COMBINED EFFECTS OF TEMPERATURE AND SALINITY ON DEVELOPMENT OF EGGS AND GROWTH OF LARVAE OF *M. MERCENARIA* AND *C. VIRGINICA*

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ABSTRACT

A study of the effect of temperature on the survival and growth of larvae of the hard clam (*Mercenaria mercenaria*) and American oyster (*Crassostrea virginica*) has shown that the rate of growth of these larvae at different temperatures was critically affected by the type of food organisms available. Both clam and oyster larvae were able to utilize naked algae, such as the chrysophytes *Monochrysis lutheri, Isochrysis galbana* and *Dicrateria* sp., and show significant growth at lower temperatures than those at which chlorophytes, such as *Chlorella* sp., which have cell walls, could be utilized. This implies that the enzyme systems required to digest naked flagellates are active at lower temperatures than are the enzyme systems required to digest cell walls.

The cells of *I. galbana* and *M. lutheri* are destroyed by temperatures of 27.5° C.–30.0° C., and growth of larvae receiving these foods at such temperatures was reduced. *Chlorella* sp. can tolerate temperatures of 33.0° C., and the rate of growth of larvae receiving *Chlorella* sp. continued to increase with each 2.5° C. increase in temperature up to 33.0° C.

Salinity also affects the temperature tolerance of clam and oyster larvae. At near optimum salinities the larvae survive and grow over a significantly wider range of temperatures than at salinities near the lower limits of their tolerance. We observed the temperature tolerances of clam and oyster larvae at a series of decreased salinities.

The development of comparatively routine methods for rearing lamellibranch larvae (Loosanoff and Davis, 1950) allowed a number of studies on the effect of various environmental factors on the development of eggs and on the survival and growth of larvae of bivalve mollusks. These studies have, in general, been conducted by varying the one factor under observation, while holding other factors as constant as possible.

Although we have reared the larvae of 20 species of lamellibranchs, at the Bureau of Commercial Fisheries Biological Laboratory in Milford, Conn., we confined experiments on the tolerances of larvae to environmental factors to two species, the American oyster, *Crassostrea virginica*, and the hard clam, *Mercenaria* (= *Venus*) *mercenaria*. We studied the effects, upon the eggs and larvae of these species, of turbidity, salinity, temperature, the kind and quantity of food, the concentration of eggs and larvae, and a variety of dissolved materials (Loosanoff and Davis, 1963).

Informative as such studies were, the results might not apply absolutely to these organisms in nature where more factors must be considered. We know little about the combined effect of two or more environmental factors on lamellibranchs. Medcof and Needler (1941) attempted to deduce the interaction of temperature and salinity on the condition index of oysters, *C. virginica*, in natural waters, and Costlow, Brookhout, and Monroe (1960, 1962) have obtained laboratory data on the combined effects of temperature and salinity on development of eggs and larvae of the decapod crustaceans *Sesarma cinereum* and *Panopeus herbstii*. Kinne (1963) has reviewed the present

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knowledge of the effects of temperature and salinity on marine and brackish water fish and has emphasized the fact (p. 302) that "monofactorial analysis may lead to conclusions that are ecologically invalid," and "should be replaced wherever possible by bi, tri or polyfactorial approach." We undertook the present study to determine how variations in such factors as food and salinity affected the temperature tolerance of eggs and larvae of the American oyster, *C. virginica*, and the hard clam, *M. mercenaria*.

METHODS

Adult clams and oysters were induced to spawn in the laboratory. Induction was by thermal stimulation and the addition of a water suspension of ripe gametes as previously described (Loosanoff and Davis, 1963). To determine the effects of experimental conditions on development of eggs, 10,000 to 15,000 recently fertilized eggs were placed in each 1-liter beaker. To determine the effects of the same conditions on survival and growth of larvae, three to six 15-liter cultures, each containing approximately 500,000 eggs, were started using our normal sea water (salinity 27.5 parts per thousand (p.p.t.)) at 24.0°±1.0° C. After 48 hours the eggs developed into normal straight-hinge larvae. These larvae were then screened from all containers and resuspended in a single 4-liter container. We measured the number of larvae per unit volume and placed the desired number of larvae (usually 8,000 to 15,000) in each of the 1-liter beakers.

In preliminary experiments to find the effects of different food organisms on survival and growth of larvae at different temperatures, duplicate cultures of 48-hour larvae were set up for each food, or combination of foods, and replicate sets were prepared for each of the six different temperatures tested in a single experiment.

In the experiments to study the combined effects of temperature and salinity on egg development and on survival and growth of larvae, duplicate cultures of eggs were established in each of the six salinities tested and replicate sets were prepared for each of the six different temperatures tested, giving a total of 72 cultures in each experiment.

To learn the effect of the several combinations of salinity and temperature on egg development 48 hours after the eggs were placed in the experimental cultures, the larvae in each culture were collected, on a stainless steel screen of small enough mesh to retain them, and resuspended in a 250-ml. graduated cylinder. After thoroughly mixing them, we withdrew and preserved a 4-ml. quantitative sample. We examined these samples under a compound microscope (×110) and counted the eggs developing into normal living larvae. To compare the numbers developing normally in successive experiments, we tabulated the results as a percentage of the maximum number developing under any combination of temperature and salinity in that experiment.

In experiments to determine the combined effect of temperature and salinity on survival and growth of larvae, we changed the water in the cultures every second day to eliminate waste products of larval metabolism and reestablish experimental conditions. In most of our work we added supplemental food daily (Davis and Guillard, 1958), but to keep our salinities constant in the experiments involving reduced salinities, we added food only every second day, when the water was changed and the salinities were adjusted.

To determine the percent survival and growth rate of clam larvae, after 10 days at the experimental conditions or when the larvae were 12 days old we took quantitative samples, in a manner similar to that used at 48 hours. The majority of larvae kept at or near optimum conditions had reached setting size by this time, but even those that had metamorphosed were collected on the screen, resuspended, and included in the quantitative samples.

We took samples for comparing growth rates and survival of oyster larvae after 8 days of experimental treatment or when the larvae were 10 days old. Under optimum conditions, some of the larvae were setting between the 10th and 12th days. Since recently metamorphosed oysters cannot be collected on the screen and resuspended, a random quantitative sample cannot be taken once setting starts.

We examined separate preserved samples under a compound microscope, and counted the larvae that survived the experiment. In addition, we measured 50 clam larvae or 100 oyster larvae from each sample, and calculated the increase in mean length during the experiment as a percentage of

the increase in size of larvae in the most rapidly growing culture.

RESULTS

Type of Food and Growth of Larvae at Different Temperatures

Previous experience had indicated that the failure of larvae to grow at 30.0° C. in some experiments might be an indirect effect of the temperature on the marine algae used as foods rather than a direct effect on the larvae themselves. This was supported by Ukeles (1961) who reported that the cells of the chrysomonads *Isochrysis galbana* and *Monochrysis lutheri*, the two food organisms used in these early experiments, were destroyed by temperatures above 27.0° C.

Because we were interested in determining the direct effects of temperature and salinity on larvae, we designed preliminary experiments to demonstrate the effect of different foods on growth of larvae at different temperatures. In the first experiment, we kept oyster larvae at temperatures of 10.0°, 15.0°, 20.0°, 25.0°, 30.0°, and 33.0° C. with one culture at each temperature receiving *Dunaliella euchlora*, another *Chlorella* sp. (580),[1] and the third a mixture of *Chlorella* sp. (580), *Dicrateria* sp. (BII),[2] *I. galbana* and *M. lutheri*. The results are shown in figure 1. At 10.0° and 15.0° C. the larvae did not grow appreciably on any of these three foods. At 20.0° C., however, larvae fed the mixture of algae grew appreciably but those receiving the motile green chlorophyte, *D. euchlora*, showed only slight growth and larvae given only the nonmotile chlorophyte, *Chlorella* sp. (580), which has a distinct cell wall, grew hardly at all.

