), the larvae kept at 30.0° C did not die but showed healthy and rapid growth, some of them reaching the setting stage during the seventh day after fertilization, i. e., considerably ahead of the cultures kept at lower temperatures.

Subsequent studies (Figs. 2, 3 and 4) showed that the results of Experiment 1 were somewhat atypical. Nevertheless, they are included here not only for comparison and discussion but partly to demonstrate the possible deviations that may occur in this type of study. Naturally, we sought for an explanation of the peculiar results observed in Experiment 1. Critical analysis of the methods and procedures used during the experiment showed that the only factors that varied from culture to culture were temperature, and possibly aeration. Other factors, such as number of larvae, quantity of food, salinity, pH, amount of light, etc., were identical in all cultures. Of course, the differences in the temperatures were the basic part of the experiment. The differences in aeration, the only factor that was not rigidly controlled, had to be properly evaluated. The following experiment was designed to evaluate the importance of such differences.

Several cultures of larvae were started under identical conditions except for the degree of aeration. Some crocks were aerated very vigorously, the water surface resembling that of boiling water in a bucket; other crocks were aerated only feebly; the third received no aeration whatsoever with exception of unavoidable splashes that occurred during a water change every second day. It was found that vigorous aeration of the cultures was unnecessary after the larvae had reached the straight hinge stage. Actually, clean and carefully attended cultures were brought to the setting stage without continuous aeration. The larvae of the unaerated cultures showed approximately the same percentage of survival, grew at the same rate and began setting at the same time as the feebly or strongly aerated cultures which, in turn, showed no significant differences. Obviously, since the differences in degree of aeration in the cultures of Experiment 1 were incomparably smaller than those of the cultures of the specially designed experiment, we may conclude that aeration was not responsible for differences in the rates of growth of larvae in the different cultures of Experiment 1.

Experiments 2, 3 and 4 showed agreement in general, the rate of growth of the larvae being more rapid at high than at low temperatures (Figs. 2, 3 and 4). However, even in these experiments certain discrepancies were noted. For example, in Experiment 2, on the sixth day, the mean length of the larvae grown at 27.0° C was somewhat greater than that of the 30.0° C group (Fig. 2). In Experiment 3, until the sixth day, the mean rate of growth of the larvae was also

TABLE I. Number of Days Needed after Fertilization for the Beginning and for the Completion of Setting of Larvae at Different Temperatures in Each of Four Experiments; Range in Days between the Beginning and Completion of Setting, and the Maximum Number of Days during Which Setting May Extend in Cultures Grown at the Same Temperatures

Temp. °C	*Days*				*Range in days*	*Number of days*
	Expt. 1	*Expt. 2*	*Expt. 3*	*Expt. 4*		
18.0	16–28	24–30	19–30	—	16–30	14
21.0	14–24	20–26	17–22	18–28	14–28	14
24.0	14–22	17–22	11–14	14–22	11–22	11
27.0	14–20	13–18	9–14	10–14	9–20	11
30.0	7–12	9–16	7–14	7–12	7–16	9

more rapid at 27.0° C than that at 30.0° C (Fig. 3). Furthermore, even the 24.0° C group of this experiment was, for some time, growing more rapidly than the 30.0° C group.

To summarize the results of all four experiments, the arithmetic means of all the length measurements for each temperature at definite times were taken and the curves fitted by the method of moving averages (Fig. 5). The curves showed that growth of the larvae was more rapid at high than at low temperatures. However, the upper portions of the curve did not suggest that the distances between the terminal points of the curves significantly increased as the temperature decreased, as was shown by Medcof (1939) for oyster larvae.

At 30.0° C, setting of larvae in three experiments began as early as the seventh day after fertilization, but in one it was delayed until the ninth (Table I). The entire population of the cultures kept at this temperature metamorphosed within five to seven days after the beginning of setting (Table II). However, the range in days between the beginning and completion of setting at this temperature, as based on all four experiments, extended from the seventh to the 16th day after fertilization and covered a period of nine days (Table I).

