HOW SOME POLLUTANTS AFFECT EMBRYOS & LARVAE OF AMERICAN OYSTER & HARD-SHELL CLAM

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This article reports the effects of detergents, pH, and pesticides on development of embryos and survival and growth of larvae of the American oyster and hard-shell clam. Although LAS detergents are more readily biodegraded than ABS detergents, results indicate that the former are at least as toxic to oyster larvae as ABS compounds. For successful recruitment of clams and oysters, the pH of estuarine waters must not fall below 7.00 for clams or 6.75 for oysters. Neither species could reproduce successfully in waters where the pH remained appreciably above 9.00. Most of the pesticides tested affected embryonic development more than survival or growth of larvae. Some, however, drastically reduced growth of larvae at concentrations that had relatively little effect on embryonic development.

The effects of pollutants on mollusks and on the survival and growth of their larvae are of interest to biologists and of considerable importance to shellfish producers. Since several of the most important commercial species are essentially inhabitants of the shallow waters of bays and estuaries, almost all grounds used for cultivation of shellfish are frequently subjected to both domestic and industrial pollutants. Scientists at the NMFS Milford (Conn.) laboratory have studied the effects of various pollutants on development of embryos and survival and growth of larvae of the American oyster, Crassostrea virginica, and the hard-shell clam, Mercenaria mercenaria. The effects of detergents, pH, and pesticides were especially studied.

METHODS

In all these experiments, a standard procedure for determining the effects of pollutants was used. To determine the effects of various pollutant concentrations on the development of bivalve embryos into normal, straight-hinge larvae, about 13,000 embryos were placed into each of a series of 1-liter beakers. To determine the effects of the same conditions on survival and growth of larvae, usually 8,000 to 12,000 larvae reared to the 48-hour straight-hinge stage under normal conditions were placed into 1-liter beakers. Duplicate cultures were maintained at each concentration of the pollutant, and two cultures were left untreated as controls. The beaker cultures with larvae were changed every second day for 10 to 12 days to eliminate metabolic waste products, and the experimental conditions were reestablished. Supplemental food was added daily and, in some experiments, 50 mg/l of Sulmet were added to each culture every second day to minimize mortality due to factors other than toxicity of the pollutant.

The effects of the pollutants on embryonic development were determined by taking a 4/250 ml aliquot of the total sample after 48 hours. The number of embryos developing normally was counted and the results expressed as a percentage of the number developing in the controls. To determine percentage of survival and increase in size of larvae, the cultures were sampled quantitatively in a manner similar to the experiments with embryos. Survival was expressed as a percentage of survival in the controls, and increase in size was determined as a percentage of the increase in mean length of larvae in the control cultures.

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DETERGENT STUDIES

An industry-wide conversion from the alkyl benzene sulfonate (ABS) type detergents, which are only very slowly degraded by bacterial action, to the biodegradable linear alkylate sulfonate (LAS) type detergents was completed on June 30, 1965. Since these detergents are almost completely degraded in an efficient sewage-treatment plant, most of the detergent will occur in the effluent as degradation products, rather than as active detergent.

A study of detergents determined the toxicity to oyster embryos and larvae of a reference sample of undegraded LAS and a commercial liquid biodegradable detergent (1). The sample of LAS contained 60.8% active LAS; the liquid detergent was of unknown composition. Test concentrations were from 0.0025 to 2.5 mg/1. For tests of degradation products of LAS, effluents were obtained from the U.S. Testing Company in which almost 100% of the 20.0 mg/1 of added LAS had been degraded. Control effluent that contained no detergent degradation products was also used. Liter cultures were set up with effluent containing degradation products equivalent to original LAS concentrations of 0.1, 0.2, 2.0, 3.0, 4.0, and 5.0 mg/1. This meant that it was necessary to add 5, 10, 100, 150, 200, and 250 ml of effluent, respectively. Another series of cultures was set up with equivalent volumes of effluent that contained no detergent degradation products to determine whether the degraded products of the detergents of the effluent affected the embryos and larvae.

