THE pH TOLERANCE OF EMBRYOS AND LARVAE OF MERCENARIA MERCENARIA AND CRASSOSTREA VIRGINICA

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The tidal estuarine waters that form the principal habitat of most commercial mollusks are some of the most complex environments in nature. Of the various interacting biological, physical, and chemical factors that affect commercial mollusks in these waters, pH has received less attention than any other major factor. Prytherch (1928) measured the pH at several stations in Milford Harbor and the Milford area of Long Island Sound. He found a pH range during the day from 7.2 to 8.4 and observed that oysters spawned at pH 7.8 to 8.2. Prytherch concluded that low pH inhibited oyster spawning and that oysters in Milford Harbor spawned at high tide because this was the only tidal stage at which the pH was between 7.8 and 8.2. Korringa (1940) quoted Gaarder (1932) and Gaarder and Spärck (1932) who found that larvae of *Ostrea edulis* died when the pH in their oyster polls exceeded 9.0.

In laboratory experiments, Loosanoff and Tommers (1947) found that adult American oysters, *Crassostrea virginica,* kept in pH 4.25 remained open, on an average, 76% of the time, but pumped only 10% as much water as did the controls. Oysters kept at pH 6.75 and 7.00 initially pumped more vigorously than the controls but the rate of pumping later decreased to less than that of the controls.

Although the pH of the open ocean usually ranges from 7.5 to 8.5 (the higher values are at the surface during active photosynthesis), the pH in tidepools, bays, and estuaries may decrease to 7.0 or lower due to dilution and production of H_2S (Sverdrup, Johnson and Fleming, 1942). These inshore areas constitute a major portion of the habitat of commercial bivalves, and Davis and Calabrese (1964) suggested that these regions may be exceedingly important also as the nursery grounds for the larval stages. Since clam and oyster larvae must, at times, encounter a wide range of pH in their natural habitat, it is possible that success or failure of recruitment in some areas may be determined by variations in pH. The present studies were designed to determine the pH tolerance of the embryonic and larval stages of hard clams (*Mercenaria mercenaria*) and American oysters (*Crassostrea virginica*) under laboratory conditions.

METHODS

The methods at this laboratory for maintaining spawners and obtaining fertilized eggs throughout the year have been described previously (Loosanoff and Davis, 1963). The effect of pH on the percentage of eggs of clams or oysters that develop into normal straight-hinge larvae was determined by placing a known number of fertilized eggs (usually 10,000 to 15,000) into each of a series of 1-liter polypropylene beakers of filtered, ultraviolet-treated sea water (salinity 27 $\pm$ 0.5‰).

The pH of duplicate cultures was adjusted with HCl or NaOH to each of the following levels: 6.00, 6.25, 6.50, 6.75, 7.00, 7.50, 8.00, 8.25, 8.50, 8.75, 9.00, 9.25, and 9.50. Finally, one pair of cultures retained at the pH of our laboratory sea water (7.40–7.70) served as controls. All cultures were kept in a constant-temperature bath at 25° ± 1° C. After 48 hours at the experimental conditions, the larvae from each culture were collected on a stainless steel screen. The larvae were resuspended in a 250-ml. graduated cylinder and, after thorough mixing, a