At 18.0° C, the earliest beginning of setting was recorded 16 days after fertilization, the latest beginning 24 days after fertilization (Table I). The range in days between the beginning and completion of setting at this temperature extended from the 16th to the 30th day after fertilization, thus covering a period of 14 days. While in Experi-

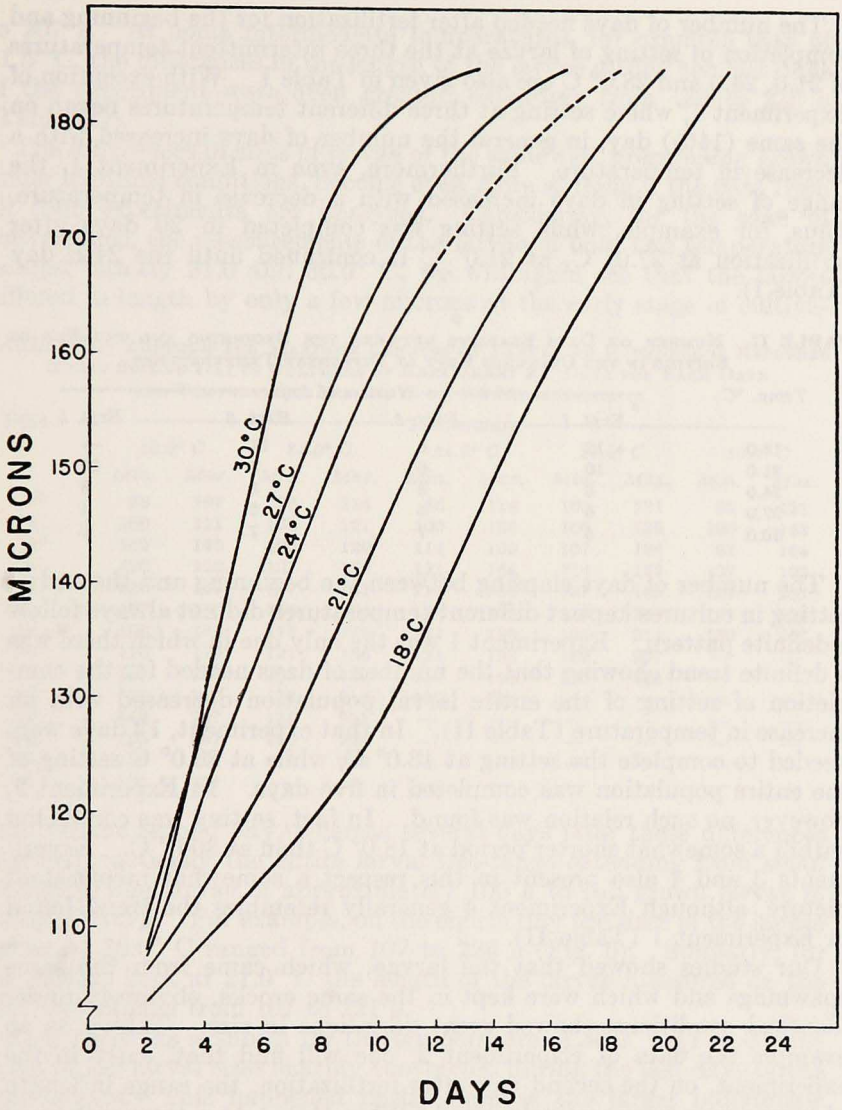

Figure 5. General trend of increase in length of larvae grown at different temperatures, as based on measurements made in four experiments.

ments 1 and 3 setting at 18.0° C continued for 12 and 11 days respectively, in Experiment 2 it was completed in only six days, thus indicating considerable variation in the behavior of a population of clam larvae, presumably kept under identical conditions.

The number of days needed after fertilization for the beginning and completion of setting of larvae at the three intermittent temperatures of 21.0, 24.0 and 28.0° C are also given in Table I. With exception of Experiment 1, where setting at three different temperatures began on the same (14th) day, in general the number of days increased with a decrease in temperature. Furthermore, even in Experiment 1, the range of setting in days increased with a decrease in temperature. Thus, for example, while setting was completed in 20 days after fertilization at 27.0° C, at 21.0° C it continued until the 24th day (Table I).