Oyster embryos had a very low tolerance to active LAS detergents (Table 1). A concentration of 0.10 mg/1 permitted only 64% of the embryos to develop and many of them were abnormal either in shape or size. Development of 47% was reached at 0.50 mg/1 of ABS, which was 54.8% active (2). It would appear that LAS is somewhat more toxic than ABS while in the active state. In experiments with the commercial liquid biodegradable detergent, oyster embryos tolerated a concentration up to 0.25 mg/1 of the gross product. The percentage of active LAS in this product was not known; therefore, it is quite probable that its lower toxicity was the result of a lower concentration of active LAS. Embryos developed at a concentration as high as 1.0 mg/1 using the similar brand product with an ABS base; again this indicated that LAS is somewhat more toxic than ABS while in its active state.

The sewage-treatment effluent, both with and without degraded LAS, had about the same toxicity. Even though a comparatively high percentage of embryos developed at a concentration of 5.0 mg/1, many larvae were abnormal. Cultures treated with active LAS were compared with those treated with sewage effluent containing either no LAS or degraded LAS. It was apparent that the LAS had lost most of its toxicity through degradation treatment. Any remaining toxicity of the LAS or its degraded products was marked by the toxicity of the effluent itself.

Oyster larvae were somewhat more tolerant to both standard LAS and the commercial product than the embryos (Table 2). A significant reduction in survival of larvae occurred at concentrations of 0.5 to 1.0 mg/1 for the LAS and between 1.0 to 2.5 mg/1 for the commercial product. Although the percentage survival of larvae in the control cultures was somewhat greater than in cultures receiving the effluent, with or without degraded LAS, it required a degradation product concentration equivalent to 4.0 mg/1 of the undegraded LAS before a drastic reduction in survival occurred. The similarity of results of effluent with and without detergent degradation products suggested that the toxicity was primarily that of the effluent itself.

As indicated by the percentage of increase in mean length, growth of oyster larvae was normal at LAS concentrations of 0.0025 to 0.25 mg/1, but showed a very sharp break between 0.25 mg/1 and 0.5 mg/1 of active LAS (Table 3). The liquid detergent was somewhat less toxic, but a decline in growth of larvae occurred between detergent concentrations of 1.0 and 2.5 mg/1. It should be remembered, however, that the percentage of active LAS in this commercial detergent was not available, and the lesser toxicity probably was due to a lower concentration of active LAS. Approximately normal growth of larvae was achieved at concentrations up to 2.0 mg/1 in both the control sewage effluent and that containing degraded LAS. Slight growth did occur at a degradation product concentration equivalent to 4.0 mg/1 of undegraded LAS in the cultures receiving effluent with degraded LAS.

We found that effluent containing LAS degradation products equivalent 20.0 mg/1 of LAS is little, if any, more toxic to oyster embryos and larvae than an equal volume of effluent containing no such degradation products. From these experiments it seems apparent that, if sewage-treatment plants are effective, it would be necessary to introduce enough effluent to constitute 15% or more of the total volume of a body of water to affect the development, survival, and growth of oyster larvae seriously. In most areas, this is probably a higher concentration of effluent than would normally be present. But in some areas, where sewage effluent approaches 15% of the total volume of the body of water it enters, it could be expected to affect recruitment of oysters.

pH STUDIES

The tidal estuarine waters that form the principal habitat of most commercial mollusks is one of the most complex environments in nature. Yet of the various interacting biological, physical, and chemical factors affecting commercial mollusks, pH has received less attention than any other major factor. While the pH of the open ocean usually ranges from 7.5 to 8.5, the pH in tidepools, bays, and estuaries may decrease to 7.0 or lower due to dilution, H_2S production, and pollution (3). 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 of these mollusks in some areas may be determined by variations in pH. With this in mind, a study was initiated to determine the effect of pH on embryos and larvae of clams and oysters (4).

The experimental setup was described before, but in this case the pH levels in the beaker cultures were adjusted from 6.0 to 9.5 by the addition of HCl or NaOH.

There was no significant decrease in the number of clam embryos developing normally within the pH range from 7.0 to 8.75 or of oyster embryos from pH 6.75 to 8.75 (Fig. 1). The number of both clam and oyster embryos developing normally at pH 9.0 was greatly reduced, and at pH 9.25 to 9.5 there was virtually no development. Clam embryos apparently were not able to tolerate as low a pH as did oyster embryos: at pH 6.75, more than three times as many oyster embryos as clam embryos developed normally.