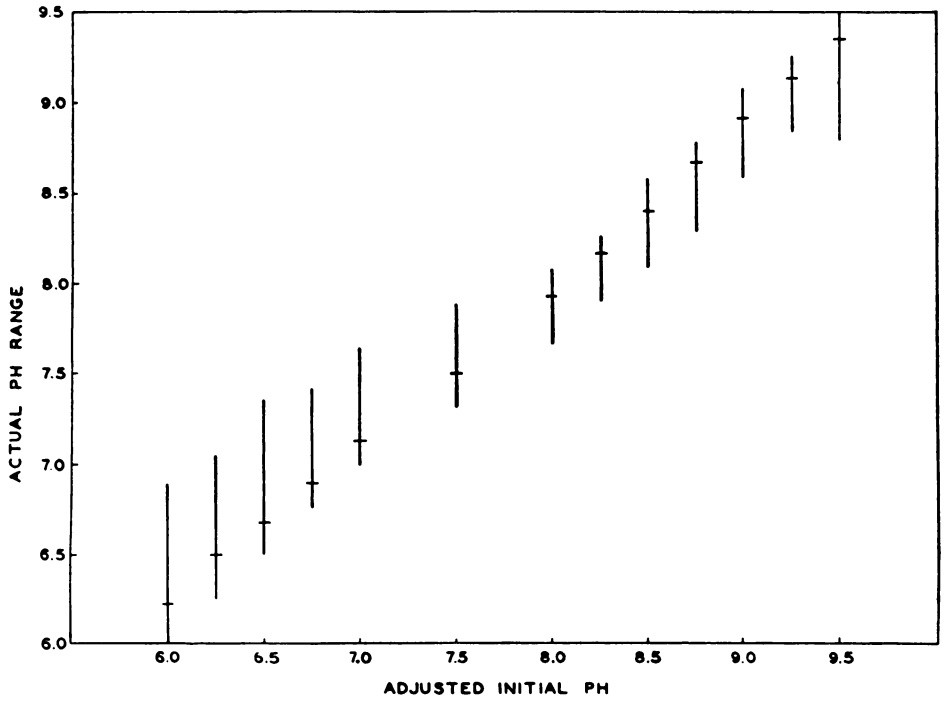

FIGURE 1. Maximum range of pH (vertical line) and average pH (horizontal bar) for each initial pH. The "adjusted initial pH" was established at the beginning of each experiment, and readjusted to this level at 12-hour intervals, by the addition of HCl or NaOH.

4-ml. sample was withdrawn and preserved in 5% neutral formalin. The larvae from each sample were then counted and the number of larvae developing normally at each pH was calculated as a percentage of the number of larvae developing normally in control cultures.

To ascertain the effect of pH on survival and growth, a known number of larvae (usually 8000 to 12,000), which had been reared to the 48-hour straight-hinge stage in our normal sea water (pH 7.40–7.70), was placed into each of the series of cultures. The sea water in these beakers was changed every second day and supplemental food, consisting of a mixture of *Isochrysis galbana, Monochrysis lutheri,* and *Chlorella* sp. 580,[1] was added to each beaker daily. The pH of each

[1] *Chlorella* sp. (Indiana U. Collection #580).

culture was adjusted to the desired level immediately after each change of sea water by the addition of an appropriate amount of HCl or NaOH. In experiments with clam larvae it was necessary to add a standard dose (50 ppm.) of Sulmet [2] at each change of sea water to prevent disease-induced mortality that was not a direct result of the pH being tested. Since buffers were not used, it was necessary to measure and readjust the pH at approximately 12-hour intervals using a line-operated, solid-state pH meter [3] having a readability of 0.02 pH unit and a repeatability of 0.01 pH unit. The range and average for each initial pH are shown in Figure 1.

Experiments with clam larvae were terminated after 10 days at the experimental pH levels, when the larvae were 12 days old, because at favorable pH the majority of the larvae had completed larval development and metamorphosis. For similar reasons experiments with oyster larvae were discontinued after 12 days at experimental pH levels, when the larvae were 14 days old.

Quantitative samples were taken from each culture at the termination of an experiment to determine the percentage of larvae surviving and their increase in mean length. In each of these samples, all survivors were counted and 50 clam larvae or 100 oyster larvae from each sample were measured to the nearest 5 μ. The increase in mean length of larvae during the test period was calculated for each pH as a percentage of the increase in mean length of larvae in the control cultures.

The method for determining the number of larvae surviving or the percentage of bivalve eggs developing into normal straight-hinge larvae is accurate to approximately $\pm$ 10% (Davis, 1958). Differences of less than 20% in the percentage of eggs developing normally or of larvae surviving a treatment are, therefore, considered insignificant.