TABLE II. Number of Days Elapsing between the Beginning and the End of Setting in the Cultures Kept at Different Temperatures

Temp. °C	*Number of days*			
	Expt. 1	*Expt. 2*	*Expt. 3*	*Expt. 4*
18.0	12	6	11	—
21.0	10	6	5	10
24.0	8	5	3	8
27.0	6	5	5	4
30.0	5	7	7	5

The number of days elapsing between the beginning and the end of setting in cultures kept at different temperatures did not always follow a definite pattern. Experiment 1 was the only one in which there was a definite trend showing that the number of days needed for the completion of setting of the entire larval population decreased with an increase in temperature (Table II). In that experiment, 12 days were needed to complete the setting at 18.0° C, while at 30.0° C setting of the entire population was completed in five days. In Experiment 2, however, no such relation was found. In fact, setting was completed within a somewhat shorter period at 18.0° C than at 30.0° C. Experiments 3 and 4 also present in this respect a somewhat inconsistent picture, although Experiment 4 generally resembles the trend found in Experiment 1 (Table II).

Our studies showed that the larvae, which came from the same spawnings and which were kept in the same crocks, obviously under identical conditions, showed great variations in size. Taking as an example the data of Experiment 2, one will find that, early in the experiment, on the second day after fertilization, the range in length of larvae was comparatively small (Table III). At a temperature of 18.0° C it ranged between 93 and 107 μ, while at 30.0° C it extended from 93 to 121 μ. However, as the experiment progressed, the difference between the minimum and maximum sizes increased. Toward the end of the experiment the length range of the larvae in the 18.0° C culture extended from 150 to 221 μ and in the 30.0° C culture from 150

to 207 μ. At some intermediate temperatures, for example that of 21.0° C, the differences in the length of the larvae during the last days of the experiment were even greater, extending from 136 to 236 μ (Table III).

The differences in sizes of larvae of the same age, presumably grown under identical conditions, become even more striking if the data from all four experiments are analyzed. Considering, for the sake of convenience, the measurements of the larvae of only two temperature groups, namely 21.0 and 30.0° C, we will again see that the larvae differed in length by only a few microns at the early stage of cultiva-

TABLE III. MINIMUM AND MAXIMUM LENGTHS OF LARVAE OF *V. mercenaria* RECORDED EVERY SECOND DAY IN CULTURES OF EXPERIMENT 2. DATA FOR EACH DATE AND TEMPERATURE ARE BASED ON 100 MEASUREMENTS

Days	*Temperature*									
	18.0° C		*21.0° C*		*24.0° C*		*27.0° C*		*30.0° C*	
	Min.	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*
2	93	107	93	114	86	114	100	121	93	121
4	100	121	100	121	100	136	100	136	100	143
6	100	143	100	129	114	150	107	164	93	164
8	100	129	107	136	121	164	114	172	107	193
10	107	143	107	157	114	164	107	186	129	207
12	107	150	114	164	114	172	114	207	121	214
14	107	164	114	179	121	186	136	214	150	207
16	114	179	121	186	121	193	129	207	—	—
18	107	200	107	193	150	193	136	214	—	—
20	121	200	136	214	129	214	—	—	—	—
22	143	207	136	221	—	—	—	—	—	—
24	129	207	136	228	—	—	—	—	—	—
26	150	221	136	236	—	—	—	—	—	—
28	150	221	—	—	—	—	—	—	—	—

tion, on the second day, whereas several days later these differences covered practically the entire larval size-range extending from small straight hinge larvae of approximately 100 μ to full grown larvae in excess of 200 μ. For example, on the eighth day the sizes of the larvae grown at 30.0° C ranged from 107 to 226 μ (Fig. 6). Similarly, for the culture kept at 21.0° C the length of the larvae measured on the 18th day ranged from 107 to 221 μ.

Sometimes, as is shown for the temperature of 30.0° C (Fig. 6), the range of the larval sizes sharply contracted during the last days of the experiments. This happened partly because the larger individuals were rapidly setting and disappearing from the free-swimming population and partly because the undeveloped slow-growing larvae, usually defective in some respects, were dying off just as rapidly.

