EFFECTS OF SOME PESTICIDES ON EGGS AND LARVAE OF OYSTERS (CRASSOSTREA VIRGINICA) AND CLAMS (VENUS MERCENARIA)

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SUMMARY

The effects of several concentrations of 31 compounds, on egg development and survival and growth of bivalve larvae, have been determined. Some compounds were tested on eggs and larvae of both oysters, Crassostrea virginica, and clams, Venus mercenaria (Mercenaria mercenaria), but the majority were tested on only one or the other of these species of bivalves. The compounds included were 14 insecticides, 4 weedicides, 4 oils and organic solvents, and 9 compounds that are used as antibiotics, bactericides, or disinfectants.

Within each group of compounds there were great differences in toxicity to bivalve larvae. Thus DDT, one of the most toxic of the commonly used insecticides, at a concentration of 0.05 parts per million (p.p.m.), caused over 90 percent mortality of oyster larvae and almost completely prevented growth, whereas growth of clam larvae in 5.0 p.p.m. of lindane, the least toxic of the commonly used insecticides, was somewhat faster than that of larvae in control cultures. Even at 10.0 p.p.m., essentially a saturated solution of lindane, there was no appreciable mortality of clam larvae. It is suggested that, in enclosed bodies of water, lindane could possibly be used to control crustaceans without harming bivalve larvae or their food organisms.

Certain concentrations of sulmet, phenol, chloramphenicol, and dowicide "A," among the antibiotic, bactericide, and disinfectant compounds, improved the rate of growth of larvae appreciably. This is attributed to the inhibition of growth of toxic bacteria. Acetone and trichlorobenzene among the solvents, monuron and fenuron among the weedicides, and lindane and guthion among the insecticides, at certain concentrations, also improve the rate of growth of larvae to some extent and it is suggested that these compounds may also partially inhibit toxic bacteria.

With almost every compound tested, slowing of the rate of growth of larvae was the first evidence of toxicity. Appreciable mortality usually occurred only when concentrations were increased enough to reduce the rate of growth by 50 percent or more.

BACKGROUND

Work on the effects of antifungal and antibacterial agents was started at Milford Laboratory several years ago in an attempt to find compounds that could be used routinely under laboratory or hatchery conditions to control fungi and bacteria known to be pathogenic or toxic to larvae of oysters, Crassostrea virginica, and clams, Venus mercenaria (Mercenaria mercenaria). Our earlier work on the effects of some antibiotics on these larvae has been reported along with the effects of some other dissolved substances (Davis and Chanley 1955). Walne (1958a, 1958b, 1959) has emphasized the harmful effects of bacterial growths in laboratory cultures of larvae of European oysters (Ostrea edulis) and described his results using antibiotics and ultraviolet light to control bacteria in these cultures.

In the present series of experiments we have tested several additional bactericides and fungicides and some of the compounds evaluated for use in combating shellfish predators. In response to requests for information on the effects, on oysters and clams of different ages, of pesticides used in marsh areas to control mosquitoes, other insects, weeds and brush, we have expanded this work to include the effects of some common insecticides and weedicides.

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Doudoroff et al (1953) from laboratory tests of soils collected from toxaphene-treated fields have concluded that stream waters can be rendered toxic to fish by drainage from such fields and state "application of some of these insecticides to crops apparently has resulted in serious pollution of streams and reservoirs into which these materials have been washed from the soil by heavy rains" (page 840). Harrington and Bidlingmayer (1958) made observations of a salt marsh in Florida following aerial treatment with dieldrin that averaged 0.13 to 0.4 parts per million (p.p.m.) of the active ingredient depending on depth of water. They state "the fish kill was substantially complete" but that "mollusks (snails, nudibranchs, tethyoids, and oysters) seemed to be unharmed by dieldrin. Crustaceans were virtually exterminated throughout the area" (page 81). Hooper (1959) has reported that toxaphene at 0.1 p.p.m. used in two Michigan fresh-water lakes, in addition to killing fish, seemed to kill most of the invertebrates except mollusks and oligochaetes.

The results reported in this article have been obtained over a period of several years. Many of the compounds have been tested at a series of concentrations in only a single experiment. In each case, however, the results represent the average of duplicate cultures at each concentration and, in most cases, both the agreement between duplicate cultures and the gradation of effect with increasing concentration of the substance tested indicate the reliability of the results. Since the compounds vary widely in their toxicity to larvae, the range of concentrations tested is not the same for all compounds.

The tests have been run using our standard methods of larval culture (Davis 1953) which include ultraviolet treatment of all sea water used. A series of one-liter cultures in 1,500 ml. pyrex beakers was used. All the cultures were kept in the same constant temperature bath and each culture received an equal quantity of the same foods each day. In testing the effects on egg development an equal number of eggs was placed in each beaker of the series. One pair of these cultures served as controls and an additional pair was used for each concentration of the compounds to be tested. Quantitative samples were taken 48 hours after fertilization to determine the number of eggs that had developed to the straight hinge larval stage. The results are given as the relative percentage (R) of eggs developing to the straight hinge stage calculated as follows:

$$R = \frac{\text{Average number of larvae in experimental cultures}}{\text{Average number of larvae in control cultures}} \times 100$$

Due to errors inherent in the setting up of cultures and sampling, the percentage of eggs developing to straight hinge larval stages and the survival percentages are accurate only to about ±10 percent. Differences of less than 20 percent are, therefore, of doubtful significance.

The effect of the compounds on survival and growth of larvae was tested using larvae that had developed to the straight hinge stage (48 hours) under normal conditions. An equal number of these larvae was placed in each of a series of beakers. Two such beaker cultures were used as controls and two were used for each concentration of the compounds to be tested. Quantitative samples were taken only on the 12th day in experiments using clam larvae, and on the 14th day in experiments using oyster larvae. Nevertheless, the larvae were examined, and their condition and the estimated mortality noted, every second day when the sea water in the cultures was changed and the different concentrations of the toxicants reestablished. Growth (G), expressed as a percentage of that in control cultures, was calculated as follows:

$$G = \frac{\text{Mean length of experimental larvae - mean length at 48 hours}}{\text{Mean length of control larvae - mean length at 48 hours}} \times 100$$

Mortality is shown only in those instances where it was obviously significant. A list of the chemicals tested, with their chemical names and solubilities, where known, is given in Appendix I (see page 10).

BACTERICIDES AND DISINFECTANTS

Although oyster larvae appear to be more susceptible to toxins produced by algae and bacteria than are clam larvae, only rarely have we observed bacteria or fungi that appeared