Five experiments were with clam larvae and four with oyster larvae. In the first three experiments with clam larvae a standard technique was being developed and various buffer systems were being tested. In these initial experiments citric acid, monobasic potassium phosphate, dibasic sodium phosphate, and Tris (hydroxymethyl aminomethane) were used as buffers in an attempt to stabilize the pH at desired levels. The phosphates and citric acid were not effective in maintaining pH levels below 7.00; these buffers also appeared somewhat toxic to clam larvae. When concentrations of these buffers were high, a white flocculent precipitate was formed in the cultures. Tris was of some help in maintaining relatively stable pH levels above 7.00, but was toxic at pH 8.50 or higher, even though apparently nontoxic at levels below 8.50. A precipitate which adhered to the sides and bottom of the beakers was formed at pH 9.50, with or without Tris. The results of these preliminary experiments are not included in our graphs.

Effect of pH on embryonic development of clams and oysters

The number of clam eggs developing normally within the pH range from 7.00 to 8.75 or of oyster eggs within the range from 6.75 to 8.75 did not vary significantly (Fig. 2). The number of both clam and oyster eggs that developed normally at pH 9.00 was greatly reduced and at 9.25 to 9.50 almost none developed.

[2] Sulmet (Sodium sulfamethazine)—Trade name of American Cyanamid.

[3] Instrumentation Laboratory's Model 165 LAB-omatic.

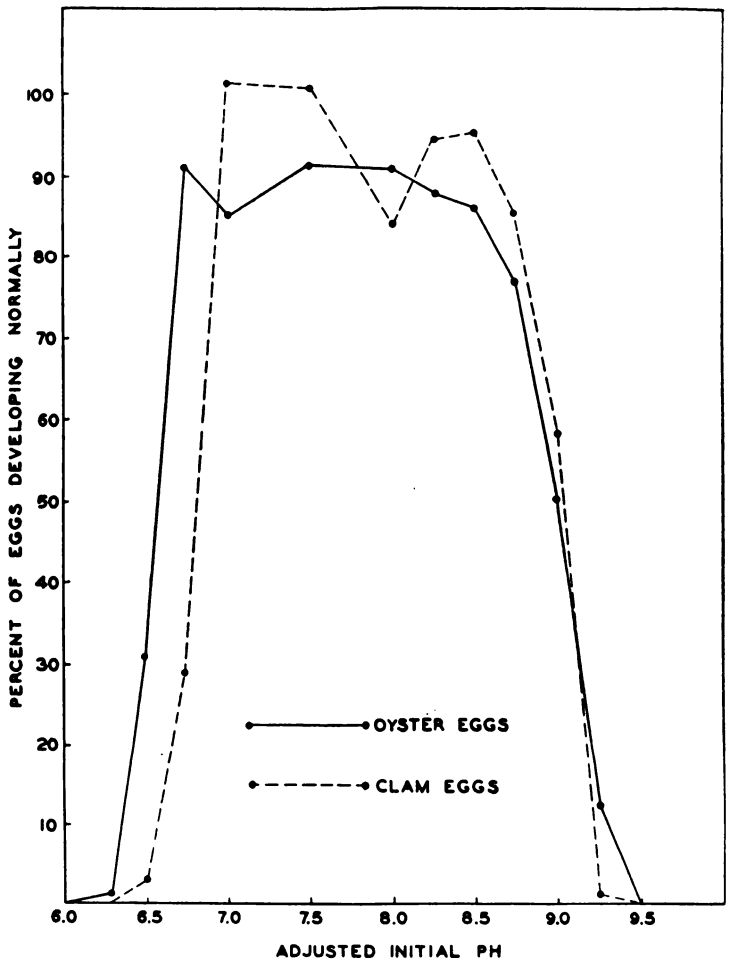

FIGURE 2. Percentage of clam and oyster eggs that developed into normal straight-hinge larvae at different pH levels, expressed as a percentage of the number developing into normal larvae in control cultures.

Clam eggs apparently were not able to tolerate pH values as low as did oyster eggs. At pH 6.75 only 29.5% of the clam eggs developed but 92.4% of the oyster eggs developed normally.