The data offered in Fig. 6 give only the extremes of the larval sizes without showing the prevalence of certain size-groups in the larval population. An example of such length-frequency distributions of

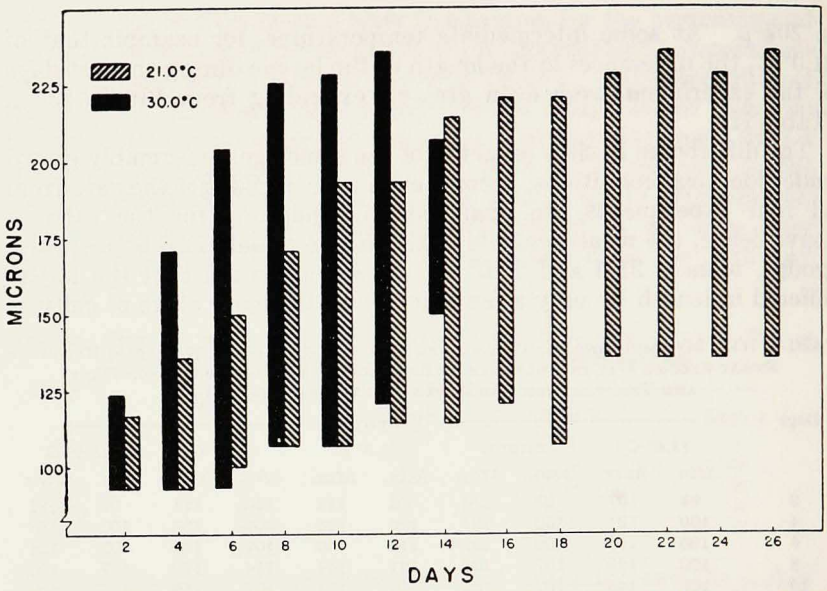

Figure 6. Extent of variations in length of larvae on different days of cultivation. Based on measurements of larvae grown at 21.0 or 30.0° C in all four experiments.

larvae is given in Fig. 7, which is based upon the measurements of the larvae grown at 18.0, 24.0 and 30.0° C in Experiment 2. The temperature selected represented the lowest, the highest, and the average of the five temperature classes. Experiment 2 was chosen as the example because it approaches most closely the mean of all four experiments. At the end of two days the length-frequency distribution of the larvae of the three cultures was relatively uniform, occupying a comparatively narrow range and showing a modal class of approximately 107 μ. Between the fourth and sixth days, however, the differences between the length-frequency distribution of the larval population of the three cultures had already become prominent. The difference was especially evident in the case of the modal classes. While for the 18.0° C group this class was 114 μ, for the 24.0 and 30.0° C groups these classes were 129 and 143 μ respectively (Fig. 7).

As the experiment progressed, the differences in length-frequency distribution of the larvae from the three cultures remained significant. In all instances the lengths of the modal classes of cultures grown at higher temperatures were greater than those at lower ones.

On the 18th day the comparison could be continued only between the 18.0 and 24.0° C cultures because the larvae in the culture of 30.0°

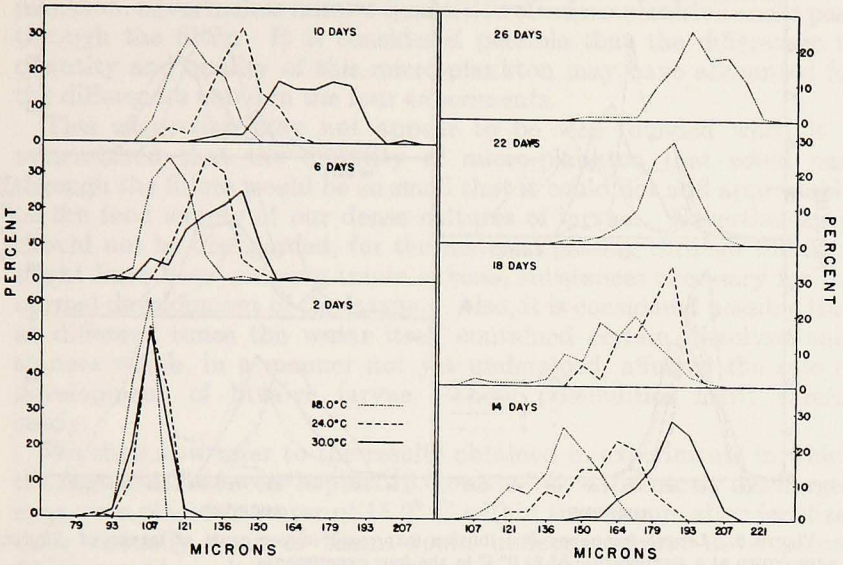

Figure 7. Length-frequency distribution (expressed in per cent) of larvae of different ages grown at temperatures of 18.0, 24.0 or 30.0° C. Experiment 2.

C had already set. For a similar reason the length-frequency distribution of the larvae after the 22nd day was available only for the 18.0° C culture.