Both clam and oyster larvae showed about normal survival throughout the pH range from 6.25 to 8.75 (Fig. 2). Oyster larvae, however, were somewhat more tolerant of low pH levels than clam larvae. At pH 6.0, for example, 21.5% of the oyster larvae survived, but none of the clam larvae. At pH 9.0, some larvae lived for a few days and showed some growth, although eventually more than 50% died; at 9.25 and higher, there was no survival of either species.

The pH range for normal growth of clam larvae was 6.75 to 8.50 and for oyster larvae 6.75 to 8.75 (Fig. 3). The pH range for normal growth was, therefore, slightly narrower than that for normal survival. The rate of growth of clam larvae was most rapid at pH 7.5 to 8.0, while oyster larvae grew most rapidly at pH 8.25 to 8.5. Although oyster embryos and larvae survive at lower pH levels than clam embryos and larvae, the optimum pH for growth of oyster larvae is somewhat higher than the optimum for clam larvae. The rate of growth decreased rapidly below 6.75 and above pH 8.75 for both clams and oysters.

It should be emphasized that clam larvae can survive at pH 6.25, which is lower than the pH 7.0 at which clam embryos develop normally; but at pH levels below 7.0 failure of clam embryos to develop normally would be the factor that would limit recruitment of this species (Fig. 4). The percentage of clam embryos developing normally, larval survival, and increase in mean length all decrease precipitously at about pH 9.0; these three factors would limit recruitment of this species.

Oyster larvae, like clam larvae, can survive at lower pH levels than those at which embryos can develop. At pH 6.25, there was a sharp increase in the survival of oyster larvae and only a negligible increase in development of oyster embryos (Fig. 5).

In experiments with adult oysters (5), it was concluded that the minimum and maximum pH levels at which they would spawn are 6.0 and 10.0, respectively. The percentage of oysters that spawned at pH 6.0 and 10.0 was considerably lower than the percentage that spawned at the normal pH (7.8) of laboratory sea water. In all tests, male oysters spawned more readily than females,

TABLE 1. Percentage of oyster eggs developing to straight-hinge larvae in various concentrations of LAS detergent and sewage effluent /after (1)/.

Concentration (mg/l)	LAS[1]	Liquid biodegradable[2] detergent	Effluent with degraded LAS	Effluent[3] without degraded LAS	Volume of effluent (ml/l)
0.0 (Controls)	100	100	100	100	0 (Controls)
0.0025	92	99	-	-	-
0.005	88	108	-	-	-
0.010	85	108	-	-	-
0.025	66	118	-	-	-
0.05	51[4]	115	-	-	-
0.10	64[4]	68	-	-	-
0.20	-	-	127	78	10
0.25	0	63	-	-	-
0.50	0	14	-	-	-
1.00	0	0	-	-	-
2.00	-	-	97	109	100
2.50	0	0	-	-	-
3.00	-	-	-	-	-
4.00	-	-	-	-	-
5.00	0	0	99[4]	66[4]	250
10.00	0	0	-	-	-

1 Concentrations listed are of active LAS; gross product contained 60.8% active LAS

2 Concentrations listed are of gross product; percentage of active LAS unknown

3 Values listed based on single experiment

4 Many larvae in these cultures abnormal in size or shape, or both

TABLE 2. Percentage of oyster larvae surviving in various concentrations of LAS detergent and sewage effluent /after (1)/.

Concentration (mg/l)	LAS[1]	Liquid biodegradable[2] detergent	Effluent with degraded LAS	Effluent[3] without degraded LAS	Volume of effluent (ml/l)
0.0 (Controls)	100	100	100	100	0 (Controls)
0.0025	76	96	-	-	-
0.005	106	88	-	-	-
0.010	93	102	-	-	-
0.025	71	104	-	-	-
0.05	96	84	-	-	-
0.10	104	120	78	-	5
0.20	-	-	76	75	10
0.25	95	95	-	-	-
0.50	63	82	-	-	-
1.00	0	87	-	-	-
2.00	-	-	69	103	100
2.50	0	42	-	-	-
3.00	-	-	63	-	150
4.00	-	-	35	-	200
5.00	0	0	10	19	250
10.00	0	0	-	-	-

[1] Concentrations listed are of active LAS; gross product contained 60.8% active LAS

[2] Concentrations listed are of gross product; percentage of active LAS unknown

[3] Values listed based on single experiment

TABLE 3. Percentage increase in mean length of oyster larvae reared in various concentrations of LAS detergent and sewage effluent /after (1)/.