Above 20.0° C., the growth rate of larvae receiving *Chlorella* sp. (580) increased with each increase in temperature. The growth rate of larvae receiving *D. euchlora*, on the other hand, was similar at 25.0°, 30.0°, and 33.0° C., and the growth rate of larvae receiving the mixed algae increased progressively only up to 30.0° C. At 33.0° C., the growth rate was somewhat slower than at 30.0° C.

A second experiment was run to clarify the upper and lower temperature limits for growth of oyster larvae (fig. 2). Again, larvae fed the

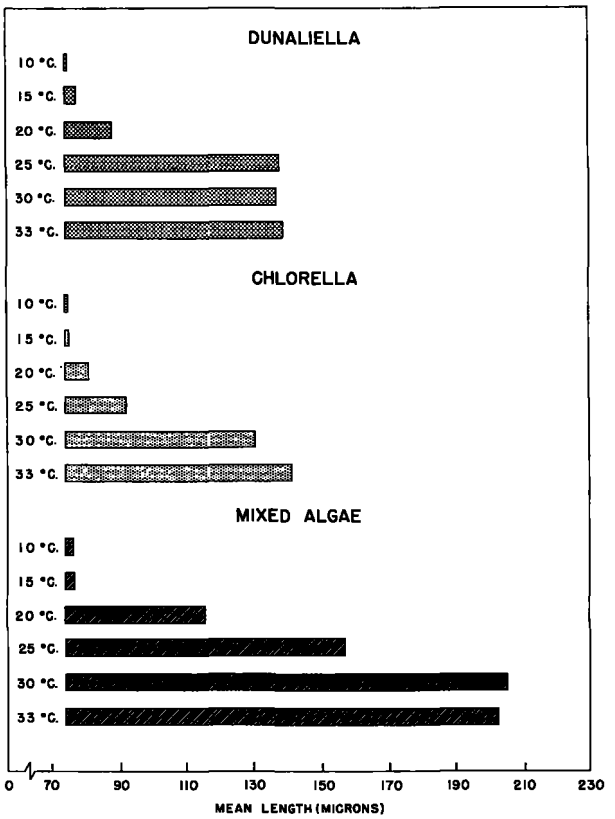

FIGURE 1.—Growth of oyster larvae receiving different foods and reared at different temperatures. Plots are based on mean length of 100 larvae from each temperature at each measuring period.

mixture of algae and kept at 15.0° C. did not grow. Growth of larvae kept at 17.5° C. was minimal, but at 20.0° C. the larvae grew faster, although at 10 days these larvae were still much smaller than those kept at 30.0° or 33.0° C. At 35.0° C., the rate of growth was also much slower than at 30.0° or 33.0° C. and all the larvae died before the end of the experiment.

Since Loosanoff, Miller, and Smith (1951) studied the effect of temperature on the growth rate of clam larvae, we conducted only a single experiment on the effect of different foods on growth of these larvae at different temperatures. Only the *Chlorella* sp. (580) and the mixed algae were tested as foods.

As with oyster larvae, there was a pronounced difference in the ability of clam larvae to utilize the two foods at low temperatures (fig. 3). Although larvae kept at 10.0° C. ingested food, they did not grow and their digestive glands had no

[1] *Chlorella* sp. (Indiana U. collection #580).
[2] *Dicrateria* sp. (Plymouth collection BII).

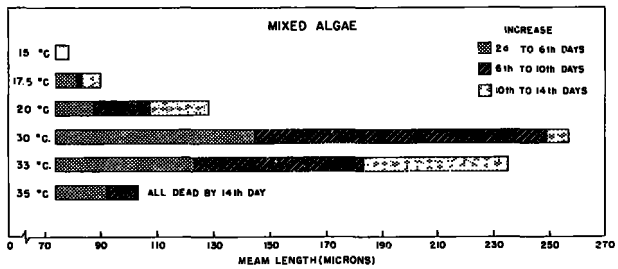

FIGURE 2.—Growth of oyster larvae receiving the mixture of algae as food and reared at high and low temperatures. Plots based on mean length of 100 larvae from each of duplicate cultures at each temperature at each measuring period. Many of the larvae kept at 30.0° and 33.0° C. set between the 10th and 14th days and were not included in the 14-day samples.

coloration, indicating their inability to digest either food. Larvae receiving *Chlorella* sp. (580) and kept at 15.0° C. likewise did not grow and showed no evidence of digesting this food. The digestive glands of larvae kept at 15.0° C. but receiving mixed algae as food did grow, and their digestive glands had normal color, indicating that some of the species of food organisms added were being digested.

All larvae kept at 20.0° C. grew and showed coloration of the digestive gland. Those receiving *Chlorella* sp. (580), however, averaged only 125.45 μ in mean length by the 12th day, while the larvae receiving the mixed algae grew to a mean length of 198.47 μ, i.e., they had reached setting size. At 30.0° C., larvae receiving *Chlorella* sp. (580) were only approaching setting size by the 12th day (mean length 170.0 μ), while those receiving mixed algae already had metamorphosed and averaged 241.45 μ in length.

Effects of Different Salinities on Temperature Tolerance of Eggs and Larvae of Clams

Our methods of determining the percentage of bivalve eggs developing into normal straight-hinge larvae are accurate to about ± 10 percent. Differences of less than 20 percent in the percentages of eggs developing under different conditions, therefore, are of doubtful significance. At our normal salinity, about 27.0 p.p.t., there was no significant difference in the percentage of clam eggs developing normally within the temperature range from 17.5° to 30.0° C. (table 1). At 32.5° and at 15.0° C., however, the percentage was decreased drastically and at 12.5° C. very few normal clam larvae developed.

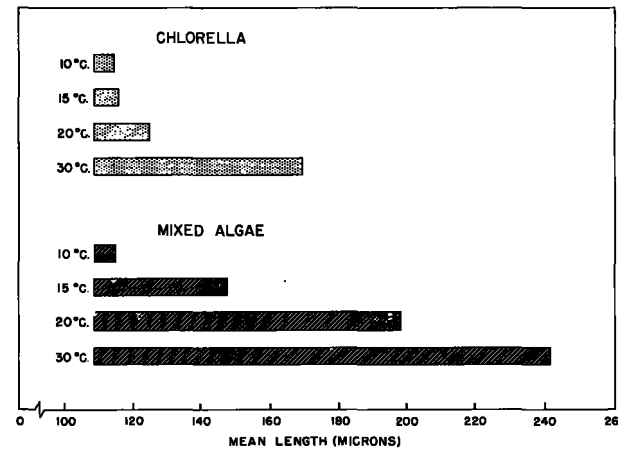

FIGURE 3.—Growth of clam larvae receiving different foods and reared at different temperatures. Plots are based on mean length of 50 larvae from each of duplicate cultures at each temperature. In cultures fed the mixture of algae and grown at 30.0° C. many larvae had already set by the end of the 10-day experimental period but were included in the samples.

At each temperature, fewer eggs developed normally at a salinity of 22.5 p.p.t. than at 27.0 $\pm$ p.p.t. Temperatures from 22.5° to 25.0° C. appear to be optimum at the salinity of 22.5 p.p.t. Few of the eggs kept at 32.5° or at 15.0° C. developed normally at this salinity. Moreover, 22.5 p.p.t. was the lowest salinity at which any appreciable number of clam eggs developed.