The length-frequency distributions of larvae grown at the same temperatures but in four different experiments did not always closely resemble each other. For example, examining the length measurements of larvae grown at a temperature of 24.0° C in each of the four experiments, we found that the differences in the modal classes of the four cultures became apparent as early as the fourth day (Fig. 8). At eight days these differences became even more sharply defined. On the 12th day, while the cultures of Experiments 1 and 4 showed considerable agreement, and while the culture of Experiment 2 was not greatly different from those two, the culture of Experiment 3 definitely deviated from the others. The difference was not only in the size-range of larvae but also in the size of the modal class which measured 207 μ, as compared with 164 μ of the modal classes in the cultures of Experiments 1 and 4.

The culture of Experiment 3 set on the 14th day; therefore the comparisons made on 16 and 20 days are based only on the cultures of Experiments 1, 2 and 4 (Fig. 8). At 16 days the cultures of Experiments 1 and 4 showed close agreement, while the culture of Experiment

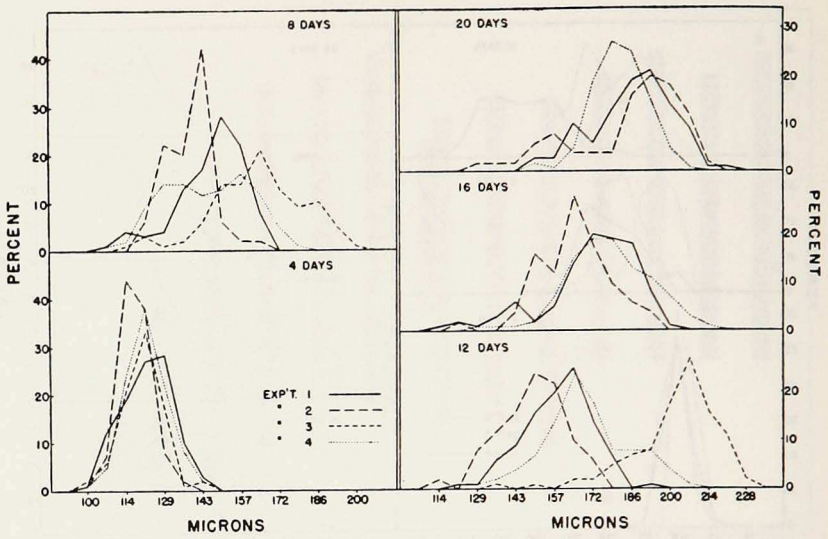

Figure 8. Length-frequency distribution (expressed in per cent) of larvae of different ages grown at a temperature of 24.0° C in the four experiments.

2 differed somewhat from the other two. However, at 20 days the agreement which existed previously between the cultures of Experiments 1 and 4 disappeared because, while the modal class of the culture of Experiment 1 showed a considerable increase, that of the culture of Experiment 4 remained practically the same. Meanwhile, the culture of Experiment 2 displayed rapid growth, thus catching up with the culture of Experiment 1.

On the basis of this comparison it may be concluded that the length-frequency distribution of the larvae grown at the same temperature in the four experiments, and otherwise kept under identical conditions, nevertheless showed considerable differences. The question naturally arises as to whether, regardless of all the precautions taken, there might have been some not-easily-identifiable differences that existed during the conduct of the four experiments which caused the lack of uniformity in the results. As far as the methods and procedures were concerned, it is certain that they were the same in all experiments. The same holds true in regard to salinity, pH, and other presumably important qualities of the water used. However, since the experiments were conducted one after another during several months rather than simultaneously, it is possible that the water used in the different experiments did differ in some respects. Even if the water were always filtered through a thick layer of cotton, thus removing most of the

plankton, nevertheless minute quantities of micro-plankton could pass through the filter. It is considered possible that the differences in quantity and quality of this micro-plankton may have accounted for the differences noted in the four experiments.

This suggestion may not appear to be well founded when it is remembered that the quantity of micro-plankton that could pass through the filters would be so small that it could not add appreciably to the food supply of our dense cultures of larvae. Nevertheless, it should not be disregarded, for the material passing through the filter might have been carrying traces of some substances necessary for the normal development of the larvae. Also, it is considered possible that at different times the water itself contained certain dissolved substances which, in a manner not yet understood, affected the rate of development of bivalve larvae. These possibilities merit careful study.

