Effects of Turbidity-Producing Substances in Sea Water on Eggs and Larvae of Three Genera of Bivalve Mollusks

BY

HARRY C. DAVIS

AND

HERBERT HIDU [1]

Bureau of Commercial Fisheries, Biological Laboratory, Milford, Connecticut 06460

(7 Text figures)

INTRODUCTION

The effect of silt or other turbidity-producing substances on shellfish generally, and particularly on oysters and their larvae, has long been of interest to biologists and to shellfish producers. In addition to the obvious damage to oyster beds silted over in harbor dredging and in building bridges and roads in estuarine areas, it has been suspected that turbidity from these sources affected larval development and setting of oysters. Lunz (1938), however, found no harmful effects from high turbidities on adult oysters or on spawning and setting, in his field study during the dredging of the Intra-Coastal Waterway of South Carolina, except where the oysters were actually buried by the spoil.

Only a few studies of the effects of turbidity have been made under controlled conditions. Loosanoff & Tommers (1948) who, in laboratory experiments, studied the effects of a series of concentrations of turbidity-producing materials on adult oysters found that 0.1 gm of silt per liter of sea water reduced the pumping rate of adult oysters by 57% and that concentrations of 3 and 4 gms per liter reduced pumping by 94%. They reported generally similar results with kaolin and chalk, and that 0.5 gm of Fuller's earth per liter reduced pumping by 60%.

More recently, Davis (1960) determined quantitatively the effects of a series of concentrations of several turbidity-producing substances on the percentage of eggs of the hard clam (*Mercenaria (= Venus) mercenaria*) that developed into normal straight-hinge larvae and on the survival and growth of the larvae. In the work reported here the same techniques were used to determine the effects of several concentrations of silt, clay, and Fuller's earth on embryonic development of the American oyster (*Crassostrea virginica*) and on survival and growth of larvae of the American oyster and the European oyster (*Ostrea edulis*).

In addition, a series of experiments was run to compare the effects of different-sized particles of pure silicon dioxide on embryos and larvae of hard clams and American oysters, in an attempt to determine the effect of particle size of a suspended material, as opposed to possible effects of its chemical composition.

MATERIALS AND METHODS

The turbidity-producing materials used – silt, clay (kaolin N. F. VII Mallinckrodt)[2], and Fuller's earth (dusting powder, McKesson), – were the same as those used in the experiments on clam larvae (Davis, 1960). The silt was collected from the bed of Milford Harbor just upstream from the laboratory. It was washed through a 325-mesh screen to remove larger particles and debris, after which it was collected in a Buchner funnel and washed with distilled water to remove the salts. It was then dried at 200° C and the dried cake was ground in an all-ceramic jar mill. The Fuller's earth was similarly ground.

Fisher's flotated silica powder (about 240 mesh) was used for the experiments to determine the effect of particle

[1] Present address: University of Maryland, Natural Resources Institute, Box 38, Solomons, Maryland 20688

[2] Mention of trade names does not imply endorsement of commercial products by the Bureau of Commercial Fisheries.

size. It was suspended in distilled water and allowed to settle in a tall column. The settled plug was pushed out and cut into sections. The sections used were chosen to give particle sizes within the ranges: $< 5\mu$, $5 - 25\mu$, and $25 - 50\mu$. Particle-size determinations, made by Dr. Keith E. Chave, Director, Marine Science Center, Lehigh University, are given in Table 1.

Table 1

Percentages by weight of particles of different sizes composing the materials tested [3]

Particle Sizes	Silt	Kaolin	Fuller's Earth
> 62.5μ	8.24	12.06	8.87
62.5 - 15.6μ	28.36	6.03	18.16
15.6 - 3.9μ	39.10	10.72	26.10
3.9 - 0.98μ	9.85	44.87	21.27
< 0.98μ	14.54	26.32	25.60
	Silicon Dioxide		
	< 5μ	5 - 25μ	25 - 50μ
125 - 64μ	—	trace	1.8
62.4 - 31.2μ	—	1.3	17.3
31.2 - 15.6μ	—	9.3	64.9
15.6 - 7.8μ	trace	84.8	16.0
7.8 - 3.9μ	4.1	2.4	trace
3.9 - 1.95μ	56.9	2.3	—
1.95 - 0.98μ	18.3	trace	—
0.98 - 0.49μ	17.2	—	—
< 0.49μ	3.5	—	—

[3] Particle size determinations made by Dr. Keith E. Chave, Director, Marine Science Center, Lehigh University, Bethlehem, Pennsylvania

In our larval cultures appreciable quantities of kaolin and Fuller's earth, particularly at low concentrations, flocculated as particles 100μ or more in size. Dr. Chave reported similar flocculations in his particle size determinations and believed, "the significant percentages of material greater than 62.5μ probably represent flocculation of particles in the water" (personal communication).

Concentrations of all materials are expressed as grams of dry powder per liter. In making up a suspension, a weighed quantity of the powdered material was thoroughly mixed with sea water and the various experimental concentrations were prepared by serial dilution.