At 20.0 p.p.t. none of the eggs developed into normal larvae at any temperature except in one experiment where a few normal larvae were found at 20.0° and 25.0° C. It should be emphasized, however, that clam larvae can survive at far lower

TABLE 1.—*Percentage of clam eggs developing to the straight-hinge stage at different combinations of temperature and salinity, Milford, Conn.*[1]

Salinity (P.P.T.)	Temperature (° C.)								
	32.5	30.0	27.5	25.0	22.5	20.0	17.5	15.0	12.5
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
27.0±	39	81	93	95	92	95	94	24	0
22.5	<1	36	65	79	73	56	52	1	<1
20.0	0	0	0	5	0	<1	0	0	0
17.5	0	0	0	0	0	0	0	0	0
15.0	0	0	0	0	0	0	0	0	0
12.5	0	0	0	0	0	0	0	0	0

[1] Percentages given are the mean of duplicate cultures in each of two or more replicate experiments. The highest number of eggs developing at any combination of temperature and salinity in each experiment was considered 100 percent for that experiment. Only at temperatures and salinities above the broken line was development of eggs comparatively normal.

salinities than the minimum salinity at which clam eggs can develop. From 20.0° to 30.0° C., survival of clam larvae does not decrease significantly until the salinity falls below 17.5 p.p.t. (table 2). Even at 15.0 p.p.t. approximately 50 percent of the larvae kept at temperatures from 17.5° to 25.0° C. survived.

The decrease in survival of clam larvae with a decrease in salinity was most drastic at high temperatures. At 32.5° C., less than 1 percent survived at a salinity of 17.5 p.p.t. At temperatures of 30.0° and 27.5° C., survival dropped from 75 percent at 17.5 p.p.t. to 25 percent or less at 15.0 p.p.t. In contrast, survival of clam larvae, at 12.5° C., although only 56 percent at our normal salinity of 27.0± p.p.t., still averaged 39 percent after 10 days at a salinity of 12.5 p.p.t.

The growth rate of clam larvae was most rapid at 30.0° C. and at a salinity of 27.0± p.p.t. (table 3). In fact 30.0° C. appeared the best temperature for growth of larvae at salinities of both 27.0± and 22.5 p.p.t., although, at all salinities above 17.5 p.p.t., the growth rate was similar at 30.0°, 27.5° and 25.0° C. A temperature of 32.5° C. was obviously above optimum at all salinities.

The temperature range for satisfactory growth of clam larvae narrowed as the salinity decreased. At 27.0± p.p.t. growth was satisfactory, i.e., at least some larvae approached setting size by the 12th day, at all temperatures from 15.0° to 32.5° C. At 17.5 p.p.t., however, satisfactory growth was limited to the temperature range from 20.0° to 30.0° C.; at 15.0 p.p.t., except at 25.0° C., there was no significant growth of larvae.

When clam larvae kept in various salinities at 12.5° C. were transferred to 24.0° C. water of normal salinity (27.0± p.p.t.), those previously in salinities of 17.5 p.p.t. or higher began to grow normally. Larvae previously kept at 12.5° C. in salinities below 17.5 p.p.t., however, did not recover after the transfer to the higher temperature and salinity and all died within 6 days.

TABLE 2.—*Percentage of clam larvae surviving after 10 days, at different combinations of temperature and salinity, Milford, Conn.*[1]

Salinity (P.P.T.)	Temperature (° C.) 32.5	30.0	27.5	25.0	22.5	20.0	17.5	15.0	12.5
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
27.0±	77	83	81	75	87	75	71	61	56
22.5	48	84	87	83	88	76	70	56	46
20.0	16	72	84	76	77	78	62	50	40
17.5	<1	76	74	83	85	69	45	50	49
15.0	0	25	22	57	53	43	58	36	47
12.5	0	<1	0	12	0	9	12	19	39

[1] Percentages given are the mean of duplicate cultures in each of two or more replicate experiments. The highest number of larvae surviving at any combination of temperature and salinity in each experiment was considered 100 percent for that experiment. The broken line indicates the ranges of temperature and salinity at which the largest percentage of larvae survived and shows the significant decrease in survival below 17.5 p.p.t.

TABLE 3.—*Percentage increase in mean length of clam larvae kept at different combinations of temperature and salinity for 10 days, Milford, Conn.*[1]

Salinity (P.P.T.)	Temperature (° C.) 32.5	30.0	27.5	25.0	22.5	20.0	17.5	15.0	12.5
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
27.0±	65	98	83	93	83	71	53	30	16
22.5	61	91	85	88	83	68	48	25	12
20.0	54	85	87	82	80	60	39	17	5
17.5	15	63	68	66	59	36	21	5	<1
15.0	0	12	17	31	20	9	3	<1	0
12.5	0	0	0	2	0	0	0	0	0

[1] Percentages given are the mean of duplicate cultures in each of two or more replicate experiments. The mean increase in length of larvae in the most rapidly growing set of duplicate cultures in each experiment was considered 100 percent for that experiment. The combinations of salinity and temperature above the broken line were the combinations at which the larvae had the most satisfactory growth and shows the similarity of the growth rates at these combinations.

Effects of Different Salinities on Temperature Tolerance of Eggs and Larvae of Oysters

In some experiments oyster eggs developed normally at salinities from 20.0 p.p.t. to 27.0± p.p.t. and temperatures from 17.5° to 32.5° C. In other experiments, however, far fewer normal larvae developed from eggs incubated at the marginal temperatures of 32.5° and 17.5° C. This tendency for poor development at the extremes of the above temperature range became even more pronounced when the eggs were incubated at a salinity of 17.5 p.p.t. (table 4). Of the eggs kept in a salinity of 15.0 p.p.t. only in the cultures kept at 27.5° C. did 30 percent or more of the eggs develop, and at 12.5 p.p.t. only an occasional normal larva developed at any temperature. As shown by Davis (1958), the minimum salinity at which oyster eggs will develop normally is determined in part by the salinity at which the parent oysters were kept prior to spawning.

The survival values for oyster larvae (table 5) indicate that satisfactory survival rates (70 percent

TABLE 4.—*Percentage of oyster eggs developing to the straight-hinge stage at different combinations of temperature and salinity, Milford, Conn.*[1]

Salinity (P.P.T.)	Temperature (° C.)					
	32.5	30.0	27.5	22.5	20.0	17.5
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
27.0±	58	86	91	84	83	67
25.0	60	78	88	86	84	80
22.5	58	84	82	88	87	84
20.0	58	77	81	90	86	58
17.5	29	69	89	81	57	10
15.0	1	17	39	24	2	0
12.5	0	0	0	0	0	0
10.0	0	0	0	0	0	0
7.5	0	0	0	0	0	0

[1] Percentages given are the mean of duplicate cultures in each of two or more replicate experiments. The highest number of eggs developing at any combination of temperature and salinity in each experiment was considered 100 percent for that experiment. Only at the combinations above the broken line was development comparatively normal.

or better) were limited to temperatures of 27.5° to 32.5° C. and salinities of 10.0 to 27.5 p.p.t. Survival at 22.5° C. was slightly less. Moreover, almost 50 percent of the larvae survived at all salinities and temperatures tested, except that only 23 percent survived in a salinity of 7.5 p.p.t. at 32.5° C., and 22 percent in a salinity of 27.0± p.p.t. at 17.5° C.

As indicated by the percentage increase in mean length at 10 days (table 6), the growth rate of oyster larvae, at all salinities, shows a very sharp break between 27.5° and 22.5° C. Except at

TABLE 5.—*Percentage of oyster larvae surviving after 8 days at different combinations of temperature and salinity, Milford, Conn.*[1]

Salinity (P.P.T.)	Temperature (° C.)					
	32.5	30.0	27.5	22.5	20.0	17.5
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
27.0±	75	70	76	56	49	22
25.0	68	64	71	54	43	45
22.5	71	66	68	54	45	42
20.0	75	58	66	60	48	37
17.5	78	71	83	65	37	53
15.0	81	74	81	71	67	42
12.5	86	90	73	67	41	40
10.0	67	79	71	66	49	49
7.5	23	38	48	54	53	38

[1] Percentages given are the mean of duplicate cultures in each of two or more replicate experiments. The highest number of larvae surviving at any combination of temperature and salinity in each experiment was considered 100 percent for that experiment. Only at the combinations above the broken line was survival of larvae over 70 percent.