Appendix I

Common Name	Chemical Name	Solubility in Water and Org. Solvents
INSECTICIDES:		
Aldrin	hexachloro hexahydro-endo, exo dimethanonaphthalene	insol. in H_2O (?) 1,590,000 p.p.m. in acetone
Niagara Compound N-3452	alkyl (C_8-C_{18}) dimethyl benzyl ammonium chloride	
Niagara Compound N-3514	2-chloro-1-nitropropane	8,000 p.p.m. in H_2O
DDT	1, 1, 1-trichloro-2, 2-bis(p-chlorophenyl) ethane	0.0002 p.p.m. in H_2O 0.2 p.p.m. as colloid 590,000 p.p.m. in acetone
Dicapthon	O-O-dimethyl-O (2 chloro-4 nitrophenyl) phosphorothioate	very low sol. in H_2O (?) v. s. in acetone
Dieldrin	hexachloro epoxy octahydro-endo, exo dimethanonaphthalene	insol. in H_2O (?) 540,000 p.p.m. in acetone
Dipterex	O, O-dimethyl-1-hydroxy-2, 2, 2 trichloroethyl phosphonate	130,000 p.p.m. in H_2O
Endrin	hexachloro epoxy octahydro-endo, endo-dimethanonaphthalene	(?)
Guthion	O, O-dimethyl-S-(4 oxobenzotriazino-3 methyl) phosphorodithioate	33 p.p.m. in H_2O
Lindane	1, 2, 3, 4, 5, 6 hexachlorocyclohexane	10 p.p.m in H_2O 440,000 p.p.m. in acetone
Parathion	O, O-diethyl-O-p-nitrophenyl thiophosphate	20 p.p.m. in H_2O
Tepp	tetraethyl pyrophosphate	misc. in H_2O (hydrolyzes) misc. in acetone
Toxaphene	mixture of polychloro bicyclic terpenes with chlorinated camphene predominant	1.5 p.p.m. in H_2O 4,500,000 p.p.m. in acetone
Sevin	1 naphthyl-N-methylcarbamate	1,000 p.p.m. in H_2O 300,000 p.p.m. in acetone
WEEDICIDES:		
Diuron	3-(3, 4-dichlorophenyl)-1, 1-dimethylurea	42 p.p.m. in H_2O
Fenuron	3 phenyl-1, 1-dimethylurea	2,900 p.p.m. H_2O
Monuron	3-(p-chlorophenyl)-1, 1-dimethylurea	230 p.p.m. in H_2O
Neburon	1-n-butyl-3-(3, 4-dichlorophenyl)-1-methylurea	4.8 p.p.m. in H_2O
OILS & SOLVENTS:		
Acetone	acetone	misc. in H_2O
Allyl alcohol	2 propene-1-o1	misc. in H_2O
O-dichlorobenzene	o-dichlorobenzene	130 p.p.m. in H_2O misc. in acetone
Trichlorobenzene	trichlorobenzene	25 p.p.m. in H_2O misc. in acetone
ANTIBIOTICS & BACTERICIDES:		
Chloramphenicol	(chloromycetin) D-(-)-threo-2-dichloroacetamido-1-p nitrophenyl -1, 3-propanediol	2,500 p.p.m. in H_2O v.s. in acetone
Delrad (alqacide)	dehydro-abietylamine acetate	v.s. in water
Dowicide "A"	sodium-o-phenylphenate . 4 H_2O	1,220,000 p.p.m. in H_2O
Dowicide "G"	sodium pentachlorophenate	330,000 p.p.m. in H_2O
Nabam	disodium ethylene bis(dithiocarbamate)	v.s. in water
Nemagon	1, 2-dibromo-3-chloropropane	1,000 p.p.m. in H_2O
Phenol	phenol	v.s. in H_2O
Roccal	alkyl(C_8H_{17}-$C_{18}H_{37}$) dimethylbenzyl ammonium chloride	10% solution v.s. in H_2O
Sulmet	(sodium sulfamethazine) 2-sulfanilimido, 4-6-dimethyl pyrimidine sodium	v.s. in H_2O

to be actively pathogenic to oyster larvae. By contrast, clam larvae are frequently seen that appear to have been invaded by active pathogens. In the tests of bactericides and disinfectants we have consequently used clam larvae, although some of these compounds have subsequently been used successfully to control bacteria that produce metabolites harmful to oyster larvae. Since the effects of toxic or pathogenic bacteria and fungi usually do not appear until after eggs have developed into shelled larvae, some of the compounds, earlier in the program, were not tested for their effect on egg development.

The effects of four bactericides (phenol, roccal, dowicide "A," and dowicide "G"), a soil fumigant (nemagon), and a fungicide (nabam) on development of clam eggs indicate the great differences between these compounds (fig. 1). Thus, nabam and dowicide "G" prevented normal development of clam eggs at each concentration tested. Roccal at a concentration of 0.1 p.p.m. permitted normal development, at 0.2 p.p.m. it reduced the number of normal larvae

by 55 percent and at 1.0 p.p.m. and higher concentrations it entirely prevented normal egg development. In contrast to these highly toxic compounds dowicide "A" and nemagon permitted a normal percentage of clam eggs to develop in concentrations up to 5.0 p.p.m., and phenol was not toxic at concentrations below 10.0 p.p.m. At low concentrations (0.025 to 0.2 p.p.m.) phenol, possibly through a slight bacteristatic effect, appeared to have increased the percentage of clam eggs developing normally.

While the compounds still had the same general order of toxicity, when tested on survival and growth of clam larvae, certain differences were apparent (fig. 2). Dowicide "G" was again lethal at all concentrations tested but nabam, which entirely prevented normal egg development at all concentrations, did not significantly affect survival of larvae through the 12 days of the experiment at concentrations of 0.5 and 1.0 p.p.m. although it did almost completely prevent growth. Roccal, dowicide "A," and nemagon conversely appeared to be

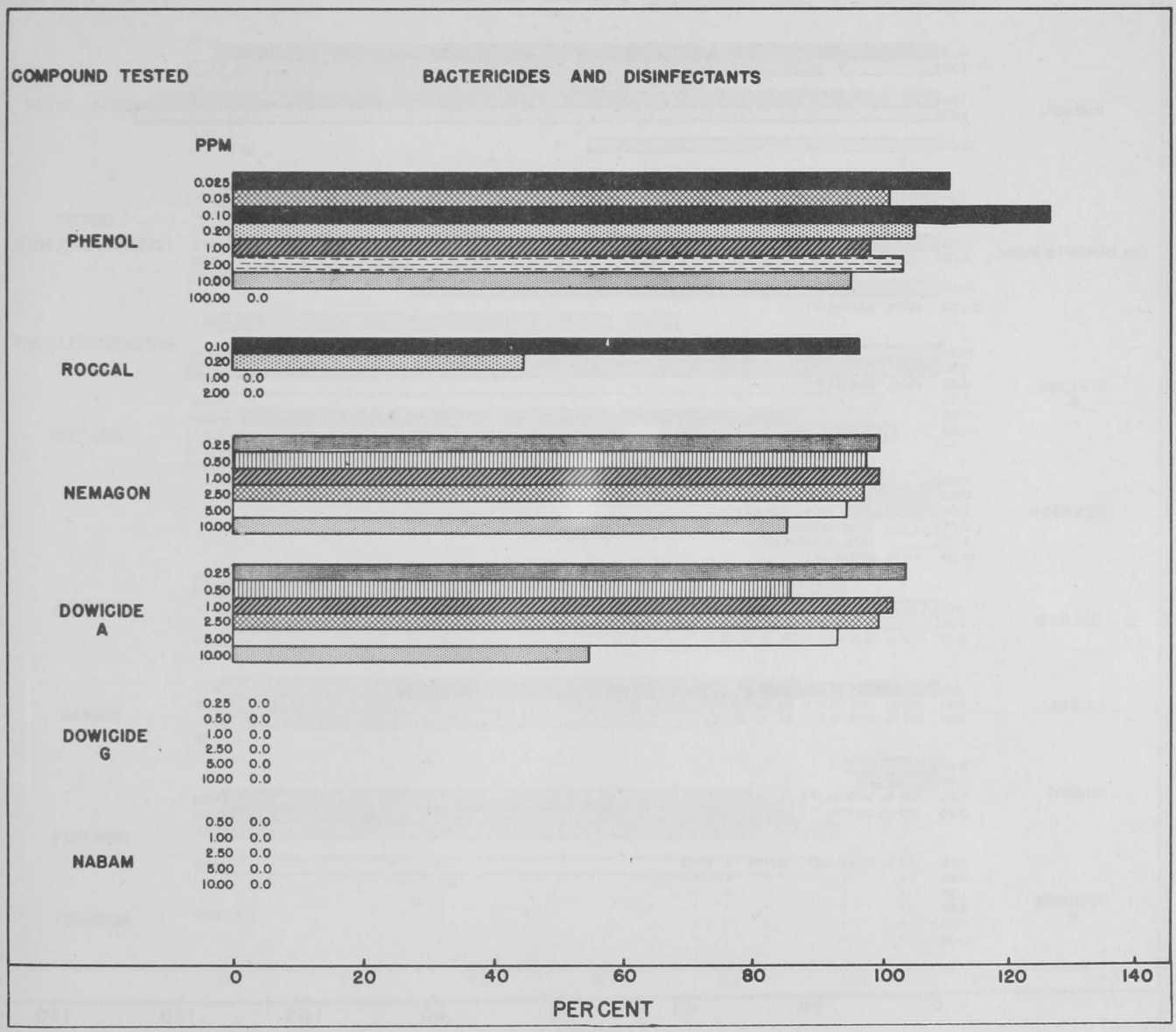

Fig. 1 - The effects of several concentrations of some bactericides and disinfectants on the relative percentage (R) of clam eggs developing to the straight hinge stage. The percentages shown are averages of duplicate cultures at each concentration calculated as follows:

$$R = \frac{\text{average number of larvae in experimental cultures}}{\text{average number of larvae in control cultures}} \times 100$$

more toxic to larvae than to the eggs, i.e. some eggs developed in 0.2 p.p.m. of roccal, but this concentration eventually killed all larvae. Similarly, although a normal percentage of eggs developed to straight hinge larval stages in 5.0 p.p.m. of dowicide "A," all concentrations of 1.0 p.p.m. and over were lethal to larvae.