We shall now refer to the results obtained in experiments in which the eggs or larvae were kept at 15.0 or 33.0° C. If recently discharged eggs were placed in water of 15.0° C within three hours after fertilization, virtually none of them would undergo normal development which would result in the formation of straight hinge larvae. Some, however, would develop into trochophore larvae, usually of abnormal appearance.

If the eggs were kept at a room temperature of about 20.0–21.0° C for three to four hours after fertilization and then placed in water of 15.0° C, a few would develop into straight hinge larvae. However, the majority of these larvae would be abnormal and would soon die.

Fertilized eggs kept at room temperature for six or nine hours before being subjected to a temperature of 15.0° C gave a greater number of individuals reaching the straight hinge larval stage, but nevertheless, the majority developing that far would be abnormal and would soon perish. However, if the eggs and the resulting larvae were kept at room temperature for two days until the straight hinge stage was well formed, after which the larvae were then placed in water of 15.0° C, many of these larvae would survive. Regardless of slow growth, it is possible that, under certain conditions, some of these larvae would reach the stage of metamorphosis.

The experiments demonstrated rather conclusively that the zygotes of *V. mercenaria* in early stages of cleavage, for normal development and survival, require a somewhat higher temperature than the zygotes or larvae in later stages.

Our results of culturing eggs and larvae of *V. mercenaria* at a relatively low temperature are in close agreement with those of Seno, et al. (1926); they found that only a few of the eggs of *Ostrea gigas* kept at

about 16.0° C developed into shelled larvae, most of which would be abnormal and would soon die. At about 14.0° C, however, the segmentation of the eggs was so abnormal that none developed into straight hinge larvae.

At the other end of our temperature range, 33.0° C, an abnormal development and heavy mortality occurred if fertilized eggs were immediately transferred to water of such a high temperature. However, if the eggs and larvae that hatched were allowed to develop at room temperature for 48 hours and were then transferred to water of 33.0° C, a rapid normal development of larvae followed similar to that noticed in the cultures kept at 30.0° C. In general, these observations, as well as those made at 15.0° C, fully coincide with the view expressed many years ago by Pelseneer (1901) that young cleavage stages of molluscan eggs require a narrower temperature range than the later stages.

In the course of our experiments, many thousands of measurements were made of the length and width of the veliger larvae of different ages and sizes. Part of these data, consisting of measurements of 1,250 larvae grown at a temperature of 21.0° C in Experiments 1, 2 and 3, were used for determining the length-width relationship from early straight hinge stage to metamorphosis. This relationship is shown by the curve given in Fig. 9. The numerals through which the curve is drawn indicate the frequency of occurrence of larvae of certain lengths and widths. The character of the curve closely approaches a straight line. The data used in the construction of the curve fall into distinct modal groups with very little degree of dispersion. As already pointed out, some of the dispersion may be due to the position of the larvae on the slide during the measurement.

The size of the smallest straight hinge larvae incorporated in the curve was 86 x 64 μ, the largest 236 x 228 μ (Fig. 9). We may mention that, in addition to the 1,250 measurements used for the construction of this curve, we have the measurements of over 20,000 other larvae grown in these experiments and in many other experiments conducted since 1948. Regardless of the extremely varied ecological conditions under which these experiments were conducted, we have never found a larva more than 240 μ in length. This makes it highly improbable that the larvae of *V. mercenaria* may grow up to 448.5 μ long before metamorphosis, as reported by Stafford (1912). Furthermore, the shapes and dimensions of larvae of different sizes given by Stafford as those of *V. mercenaria*, when compared with those of larvae grown in our laboratory from the eggs of adult clams made to spawn under controlled conditions, show beyond all doubt that Stafford mistook the larvae of some other bivalves for those of *V. mercenaria*.