Concentration (mg/l)	LAS[1]	Liquid biodegradable[2] detergent	Effluent with degraded LAS	Effluent[3] without degraded LAS	Volume of effluent (ml/l)
0.0 (Controls)	100	100	100	100	0 (Controls)
0.0025	96	100	-	-	-
0.005	97	101	-	-	-
0.010	98	101	-	-	-
0.025	106	100	-	-	-
0.05	94	102	-	-	-
0.10	96	103	111	-	5
0.20	-	-	105	92	10
0.25	88	101	-	-	-
0.50	31	103	-	-	-
1.00	Dead	100	-	-	-
2.00	-	-	85	71	100
2.50	Dead	44	-	-	-
3.00	-	-	55	-	150
4.00	-	-	32	-	200
5.00	Dead	Dead	-[4]	-[4]	250
10.00	Dead	Dead	-	-	-

[1] Concentrations listed are of active LAS; gross product contained 60.8% active LAS

[2] Concentrations listed are of gross product; percentage of active LAS unknown

[3] Values listed based on single experiment

[4] Number surviving too small for accurate determination of mean length

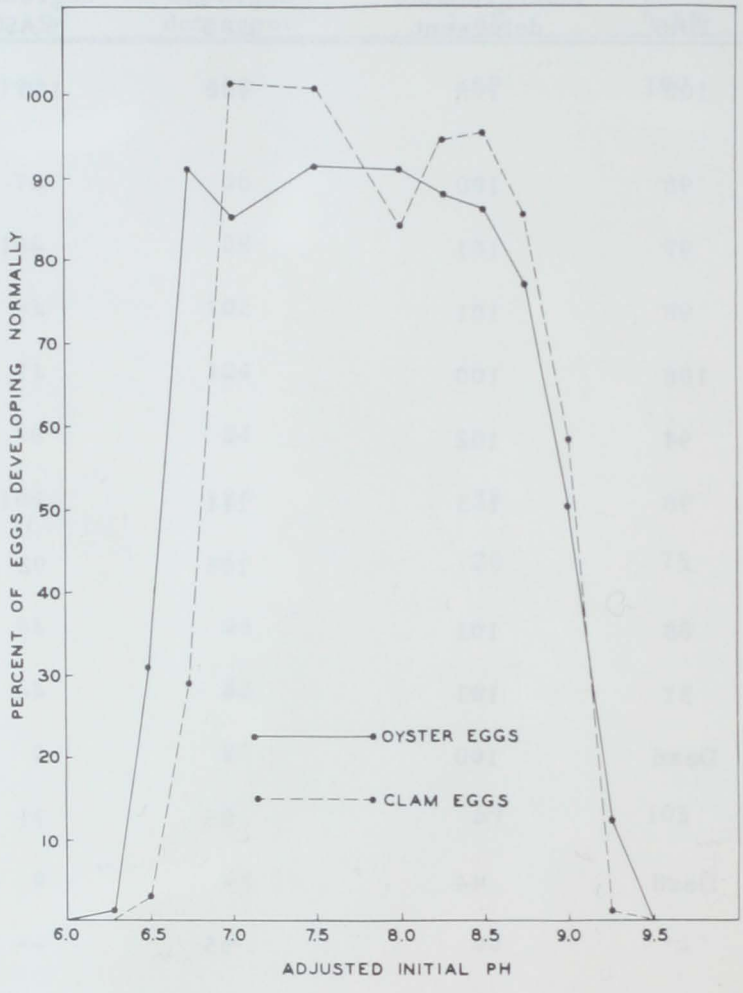

Fig. 1 - Percentage of clam and oyster eggs that developed into normal straight-hinge at different pH levels, expressed as a percentage of the number developing into normal larvae in control cultures [after (4)].