TABLE 6.—*Percentage increase in mean length of oyster larvae kept at different combinations of temperature and salinity for 8 days, Milford, Conn.*[1]

Salinity (P.P.T.)	Temperature (° C.)					
	32.5	30.0	27.5	22.5	20.0	17.5
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
27.0±	89	92	76	35	19	9
25.0	84	87	74	32	21	11
22.5	88	91	76	32	21	13
20.0	90	94	76	31	22	12
17.5	85	92	77	31	19	10
15.0	79	87	67	28	18	8
12.5	49	69	52	21	11	4
10.0	30	36	36	14	6	1
7.5	2	11	19	6	2	<1

[1] Percentages given are the mean of duplicate cultures in each of two or more replicate experiments. The mean increase in length of larvae in the most rapidly growing set of duplicate cultures in each experiment was considered 100 percent for that experiment. The broken line shows the ranges of temperature and salinity at which the larval growth rate was 70 percent or better.

temperatures above 22.5° C. and salinities above 12.5 p.p.t., a larval growth rate equivalent to 70 percent of the maximum rate was achieved only in a salinity of 12.5 p.p.t. and temperature of 30.0° C. At salinities of 10.0 p.p.t. or lower, the growth rate was less than 50 percent at all temperatures; at temperatures of 22.5° C. or lower the growth rate was less than 50 percent at all salinities.

To determine the time required for larvae to reach metamorphosis at different temperatures, oyster larvae were reared at 20.0°, 22.5°, 25.0°, 27.5°, 30.0° and 32.5° C. and at our normal salinity of 27.0± p.p.t. The experiment continued until all larvae set. The results are shown in table 7.

Because of the slow growth of larvae at low temperatures and low salinities, we did not rear larvae to metamorphosis at all combinations of temperature and salinity.

TABLE 7.—*Number of days for oyster larvae to reach metamorphosis at combinations of different temperatures and 27.0± p.p.t. salinity, Milford, Conn.*

Temperature	Days after fertilization to—	
	Beginning of setting	End of setting
° C.		
30.–32.5	10–12	18–20
27.5	14–16	28–30
25.0	24–26	38–40
22.5	28–30	44–46
20.0	36–40	64–68

To determine (1) the minimum temperature at which oyster larvae, grown to setting size at temperatures of 27.0±1.0° C., could set; and (2) the effect of lowered temperatures on intensity of setting, we conducted five experiments. In each 1-liter beaker, we placed approximately 2,000 oyster larvae reared to 80-mesh size (250–300 μ in length) at a temperature of 27.0°±1.0° C.; two of these cultures were kept at each temperature. Two clean oyster shells in each culture, were examined every second day to determine the occurrence and relative intensity of setting. We counted only those spat occurring on the white inner surface of the test shells. The average numbers of spat per culture recorded are shown in table 8.

In the third experiment, the larvae initially were smaller than in the other experiments, and, although none set at 15.0° or 12.5° C., some still lived 20 days after transfer to these temperatures. It appeared, from these experiments, that at temperatures of 15.0° and 12.5° C. the only larvae that set were those that were mature and ready to set at the time of transfer to these temperatures. Smaller immature larvae, although living more than 20 days after transfer, apparently were unable to develop to the setting stage.

At temperatures from 25.0° to 17.5° C., the number of larvae completing metamorphosis declined with each decrease in temperature, and the time required for setting increased. The decrease in intensity of setting with decreasing temperature within this range is probably the result of the cumulative mortality of larvae as a result of the slower growth rate and consequent lengthening of the larval period.

TABLE 8.—*Average numbers of spat at combinations of different temperatures and 27.0± p.p.t. salinity, Milford, Conn.*[1]

Temperature	Average spat per culture				
	1st experiment	2d experiment	3d experiment	4th experiment	5th experiment
° C.	*Number*	*Number*	*Number*	*Number*	*Number*
25.0	293	723	723	920	604
22.5	181	723	---	---	---
20.0	170	519	---	---	---
17.5	---	397	---	---	---
15.0	---	---	0	67	---
12.5	---	---	0	33	1
10.0	---	---	---	---	0

[1] Oyster larvae used in the individual experiments were grown to setting size at temperatures of 27.0±1.0° C. and a normal salinity of 27.0±p.p.t.

DISCUSSION

The failure of bivalve larvae to grow at low temperatures appears to be the result of their inability to digest available food. Our experiments demonstrated that larvae survived for long periods and ingested food at temperatures below the minimum temperature at which they grow. Moreover, since larvae can utilize some foods at lower temperatures than other foods (figs. 1 and 3), perhaps the enzyme systems needed to digest algae without cell walls, such as the chrysomonads, are active at lower temperatures than are the enzyme systems required to digest algae that have cell walls, such as *Chlorella*. The increase, with increase in temperature, in growth rate of larvae fed any one food probably results from increased activity of the enzyme system at higher temperatures.

From our knowledge of the temperature and salinity tolerances of the food organisms used in these experiments, there is no reason to suspect that the reduced growth rate of larvae at low temperatures and salinities results from an indirect effect on the food organisms. Ukeles (1961), however, showed that at temperatures of 27.0° C. or higher the cells of *I. galbana* and *M. lutheri* are destroyed, while cells of *Chlorella* sp. (580) survive even at 33.0° C.

The growth rate of oyster larvae receiving *Chlorella* continued to increase with each increase in temperature up to 33.0° C., while larvae fed the mixture of algae showed no increase between 30.0° and 33.0° C. (fig. 1). It seems probable that in the latter case any increase in activity of the enzyme systems of the larvae at 33.0° C. was offset by a reduction in the amount of food available due to the destruction of cells of *I. galbana* and *M. lutheri*. We believe, therefore, that our results at higher temperatures may be partly due to an indirect effect on the larvae through the food chain. Nevertheless, the drastic reduction in rate of growth at 35.0° C. and the heavy mortality of larvae (fig. 2) almost certainly reflect a direct effect of temperature on the larvae themselves.

Both clam and oyster larvae survived in fair numbers and grew appreciably in salinities significantly lower than the minimum salinity at which eggs develop normally. There are, however, striking differences in the tolerances of the

larvae of these two species of lamellibranchs. Virtually none of the clam eggs kept at 12.5° C., the minimum temperature at which clam larvae will grow, developed normally (table 1). In contrast, an approximately normal number of oyster eggs developed at 17.5° C. (table 4), which is the lowest temperature at which oyster larvae show appreciable growth.

The most notable interspecific differences were to the effects of temperature and salinity on growth of larvae. Growth of clam larvae was comparatively little affected by temperature differences within the range from 20.0° to 30.0° C., whereas growth of oyster larvae was markedly affected by every increase in temperature from 17.5° to 30.0° C. The optimum temperature for growth of clam larvae was not well defined but appeared to drop from 30.0° C. at salinities of 22.5 p.p.t. and higher to 27.5° C. at salinities of 17.5 and 20.0 p.p.t. and to 25.0° C. at a salinity of 15.0 p.p.t. Differences in the growth rate of clam larvae at temperatures from 22.5° to 30.0° C. appeared to be almost random, and the optimum growth rate occurred at temperatures other than 30.0° C. at each salinity tested in one or more experiments (table 9).

In contrast, there is a marked stepwise increase in the growth rate of oyster larvae with each increase in temperature up to 30.0° C. (table 6). There is a fairly well defined optimum between 30.0° and 32.5° C. for all salinities except 7.5 p.p.t. where the optimum is 27.5° C. Differences between the growth rate of oyster larvae at 30.0° and at 32.5° C. were usually small with sometimes one and sometimes the other temperature giving the maximum growth rate, indicating that the optimum might well lie between these two temperatures.