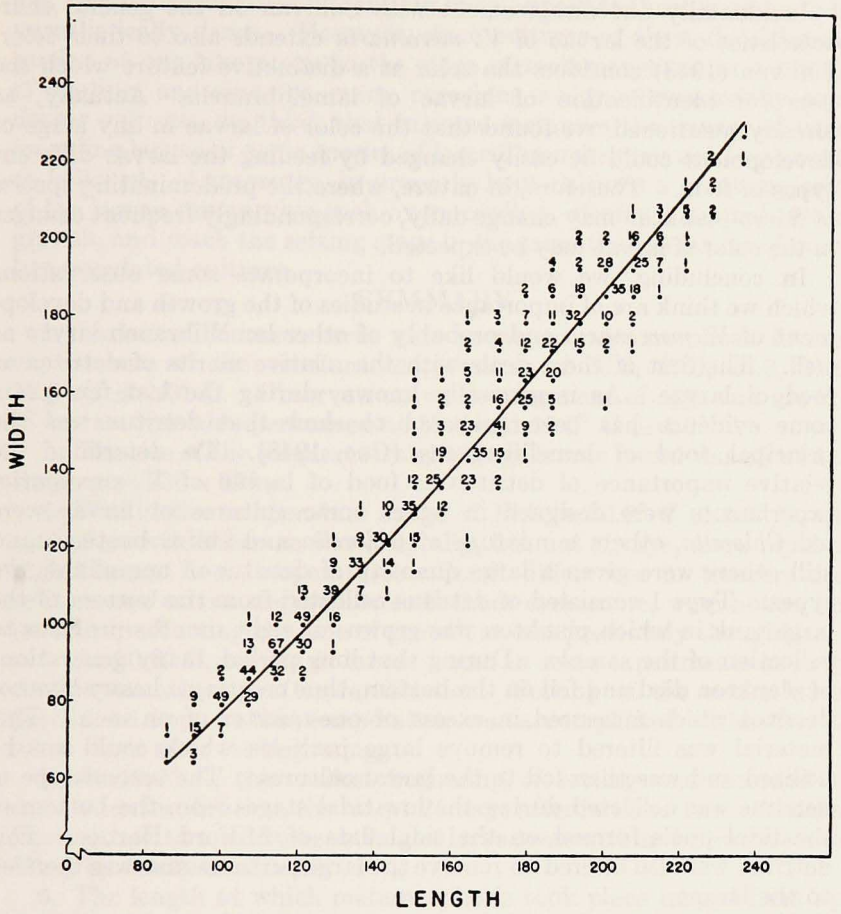

Figure 9. Length-width relationship of larvae of different sizes. Based on measurements of 1,250 larvae. Measurements in microns.

Similar remarks may be made about the conclusions of Sullivan (1948) on the size and shape of larvae of *V. mercenaria*. However, in a recent letter, Miss Sullivan clarifies the issue by writing as follows:

> It seems likely then that I have confused the larvae of *P. morrhuana* and *V. mercenaria*. This mistake is attributable to the fact that the newly settled spat of both types were very scarce in my collections. It was, furthermore, difficult to distinguish between the two types of very young spat or to identify either type with certainty.
>
> If it is the case then that my larva, *P. morrhuana*, is really *V. mercenaria*, it follows that the settling size of *Venus* larvae in Malpeque Bay corresponds reasonably well with that of *Venus* larvae in your cultures.

Incidentally our disagreement with Sullivan on the general characteristics of the larvae of *V. mercenaria* extends also to their color. Sullivan (1948) considers the color as a distinctive feature which she uses for identification of larvae of lamellibranchs. Actually, as already mentioned, we found that the color of larvae in any stage of development could be easily changed by feeding the larvae different types of food. Therefore, in nature, where the predominating species of micro-plankton may change daily, correspondingly frequent changes in the color of larvae may be expected.

In concluding, we would like to incorporate some observations which we think are of importance in studies of the growth and development of *V. mercenaria* and probably of other lamellibranch larvae as well. The first of these deals with the relative merits of detritus as food of larvae. As is generally known, during the last few years some evidence has been gathered to show that detritus was the principal food of lamellibranchs (Coe, 1948). To determine the relative importance of detritus as food of larvae of *V. mercenaria*, experiments were designed in which some cultures of larvae were fed *Chlorella*, others a mixture of *Chlorella* and sulfur bacteria, and still others were given a large quantity of detritus of one of the two types. Type 1 consisted of detritus collected from the bottom of the large tank in which plankton was grown for eight months previous to collection of the sample. During that long period, many generations of plankton died and fell on the bottom, thus creating a heavy layer of detritus which measured in excess of one-quarter of an inch. This material was filtered to remove large particles which could not be utilized and was then fed to the larval cultures. The second type of detritus was collected during the low tidal stages from the bottom of the tidal pools formed on the tidal flats of Milford Harbor. This detritus was also filtered to remove the large particles and was then fed to the larvae.

