Inhibition of Embryonic Development of the Hard Clam, *Mercenaria mercenaria*, by Heavy Metals

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Heavy metals have long been recognized as serious pollutants of the aquatic ecosystem, with deleterious effects on the associated organisms. Limited information is available for marine organisms and those studies that have been reported deal primarily with adult organisms; relatively little is known about the effects of metals on invertebrates, especially their embryonic stages (CALABRESE et al. in press; KOBAYASHI 1971; OKUBO and OKUBO 1962).

With the development of techniques for rearing bivalve mollusks in the laboratory (LOOSANOFF and DAVIS 1963), further incentive has been provided for studies of the effect of pollutants on embryos of shellfish (DIMICK and BREESE 1965; WOELKE 1967). The present study was undertaken to determine the effect of mercury, silver, zinc, nickel and lead on the survival and subsequent development of embryos of the hard clam, Mercenaria mercenaria.

MATERIALS AND METHODS

Adult clams were induced to spawn in natural seawater in the laboratory by thermal stimulation and the addition of a water suspension of stripped sperm from a sacrificed male (LOOSANOFF and DAVIS 1963). After the clams began spawning in natural seawater, they were transferred to synthetic seawater. The gametes were thus collected in this synthetic medium. The effect of heavy metals on the survival of clam embryos was determined by placing a known number of fertilized eggs (usually 15,000 to 17,000) into each of a series of 1-liter polypropylene beakers containing synthetic seawater (salinity 25 °/oo) at 26 ± 1°C. Duplicate cultures were established at each of the test concentrations and four untreated cultures served as controls in each experiment. The synthetic seawater formulation (ZAROOGIAN et al. 1969) was made up of technical and analytical grade reagents dissolved in well water. This medium was used in place of natural seawater the composition of which varies, especially in regard to the presence of trace metals, dissolved organics and particulate matter.

The metals, tested as inorganic metallic salts, were as follows: lead nitrate [$Pb(NO_3)_2$]; mercuric chloride ($HgCl_2$); nickel chloride ($NiCl_2.6H_2O$); silver nitrate ($AgNO_3$); and zinc chloride ($ZnCl_2$). These metallic salts were chosen because of their solubility factor. In preliminary tests several ranges

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of concentrations were used for each metal to arrive at a range of concentrations to be used in final tests. Ten concentrations of each metal were used in final tests.

The background concentrations of heavy metals (in parts per million) in the synthetic seawater medium itself, as tested by an independent laboratory using atomic absorption spectrophotometry, were as follows: Hg - 0.00003, Ag - 0.003, Zn - 0.029, Ni - 0.0065 and Pb - 0.007.

The pH levels in all test containers, with metal salts added, remained between 7.0 and 8.5, the pH range for optimal development of oyster embryos (CALABRESE and DAVIS 1966).

Static tests were conducted throughout this study and all tests were initiated within one hour after the eggs were fertilized. Tests were terminated after 42 to 48 hours because known embryonic development, under normal conditions, is completed by this time and the embryos develop into straight-hinge larvae. To determine the effect of metal toxicity on embryonic development, the embryos that survived and developed into larvae in each culture were collected on a 36-micron nylon screen. The larvae were resuspended in a 250-ml graduated cylinder and, after thorough stirring to insure uniform distribution of the larvae, a 4-ml quantitative sample was removed and preserved in 5% neutral formalin. The samples were examined under a compound microscope and the number of embryos that had survived and developed into larvae was counted. The counts of samples from duplicate cultures were averaged and the results expressed as a percentage of the average number surviving in control cultures. The results of successive tests were then averaged and the LC_{50} value was determined by straight line graphical interpolation using logarithmic probablity paper: percent survival vs log concentration of metal. Under normal conditions in the laboratory, development of shellfish embryos in repetitive tests is somewhat variable, thus it was not unexpected to find that only two of the metals tested satisfied tests of linearity. It was not feasible, therefore, to use the probit method of analysis for determining confidence limits of the LC_{50} values. The ranges of the LC_{50} values arising from the individual metal experiments were then determined using the same procedures as with the LC_{50} values. The LC_0 and LC_{100} values were derived from actual observation. The results are based on five tests conducted with lead, mercury and silver and six with nickel and zinc.

RESULTS AND DISCUSSION

It must be understood that the metal toxicity discussed below is valid only for those forms of inorganic metallic salts tested and does not take into account what form the metals were when in solution. The acute toxicity of heavy metals, as inorganic salts, to embryos of the hard clam is shown in Table 1. These concentrations, not including

background levels, are reported in parts per million added to the synthetic seawater medium at the start of each experiment. Of the metals tested, mercury and silver were the most toxic. Mercury was 100% lethal at 0.0075 ppm and silver at 0.045 ppm. The estimated LC_{50} value for mercury was 0.0048 ppm, while for silver it was 0.021 ppm. Zinc and nickel, although not as toxic as mercury and silver, were 100% lethal to clam embryos at 0.25 and 0.60 ppm, respectively, while the estimated LC_{50} values were 0.166 and 0.31 ppm. Lead was the least toxic of the metals tested, although the toxicity was still great in that it was lethal at 1.2 ppm.