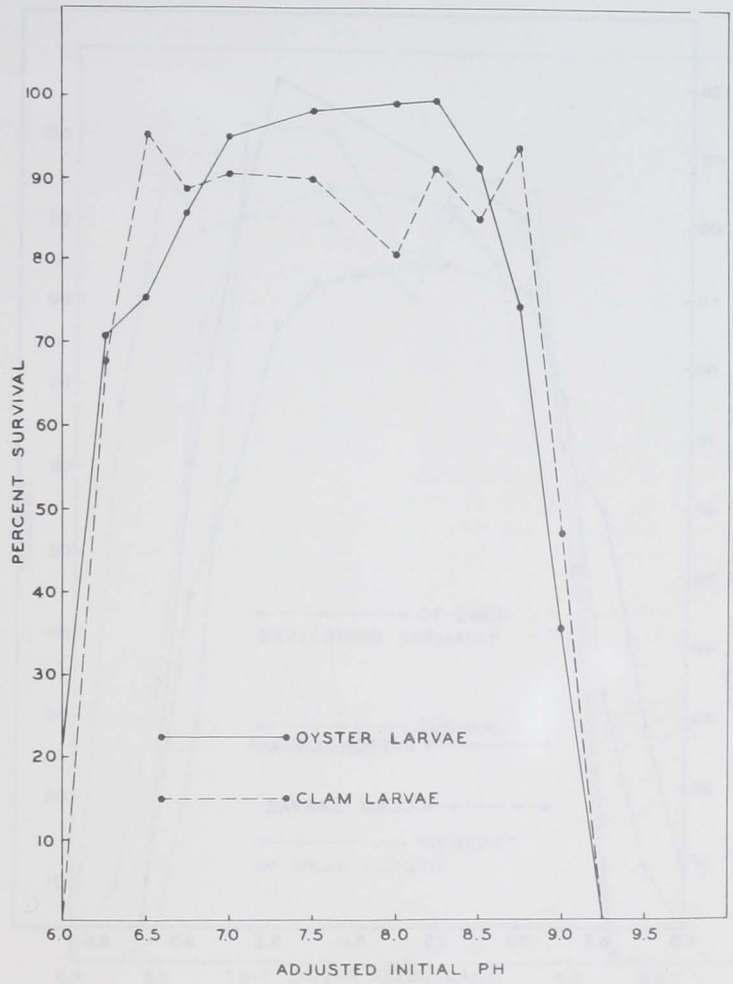

Fig. 2 - Percentage of clam and oyster larvae that survived at different pH levels, expressed as a percentage of survival in control cultures [after (4)].

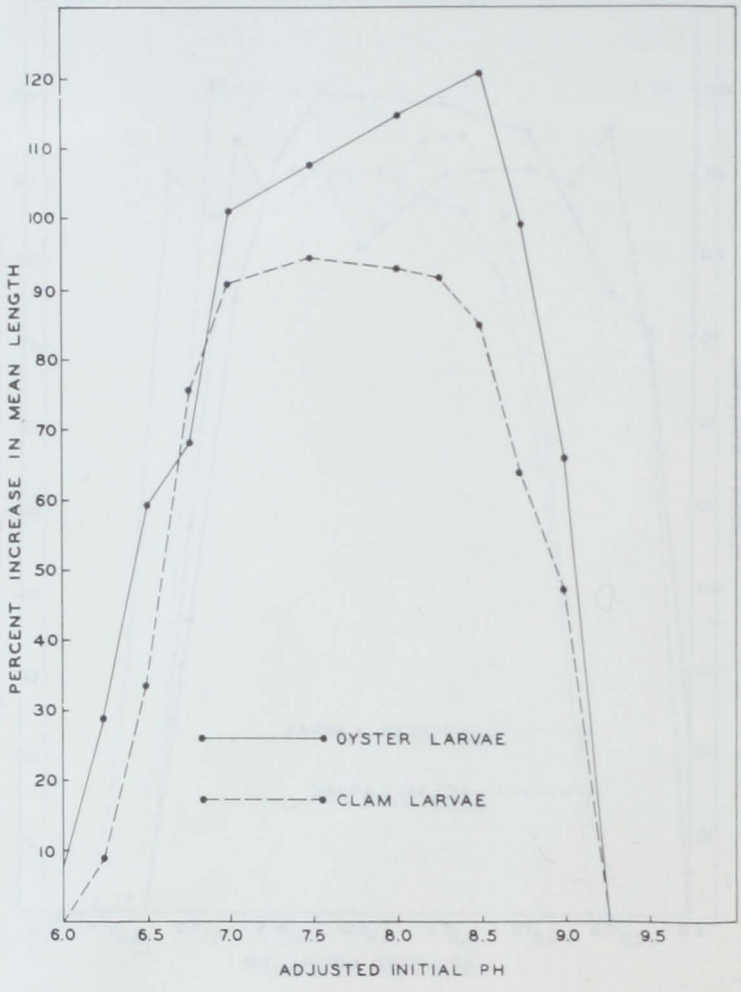

Fig. 3 - Increase in mean length of clam and oyster larvae at different pH levels, expressed as a percentage of the increase in mean length of larvae in control culture [after (4)].

