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TOXIC EFFECTS OF MERCURY ON THE HARD CLAM, *MERETRIX LUSORIA*, IN VARIOUS SALINITIES

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Abstract—1. The 96-hr LC_{50} values for juvenile hard clams, *Meretrix lusoria*, were 328, 392 and 194 μg/l Hg in 10, 20 and 30 ppt salinities at 25 ± 1°C, respectively; for adult hard clams 341 and 140 μg/l Hg in 20 and 30 ppt salinities, respectively.

2. Acclimatizing the adult clams to low salinity of 10 ppt lessened the toxicity of mercury. However, juvenile animals appeared to be more sensitive to mercury poisoning after 96 hr exposure in 10 ppt salinity.

3. All embryos exposed to 40 μg/l Hg and above died within 30 hr. In the control, 44% of hatched embryos had developed into D-stage larvae, while those exposed to 20 μg/l Hg were still in the trochophore stage. Most of the retarded larvae developed into abnormal forms within 30 hr at 28°C in 15 ppt salinity.

4. In order to maintain water quality and protect natural resources, the recommended safe level of mercury is 0.046 (0.039–0.053) μg/l Hg, based on the estimated 30-hr EC_{50} for the clam embryos, with an application factor of 0.01.

INTRODUCTION

Heavy metals have long been recognized as serious pollutants in the aquatic ecosystem, showing deleterious or even toxic effects on associated organisms. For example, green oyster, caused by a high accumulation of copper, was observed in Taiwan at the Charting coastal area (Hung *et al.*, 1989). Minamata Disease, caused by the consumption of fish and shellfish containing a high concentration of mercury, has also been well documented (Irukayama *et al.*, 1962; Yoshida *et al.*, 1967). The hard clam, *Meretrix lusoria*, is one of the most important edible bivalve molluscs in Taiwan. Mass mortality of cultivated clams in coastal waters, which has occurred during the months of April and May each year since 1969, has mainly been attributed to the toxic effect of polluted water discharged from the industrial plants in the upstream areas of rivers. (Tseng, 1976; Chen, 1984).

Mercury, one of the group II B metals, has been recognized as being a highly toxic element to aquatic fauna (Chen, 1980; Hsieh and Chen, 1980; Chen and Chin, 1983). The total production of mercury for use in industrialization has greatly increased during this century. It is mainly used in the manufacturing of electrical equipment and in the electrolytic production of chlorine and caustic soda (Moore and Ramamoorthy, 1984). After the outbreak of Minamata Disease (Kitamura, 1968; Förstner and Wittmann, 1981), the consequences of mercury contamination in the aquatic environment came under closer scrutiny. In Taiwan, estuarine seawater polluted with mercury has been surveyed in the Lu-Ar-Man River, Tainan (Chen and Lin, unpublished data). Whereas many studies point out that sub-lethal concentrations of mercury have minimal effects on organisms in optimum conditions, it becomes more toxic under stress conditions with extreme temperatures and salinity (Vernberg and Vernberg, 1972; Jones, 1973; Nelson *et al.*, 1977; Denton and Burdon-Jones, 1981). Interestingly, little is known about the toxic effects of mercury on the hard clam, *Meretrix lusoria*. The present study was therefore undertaken to determine the effect of mercury on the mortality and embryonic development of the hard clam, *Meretrix lusoria*, at different salinities.

MATERIALS AND METHODS

Adult and juvenile hard clams, *Meretrix lusoria*, were obtained from a commercial shellfish farm located in the Taishi area and were acclimatized to laboratory tanks at different salinities (10, 20 and 30 ppt) for one week before use. The adult clams used had an average shell length of 2.97 ± 0.15 cm, shell height of 2.47 ± 0.14 cm and wet weight of 6.552 ± 0.963 g. The juveniles tested had an average shell length of 1.06 ± 0.08 cm, shell height of 0.88 ± 0.07 cm and wet weight of 2.777 ± 0.144 g. During the period of acclimation, the clams were not fed but supplied with aeration. Water temperatures were maintained at 25 ± 1°C.

Mercury stock solutions were prepared by dissolving 1.36 g of mercuric chloride ($HgCl_2$, Merck reagent grade) in 1 l of distilled water containing 1 ml concentrated HNO_3 to make 1000 mg/l Hg. Before commencing the experiments, they were diluted to desired concentrations using filtered seawater (0.45-μ Millipore filter). For adult clams, the nominal

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concentrations studied were 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/l Hg. Thirteen adult clams were placed into each of a series of 3-l polypropylene containers having different Hg concentrations. For juvenile clams, triplicate cultures were also established for each test concentration and control, with nominal concentrations of 0.1, 0.2, 0.4, 0.8 and 1.6 mg/l Hg. Ten clams were placed into each of a series of 1-l beakers, similar to those used for the adults. Triplicate cultures were established for each test concentration and triplicate untreated cultures served as controls. In the toxicity test, static method without aeration was conducted throughout this study and all test solutions were renewed daily (Buikema *et al.*, 1982; Apha *et al.*, 1985). During the experiment, the clams were not fed. In all test solutions, water temperatures were maintained at 25 ± 1°C, dissolved oxygen and pH varied from 3.5 to 7.0 mg/l and from 7.75 to 8.36, respectively. Death was determined when both valves continued to gape and showed no sign of response after mechanical agitation.