Effect of pH on survival of clam and oyster larvae

Survival of both clam and oyster larvae was approximately normal throughout the pH range from 6.25 to 8.75 (Fig. 3). Oyster larvae were somewhat more tolerant, however, of low pH than clam larvae. At pH 6.00, for example, 21.5% of the oyster larvae survived, but all of the clam larvae died. Survival of both clam and oyster larvae increased sharply from 20% or less at pH 6.00 to approxi-

mately 70% at 6.25 and decreased sharply from 70% or better at pH 8.75 to approximately 40% at 9.00. Most of the larvae lived a few days at pH 9.00 although eventually more than 50% died. No larvae of either species survived at 9.25 and higher.

Effect of pH on growth of clam and oyster larvae

The pH range for normal growth was 6.75 to 8.50 for clam larvae and 6.75 to 8.75 for oyster larvae (Fig. 4). The range for normal growth was, therefore, slightly narrower than the range for normal survival. The rate of growth of clam larvae was most rapid at pH 7.50 to 8.00, whereas oyster larvae grew most rapidly at 8.25 to 8.50. Although oyster eggs and larvae survive at lower pH levels than clam eggs and larvae, the optimum pH for growth of oyster larvae was somewhat

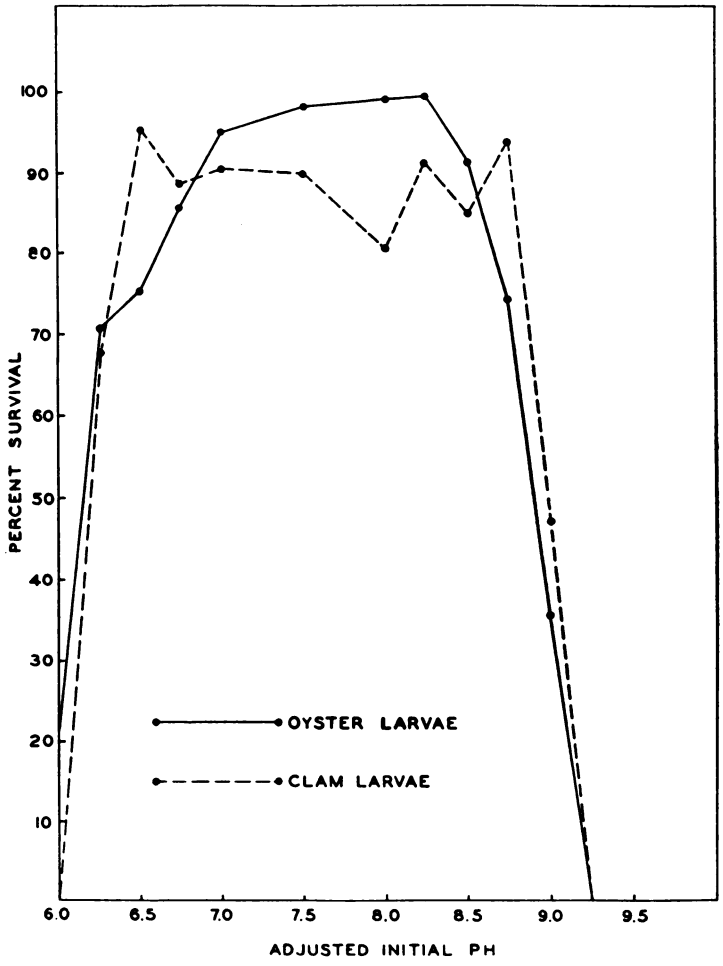

FIGURE 3. Percentage of clam and oyster larvae that survived at different pH levels, expressed as a percentage of survival in control cultures.