The methods for obtaining fertilized oyster eggs out of season have been described, as have methods for determining the percentage of eggs developing to the straight-hinge larval stage, and for determining the survival and rate of growth of larvae (Loosanoff & Davis, 1963).

In all experiments the sea water in the larval cultures was changed every second day and larvae in all cultures were fed a mixture of *Isochrysis galbana* and *Monochrysis lutheri* daily. The quantity and quality of food were the same for all cultures within an experiment since each culture received an equal volume of algal food. The quantity and quality of food in successive experiments, however, were not necessarily equal, due to changes in the algal cultures and sea water. Duplicate cultures were used in each experiment at each concentration of suspended material. Temperatures of all cultures were maintained between 23° C and 25° C and salinity between 26 and 27.5‰.

European oyster larvae may start setting 8 days after release under optimum conditions and are, thereafter, not available for quantitative sampling. Consequently, samples for determining percentage survival and rate of growth of these larvae were taken on the 7th day after the larvae were released, i.e., after 7 days at experimental conditions. Few American oyster larvae set before the 14th day even under near-optimum conditions. Samples of American oyster larvae, therefore, were taken on the 14th day after fertilization or after 12 days at the experimental conditions. Some clam larvae may reach setting size and undergo metamorphosis as early as the 8th day, but even these early post-setting stages can be suspended and accurate quantitative samples can be taken as late as the 12th day after fertilization, i. e., after 10 days at the experimental conditions.

EFFECTS ON EMBRYONIC DEVELOPMENT

Silt was more harmful to American oyster embryos than either clay (kaolin) or Fuller's earth. As little as 0.188g/l of silt had a significant effect on the percentage of oyster eggs developing to the straight-hinge larval stage, whereas Fuller's earth had no significant effect until concentrations exceeded 1g/l and kaolin had no significant effect until concentrations exceeded 2g/l (Table 2). Moreover, in only one experiment did any (3%) of the oyster eggs develop normally in 1g/l of silt, whereas the average number developing normally, for all experiments in 4g/l was 26% for Fuller's earth and 76% in kaolin.

Davis (1960) found that a normal number of clam eggs developed in silt concentrations as high as 0.75g/l, but it required only 0.125g/l of Fuller's earth or 0.5g/l of kaolin to cause a significant reduction in the number of clam eggs developing normally. The experiments reported here show that oyster embryos are less tolerant of silt than are clam embryos, but oyster embryos are considerably more tolerant of kaolin and Fuller's earth than are clam embryos. Results with clam eggs and with oyster eggs were similar, however, in that no embryos of either species developed normally at the higher concentrations of silt, whereas a significant number of embryos of both species did develop normally in even the highest concentrations (4g/l) of kaolin and Fuller's earth.

Table 2

Percentage of clam and American oyster eggs developing to the straight-hinge larval stage in different concentrations of suspended materials. The number of eggs developing to the straight-hinge stage in control cultures was considered 100%

Concentration grams/liter	Silt Clam[4]	Silt Oyster	Kaolin Clam[4]	Kaolin Oyster	Fuller's Earth Clam[4]	Fuller's Earth Oyster
0.0 (controls)	100	100	100	100	100	100
0.125	95	95	82	100	75	104
0.188	90	78	—	—	—	—
0.250	96	73	82	100	61	103
0.375	93	66	—	—	—	—
0.500	99	31	52	104	41	102
0.750	92	9	—	—	—	—
1.000	79	3	37	108	57	98
1.500	65	0	—	—	—	—
2.000	39	0	49	94	50	79
3.000	0	0	—	—	—	—
4.000	0	0	42	76	45	26

	Silicon Dioxide Particles					
	< 5μ		5 - 25μ		25 - 50μ	
	Clam	Oyster	Clam	Oyster	Clam	Oyster
0.0 (controls)	100	100	100	100	100	100
0.125	106	94	106	105	114	112
0.250	105	109	96	104	98	94
0.500	105	93	98	106	102	100
1.000	99	107	94	103	98	111
2.000	85	114	92	109	96	109
4.000	69	123	103	96	92	117

[4] from DAVIS, 1960

The experiments with silt, kaolin, and Fuller's earth led us to conclude, tentatively, that the larger particles present in silt and, to a lesser extent, in kaolin and Fuller's earth were primarily responsible for the deleterious effects on oyster eggs, whereas the smaller particles, most numerous in kaolin, were probably responsible for the effect on clam eggs.

The percentage of oyster eggs developing normally did not decrease significantly, however, in any of the various concentrations of silicon dioxide tested, regardless of particle size. Moreover, only at the highest concentrations of the smallest particles of silicon dioxide did the percentage of clam eggs developing normally decrease significantly. Obviously, then, the decreases in the percentage of clam and oyster eggs developing in the higher concentrations of silt, kaolin, and Fuller's earth, and the differences between their effects are not wholly the result of differences in particle size. Although silicon dioxide has a higher density than the other substances tested and fewer particles of any given size per unit of weight, it seems unlikely that this difference alone can account for the difference in results. Although 4g/l of silicon dioxide gave no reduction in the percentage of oyster eggs developing normally, only 1g/l of silt reduced the number by 97%. Even if the silicon dioxide were 4 times as dense as the silt, there should be at least as many particles in 4g of silicon dioxide as in 1g of silt.