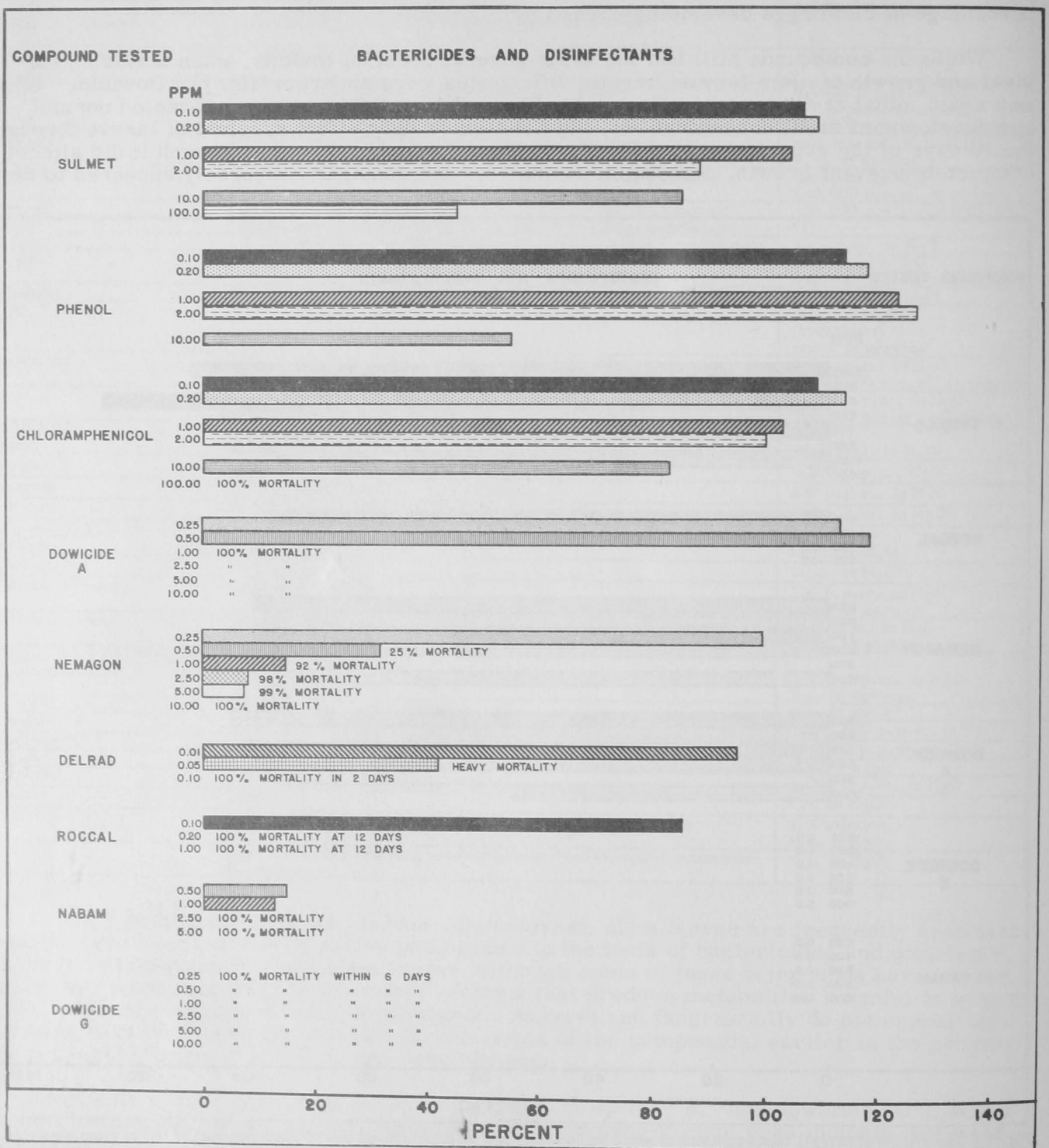

Fig. 2 - The effects of several concentrations of some bactericides and disinfectants on the growth of clam larvae. The percentages (G) plotted are averages of duplicate cultures at each concentration calculated as follows:

$$G = \frac{\text{mean length of experimental larvae at 12 days - mean length at 48 hours}}{\text{mean length of control larvae at 12 days - mean length at 48 hours}} \times 100$$

Likewise, nemagon even at 10.0 p.p.m. did not seriously affect egg development, but growth and survival of larvae were normal only at 0.25 p.p.m.; all higher concentrations drastically reduced growth and survival. Delrad, an algacide, was also quite toxic to larvae at concentrations high enough to be an effective algacide. Phenol, at 10.0 p.p.m., although it had little effect on eggs, significantly reduced the rate of growth of clam larvae. At lower concentrations, however, phenol along with low concentrations of dowicide "A" and certain concentrations of sulmet and chloramphenicol increased the rate of growth of clam larvae significantly above that of larvae in control cultures. Nevertheless, even with these compounds too high a concentration can significantly reduce growth and survival of clam larvae (fig. 2).

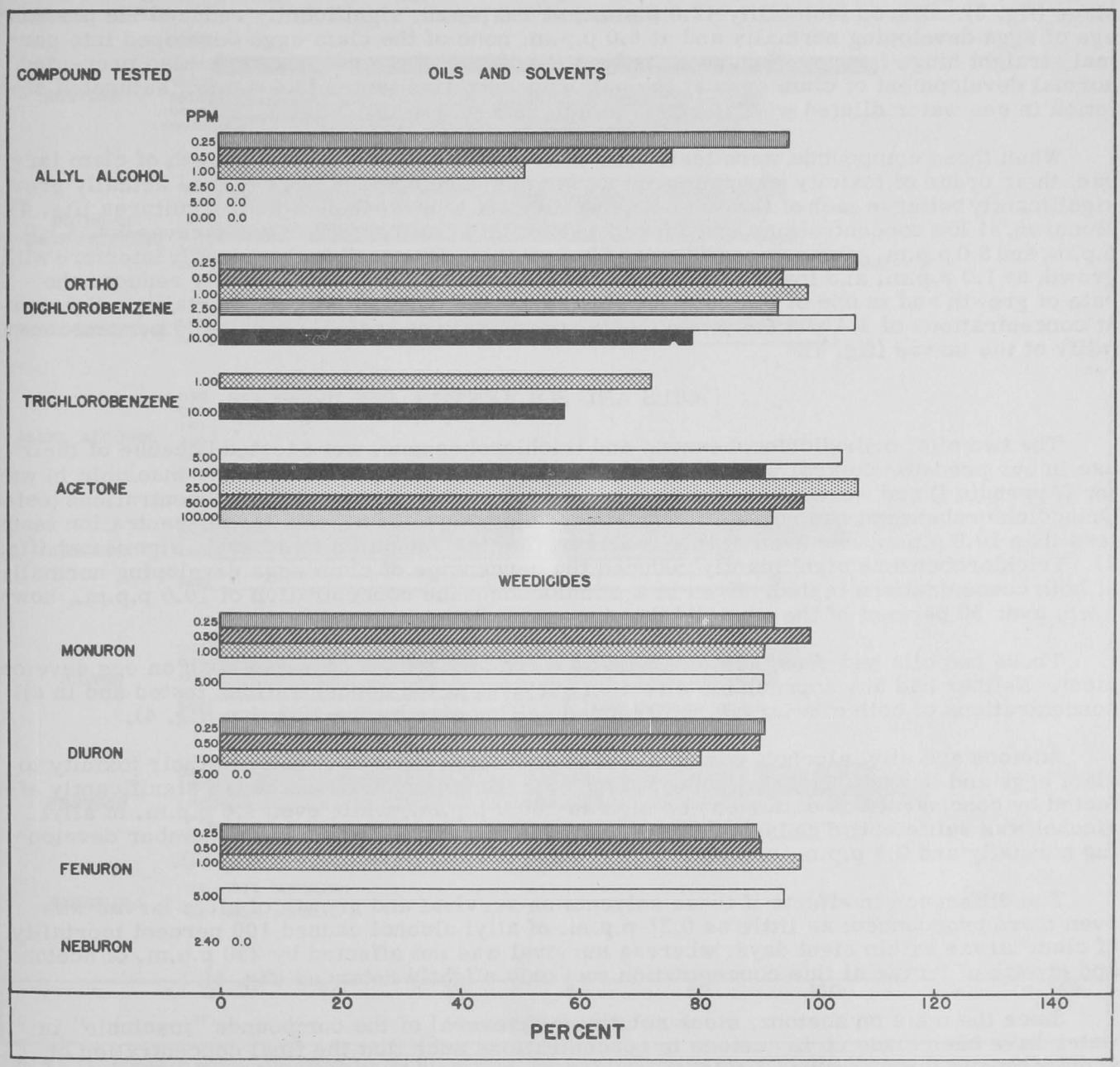

Fig. 3 - The effects of several concentrations of oils, solvents and weedicides on the relative percentage (R) of clam eggs developing to the straight hinge stage. The percentages shown are averages of duplicate cultures at each concentration calculated as follows:

$$R = \frac{\text{average number of larvae in experimental cultures}}{\text{average number of larvae in control cultures}} \times 100$$

Sulmet is now used routinely at our laboratory to prevent growth of harmful bacteria in larval cultures, and phenol, chloramphenicol, and perhaps even dowicide "A" can be used in conjunction with other antibiotics to aid in control of epidemics caused by bacteria not amenable to control with sulmet.