Clam and oyster larvae differed also in their growth response to different salinity levels, but whereas oyster larvae were more sensitive than clam larvae to differences in temperature, the latter appeared more sensitive to salinity differences. Growth of clam larvae, at almost all temperatures, decreased stepwise with each decrease in salinity (table 3), and the maximum salinity (27.0± p.p.t.) was the most nearly optimum of the salinities tested. The growth rate of oyster larvae, however, was relatively constant at each temperature within the salinity range from 15.0 to 27.5 p.p.t. and there was no apparent optimum salinity (table 6).

Kinne and Kinne (1962) stated that extreme conditions of temperature, salinity, and oxygen could induce developmental arrest, and that "such arrest may remain reversible if conditions are normalized within hours or a few days; longer periods cause irreversible damage; they are lethal." In our experiments the "developmental arrest" of clam larvae kept at 12.5° C. and at salinities above 17.5 p.p.t. was reversible, but irreversible at salinities of 15.0 p.p.t. or lower.

The data on clam eggs and larvae were remarkably consistent, i.e., there were no major differences between successive experiments in the percentage of eggs developing to the straight-hinge larval stage, in the survival of larvae or in the growth rate of larvae (tables 9 and 10). In experiments with oyster eggs and larvae, however, there were large differences between experiments on the effect of salinity. Moreover, while the data on the salinity tolerances of clam eggs and larvae agreed quite well with Davis (1958), the data on salinity tolerances of eggs and larvae of oysters did not.

In the first experiment with oyster eggs and larvae, for example, the optimum salinity for growth of oyster larvae at all temperatures was either 25.0 or 27.0±p.p.t. (exp. 1, table 11). In experiments 2 and 3, however, the optimum salinity at most temperatures was between 15.0 and 20.0 p.p.t. Because we suspected that better growth at lowered salinities might be a result of diluting toxic external metabolites of algal blooms that sometimes occur in Milford Harbor (Davis and Chanley, 1955), in the fourth experiment we used sea water collected from a considerable distance offshore in Long Island Sound. The egg development data from this experiment (exp. 4, table 12) showed unexpectedly that dilution of dissolved toxins in sea water may indeed be responsible for better results in lowered salinities. None of the eggs kept in the undiluted offshore sea water developed into normal larvae, indicating that our offshore sea water contained a considerable amount of toxins. Although an approximately normal percentage of straight-hinge larvae developed at some temperatures in 20.0 p.p.t., a normal percentage did not occur at all temperatures except in cultures where this sea water had been diluted to a salinity of 17.5 p.p.t. or lower.

In experiments 5 and 7 (tables 11 and 12),

TABLE 9.—*Growth of clam larvae at 12 days, at different combinations of salinity and temperature, Milford, Conn.*
[Data from individual experiments using clam eggs and larvae]

Salinity (p.p.t.) and temperature (° C)	Mean length of larvae at 12 days					Increase in mean length of larvae (2d to 12th days)				
	Experiment				Average	Experiment				Average
	1	2	3	4		1	2	3	4	
27.5 p.p.t.:	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
12.5°			123.8	126.4	125.1			16.0	15.2	15.6
15.0°			141.7	142.4	142.1			31.3	27.7	29.5
17.5°			172.5	168.0	170.3			57.6	47.8	52.7
20.0°	188.9	193.4	188.8	195.2	191.5	63.9	78.4	71.5	69.1	70.7
22.5°	213.9	198.4			206.2	84.3	83.1			82.7
25.0°	213.1	203.6	222.2	234.7	218.4	83.7	88.0	100.0	100.0	92.9
27.5°	211.3	200.0			205.7	82.2	84.6			83.4
30.0°	233.3	216.4	218.2	230.9	224.9	100.0	100.0	96.6	97.0	98.4
32.5°	188.0	181.2			184.6	63.3	67.0			65.1
22.5 p.p.t.:										
12.5°			120.9	121.0	121.0			13.6	11.0	12.3
15.0°			135.7	137.5	136.6			26.1	23.9	25.0
17.5°			164.3	164.3	164.3			50.6	44.9	47.7
20.0°	190.6	192.5	184.9	185.5	188.4	65.5	77.6	68.2	61.5	68.2
22.5°	207.2	201.7			204.5	78.9	86.2			82.5
25.0°	211.6	202.1	212.2	223.7	212.8	82.4	86.6	91.5	91.4	88.0
27.5°	212.3	203.3			207.8	83.0	87.7			85.3
30.0°	216.7	198.9	215.0	233.1	215.9	86.9	83.6	93.9	98.7	90.8
32.5°	177.4	182.0			179.7	54.8	67.8			61.3
20 p.p.t.:										
12.5°			110.7	112.7	111.7			4.9	4.5	4.7
15.0°			124.5	129.8	127.2			16.6	17.9	17.3
17.5°			152.3	154.9	153.6			40.4	37.5	39.0
20.0°	181.3	184.6	172.6	177.3	179.0	58.0	70.2	57.7	55.1	60.2
22.5°	199.5	203.9			201.6	72.6	88.3			80.4
25.0°	206.0	204.3	203.1	210.4	205.9	77.9	88.6	83.7	81.0	82.3
27.5°	217.9	200.8			209.4	87.6	85.4			86.5
30.0°	212.8	207.5	198.2	218.1	209.2	83.4	91.8	79.5	87.0	85.4
32.5°	169.0	173.9			171.4	48.0	60.2			54.1
17.5 p.p.t.:										
12.5°			105.9	106.1	106.0			.8	.0	.4
15.0°			111.6	112.9	112.3			5.6	4.6	5.1
17.5°			132.6	131.7	132.2			23.5	19.3	21.4
20.0°	157.2	150.9	145.8	148.0	150.5	38.5	39.0	34.8	32.1	36.1
22.5°	184.4	170.8			177.6	60.5	57.6			59.1
25.0°	193.4	188.5	179.1	182.1	185.8	67.7	73.9	63.2	58.8	65.9
27.5°	188.2	186.6			187.4	63.5	72.1			67.8
30.0°	184.6	187.0	175.7	183.1	182.6	60.6	72.4	60.3	59.6	63.2
32.5°	.0	141.3			[1] 141.3	.0	29.6			14.8
15.0 p.p.t.:										
12.5°			104.2	104.0	104.1			.0	.0	.0
15.0°			106.3	106.9	106.6			1.1	.0	.6
17.5°			110.1	110.0	110.1			4.4	2.3	3.4
20.0°	120.9	121.4	116.0	116.5	118.7	9.1	11.0	9.4	7.4	9.2
22.5°	138.2	127.1			132.6	23.1	16.3			19.7
25.0°	153.1	142.9	140.7	139.2	179.2	35.2	31.1	30.5	25.2	30.5
27.5°	152.7	.0			[1] 152.7	34.8	.0			17.4
30.0°	125.9	.0	129.3	124.3	126.5	13.2	.0	20.7	13.5	11.9
32.5°	.0	.0			.0	.0	.0			.0
12.5 p.p.t.:										
12.5°			104.1	104.9	104.5			.0	.0	.0
15.0°			102.7	104.2	103.5			.0	.0	.0
17.5°			103.7	101.5	102.6			.0	.0	.0
20.0°	.0	.0	103.5	104.1	103.8	.0	.0	.0	.0	.0
22.5°	.0	.0			.0	.0	.0			.0
25.0°	111.7	.0	112.8	103.3	109.3	1.7	.0	6.7	.0	2.1
27.5°	.0	.0			.0	.0	.0			.0
30.0°	.0	.0	102.8	.0	[1] 102.8	.0	.0	.0	.0	.0
32.5°	.0	.0			.0	.0	.0			.0

[1] All died in one experiment. Average of single experiment in which some survived.

oyster larvae kept at 27.5° and 30.0° C. and salinities of 7.5 and 10.0 p.p.t. showed a much better rate of survival and growth than in experiment 6 of this series or recorded by Davis (1958). It is possible that in experiments 5 and 7 we happened to get genetic crosses that were better adapted to tolerate low salinities than the general population of Long Island Sound oysters. It is conceivable that the variations in growth noted in experiments 1, 2 and 3 also were genetic and that the Long Island Sound oyster population is heterogeneous in tolerance to salinity.