EFFECTS ON SURVIVAL OF LARVAE

European oyster larvae were the most hardy of the 3 species tested in that survival did not decrease significantly in concentrations up to 4g/l of any of the suspended material used, except kaolin (Figures 1a, 2a, 3a). This result is in striking contrast to that with clam larvae, which showed a fair survival in 4g/ of silt but no survival in 1g/l of either kaolin or Fuller's earth (DAVIS, 1960). American oyster larvae were less tolerant of silt than either European oyster larvae or clam larvae, for as little as 0.75g/l of silt caused a significant decrease in the percentage of American oyster larvae surviving. American oyster larvae, nevertheless, were more tolerant of kaolin and Fuller's earth than were clam larvae. Approximately 27 to 34% survived in 4g/l of either kaolin or Fuller's earth.

We noted that many clam larvae, in the presence of the smaller particles of kaolin and Fuller's earth, eventually lost their ability to reject these particles. The larvae then ingested the particles, the stomach became packed, and the larvae died. We postulated that both species of oyster larvae were better able to reject these small particles and, hence, showed a lower mortality than clam larvae in kaolin and Fuller's earth.

The experiments with silicon dioxide indicated that it is primarily the smaller particles that affect survival. Both hard clam (Figure 4a) and American oyster (Figure 5a) larvae suffered severe mortality at comparatively low (0.5g/l) concentrations of the smallest (up to 5μ) particles, whereas neither species suffered significant mortality at values as great as 4g/l of the larger particles; even at 4g/l of the smallest particles of silicon dioxide, however, mortality was not as great as with lesser concentrations of some of the other materials.

The effects of kaolin and Fuller's earth, for example, particularly at higher concentrations, on survival and growth of clam larvae were more drastic than would be expected from the effect of silicon particles of similar size. Nevertheless, the much greater mortality of clam and oyster larvae in the presence of the smallest particles of silicon dioxide, as compared to mortality in the presence of larger particles, did follow the same trend as with kaolin and Fuller's earth. The observation of numerous oyster larvae with their guts packed with silicon particles demonstrated that the American oyster larvae could not reject these small silicon dioxide particles as successfully as they did the particles of kaolin or Fuller's earth. European oyster larvae, however, were apparently capable of rejecting all suspended particles and, hence, suffered little mortality.

EFFECTS ON GROWTH OF LARVAE

The effects of suspended material on growth of both species of oyster larvae were somewhat different from their effects on clam larvae. For example, silt had no deleterious effect on growth of clam larvae until concentrations reached 1g/l, and growth was not reduced drastically until silt concentrations were in excess of 2g/l. Moreover, clam larvae showed evidence of feeding even in 4g/l of silt although growth was negligible (Davis, 1960). Larvae of American and European oysters reared in 0.75g/l of silt, however, suffered a significant reduction in growth and those in 2g/l and over did not grow at all (Figure 1b). At 3g/l and 4g/l of silt all American oyster larvae eventually died. Even though most European oyster larvae survived at all silt concentrations, reduction in growth of these larvae at concentrations of 1g/l and less was more drastic than for clam or American oyster larvae.

Conversely, both species of oyster larvae tolerated kaolin (Figure 2b) and Fuller's earth (Figure 3b) better than clam larvae did. Growth of clam larvae was drastically reduced by 0.5g/l of either kaolin or Fuller's earth and all clam larvae were killed by 1g/l of either material (Davis, 1960). Growth of American oyster larvae, on the other hand, was not significantly reduced by 1g/l of kaolin. Moreover, in at least 3 of the 4 experiments these oyster larvae showed appreciable growth in 2g/l of kaolin and growth of European oyster larvae was significantly affected only in concentrations of 4g/l of kaolin (Figure 2b).

Fuller's earth had a more deleterious effect than kaolin on growth of both species of oyster larvae. Nevertheless, both species showed significant growth and good survival at 1g/l of Fuller's earth, but American oyster larvae showed only fair survival and no appreciable growth at 2g/l. European oyster larvae still showed good survival and fair growth even at 2g/l of Fuller's earth (Figure 3b).

Growth of clam larvae was not seriously affected by concentrations up to 2g/l of either the 25 - 50μ or the 5 - 25μ particles of silicon dioxide (Figure 4b). Even in the presence of the smallest particles ($< 5\mu$) growth of the clam larvae that survived was fairly good. Results with American oyster larvae (Figure 5b) were similar. The rate of growth decreased at almost all concentrations of all particle sizes of silicon dioxide, but the largest particles interfered least with growth of these larvae. Even in the presence of the smallest particles that interfered most, with oyster larvae, as with clam larvae, those that survived showed appreciable growth.