WEEDICIDES

The weedicides tested all belong to the methyl urea group of compounds but differ greatly in their solubilities and in their toxicity to clam eggs and larvae. Fenuron and monuron are the most soluble (Appendix I) and were the least toxic. Neither, in concentrations up to 5.0 p.p.m., significantly affected the percentage of clam eggs reaching the straight hinge stage (fig. 3). Diuron (solubility 42.0 p.p.m.) at 1.0 p.p.m. significantly reduced the percentage of eggs developing normally and at 5.0 p.p.m. none of the clam eggs developed into normal straight hinge larvae. Neburon, the least soluble (4.8 p.p.m. in water), also prevented normal development of clam eggs at the only concentration tested (2.4 p.p.m.; saturated solution in sea water diluted with an equal volume of sea water).

When these compounds were tested for their effect on survival and growth of clam larvae, their order of toxicity was the same as for egg development. The larvae actually grew significantly better in each of the four concentrations of fenuron than in control cultures (fig. 4). Monuron, at low concentrations, likewise increased the rate of growth of clam larvae but at 1.0 p.p.m. and 5.0 p.p.m. gave some evidence of being toxic. Diuron did not seriously interfere with growth at 1.0 p.p.m. and lower concentrations but at 5.0 p.p.m. it drastically reduced the rate of growth and in one of the pair of cultures caused over 90 percent mortality. Neburon at concentrations of 2.4 and 4.8 p.p.m. (only concentrations tested) caused 100 percent mortality of the larvae (fig. 4).

OILS AND SOLVENTS

The two oils, orthodichlorobenzene and trichlorobenzene, were tested because of their use in our predator control work (Loosanoff et al 1960). Both are relatively insoluble in water (Appendix I) and were comparatively harmless to clam larvae at the concentrations tested. Orthodichlorobenzene had no significant effect on egg development at any concentration tested less than 10.0 p.p.m. and even at this concentration the reduction was barely significant (fig. 3). Trichlorobenzene significantly reduced the percentage of clam eggs developing normally at both concentrations tested. Even at a trichlorobenzene concentration of 10.0 p.p.m., however, over 50 percent of the eggs did develop normally.

These two oils had even less effect on survival and growth of larvae than on egg development. Neither had any appreciable effect on survival at the concentrations tested and in all concentrations of both oils larvae had reached setting size by the 12th day (fig. 4).

Acetone and allyl alcohol, commonly used solvents, differed greatly in their toxicity to clam eggs and larvae. The percentage of eggs developing normally was not significantly affected by concentrations of acetone as high as 100.0 p.p.m., while even 1.0 p.p.m. of allyl alcohol was sufficient to cause an approximate 50 percent reduction in the number developing normally and 2.5 p.p.m. completely prevented normal development (fig. 3).

The difference in effects if these solvents on survival and growth of clam larvae was even more pronounced; as little as 0.25 p.p.m. of allyl alcohol caused 100 percent mortality of clam larvae within eight days, whereas survival was not affected by 250 p.p.m. of acetone and growth of larvae at this concentration was only slightly retarded (fig. 4).

Since the tests on acetone, stock solutions of several of the compounds "insoluble" in water have been made up in acetone in concentrations such that the final concentration of acetone in any larval culture would be 100 p.p.m. or less. Tests on nemagon, aldrin, toxaphene, and sevin were run in this manner, which insures that the correct quantity of the toxicant is added to the culture even though it may not all stay in solution in the sea water.

In tests with nemagon and sevin (solubility of both 1,000 p.p.m. in water) results were the same whether the stock solutions were made up in water or in acetone.

INSECTICIDES

The effects of twelve insecticides, on survival and growth of bivalve larvae, have been tests. Six of these (guthion, sevin, lindane, toxaphene, aldrin, and dicapthon), together with Niagara Compound N-3514 (2-chloro-1-nitropropane) have been tested on clam eggs (fig. 5) and larvae (fig. 6). Seven, including guthion, parathion, DDT, dipterex, TEPP, dieldrin, and

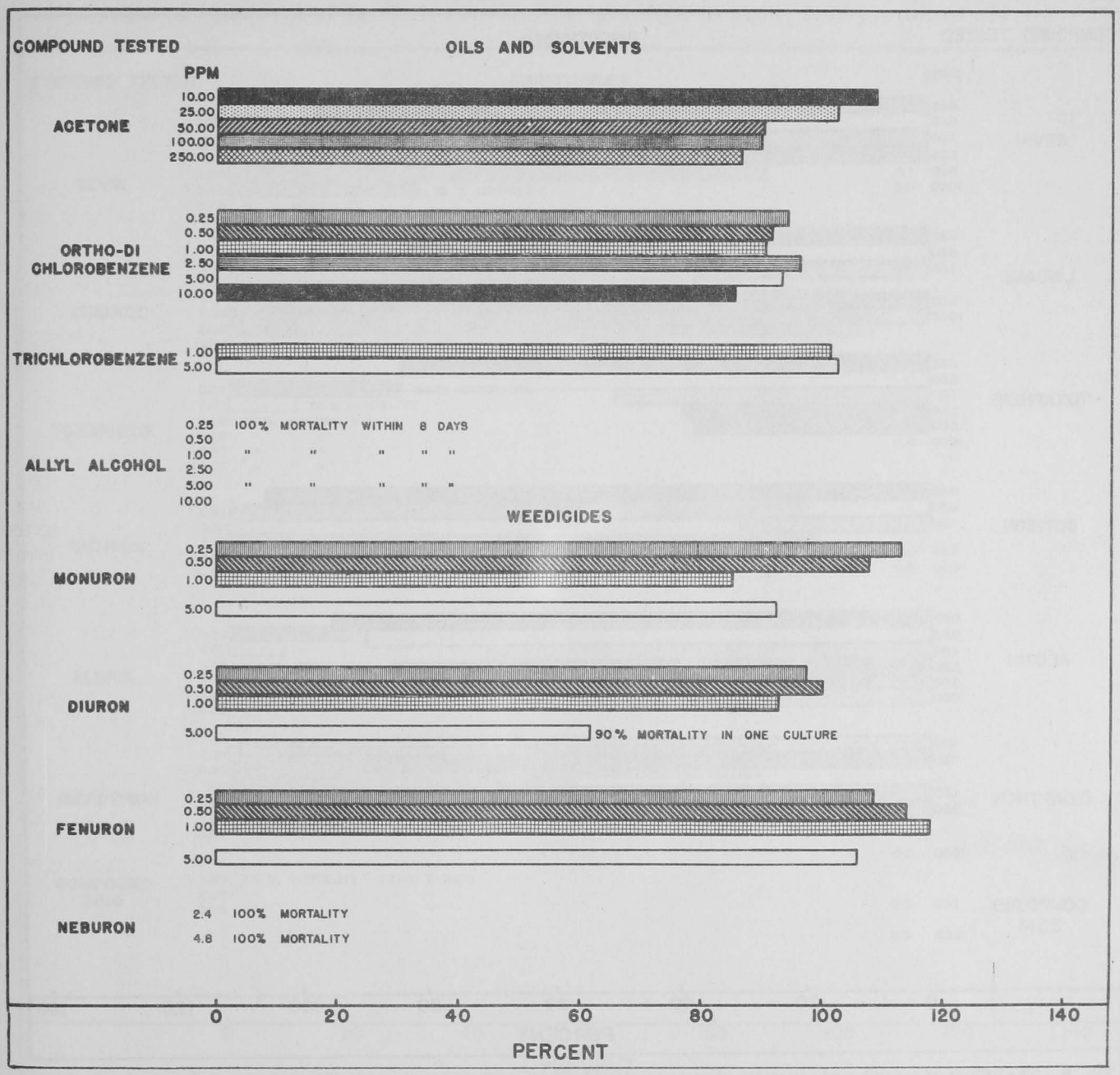

Fig. 4 - The effects of several concentrations of oils, solvents, and weedicides on the growth of clam larvae. The percentages (G) plotted are averages of duplicate cultures at each concentration calculated as follows:

$$G = \frac{\text{mean length of experimental larvae at 12 days - mean length at 48 hours}}{\text{mean length of control larvae at 12 days - mean length at 48 hours}} \times 100$$

endrin, together with Niagara Compound N-3514 and Niagara Compound N-3452 [alkyl (C_8C_{18}) dimethyl benzyl ammonium chloride[/] have been tested for their effect on survival and growth of oyster larvae (fig. 8). Guthion, endrin, dieldrin, TEPP, sevin, lindane, and Niagara Compounds N-3514 and N-3452 have also been tested on oyster eggs (fig. 7).

Lindane was the least toxic, to bivalve eggs and larvae, of any of these compounds. Approximately 60 percent of the clam eggs and 43 percent of the oyster eggs developed normally in concentrations of lindane up to 10 p.p.m., which is essentially a saturated solution. Moreover, there was no appreciable mortality of clam larvae receiving this concentration of

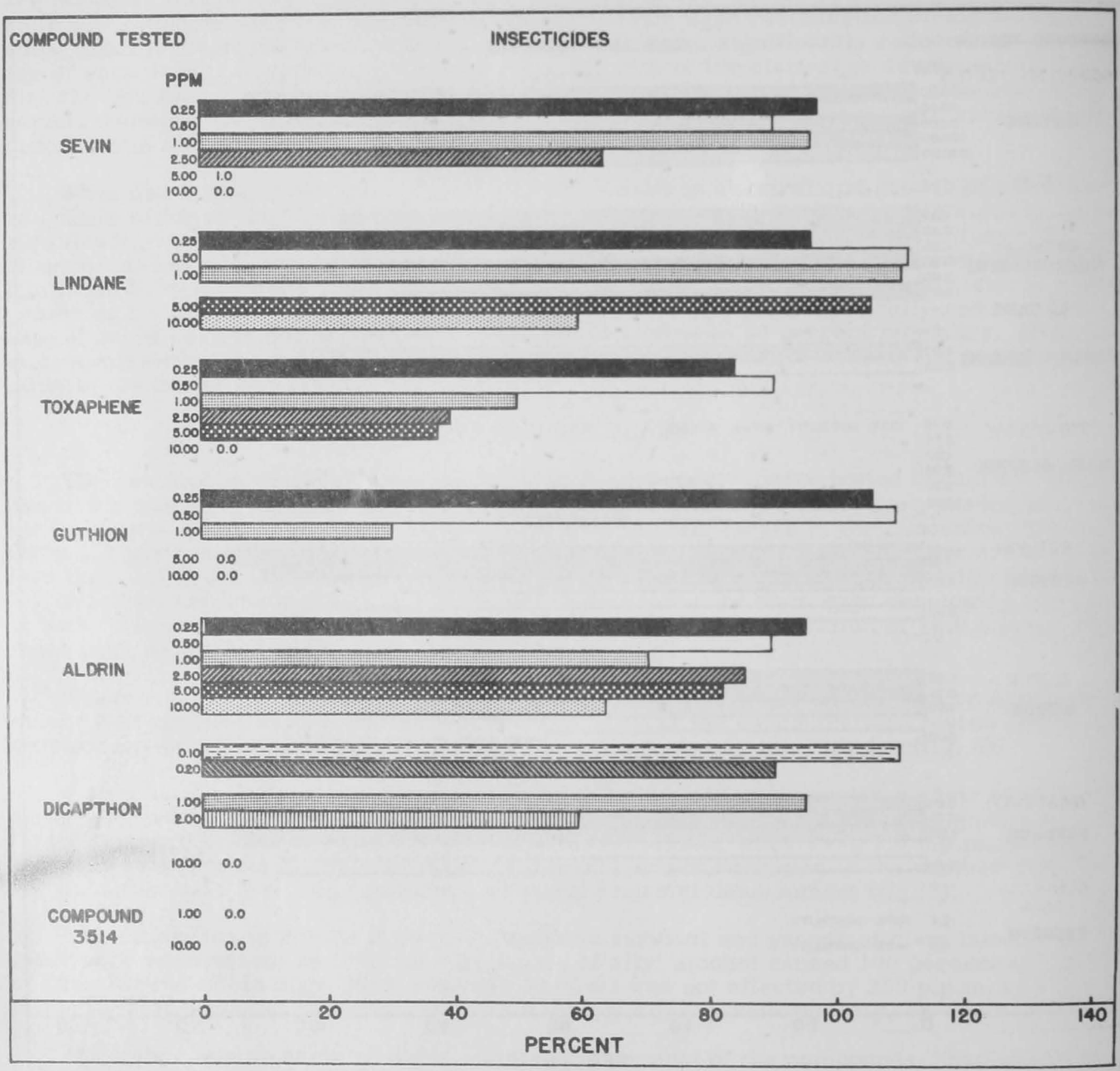

Fig. 5 - The effects of several concentrations of some insecticides on the relative percentage (R) of clam eggs developing to the straight hinge stage. The percentages shown are averages of duplicate cultures at each concentration calculated as follows:

$$R = \frac{\text{average number of larvae in experimental cultures}}{\text{average number of larvae in control cultures}} \times 100$$

lindane routinely, although growth of these larvae was significantly reduced (fig. 6). Concentrations of 5 p.p.m. or lower had little or no effect on development of clam or oyster eggs or on survival and growth of clam larvae (effect on oyster larvae not tested).

Aldrin did not appear to be very toxic to clam eggs, i.e. 64 percent developed normally at 10.0 p.p.m. (fig. 5). When aldrin was tested on clam larvae, however, it proved to be quite toxic. Growth of clam larvae was almost completely stopped by aldrin concentrations of 0.25 p.p.m. and 0.5 p.p.m. although there was no appreciable mortality of larvae at these concentrations. At all higher concentrations of aldrin, however, mortality was essentially 100 percent (fig. 6). Doudoroff et al (1953) report that the 10-day TLM (the median tolerance limit at which just 50 percent of the fish survive) for goldfish is about 0.02 p.p.m. of aldrin.