In comparing previous studies (CALABRESE et al. in press) on the effect of heavy metals on embryos of the American oyster, Crassostrea virginica, to the present studies with clam embryos (Table 2), the data indicate that clam embryos are much more sensitive than oyster embryos to lead and nickel, somewhat more sensitive to zinc, less sensitive to silver and as sensitive to mercury. Lead, for example, was 100% lethal to clam embryos at 1.20 ppm, while a concentration greater than 6.0 ppm was required to cause total mortality of oyster embryos. It is not clear why the difference in LC_{100} concentrations of lead was so great between these two species of bivalves.

Larvae of other species of bivalves, such as the mussel, Mytilus edulis, and the oyster, Crassostrea commercialis, were exposed to mercury (WISELY and BLICK 1967) and 50% died within two hours at 13.0 and 180.5 ppm, respectively. It was concluded that this high resistance was due to the ability of these organisms to withdraw their bodies into their shells, thereby reducing the penetration of the toxic material into the soft parts.

Embryos of Mytilus sp and Crassostrea sp were exposed to mercury (OKUBO and OKUBO 1962) and were not affected at a concentration of 0.01 ppm, but were affected at 0.032 ppm. These concentrations were considerably higher than those affecting M. mercenaria embryos in the present study. Zinc had no effect on Mytilus sp at 0.32 ppm, but did affect them at 1.0 ppm and had no effect on Crassostrea sp at 1.0 ppm, but did at 3.2 ppm. These concentrations were considerably higher than those affecting M. mercenaria embryos in the present study and C. virginica embryos in previous studies (CALABRESE et al. in press).

It must be pointed out that the results obtained from this study should be considered directly applicable to the synthetic medium used. Possible changes in results may have been obtained if natural seawater were used because of the existence of organic ligands for complexation of metals which could have either increased or decreased the toxicity of the metals to the clam embryos. As stated earlier, however, natural seawater was not used in these experiments because of the possible variability in composition of our natural seawater supply in regard to the presence of

TABLE 1

Toxicity of Heavy Metals, as Inorganic Salts, to Hard Clam Embryos at 26 ± 1°C in Synthetic Sea Water (25°/oo Salinity). The Concentrations are in Parts Per Million of Metal Ion Added to Medium at Start, Producing Mortality of 0, 50 and 100 Percent (LC_0 and LC_{100} are Actual Values and LC_{50} is Estimated).[1]

Metals as Inorganic Salts	LC_0	LC_{50}	Range of LC_{50}	LC_{100}
Mercuric chloride	0.0025	0.0048	0.0038-0.0056	0.0075
Silver nitrate	0.010	0.021	0.019 -0.025	0.045
Zinc chloride	0.095	0.166	0.138 -0.175	0.25
Nickel chloride	0.10	0.31	0.28 -0.33	0.60
Lead nitrate	0.40	0.78	0.72 -0.80	1.20

[1] These concentrations do not include background concentrations of heavy metals in the synthetic seawater medium. They were (in ppm): Hg - 0.00003, Ag - 0.003, Zn - 0.029, Ni - 0.0065 and Pb - 0.007.

trace metals, dissolved organics and particulate matter. It must be understood that experiments of this type require several months' duration to be completed and the use of natural seawater during this time frame would be more apt to cause distortion of the results than the use of synthetic seawater which, in itself, is a standard medium by which other researchers can compare their experimental results. The results of this study, therefore, may represent the relative, if not the absolute toxicity of the various metals tested in the natural marine ecosystem.

It would now be necessary to study the effect of these same heavy metals on larvae of the hard clam and also the combined effects of these metals on these life stages to determine whether various metals in combination act synergistically or antagonistically. 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 would serve to prolong the pelagic life of the larvae and, thus, increase their chance of loss through predation, disease and dispersion, thereby reducing recruitment into the population.

TABLE 2

Toxicity of Heavy Metals, as Inorganic Salts, to Oyster and Hard Clam Embryos at 26 ± 1°C in Synthetic Sea Water (25°/oo Salinity). The Concentrations are in Parts Per Million of Metal Ion Added to Medium at Start, Producing Mortality of 0, 50 and 100 Percent (LC_0 and LC_{100} are Actual Values and LC_{50} is Estimated)[1].

Metals as Inorganic Salts	LC_0		LC_{50}		LC_{100}	
	Clams	Oysters	Clams	Oysters	Clams	Oysters
Mercuric chloride	0.0025	0.001	0.0048	0.0056	0.0075	0.008
Silver nitrate	0.010	0.003	0.021	0.0058	0.045	0.01
Zinc chloride	0.095	0.075	0.166	0.31	0.25	0.5
Nickel chloride	0.10	0.10	0.31	1.18	0.60	3.0
Lead nitrate	0.40	0.50	0.78	2.45	1.20	>6.0

[1] These concentrations do not include background concentrations of heavy metals in the synthetic sea water medium. They were (in ppm): Hg - 0.00003, Ag - 0.003, Zn - 0.029, Ni - 0.0065 and Pb - 0.007.

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