Regardless of the possible effect of toxic pollutants and of genetic factors, the general features of the effect of lowered salinities on temperature tolerance appear clear. By every measure of temperature tolerance, the tolerated range of temperatures narrowed as the salinity decreased. As measured by the percentage of eggs developing normally and as measured by the growth of larvae, this narrowing of the range resulted from more rapid decreases at both extremes of the temperature range than at intermediate temperatures. As measured by the survival of clam larvae, the

TABLE 10.—*Development of clam eggs to straight-hinge larval stage and survival of clam larvae at 12 days, at different combinations of salinity and temperature, Milford, Conn.*

[Data from individual experiments using clam eggs and larvae]

Salinity (p.p.t.) and temperature (°C)	Eggs developing to straight-hinge larval stage					Larvae surviving to 12th day				
	Experiment				Average	Experiment				Average
	1	2	3	4		1	2	3	4	
27.5 p.p.t.:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
12.5°			0.0	0.0	0.0			31.8	79.6	55.7
15.0°			1.7	56.0	23.6			56.8	65.3	61.0
17.5°			100.0	88.1	94.1			63.6	78.2	70.9
20.0°	99.4	93.4	88.1	98.8	94.9	73.9	85.0	71.2	69.4	74.9
22.5°	92.9	90.1			91.5	74.8	100.0			87.4
25.0°	100.0	93.4	85.6	100.0	94.8	86.6	87.6	62.7	64.6	75.4
27.5°	86.5	100.0			93.3	79.9	83.3			81.4
30.0°	80.0	79.3	80.5	82.7	80.6	89.8	78.2	64.4	100.0	83.1
32.5°	78.1	.0			39.0	61.1	92.3			76.7
22.5 p.p.t.:										
12.5°			.0	1.8	.9			32.2	59.9	46.0
15.0°			1.7	0.6	1.1			34.7	78.2	56.4
17.5°			53.4	50.6	52.0			49.2	89.8	69.5
20.0°	58.7	49.6	62.7	50.6	55.4	91.9	82.5	50.8	78.9	76.0
22.5°	80.0	66.1			73.1	85.5	90.2			87.9
25.0°	80.6	60.3	94.1	79.8	78.7	91.2	88.9	60.2	93.2	83.4
27.5°	70.3	61.2			65.8	89.4	85.5			87.4
30.0°	32.9	6.6	39.8	63.1	35.6	92.6	80.3	76.3	87.1	84.1
32.5°	.0	.8			.4	55.8	39.3			47.6
20. p.p.t.:										
12.5°			.0	.0	.0			14.4	66.0	40.2
15.0°			.0	.0	.0			33.1	66.7	49.9
17.5°			.0	.0	.0			37.3	86.4	61.9
20.0°	.0	.0	.0	1.8	.5	82.0	86.8	51.7	91.8	78.1
22.5°	.0	.0			.0	86.9	66.7			76.8
25.0°	.0	.0	.8	19.6	5.1	99.3	88.5	44.9	72.8	76.4
27.5°	.0	.0			.0	86.9	82.1			84.5
30.0°	.0	.0	.0	.0	.0	89.4	42.7	66.9	89.1	72.0
32.5°	.0	.0			.0	6.0	25.2			15.6
17.5 p.p.t.:										
12.5°			.0	.0	.0			45.8	51.7	48.8
15.0°			.0	.0	.0			39.0	61.2	50.1
17.5°			.0	.0	.0			37.3	61.2	44.6
20.0°	.0	.0	.0	.0	.0	95.8	71.4	33.9	74.1	68.8
22.5°	.0	.0			.0	86.6	82.5			84.5
25.0°	.0	.0	.0	.0	.0	100.0	84.2	67.8	80.3	83.1
27.5°	.0	.0			.0	89.8	57.3			73.5
30.0°	.0	.0	.0	.0	.0	63.3	54.3	100.0	86.4	76.0
32.5°	.0	.0			.0	.0	.9			.5
15.0 p.p.t.:										
12.5°			.0	.0	.0			75.4	18.4	46.9
15.0°			.0	.0	.0			55.1	17.0	36.1
17.5°			.0	.0	0			78.0	37.4	57.7
20.0°	.0	.0	.0	.0	.0	35.3	17.5	82.2	36.1	42.8
22.5°	.0	.0			.0	76.7	28.6			52.7
25.0°	.0	.0	.0	.0	.0	62.9	22.6	92.4	51.7	57.4
27.5°	.0	.0			.0	43.5	.0			21.8
30.0°	.0	.0	.0	.0	.0	3.9	.0	63.6	32.7	25.1
32.5°	.0	.0			.0	.0	.0			.0
12.5 p.p.t.:										
12.5°			.0	.0	.0			63.6	14.3	38.9
15.0°			.0	.0	.0			30.0	8.8	19.4
17.5°			.0	.0	.0			19.5	3.4	11.5
20.0°	.0	.0	.0	.0	.0	.0	.0	24.6	9.5	8.5
22.5°	.0	.0			.0	.0	.0			.0
25.0°	.0	.0	.0	.0	.0	.7	.0	44.1	3.4	12.1
27.5°	.0	.0			.0	.0	.0			.0
30.0°	.0	.0	.0	.0	.0	.0	.0	1.7	.0	.4
32.5°	.0	.0			.0	.0	.0			.0

narrowing of the range was primarily by marked decreases in percent survival at the higher temperatures. With oyster larvae, in these experiments, apparently at even the lowest salinity tested (7.5 p.p.t.) we were just beginning to get marked effects of salinity on survival. It would appear that here, also, the narrowing of the range would be primarily by heavy mortality at the higher temperatures if the salinity were to be lowered still further.

The difference in the temperature requirements for satisfactory growth of clam and oyster larvae probably accounts for some of the difference in success of recruitment of these two species of bivalves in many shellfish-producing areas. While many clam larvae, in water at proper salinity, reached setting size by the 12th day at only 15.0° C., oyster larvae in 20.0° C. water required 36 to 40 days to begin setting. Although the actual time required in natural waters would be altered by the type and quantity of food and by other factors, the relative time required probably

TABLE 11.—*Growth of oyster larvae at 10 days, at different combinations of salinity and temperature, Milford, Conn.*

[Data from individual experiments using oyster eggs and larvae]