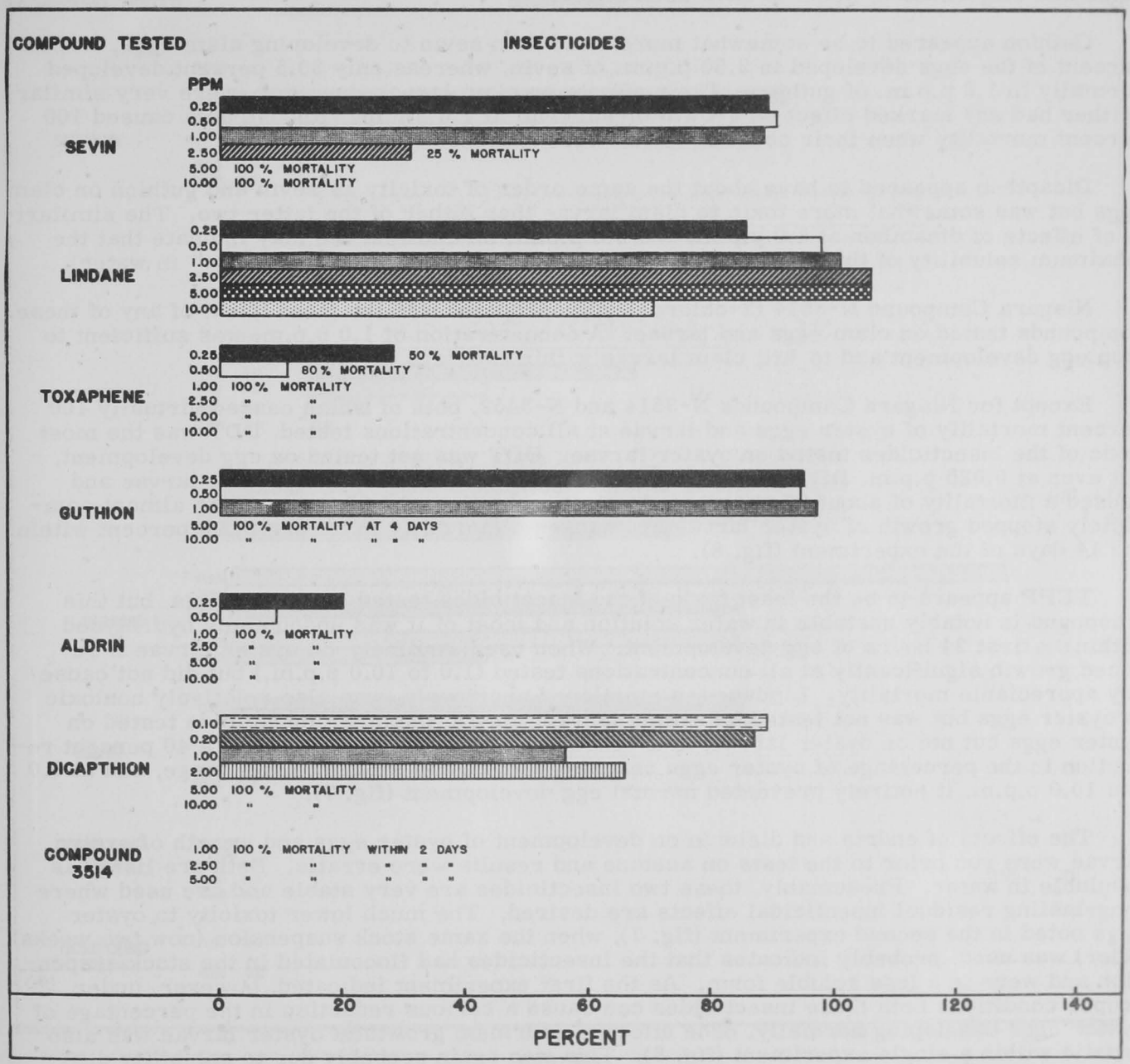

Fig. 6 - The effects of several concentrations of some insecticides on the growth of clam larvae. The percentages (G) plotted are averages of duplicate cultures at each concentration calculated as follows:

$$G = \frac{\text{mean length of experimental larvae at 12 days - mean length at 48 hours}}{\text{mean length of control larvae at 12 days - mean length at 48 hours}} \times 100$$

Toxaphene, likewise, was more toxic to clam larvae than might be expected from its effect on clam eggs. Some eggs developed normally in concentrations up to 5.0 p.p.m. of toxaphene and egg development appeared to be entirely normal at 0.25 and 0.50 p.p.m. Since the solubility of toxaphene is presumably 1.5 p.p.m., the increasing toxicity to clam eggs at 2.5, 5.0 and 10.0 p.p.m. possibly indicates that some of the other components of this mixture reach toxic levels as the concentration is increased above 1.5 p.p.m. When clam larvae were kept in solutions of toxaphene, however, 0.25 p.p.m. was sufficient to reduce growth of the larvae drastically and to cause a 50 percent mortality. Some larvae survived 12 days in 0.50 p.p.m. but growth of these larvae was negligible. Doudoroff et al (1953) report the 10-day TLM for goldfish is somewhat below 0.005 p.p.m. of toxaphene. As already noted, Hooper (1959) reports that in lakes treated with 0.1 p.p.m. fish and most invertebrates, except mollusks and oligochaetes, seem to have been killed.

Guthion appeared to be somewhat more toxic than sevin to developing clam eggs, i.e. 64 percent of the eggs developed in 2.50 p.p.m. of sevin, whereas only 30.5 percent developed normally in 1.0 p.p.m. of guthion. Their effects on clam larvae, however, were very similar; neither had any marked effect on growth or survival at 1.0 p.p.m. although both caused 100 percent mortality when their concentrations were increased to 5.0 p.p.m.

Dicapthon appeared to have about the same order of toxicity as sevin and guthion on clam eggs but was somewhat more toxic to clam larvae than either of the latter two. The similarity of effects of dicapthon at 1.0 p.p.m. and 2.0 p.p.m. on clam larvae may indicate that the maximum solubility of this compound is about 1.0 p.p.m. (solubility "very low" in water).

Niagara Compound N-3514 (2-chloro-1-nitropropane) was the most lethal of any of these compounds tested on clam eggs and larvae. A concentration of 1.0 p.p.m. was sufficient to stop egg development and to kill clam larvae within two days.

Except for Niagara Compounds N-3514 and N-3452, both of which caused virtually 100 percent mortality of oyster eggs and larvae at all concentrations tested, DDT was the most toxic of the insecticides tested on oyster larvae. DDT was not tested on egg development, but even at 0.025 p.p.m. DDT drastically reduced the rate of growth of oyster larvae and caused a mortality of about 20 percent. At a concentration of 0.05 p.p.m. DDT almost completely stopped growth of oyster larvae and caused a mortality in excess of 90 percent within the 14 days of the experiment (fig. 8).

TEPP appears to be the least toxic of the insecticides tested on oyster eggs, but this compound is notably unstable in water solution and most of it was undoubtedly hydrolyzed within the first 24 hours of egg development. When used routinely on oyster larvae it reduced growth significantly at all concentrations tested (1.0 to 10.0 p.p.m.) but did not cause any appreciable mortality. Lindane, as mentioned previously, was also relatively nontoxic to oyster eggs but was not tested on oyster larvae. Sevin, the other insecticide tested on oyster eggs but not on oyster larvae, at a concentration of 1.0 p.p.m. caused a 40 percent reduction in the percentage of oyster eggs reaching the normal straight hinge stage, and at 5.0 and 10.0 p.p.m. it entirely prevented normal egg development (fig. 7).

The effects of endrin and dieldrin on development of oyster eggs and growth of oyster larvae were run prior to the tests on acetone and results were erratic. Both are listed as insoluble in water. Presumably, these two insecticides are very stable and are used where long-lasting residual insecticidal effects are desired. The much lower toxicity to oyster eggs noted in the second experiment (fig. 7), when the same stock suspension (now two weeks older) was used, probably indicates that the insecticides had flocculated in the stock suspension and were in a less soluble form. As the first experiment indicated, however, under proper conditions both these insecticides can cause a serious reduction in the percentage of oyster eggs developing normally. The effect of endrin on growth of oyster larvae was also erratic within a single experiment (fig. 8). This was again probably due to solubility difficulties and failure to get a uniform suspension in the different test beakers.

Dipterex was not tested on oyster eggs, but caused a significant reduction in growth of oyster larvae at a concentration of only 0.025 p.p.m. The rate of growth of oyster larvae was almost the same in dipterex concentrations of 0.025 p.p.m., 0.05 p.p.m. and 1.0 p.p.m. i.e. it did not decrease appreciably with an increase in the concentration of dipterex. At 1.0 p.p.m., however, the mortality of oyster larvae reached almost 50 percent, whereas in the lower concentrations there was no appreciable mortality.