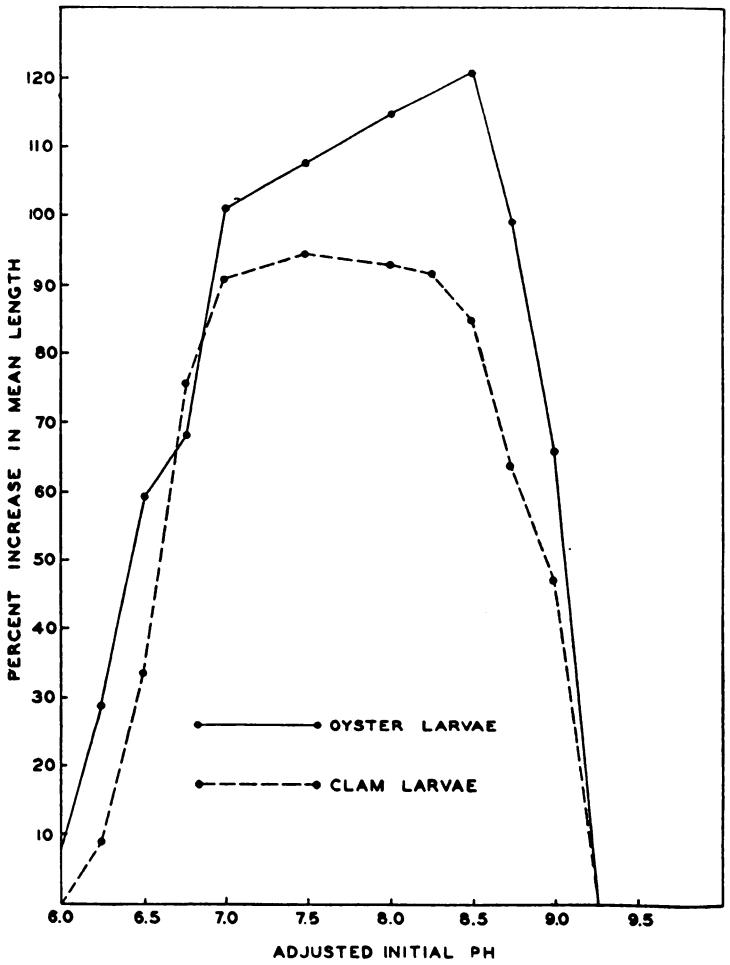

FIGURE 4. Increase in mean length of clam and oyster larvae at different pH levels expressed as a percentage of the increase in mean length of larvae in control cultures. Fifty clam or 100 oyster larvae were measured from each of duplicate cultures at each pH in each of two or more replicate experiments.

higher than the optimum for clam larvae. The rate of growth for both clams and oysters varied only slightly within the pH range 6.75 to 8.50, but below pH 6.75 the rate of growth decreased rapidly. The rate of growth also decreased rapidly at pH values above 8.75 for oysters and above 8.50 for clams. Since the empty shells of dead larvae were not dissolved at the higher pH levels, it was possible to measure them. At pH 9.00 some increase in length had taken place before the larvae died, but at 9.25 to 9.50 there had been no growth.

Implications for distribution and survival in nature

The failure of bivalve larvae to survive and grow at low pH levels did not appear to be an indirect result of the effect of pH on the algal cells added as food. That

the food cells were not destroyed by low pH and that they remained in suspension was shown by the fact that they were ingested by the larvae. Since the larvae were fed supplemental food daily, starvation was unlikely even if some algal cells were destroyed. Even those larvae that survived at low pH and had food in their stomachs, however, did not grow appreciably. Gray (1922) found that movement of gill cilia of mussels was more readily inhibited by weak acids which entered the ciliary cells than by strong acids which do not enter the cells readily and, conversely, that weak bases were more efficient restoratives of ciliary movement than strong bases. Because some food was ingested, even at our lowest pH levels, it seems unlikely that failure of these larvae to grow can be attributed to the effect of pH on ciliary movement.