VARIATIONS AMONG SUCCESSIVE EXPERIMENTS AND POSSIBLE CAUSES

Davis & Chanley (1956) pointed out that the first evidence of toxicity to larvae, either of toxins produced by living microorganisms or of synthetic chemical toxins, was a reduction in the growth rate of larvae. It was suggested by Davis (1960) that the more rapid growth of clam larvae in the lower concentrations of silt, kaolin, and Fuller's earth was due, in part, to chelation or adsorption of toxic substances present in the sea water or produced by the algae and bacterial contaminants added as foods.

In the 3 experiments with silt, the effect of silt in concentrations of 0.5g/l and lower was to increase the rate of growth of the oyster larvae. The rate of growth of the larvae differed somewhat in the 3 consecutive experiments, but the increases in length, when converted to percentages of the length increase in control cultures, were in general agreement and results of the 3 experiments were averaged to give the curve plotted in Figure 1b.

Four experiments were run with kaolin and Fuller's earth concurrently but subsequent to the experiments with silt and, consequently, the food cultures and sea water were different. Growth of larvae in the control cultures was poor in Experiments 2 and 3, of the 4 consecutive experiments, and the shapes of the curves are different from those of Experiments 1 and 4 in which growth of larvae in control cultures was normal (Figures 6, 7). We believe the poor growth of larvae in the control cultures was due to an excessive amount of toxins produced by microorganisms present in the sea water or, possibly, by toxin-producing bacteria contaminating our algal food cultures.

When the quantity of toxins appeared to be low (Experiments 1, 4) ("normal" for our laboratory cultures) growth was optimum in about 0.5g/l of kaolin, i. e., 0.5g/l was sufficient to adsorb the toxins (Figure 6). When the quantity of toxins appeared to be greater (Experiments 2, 3), about 1g/l of kaolin was required to adsorb the toxins and give optimum growth, whereas at the lower concentrations the harmful effects of suspended materials and toxins almost appeared to be additive (Figure 6).

The data from the experiments with Fuller's earth gave less clear-cut but similar results. When the concentration of toxins was low (Experiments 1, 4) 0.25g to 0.5g/l was sufficient to adsorb the toxins, whereas 0.5g/l or, perhaps, slightly more was required when the concentration of toxins was high (Experiments 2, 3, and Figure 7).

Of the materials used, only silt caused any appreciable change in the pH of our sea water. Even with silt the maximum change in pH was from 7.5 (normal for our laboratory sea water) to 6.40. Other experiments have shown that at pH 6.40 the decrease in the proportion of clam or oyster eggs developing into normal larvae and in the survival and growth of larvae of these two genera of bivalves is less drastic than is caused by silt alone (Calabrese & Davis, 1966). A portion of the effect of silt may be attributable, nevertheless, to its effect on the pH of sea water.

Other possible causes for the difference in effects of silt, kaolin, Fuller's earth, and silicon dioxide include possible soluble toxic components or, more probably, differences in their adsorptive and chelating characteristics. Silt, a mixture of organic and inorganic materials, would seem most likely to contain toxic components and highly effective chelators capable of causing mortality of embryos or larvae by over-chelation.

Our experiments indicate that bivalve larvae can tolerate turbidities higher than those normally encountered in natural waters, and that under certain circumstances low concentrations of turbidity-producing materials may be beneficial. Nevertheless, higher silt concentrations, such as those produced by dredging or filling operations, could be detrimental to bivalve larvae, both as a direct effect of the particulate matter and, indirectly, as a result of lowered pH. We also suspect that, in natural waters, disturbing the bottom may release numerous bacteria, some of which may be toxic, and organic enrichment sufficient to enable these bacteria to reproduce rapidly. The effect of dredging, therefore, may be more deleterious to bivalve larvae than would be indicated by their tolerance to turbidity-producing materials alone.

SUMMARY

1. As little as 0.188g/l of silt caused a significant decrease in the percentage of oyster eggs developing normally, as did 3g/l of kaolin or 4g/l of Fuller's earth.
2. The percentage of American oyster eggs developing normally was not affected by concentrations of silicon dioxide of 4g/l, regardless of particle size. Clam eggs were affected only at 4g/l of the smallest particles ($< 5\mu$).
3. Survival of European oyster larvae was less affected by silt, kaolin, and Fuller's earth than was survival of larvae of either American oysters or hard clams.
4. The smallest particles ($< 5\mu$) of silicon dioxide had the greatest effect on survival and growth of clam and oyster larvae. Larger particles ($5 - 25\mu$ and $25 - 50\mu$) had little effect on survival of either species or on growth of clam larvae. Growth of American oyster larvae decreased progressively as the size of silicon dioxide particles was decreased.
5. Growth of European oyster larvae was less affected by kaolin and Fuller's earth than was growth of larvae of the American oyster or hard clam, but was more strongly affected by silt than was growth of larvae of either of the other two species.
6. Bivalve larvae grew faster in low concentrations of turbidity-producing substances than in clear sea water, possibly because the suspended particles chelate or adsorb toxins present in larval cultures. The "optimum" concentration of suspended material probably depends upon the amount of toxin to be chelated or adsorbed.