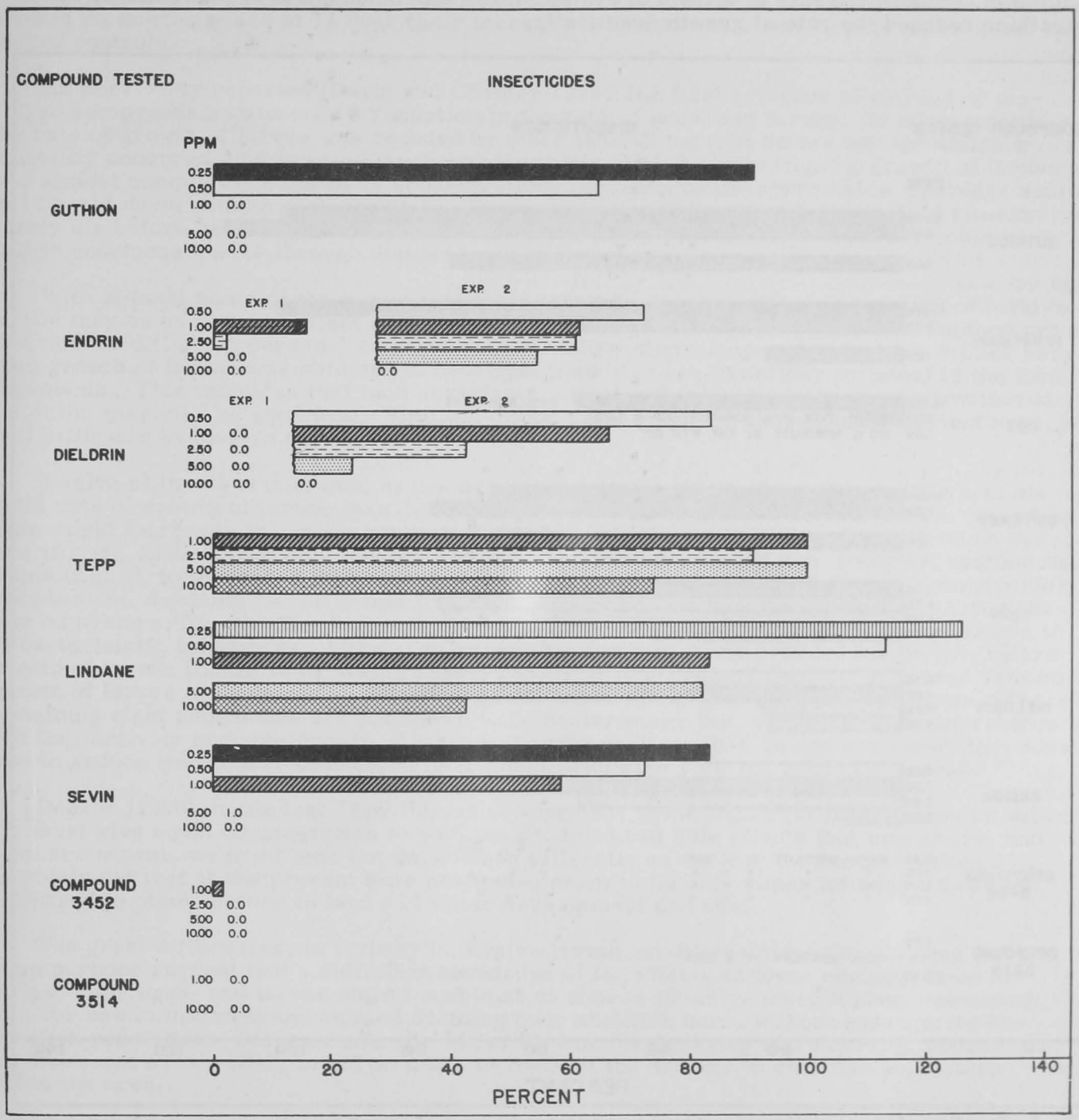

Fig. 7 - The effects of several concentrations of some insecticides on the relative percentage (R) of oyster eggs developing to the straight hinge stage. The percentages shown are averages of duplicate cultures at each concentration calculated as follows:

$$R = \frac{\text{average number of larvae in experimental cultures}}{\text{average number of larvae in control cultures}} \times 100$$

Guthion and parathion were two of the first insecticides to be tested for their effects on growth and survival of oyster larvae. Guthion has subsequently been tested for its effect on egg development. While guthion at 0.50 p.p.m. reduced the percentage of oyster eggs reaching the straight hinge stage and at 1.0 p.p.m. entirely prevented egg development (fig. 7), at very low concentrations (0.025 p.p.m. and 0.05 p.p.m.) it appeared to increase the rate of growth of oyster larvae (fig. 8). Parathion at 0.025 p.p.m. also slightly increased the rate of growth. Both compounds, however, are toxic at higher concentrations. Guthion at a concentration of 1.0 p.p.m. reduced the rate of growth of oyster larvae appreciably and at this concentration parathion reduced the rate of growth drastically.

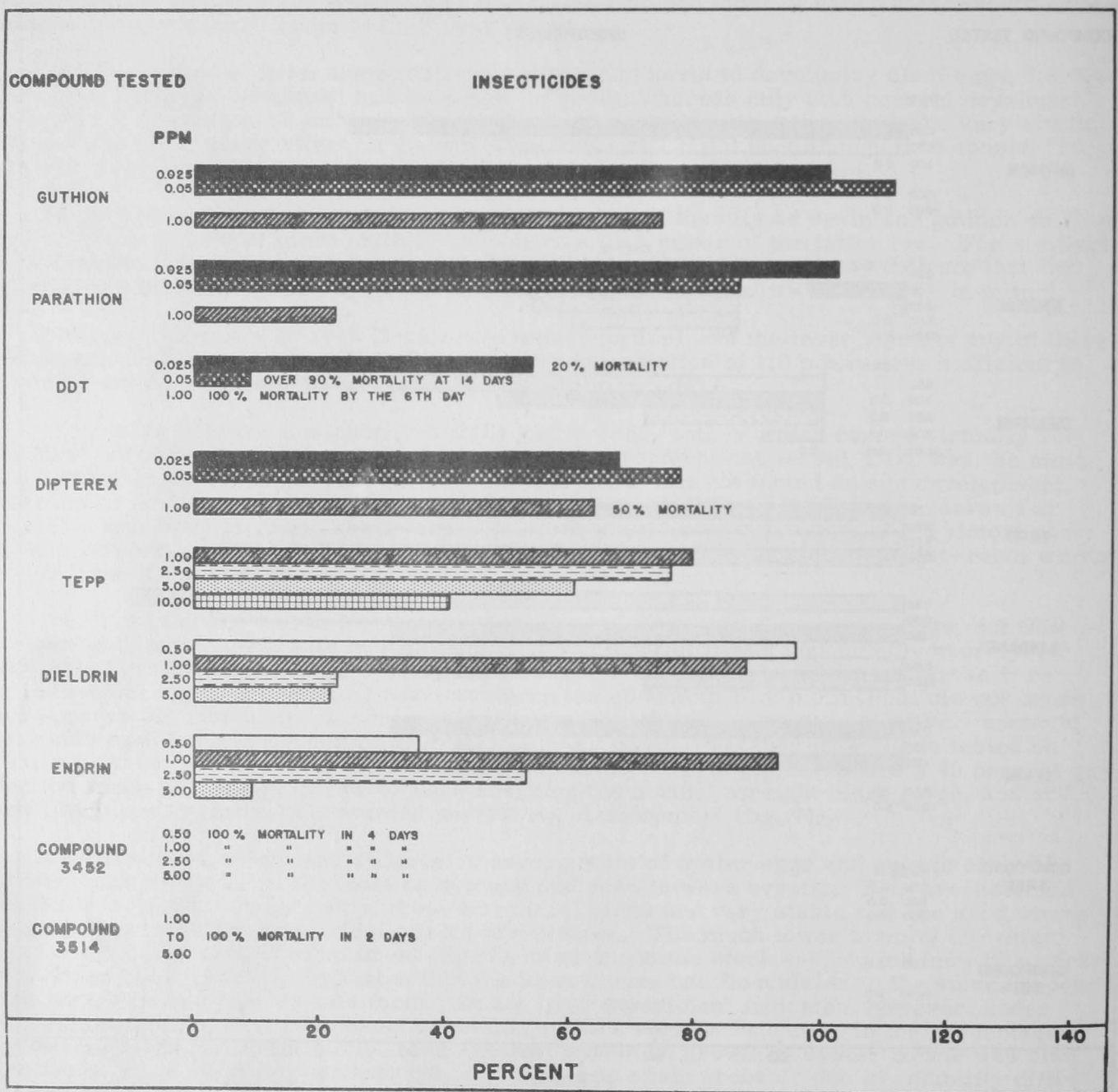

Fig. 8 - The effects of several concentrations of some insecticides on the growth of oyster larvae. The percentages (G) plotted are averages of duplicate cultures at each concentration calculated as follows:

$$G = \frac{\text{mean length of experimental larvae at 14 days - mean length at 48 hours}}{\text{mean length of control larvae at 14 days - mean length at 48 hours}} \times 100$$

DISCUSSION

In general, some eggs were capable of developing into normal straight hinge larvae in higher concentrations of almost all of the compounds tested than the larvae could tolerate and grow. Guthion was the only compound that permitted normal or near normal growth of larvae at concentrations which allowed less than 50 percent of the eggs to develop normally. Thus, at 1.0 p.p.m. of guthion only 31 percent of the clam eggs and no oyster eggs developed normally, yet clam larvae survived and grew almost as well as the controls. Oyster larvae showed no mortality and at 14 days their increase in length was about 75 percent as great as that of controls.