It should be emphasized that clam larvae can survive at pH levels lower than those at which clam eggs can develop normally (Fig. 5). The range for normal

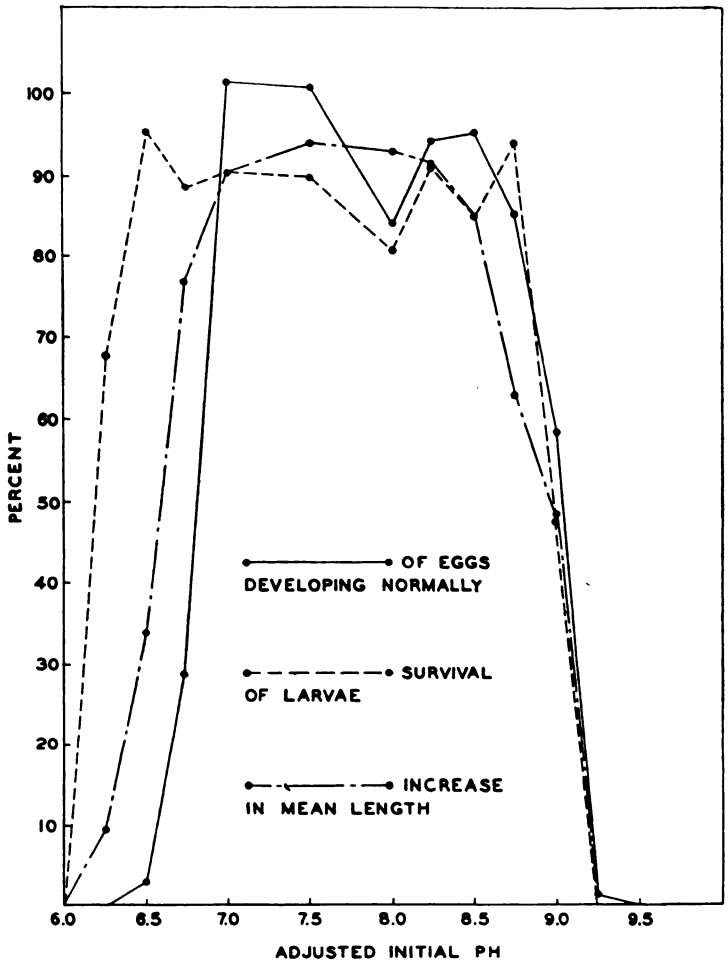

FIGURE 5. The pH tolerance of clam embryos and larvae as indicated by percentage of eggs that developed normally, survival of larvae, and increase in mean length of larvae.

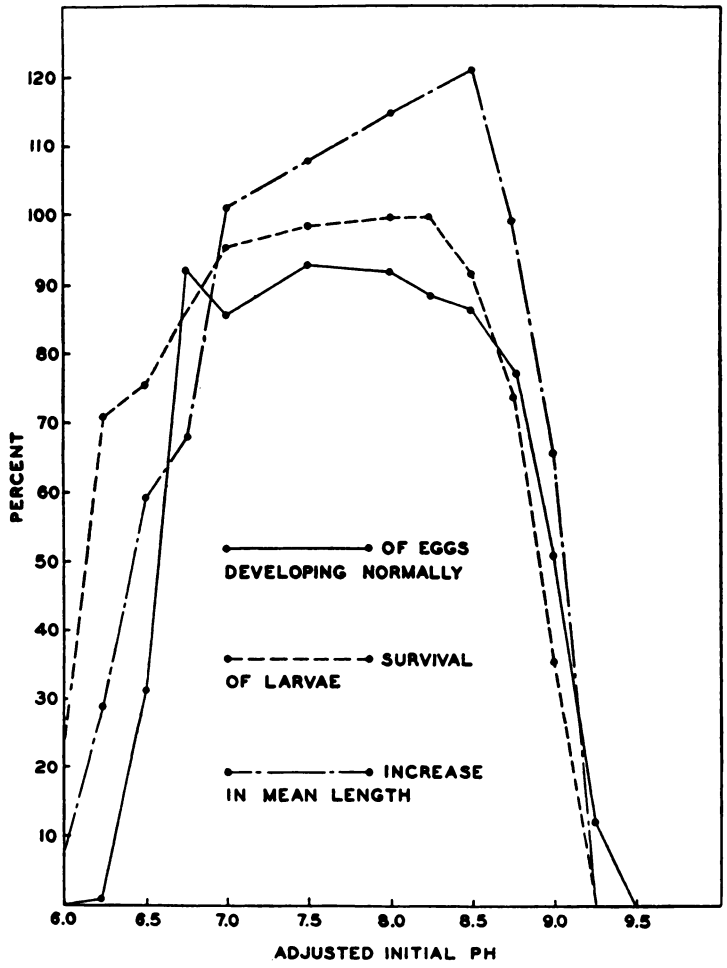

FIG. 6. The pH tolerance of oyster embryos and larvae as indicated by percentage of eggs that developed normally, survival of larvae, and increase in mean length of larvae.