The experiments showed that there was no evidence whatsoever to support the contention that the larvae of *V. mercenaria* would grow better on detritus than on living *Chlorella* or on a mixture of living *Chlorella* and sulfur bacteria. As a rule, the cultures grown on detritus were always much inferior to those fed with *Chlorella* or its mixtures with bacteria, and the larvae usually died from starvation before they reached the stage of metamorphosis.

The second observation concerns the density of the larval population in the cultures. As already mentioned, our experiments usually began with approximately 50 eggs per cc of water. This was a much heavier concentration than that practiced or advocated by other investigators who have usually emphasized the danger of overcrowd-

ing. Our concentrations, therefore, may appear to those workers to be unrealistically dense. However, since cultures of these densities are carried at our laboratory to the stage of metamorphosis as a matter of routine, and since on many occasions we grew successfully even denser cultures, we think that in some instances the danger of overcrowding larvae of some species of lamellibranchs may not be as acute as believed. Apparently, in properly kept cultures a great majority of the larvae can survive such overcrowding, display a normal rate of growth, and reach the setting stage in the same time as larvae of much less populated cultures.

SUMMARY

1. Larvae of *V. mercenaria* were grown to metamorphosis in four experiments at the constant temperature of 30.0, 27.0, 24.0, 21.0, 18.0° C ± 1.0° C. Observations on development of eggs and growth of larvae were also performed at 33.0 and 15.0° C ± 1.0° C.

2. The size of the smallest straight hinge larvae found was 86 x 64 μ, the largest 236 x 228 μ.

3. Within the temperature range of 18.0 to 30.0° C ± 1.0° C, the rate of growth of larvae was generally, but not always, more rapid at high than at low temperatures. Small differences in temperature, such as one or two degrees, or sometimes even more, were not as important in affecting the rate of growth as was previously thought.

4. At 30.0° C, setting of larvae began in some experiments as early as the seventh day after fertilization. Setting of the entire larval population kept at this temperature was accomplished within five to seven days after its beginning.

5. At 18.0° C, the earliest beginning of setting was recorded 16 days after fertilization, the latest 24 days after fertilization. At other intermittent temperatures setting of larvae was within the bounds indicated by the two extremes.

6. The length at which metamorphosis took place ranged from approximately 175 to 236 μ, occurring most commonly between 200 and 210 μ. There was no indication that the larvae grown at lower temperatures were reaching larger size before setting than those grown at higher temperatures.

7. Larvae which came from the same source and which were kept under identical conditions showed great variation in size. In some instances the length of the larvae in an old culture ranged from about 100 μ to that of full grown larvae measuring in excess of 200 μ.

8. The length-frequency distribution of larvae grown at the same temperature but in four different experiments showed considerable variation. These variations were found in the range of larval lengths and also in the lengths of the modal classes.

9. If, immediately after fertilization, the eggs of clams were placed in water of a temperature of 15.0° C ± 1.0° C, virtually none of them would undergo normal development leading to the straight hinge stage. However, if the eggs were kept at room temperature for nine hours before they were subjected to the above temperature, some of them would develop into straight hinge larvae.

10. Eggs placed in water of 33.0° C ± 1.0° C soon after fertilization would show abnormal development and heavy mortality. However, if the zygotes were kept at room temperature of about 22.0° C for about two days after fertilization and were then transferred to the higher temperature, a rapid normal development of larvae would follow.

11. Young cleavage stages of clam eggs occur within a narrower temperature range than the later stages.

12. The color should not be considered as a distinctive feature to be used in the identification of lamellibranch larvae, because it changes easily depending on the color of the organisms eaten by the larvae.

13. No evidence was found to support the contention that organic detritus is a better food for larvae than living phytoplankton. Two types of detritus, one composed mostly of dying and decomposing plankton grown under laboratory conditions, the other collected from the bottom of tidal pools, were fed to the larvae, but such feeding caused their slow starvation and death.

14. The degree of aeration was found to be unimportant in affecting the rate of survival and rate of growth of clam larvae. Clean and carefully attended cultures could be brought to the setting stage without continuous aeration.

15. Overcrowding of larval cultures of *V. mercenaria* is not too easily achieved. As a rule, our cultures contained 50 larvae per cc of water, and several cultures, in which the concentration of larvae was more than 100 per cc of water, were grown to metamorphosis.

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