Salinity (p.p.t.) and temperature (° C)	Mean length of larvae at 10 days								Increase in mean length of larvae (2d to 10th days)							
	Experiment							Average	Experiment							Average
	1	2	3	4	5	6	7		1	2	3	4	5	6	7	
27.5 p.p.t.:	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
17.5°	94.70	81.20	82.65	83.07	----------	83.34	87.53	85.42	22.2	6.7	4.9	4.2	--------	7.0	8.5	8.7
20.0°	112.76	86.76	86.60	102.85	----------	92.23	106.46	97.93	41.8	12.7	7.5	14.5	--------	15.4	19.8	18.9
22.5°	130.94	99.74	102.50	141.21	----------	116.15	130.37	120.15	63.1	26.7	17.8	34.4	--------	34.7	34.1	35.1
27.5°	161.05	121.32	185.94	224.38	----------	170.45	208.23	178.59	97.1	49.9	71.8	77.6	--------	80.4	80.7	76.3
30.0°	163.59	142.46	223.75	242.26	----------	190.17	240.50	200.35	100.0	72.7	96.3	86.8	--------	97.0	100.0	92.1
32.5°	152.73	138.86	229.45	243.77	----------	193.75	220.00	196.33	87.9	68.8	100.0	87.6	--------	100.0	87.7	88.9
25.0 p.p.t.:																
17.5°	99.50	79.44	83.98	88.56	----------	----------	----------	87.87	27.1	4.8	5.8	7.0	--------	--------	--------	11.2
20.0°	107.83	91.33	89.48	107.25	----------	----------	----------	98.97	37.1	17.6	9.4	19.3	--------	--------	--------	20.9
22.5°	120.83	103.97	105.15	141.73	----------	----------	----------	117.92	51.7	31.2	11.1	34.6	--------	--------	--------	32.2
27.5°	150.65	130.37	185.88	229.00	----------	----------	----------	173.95	85.8	59.7	71.8	80.0	--------	--------	--------	74.3
30.0°	150.98	139.71	224.55	251.44	----------	----------	----------	192.75	91.4	69.8	96.9	91.6	--------	--------	--------	86.6
32.5°	156.00	140.98	226.25	239.99	----------	----------	----------	191.87	82.5	71.1	97.7	85.7	--------	--------	--------	84.3
22.5 p.p.t.:																
17.5°	97.99	86.96	82.88	91.99	----------	----------	----------	89.96	26.0	12.9	5.1	8.8	--------	--------	--------	13.2
20.0°	108.02	93.36	90.20	110.00	----------	----------	----------	100.39	37.3	19.8	10.2	18.2	--------	--------	--------	21.4
22.5°	114.84	103.57	101.68	144.02	----------	----------	----------	116.03	45.0	30.8	17.3	35.8	--------	--------	--------	32.2
27.5°	157.36	137.85	164.98	238.27	----------	----------	----------	174.62	93.0	67.2	58.3	84.8	--------	--------	--------	75.8
30.0°	160.29	156.25	213.18	256.61	----------	----------	----------	194.58	96.3	83.3	89.5	94.3	--------	--------	--------	90.9
32.5°	152.63	149.97	226.90	240.40	----------	----------	----------	193.48	87.6	80.8	98.4	85.9	--------	--------	--------	88.2
20.0 p.p.t.:																
17.5°	94.47	85.49	85.00	90.09	----------	----------	----------	88.76	22.0	11.3	6.5	7.8	--------	--------	--------	11.9
20.0°	107.97	92.59	95.60	110.32	----------	----------	----------	101.62	37.2	19.0	13.3	18.3	--------	--------	--------	22.0
22.5°	113.60	98.74	109.23	137.96	----------	----------	----------	114.88	43.6	25.6	22.2	32.7	--------	--------	--------	31.0
27.5°	139.83	136.62	191.13	244.42	----------	----------	----------	178.00	73.2	66.4	75.2	88.0	--------	--------	--------	75.7
30.0°	153.80	156.25	230.80	267.62	----------	----------	----------	202.12	88.9	87.6	100.0	100.0	--------	--------	--------	94.1
32.5°	150.25	160.23	220.85	243.40	----------	----------	----------	193.68	85.0	91.9	94.4	88.0	--------	--------	--------	89.8
17.5 p.p.t.:																
17.5°	93.93	85.83	84.55	89.73	----------	79.00	85.81	86.48	20.8	11.7	6.2	7.6	--------	3.4	7.4	9.5
20.0°	100.38	95.05	97.03	112.56	----------	92.79	102.39	100.03	28.6	21.6	14.3	19.5	--------	15.0	17.3	19.4
22.5°	108.43	100.95	111.50	136.87	----------	113.59	124.58	115.99	37.7	28.0	23.6	32.1	--------	32.5	30.6	30.8
27.5°	131.58	147.92	198.18	241.35	----------	166.98	197.12	180.52	63.9	78.6	79.8	86.4	--------	77.5	74.0	76.7
30.0°	143.45	160.47	223.58	265.29	----------	189.30	224.35	201.73	77.3	92.1	96.2	98.8	--------	96.3	90.3	91.8
32.5°	144.53	164.52	217.99	231.93	----------	175.10	204.43	189.75	78.5	93.7	92.6	81.5	--------	84.3	78.4	84.8
15.0 p.p.t.:																
17.5°	89.81	86.26	82.90	85.23	----------	77.96	84.42	84.43	16.7	12.1	5.1	5.3	--------	2.5	6.6	8.1
20.0°	95.28	97.63	94.75	111.40	----------	89.70	102.36	98.52	22.9	24.4	12.8	18.9	--------	12.4	17.3	18.1
22.5°	105.08	101.06	109.95	131.32	----------	104.78	126.20	113.07	34.0	28.1	22.6	29.2	--------	25.1	31.6	28.4
27.5°	120.50	144.20	181.89	243.83	----------	130.59	197.73	169.79	51.4	74.6	69.2	87.7	--------	46.8	74.4	67.4
30.0°	134.50	166.77	212.80	261.52	----------	160.77	235.62	195.33	67.2	99.0	89.2	96.8	--------	72.2	97.1	86.9
32.5°	139.08	167.75	209.59	220.44	----------	153.90	192.52	180.55	72.3	100.0	87.2	75.5	--------	66.4	71.3	78.8
12.5 p.p.t.:																
17.5°	----------	----------	----------	----------	----------	76.20	84.28	80.24	--------	--------	--------	--------	--------	1.0	6.5	3.8
20.0°	----------	----------	----------	----------	----------	86.05	95.69	90.87	--------	--------	--------	--------	--------	9.3	13.3	11.3
22.5°	----------	----------	----------	----------	----------	95.85	114.05	104.95	--------	--------	--------	--------	--------	17.6	24.3	21.0
27.5°	----------	----------	----------	----------	----------	118.47	186.42	152.45	--------	--------	--------	--------	--------	36.6	67.6	52.1
30.0°	----------	----------	----------	----------	----------	142.31	207.60	174.95	--------	--------	--------	--------	--------	56.7	80.3	68.5
32.5°	----------	----------	----------	----------	----------	145.37	138.85	142.11	--------	--------	--------	--------	--------	59.3	39.2	49.3
10.0 p.p.t.:																
17.5°	----------	----------	----------	----------	77.00	74.88	77.99	76.62	--------	--------	--------	--------	--------	.0	2.7	1.4
20.0°	----------	----------	----------	----------	83.80	80.25	86.51	83.52	--------	--------	--------	--------	--------	4.4	7.8	6.1
22.5°	----------	----------	----------	----------	103.40	86.58	103.54	97.84	--------	--------	--------	--------	--------	9.8	18.0	13.5
27.5°	----------	----------	----------	----------	159.50	103.81	151.70	138.37	--------	--------	--------	--------	--------	24.3	46.9	35.6
30.0°	----------	----------	----------	----------	172.70	106.14	150.69	143.18	--------	--------	--------	--------	--------	26.4	46.3	36.3
32.5°	----------	----------	----------	----------	138.40	109.30	124.42	124.04	--------	--------	--------	--------	--------	28.9	30.5	29.7
7.5 p.p.t.:																
17.5°	----------	----------	----------	----------	73.60	72.26	75.26	73.71	--------	--------	--------	--------	--------	.0	1.1	.6
20.0°	----------	----------	----------	----------	76.40	73.95	79.96	76.77	--------	--------	--------	--------	--------	.0	3.9	2.0
22.5°	----------	----------	----------	----------	84.50	75.54	91.10	83.71	--------	--------	--------	--------	--------	.5	10.6	5.6
27.5°	----------	----------	----------	----------	117.40	84.76	123.43	108.53	--------	--------	--------	--------	--------	8.2	29.9	19.1
30.0°	----------	----------	----------	----------	103.00	79.69	103.09	95.26	--------	--------	--------	--------	--------	3.9	17.8	10.9
32.5°	----------	----------	----------	----------	79.40	74.32	79.25	77.66	--------	--------	--------	--------	--------	.0	3.5	1.8

would be comparable. In Long Island Sound, where the temperature rarely gets above 21.0° C. and where there are strong tides, during such a greatly prolonged free-swimming period most oyster larvae might be lost by dispersal and by natural predation and mortality. The more rapidly maturing clam larvae, however, would suffer much less loss from these causes.

The fact that mature oyster larvae reared at relatively high temperature (27.0° C.) could set when transferred to temperatures as low as 12.5° C. suggests that oyster larvae that had grown to setting size in protected shallow estuaries where the temperature reached 25.0° to 30.0° C. might well be the source of mature larvae that set in the colder waters of Long Island Sound and similar areas.