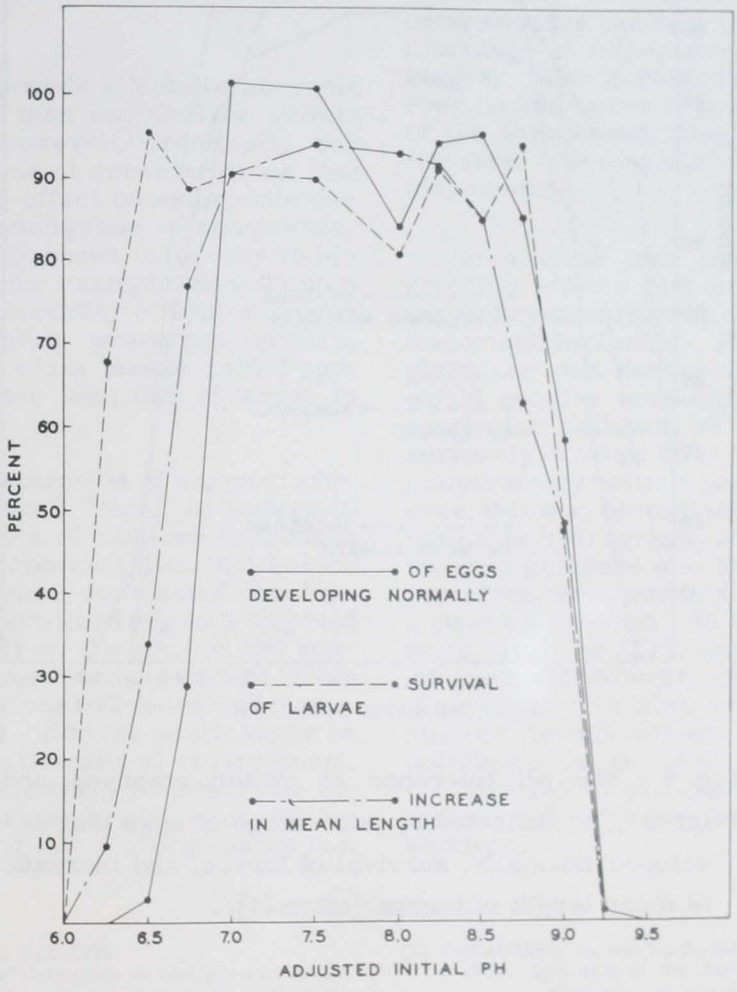

Fig. 4 - The pH tolerance of clam embryos and larvae, as indicated by percentage of eggs that developed normally, survival of larvae, and increase in mean length of larvae [after (4)].