survival of larvae was 6.25 to 8.75, whereas the range for normal embryonic development was only 7.00 to 8.75. In environments with a pH below 7.00, failure of clam eggs to develop normally would be the factor that would limit recruitment of this species. At pH levels 9.00 and above the percentage of clam eggs developing normally, the percentage of larvae surviving, and the percentage increase in mean length all decrease abruptly, so that at high pH levels all three aspects of development limit recruitment of the species. Variations in the percentages of eggs developing normally and of larvae surviving at pH levels between 7.00 and 8.75 were erratic, but all fell within the $\pm$ 10% confidence limits of our method. Although the pH ranges for normal survival of clam larvae were 6.25 to 8.75 and those for normal rate of growth were 6.75 to 8.50, the optimum for growth was 7.50 to 8.00. The differences in rates of growth at pH 6.75 to 8.50, however, were slight enough

to be negligible in recruitment of this species in nature. The pH of our laboratory sea water (7.40–7.70) was close to optimum for growth of hard clam larvae.

Oyster larvae, like clam larvae, survived at lower pH levels than those at which the eggs developed (Fig. 6). At pH 6.00 none of the oyster eggs developed normally, but 21.5% of the larvae survived. At pH 6.25 the percentage survival of larvae increased sharply, but the increase in the percentage of eggs developing normally was negligible. Most of the oyster eggs developed normally at pH 6.75, whereas a pH of 7.00 was required for most clam eggs to develop. Oysters, therefore, should be able to penetrate into areas of lower pH than clams could tolerate. The range for normal survival of oyster larvae was pH 6.25 to 8.75, and the range for a normal rate of growth of the larvae was 6.75 to 8.75. The optimum pH for growth of oyster larvae, however, was 8.25 to 8.50, *i.e.,* both the optimum and the upper limit for normal growth were somewhat higher than for clam larvae. As with clams, however, the percentage of eggs developing normally, the percentage survival of larvae, and the rate of growth all decrease rapidly at pH 9.00 and above. Since oyster larvae at pH 8.00 to 8.50 outgrew the oyster larvae in the control cultures (pH 7.40–7.70), it was apparent that the pH of our normal laboratory sea water was somewhat too low for the most rapid growth of these larvae.

It can be concluded that for successful recruitment of clams and oysters the pH of the tidal estuarine waters that form their principal habitat must not fall below 7.00 for clams or 6.75 for oysters for any appreciable time. Moreover, neither species could reproduce successfully in waters where the pH remained appreciably above 9.00.

Laboratory experiments have shown that high concentrations of silt can lower the pH of our sea water to 6.40, or below the lower limit for normal development of eggs of hard clams and oysters. It is apparent, therefore, that heavy siltation, or any pollution that can change the pH of tidal estuarine waters, could cause failure of recruitment of hard clams and oysters.

Summary

1. The pH range for normal embryonic development of oysters was 6.75 to 8.75, and for clams, 7.00 to 8.75.

2. More than 68% of the larvae of both clams and oysters survived at pH 6.25 to 8.75. The lower pH limit for survival of oyster larvae was 6.00 and for clam larvae, 6.25.

3. The pH range for normal growth was 6.75 to 8.50 for clam larvae and 6.75 to 8.75 for oyster larvae. The rate of growth of both species dropped rapidly at pH levels below 6.75.

4. The optimum pH for growth was 7.50 to 8.00 for clam larvae and 8.25 to 8.50 for oyster larvae.

5. At pH 9.00 to 9.50 the percentage of eggs that developed normally, the percentage of larvae that survived, and the percentage increase in mean length of both species decreased rapidly.

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