LITERATURE CITED

Calabrese, Anthony & Harry Carl Davis
1966. The pH tolerance of embryos and larvae of *Mercenaria mercenaria* and *Crassostrea virginica*. Biol. Bull. 131 (3): 427 - 436 (December 1966)

Davis, Harry Carl
1960. Effects of turbidity-producing materials in sea water on eggs and larvae of the clam (*Venus (Mercenaria) mercenaria*). Biol. Bull. 118 (1): 48 - 54

Davis, Harry Carl & Paul Edwin Chanley
1956. Effects of some dissolved substances on bivalve larvae. Proc. Nat. Shellfish. Assoc. 46: 59 - 74

Loosanoff, Victor Lyon & Harry Carl Davis
1963. Rearing of bivalve mollusks *In*: Advances in Marine Biology, F. S. Russell (ed.), Acad. Press, London 1: 1 - 136

Loosanoff, Victor Lyon & Frances Doretta Tommers
1948. Effect of suspended silt and other substances on the rate of feeding of oysters. Science 107: 69 - 70

Lunz, Robert George
1938. Part I. Oyster culture with reference to dredging operations in South Carolina, 1 - 135. Part II. The effects of the flooding of the Santee River in April 1936, on oysters in the Cape Romain area of South Carolina. Rep. U. S. Engineer Office, South Carolina, 1 - 33

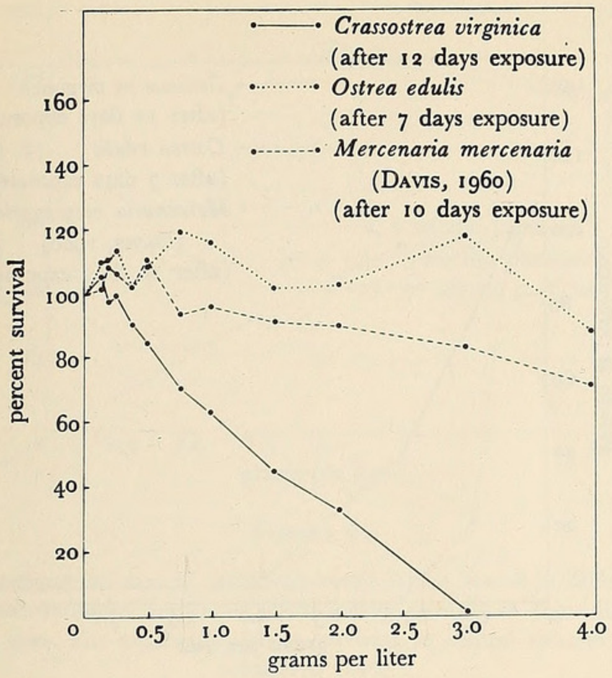

Figure 1 a

Percentage survival of three genera of bivalve larvae reared in sea water suspensions containing different concentrations of silt. The number of larvae surviving in control cultures was considered 100%.

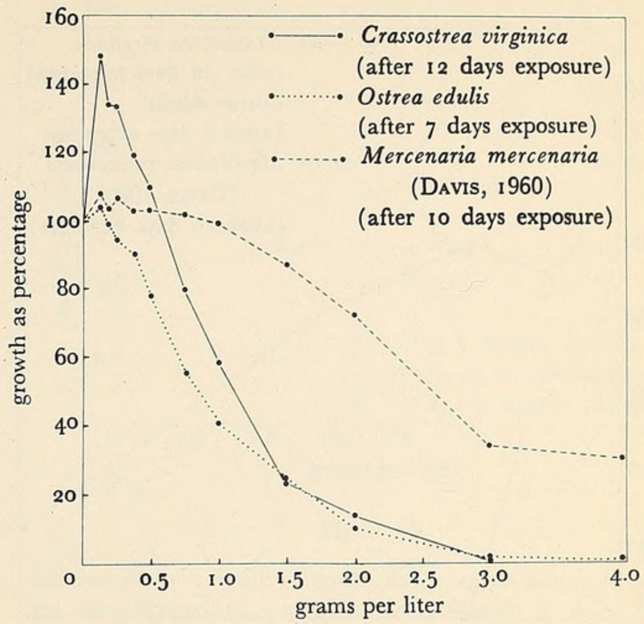

Figure 1 b

Increase in mean length of three genera of bivalve larvae, grown in different concentrations of suspended silt, plotted as percentages of the increase in mean length of larvae in control cultures.