As previously reported (Davis and Chanley 1955), the first evidence of toxicity of dissolved compounds was usually a reduction in the rate of growth of larvae. In many instances the rate of growth of larvae was reduced by more than 50 percent before any appreciable mortality occurred, and in some, such as nabam (fig. 2) and aldrin (fig. 7), growth of larvae was almost completely stopped by concentrations too low to cause appreciable mortality within 12 or 14 days. Under such conditions, however, in natural waters larvae would almost surely die before reaching metamorphosis or the greatly extended larval period would result in enormous losses through dispersion, predation, etc.

With at least some of the compounds tested the effects on growth and survival of bivalve larvae may be an indirect effect resulting from the toxicity of the compounds to the food organisms used (Ukeles personal communication). With other compounds, such as sulmet, very good growth of larvae was obtained in concentrations that are inhibitory or lethal to the food organisms. This indicates that food organisms, killed without coagulation or destruction of the cells, may still be good food. Similarly, food cells killed by ultraviolet treatment are still utilizable by bivalve larvae.

Twelve of the 31 compounds, at one or more of the concentrations tested, gave a more rapid rate of growth of larvae than did the controls. This increased rate of growth ranged from slight increases that were not statistically significant, in the case of parathion on oysters (fig. 8), lindane on clams (fig. 6), and trichlorobenzene, diuron and, possibly, acetone on clams (fig. 4), to increases that appear to be highly significant, such as phenol, sulmet, chloramphenicol, dowicide "A" on clams (fig. 2), monuron and fenuron on clams (fig. 4), and guthion on oysters (fig. 8). The four that produced the most pronounced increases are known to be bacteristatic substances. Since it is known that some bacteria produce toxins that retard growth of larvae (Davis 1953, Walne 1958a, 1958b), it is presumed that the increased rate of growth of larvae in these cases is the result of inhibiting the growth of toxic bacteria. The remaining eight compounds are not known to be bacteristatic but, since it seems improbable that they actively promote growth of larvae, it appears likely that, in some manner, they also tend to reduce the number of bacteria that would otherwise slow the growth of larvae.

Decker (1960) states that "Any impartial appraisal of the impact of insecticides on wildlife must give equal consideration to both the good and bad side effects that may occur, and if we are honest, we must look for the good as diligently as we look for the bad" (page 30). He points out that at the present time pesticides seem to be only minor influents in nature compared to other factors in land and water development and use.

The great differences, in toxicity to bivalve larvae, of different compounds used for the same purpose suggest that a sufficient knowledge of the effects of these compounds on bivalves, their eggs, and larvae should enable us to choose effective insecticides, weedicides, etc., for use in marshes and on land draining over shellfish beds, without endangering the shellfish population. For example, the use of lindane for spraying marsh areas, instead of the much more toxic DDT, might do much to restrict the damage to shellfish populations within the area.

The concentration of pesticides in estuarine waters resulting from runoff from treated land areas, of course, cannot be predicted. It would be expected to be highest where the runoff enters the estuary, but actual concentrations would only be determined by chemical analy-

sis of the water. As a guide in direct addition of a pesticide to a body of water, as in the control of aquatic weeds and mosquito control programs, assuming that all of the pesticide goes into solution and is uniformly distributed, an application of one pound per acre would give a concentration of 0.37 p.p.m. in water one foot deep. If the water were 16 feet deep, the concentration would be 0.37 ÷ 16 or 0.023 p.p.m. In the case of DDT, this would still be enough to reduce the rate of growth of oyster larvae by 40 percent and to reduce their survival by 20 percent.

Loosanoff (1947) and Waugh and Ansel (1956) found that oyster shells treated with a kerosene solution of DDT caught almost as many oyster spat as did untreated shells and that the number of barnacles that set on such treated shells was greatly reduced. Waugh et al (1952), however, reported that lindane was almost as effective as DDT in treatment of shells to prevent setting of barnacles. Since the toxicity of lindane to bivalve larvae is much lower than that of DDT, lindane would seem to be the logical choice for such usage.

Loosanoff et al (1957) also found that crustaceans of the subclass Copepoda, in mass cultures of algae, could be killed by use of 1.0 p.p.m. of guthion, dipterex, parathion, lindane, or DDT. They found that lindane at 0.05 p.p.m. also killed these crustaceans, and have unpublished data showing an 80 percent mortality of adult green crabs in 0.2 p.p.m. Ukeles (unpublished data) has shown that the five species of algae tested tolerate up to 0.5 p.p.m. of lindane. Since clam larvae showed optimum growth at 5.0 p.p.m. lindane, it suggests the possibility that in enclosed bodies of water, as in pond culture, a sufficient concentration of this chemical could be maintained to destroy all crustacean larvae, probably including those of barnacles, without affecting the growth of bivalve larvae or their food organisms.

The fact that oysters will set on shells treated with a substance as toxic to larvae as DDT indicates that chemicals, with the very low solubilities characteristic of many of these pesticides, can be safely used in controlling fouling organisms and predators if properly applied. The chlorinated benzenes, which are the basic ingredient of the chemical methods proposed for predator control by Loosanoff et al (1960) are not only relatively insoluble but also relatively nontoxic to eggs and larvae of bivalves. Moreover, the method of application proposed by these authors is designed to limit their dispersal. It should be possible, by suitable choices of chemical additives, to develop mixtures that, when used on spat collectors or on shellfish beds, will control many of the competitors and predators of shellfish without affecting the development and growth of shellfish and their foods.

LITERATURE CITED

DAVIS, H. C.
1953. On Food and Feeding of Larvae of the American Oyster (C. virginica). Biol. Bull., vol. 104, no. 3, pp. 334-350.

_____, and CHANLEY, P. E.
1956. Effects of Some Dissolved Substances on Bivalve Larvae. Proc. Natl. Shellfish. Assoc. 1955, vol. 46, pp. 59-74.

DECKER, GEORGE C.
1960. Insecticides in the 20th Century Environment. AIBS Bull., vol. 10, no. 2, pp. 27-31.

DOUDOROFF, PETER; KATZ, MAX; and TARZWELL, CLARENCE M.
1953. Toxicity of Some Organic Insecticides to Fish. Sewage and Industrial Wastes, vol. 25, no. 7, pp. 840-844.

HARRINGTON, ROBERT W., Jr., and BIDLINGMAYER, WILLIAM L.
1958. Effects of Dieldrin on Fishes and Invertebrates of a Salt Marsh. The Journ. of Wildlife Management, vol. 22, no. 1, pp. 76-82.

HOOPER, FRANK F.
1959. Use of the Newer Organic Insecticides for Fish Population Control. Institute for Fisheries Research, Michigan Department of Conservation, Ann Arbor, Mich.

LOOSANOFF, V. L.:
1947. Effects of DDT Upon Setting, Growth and Survival of Oysters. Fishing Gazette, vol. 64, no. 4, pp. 94 and 96.

_____; HANKS, J. E.; and GANAROS, A. E.
1957. Control of Certain Forms of Zooplankton in Mass Algal Cultures. Science, vol. 125, no. 3257, pp. 1092-1093.

_____; MacKENZIE, C. L., Jr.; and SHEARER, L. W.
1960. Use of Chemicals to Control Shellfish Predators. Science, vol. 131, no. 3412, pp. 1522-1523.

WALNE, P. R.
1958a. The Importance of Bacteria in Laboratory Experiments on Rearing the Larvae of Ostrea edulis (L.). Journ. Mar. Biol. Assoc. U. K., vol. 37, pp. 415-425.

1958b. Ultra-Violet Sterilization of Water Used for Rearing Oyster Larvae. Nature, vol. 181, p. 1747.

1959. Experiments on the Large Scale Culture of Oyster Larvae. International Council for the Exploration of the Sea, C. M. 1959, Shellfish Committee no. 106, pp. 1-7.

WAUGH, G. DUNCAN, and ANSELL, A.
1956. The Effect, on Oyster Spatfall, of Controlling Barnacle Settlement with DDT. The Annals of Applied Biology, vol. 44, no. 4, pp. 619-625.

_____; HAWES, F. B.; and WILLIAMS, F.
1952. Insecticides for Preventing Barnacle Settlement. The Annals of Applied Biology, vol. 39, no. 3, pp. 407-415.

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