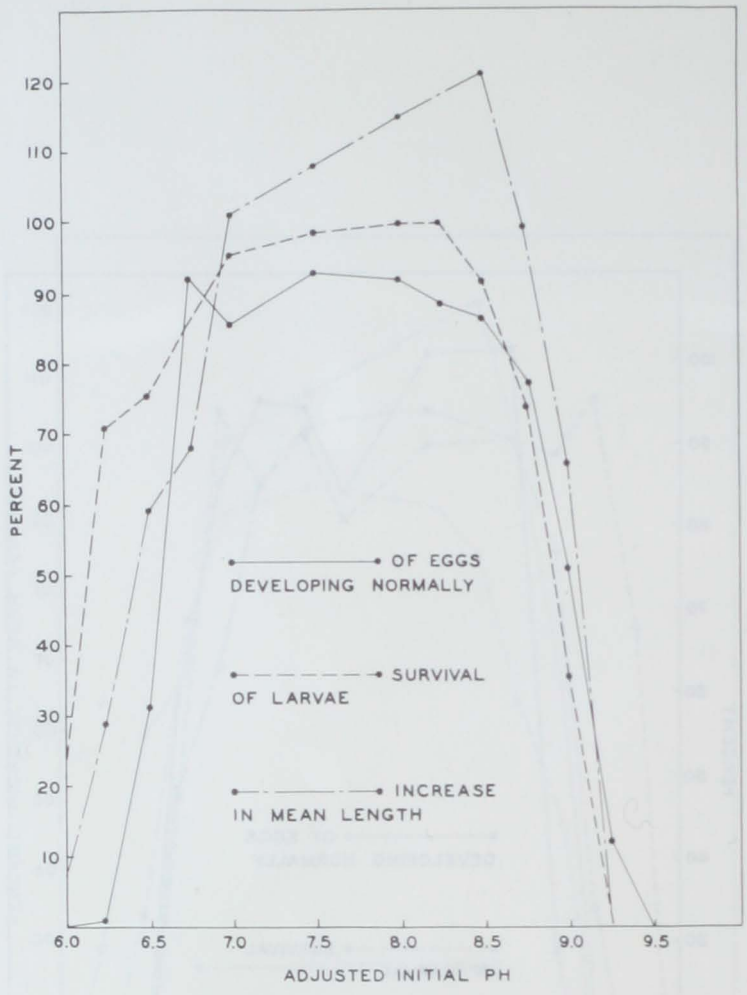

Fig. 5 - The pH tolerance of oyster embryos and larvae, as indicated by percentage of eggs that developed normally, survival of larvae, and increase in mean length of larvae /after (4)/.

and at pH 6.0 it was most difficult to induce females to spawn. Also, eggs and sperm released at pH 6.0 and 10.0 lost their viability within 2 to 4 hours.

It can be concluded that the pH of the tidal estuarine waters that form the principal habitat of the hard-shell clam and American oyster must not fall below pH 7.0 for clams or pH 6.75 for oysters, even though the larvae of both species can survive at lower pH levels. Moreover, neither species could reproduce successfully in waters where the pH remained appreciably above 9.0. Laboratory experiments have shown that high concentrations of silt can lower the pH of sea water to 6.4 or below the lower limit for normal embryonic development of 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 these clams and oysters.

PESTICIDE STUDIES

Fifty-two compounds were tested for their effects on embryos and larvae of the hard-shell clam and the American oyster, using the procedures described above (6,7). The pesticides included 17 insecticides, 12 herbicides, 1 nematocide, 4 solvents, and 18 miscellaneous bactericides, fungicides, and algicides.

Most of the compounds affected embryonic development more than survival or growth of larvae. Some, however, drastically reduced growth of larvae at concentrations that had relatively little effect on embryonic development. Within each group of compounds, there were great differences in toxicity to bivalve larvae. DDT, for example, at 0.05 ppm caused over 90% mortality of oyster larvae and almost completely prevented growth, whereas growth of clam larvae in 5.0 ppm of lindane was faster than that of larvae in control cultures.

The highest concentration of any pesticide that can be considered "safe" in waters in which valuable species of bibalves reproduce will be the highest concentration that has no appreciable effect on: 1) survival of developing embryos (from fertilized egg to 2-day-old veliger larvae), or 2) on the growth and survival of the fully formed veliger larvae (from 2-day-old feeding larvae to 2-week-old metamorphosing larvae). For the pesticide to be considered "safe" in the natural environment, it would also be necessary to determine the concentrations tolerated by the adult bivalve spawning stock and by the organisms that serve as foods for both larval and adult bivalves.

A distinction is made between developing embryos and the fully formed larvae because, quite often, the tolerance of these two pelagic stages to a given toxicant is markedly different. Moreover, growth of the fully developed larvae may be drastically retarded at concentrations of toxicant too low to cause direct mortality of either embryonic or larval stages. Such a retardation of growth, however, would serve to prolong the pelagic life of the larvae and, thus, increase the chance for loss through predation, disease, and dispersion.

An attempt was made to point out the possible effects that pollution may have on the embryos and larvae of some commercially important mollusks. From the information given one can deduce that in some cases it would require such high concentrations of a particular pollutant to affect bivalve larvae seriously in large bays and estuaries so that pollution may not be a serious problem. However, this may be misleading because, depending on the hydrography of a particular body of water, a pollutant may remain localized and, therefore, concentrated. This would then be a serious problem. In cases of other pollutants, such as DDT, only a small amount in an area will affect recruitment of bivalves. A problem that also would have to be considered is the effect of a combination of pollutants in an area; the interaction of a combination of pollutants may enhance the toxicity of any one particular pollutant to bivalves.

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