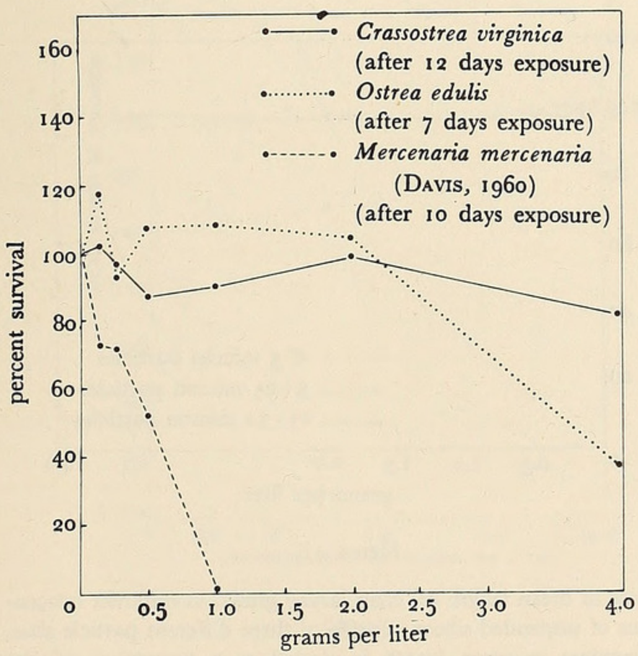

Figure 2 a

Percentage survival of three genera of bivalve larvae reared in different concentrations of suspended kaolin. The number of larvae surviving in control cultures was considered 100%.

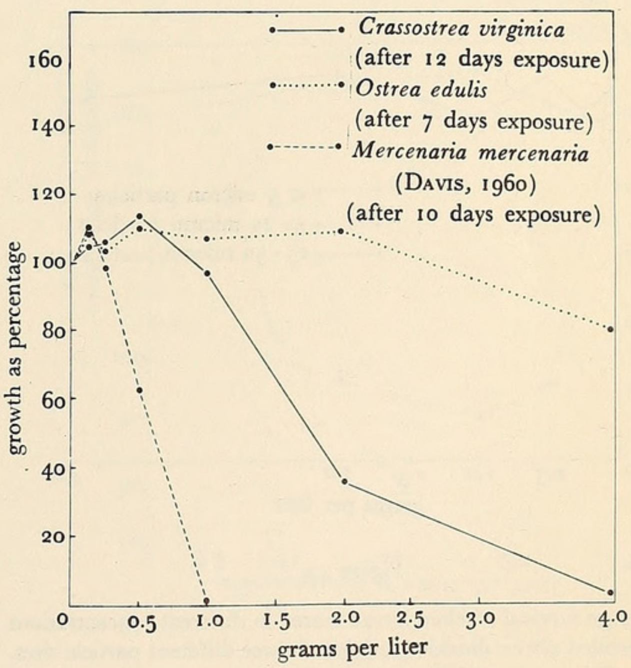

Figure 2 b

Increase in mean length of three genera of bivalve larvae, grown in different concentrations of suspended kaolin, plotted as percentages of the increase in mean length of larvae in control cultures.

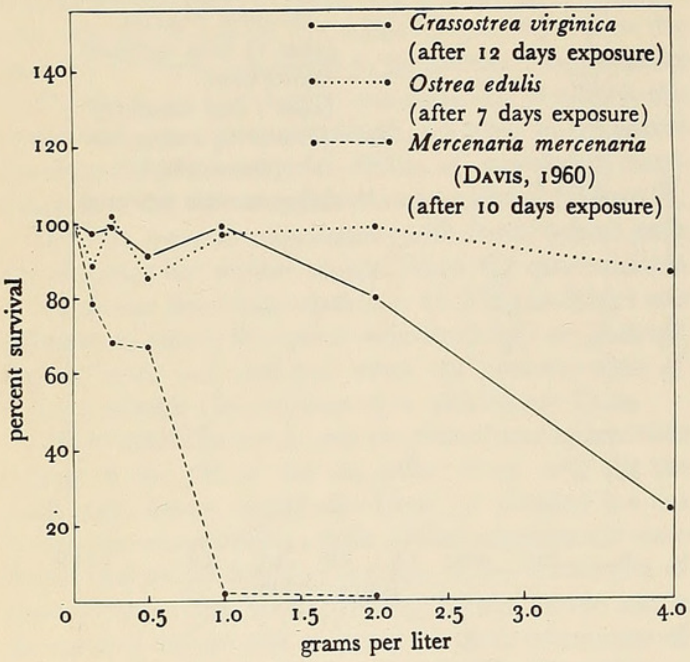

Figure 3 a

Percentage survival of three genera of bivalve larvae reared in different concentrations of suspended Fuller's earth. The number of larvae surviving in control cultures was considered 100%.