SUMMARY

1. Both clam and oyster larvae can ingest foods at significantly lower temperatures than the minimum temperatures at which they can digest and

TABLE 12.—*Development of oyster eggs to straight-hinge larval stage and survival of oyster larvae at 10 days, at different combinations of salinity and temperature, Milford, Conn.*

[Data from individual experiments using oyster eggs and larvae]

Salinity (p.p.t.) and temperature (° C.)	Eggs developing to straight-hinge larval stage								Larvae surviving to 10th day							
	Experiment							Average	Experiment							Average
	1	2	3	[1] 4	5	6	7		1	2	3	4	5	6	7	
27.5 p.p.t.:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
17.5°	90.8	70.3	79.1	0.0	30.5	38.7	90.9	66.7	23	31	81	35	----	21	38	22
20.0°	97.5	85.0	100.0	.0	77.3	72.4	66.4	83.1	39	35	86	28	----	39	66	49
22.5°	100.0	88.4	72.8	.0	88.7	51.5	100.0	83.6	46	20	87	52	----	47	86	56
27.5°	92.5	93.5	76.2	.0	90.8	100.0	94.1	91.2	64	59	100	63	----	84	84	76
30.0°	83.0	83.7	75.7	.0	88.7	88.3	97.7	86.2	49	60	79	60	----	100	79	70
32.5°	82.0	71.6	51.9	.0	20.6	20.9	98.2	57.5	65	54	94	60	----	86	90	75
25.0 p.p.t.:																
17.5°	75.5	87.0	78.1	.0	----	----	----	80.2	26	30	87	37	----	----	----	45
20.0°	87.4	85.5	81.0	.0	----	----	----	83.7	33	31	72	35	----	----	----	43
22.5°	99.2	91.0	67.0	7.6	----	----	----	85.7	40	35	83	56	----	----	----	54
27.5°	75.3	91.0	96.6	32.7	----	----	----	87.6	54	81	94	54	----	----	----	71
30.0°	80.5	75.5	78.6	1.2	----	----	----	78.2	54	64	82	56	----	----	----	64
32.5°	63.5	50.5	67.0	.0	----	----	----	60.3	52	64	86	71	----	----	----	68
22.5 p.p.t.:																
17.5°	67.8	81.5	82.5	5.8	----	----	----	83.9	20	29	83	36	----	----	----	42
20.0°	95.0	85.0	81.1	55.0	----	----	----	87.0	36	22	78	42	----	----	----	45
22.5°	99.2	91.0	81.1	71.9	----	----	----	88.4	50	32	77	56	----	----	----	54
27.5°	93.3	87.6	76.2	.0	----	----	----	82.4	46	88	84	55	----	----	----	68
30.0°	84.0	96.5	71.8	4.1	----	----	----	84.1	43	72	84	66	----	----	----	66
32.5°	62.6	50.6	61.6	.0	----	----	----	58.3	61	75	84	64	----	----	----	71
20.0 p.p.t.:																
17.5°	41.5	67.0	64.6	9.9	----	----	----	57.7	13	47	71	27	----	----	----	37
20.0°	94.2	91.8	72.3	81.3	----	----	----	86.1	24	25	88	53	----	----	----	48
22.5°	95.0	96.5	77.2	84.2	----	----	----	89.6	68	31	73	68	----	----	----	60
27.5°	82.0	97.0	63.1	100.0	----	----	----	80.7	49	63	83	67	----	----	----	66
30.0°	80.5	96.0	54.3	42.7	----	----	----	76.9	51	38	79	62	----	----	----	58
32.5°	51.6	61.4	60.2	10.5	----	----	----	57.7	65	69	97	62	----	----	----	75
17.5 p.p.t.:																
17.5°	.0	12.0	7.8	.0	.0	1.2	46.8	9.7	82	73	49	31	----	46	38	53
20.0°	63.5	55.4	38.3	60.2	20.6	70.6	88.2	56.7	67	19	61	43	----	33	57	37
22.5°	95.8	79.0	58.2	84.2	79.4	71.2	95.9	80.5	81	24	78	76	----	60	72	65
27.5°	89.0	100.0	60.2	91.8	100.0	93.3	85.4	88.5	88	65	80	100	----	81	85	83
30.0°	86.5	77.4	47.1	28.1	69.5	82.2	92.7	69.1	83	64	81	40	----	68	90	71
32.5°	29.6	25.3	22.8	4.1	6.4	38.0	74.1	28.6	87	88	73	56	----	85	81	78
15.0 p.p.t.:																
17.5°	.0	.0	.0	.0	.0	.0	.0	.0	52	57	43	28	----	29	40	42
20.0°	.8	.0	2.4	.0	.0	.0	7.7	1.6	79	88	80	47	----	55	50	67
22.5°	28.0	5.3	23.3	18.1	3.5	24.5	63.2	23.7	90	100	73	75	----	24	65	71
27.5°	34.8	13.3	31.1	29.8	35.5	55.8	73.6	39.1	100	86	80	45	----	78	97	81
30.0°	6.8	.7	8.7	14.6	8.5	28.8	48.2	16.6	81	91	72	37	----	71	90	74
32.5°	.0	.0	1.0	1.8	.0	1.2	2.3	.9	81	95	80	70	----	74	87	81
12.5 p.p.t.:																
17.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	56	23	40
20.0°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	33	49	41
22.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	68	66	67
27.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	63	83	73
30.0°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	79	100	90
32.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	84	87	86
10.0 p.p.t.:																
17.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	49	48	49
20.0°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	74	24	49
22.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	67	65	66
27.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	61	81	71
30.0°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	63	94	79
32.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	65	68	67
7.5 p.p.t.:																
17.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	42	34	38
20.0°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	64	41	53
22.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	57	50	54
27.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	32	64	48
30.0°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	21	54	38
32.5°	----	----	----	----	.0	.0	.0	.0	----	----	----	----	----	39	7	23

[1] Omitted from calculations (see text).

assimilate the food organisms used in these experiments.

2. Both clam and oyster larvae can digest and assimilate naked flagellates, such as *M. lutheri* and *I. galbana*, at lower temperatures than those at which they can utilize algae with cell walls, such as *Chlorella* sp. (580).

3. The minimum temperature for appreciable growth of clam larvae fed naked flagellates was 12.5° C. and the minimum for growth of oyster larvae was 17.5° C.

4. The optimum salinity for growth of clam larvae was 27.0 p.p.t. (the highest salinity tested) or possibly higher.

5. There was no well-defined optimum temperature for growth of clam larvae at any salinity;

maximum growth occurred at temperatures ranging from 25.0° to 30.0° C. in different experiments at almost all salinities.

6. The optimum temperature for growth of oyster larvae was between 30.0° and 32.5° C. in all experiments for all salinities except 7.5 p.p.t. where the optimum was 27.5° C.

7. There was no well-defined optimum salinity for growth of oyster larvae at any temperature; maximum growth occurred in salinities varying from 15.0 to 27.0 p.p.t. in different experiments at some temperatures and from 20.0 to 27.0 p.p.t. even at 17.5° C., where there was least variation.

8. The effect of reduced salinities on larvae of both clams and oysters was to reduce the range of temperatures tolerated. The temperature range for development of eggs and growth of larvae was shortened from both ends, whereas the range for survival of clam larvae was reduced at least primarily by heavy mortality at high temperatures.

9. The time required for oyster larvae to reach the setting stage, under our laboratory conditions, ranged from 10–12 days at 30.0° to 32.5° C. to 36–40 days at 20.0° C.

10. Oyster larvae reared to setting size at about 27.0° C. and transferred to lower temperatures could set at temperatures as low as 12.5° C., but the percentage of such larvae successfully completing metamorphosis decreased progressively with each decrease in temperature.

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