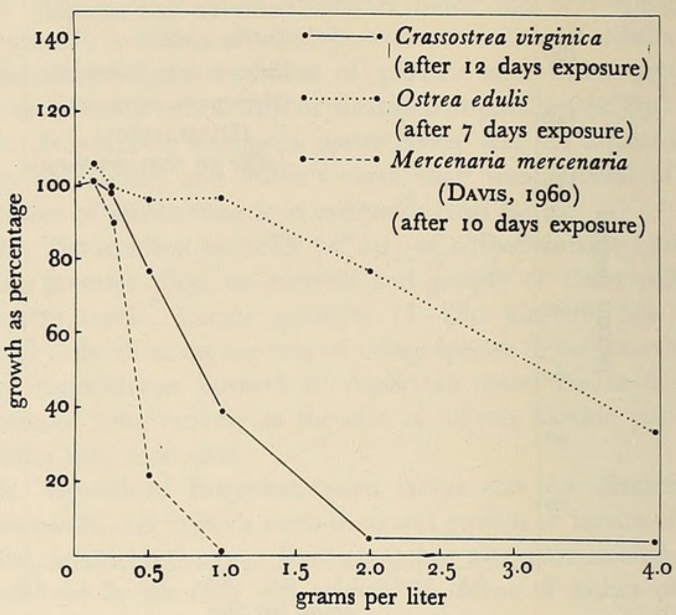

Figure 3 b

Increase in mean length of three genera of bivalve larvae, grown in different concentrations of suspended Fuller's earth, plotted as percentages of the increase in mean length of larvae in control cultures.

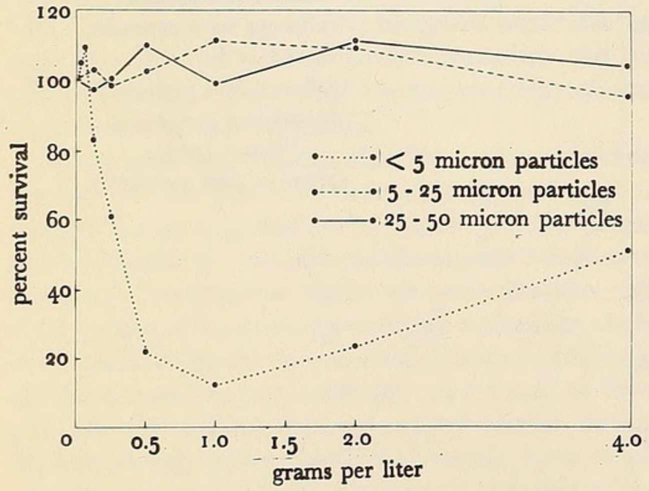

Figure 4 a

Percentage survival of clam larvae reared in different concentrations of suspended silicon dioxide particles of three different particle sizes. The number of larvae surviving in control cultures was considered 100%.

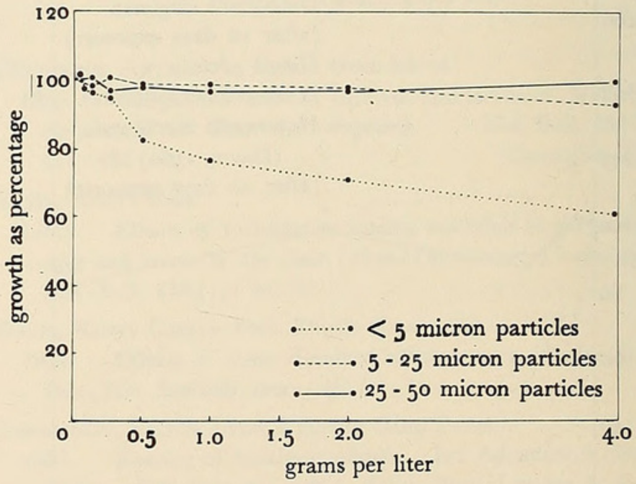

Figure 4 b

Increase in mean length of clam larvae grown in different concentrations of suspended silicon dioxide of three different particle sizes. The increase in mean length is plotted as a percentage of the increase in mean length of larvae in control cultures.

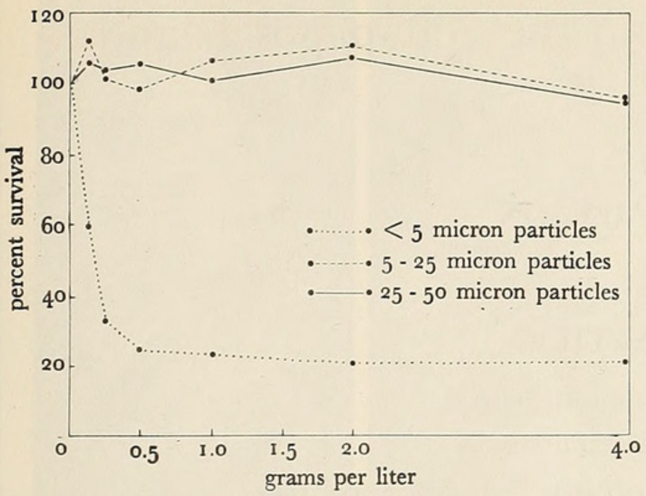

Figure 5 a

Percentage survival of American oyster larvae reared in different concentrations of suspended silicon dioxide particles of three different sizes. The number of larvae surviving in control cultures was considered 100%.

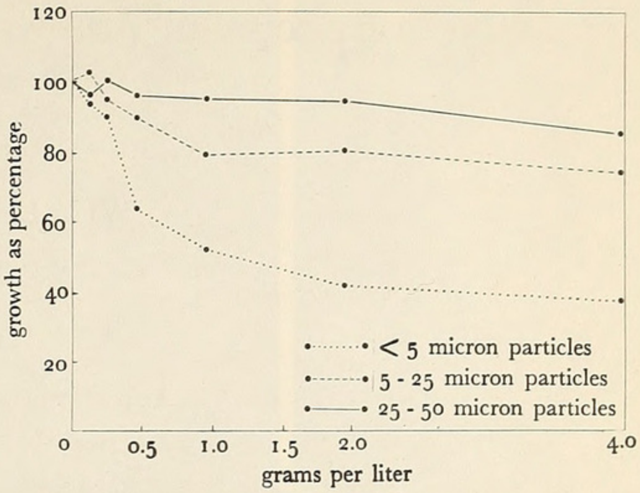

Figure 5 b

Increase in mean length of American oyster larvae grown in different concentrations of suspended silicon dioxide of three different particle sizes. The increase in mean length is plotted as a percentage of the increase in mean length of larvae in control cultures.

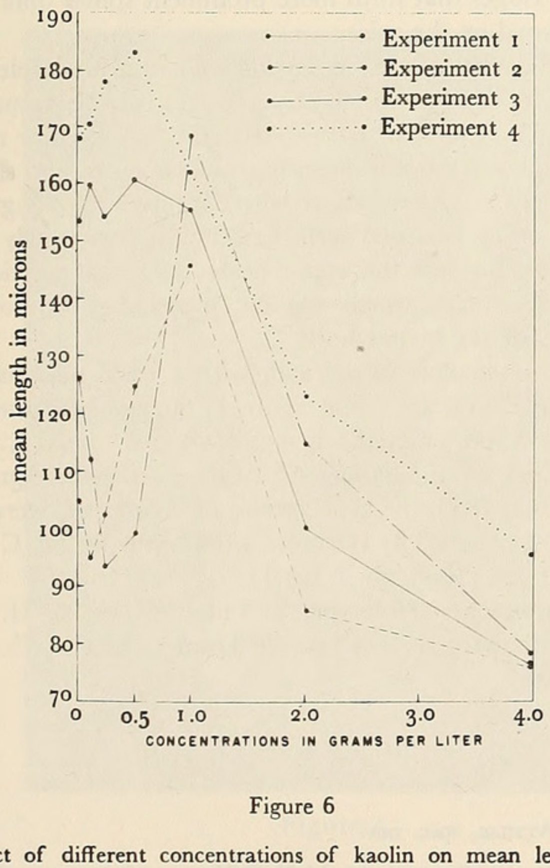

Figure 6

Effect of different concentrations of kaolin on mean length of American oyster larvae at 14 days in four separate experiments. Points plotted are averages for duplicate cultures at each concentration in each experiment.

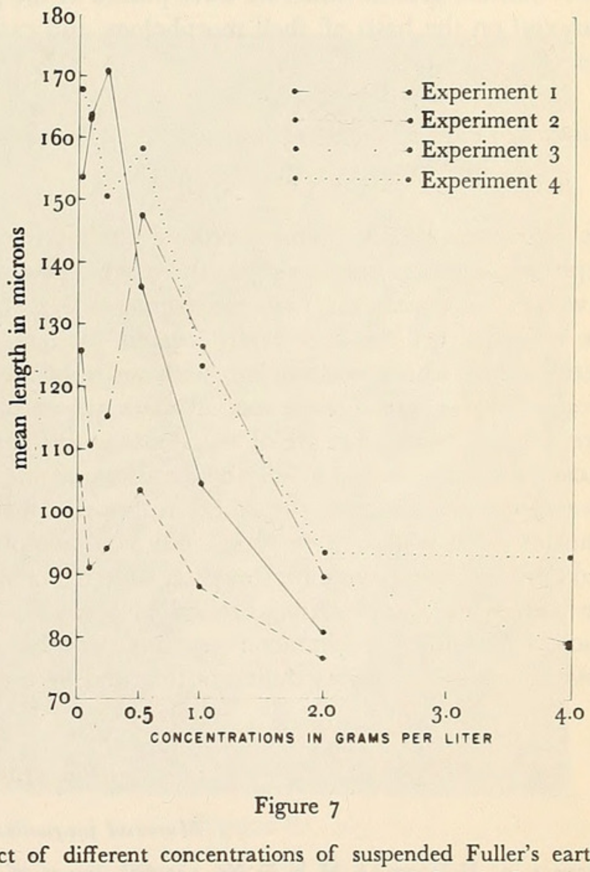

Figure 7

Effect of different concentrations of suspended Fuller's earth on mean length of American oyster larvae at 14 days in four separate experiments. Points plotted are averages for duplicate cultures at each concentration in each experiment.