Adult clams were induced to spawn in natural seawater in the laboratory by thermal stimulation (Chen and Lyuu, 1983). After the clams began spawning, the fertilized eggs were collected and transferred to filtered seawater. To determine the toxic effect of mercury on embryonic development, 1 ml filtered seawater containing fertilized eggs (about 80) was placed into each of a series of tubes containing 5 ml filtered seawater (15 ppt salinity), with various mercury concentrations, at 26 ± 1°C. The final nominal concentrations were 2.5, 5.0, 10.0, 20.0 and 40.0 μg/l Hg. Four replicates of each test concentration were used, and four untreated cultures served as controls. A static test was conducted throughout this study and initiated within 2 hr after eggs were fertilized. Tests were terminated after 30 hr because the embryonic development was completed by this time, the embryos having developed into straight hinged, D-stage larvae. At the end of the experiment, all test solutions from each tube were collected, and fixed with 10% buffered formalin. The samples were examined immediately under a microscope and the number of embryos that had developed into D-larvae were counted. The results of the counts of samples from four replicate cultures were averaged and the final result expressed as a percentage of the average number in control cultures.

The dose response of test organisms obtained from replicate tanks of each test solution was assessed by the method of probit analysis (Finney, 1971). The LC_{50} (median lethal concentration) values and EC_{50} (median effective concentration, the criterion being failure to reach D-stage larvae) values of Hg, with their 95% confidence limits, were calculated using a microcomputer program (Trevors and Lusty, 1985). The program was based on the method described by Hubert (1980). With this program, the estimated probit line and the result of a Chi-square test for goodness of fit were computed. In order to compare the survival time at fixed mercury concentrations between different life stages, the time to 50% mortality, expressed as LT_{50}, was also calculated.

RESULTS AND DISCUSSION

Acute toxicity of mercury to adult hard clams in different salinities is shown in Fig. 1. As might be expected, the higher the mercury concentration, the higher the mortality. Toxicity to the animals is also found to be affected by different salinities; mortality increases with increasing salinities. The same is true for juvenile hard clams exposed to mercury in various salinities. However, salinity's effect on the toxicity of mercury is clearer in adult hard clams than juvenile animals, especially for adults in the 10 ppt salinity range.

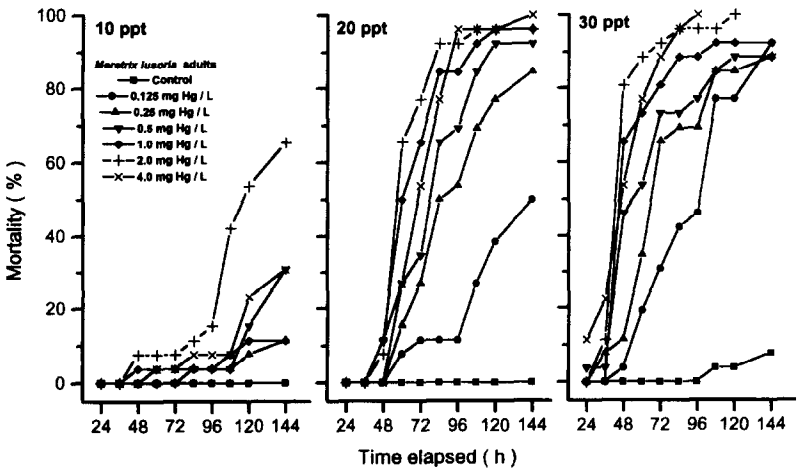

Fig. 1. Percentage mortality of *Meretrix lusoria* adults exposed to different mercury concentrations in three salinities.

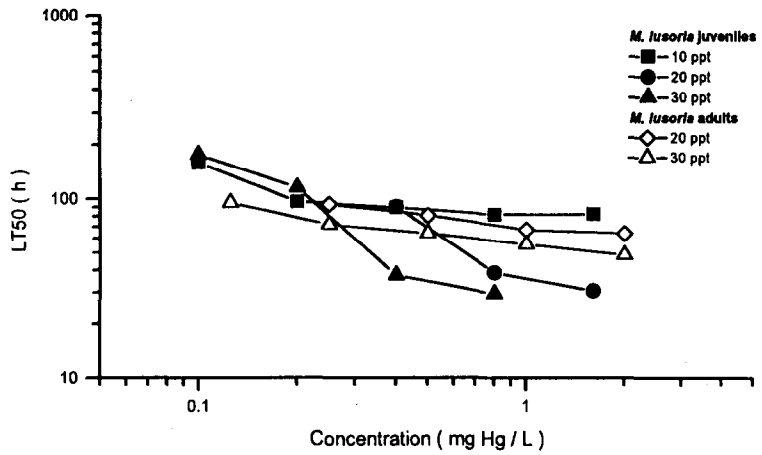

Fig. 2. Acute toxicity of mercury to *Meretrix lusoria* juveniles and adults in different salinities.

It is commonly believed that animals of different ages have differing susceptibility to pollutants, younger animals being more sensitive to pollution than adults (Chen, 1980). Figure 2 shows the comparison of mercury toxicity to juveniles and adults by plotting their LT_{50} values against the mercury concentration used. It can be seen that the adults are more resistant to mercury poisoning at concentrations above 0.4 mg/l Hg in the same salinity. However, inversely, at low concentrations of 0.4 mg/l Hg or below, juvenile hard clams can survive longer than adults clam. The LC_{50} and their 95% confidence limits for embryo, juvenile and adult hard clams are shown in (Table 1).

The hard clam is a euryhaline species; its optimal salinity for survival and growth decrease with advanced life stages. Adult hard clams prefer low salinities of 10–15 ppt to normal salinity, while juvenile clams need optimal salinity of 20 ppt (Chen, 1984; Yang and Ting, 1984). These facts suggest that a stressful condition may accelerate the toxicity of mercury. Dillon (1977) reported that mercury appeared to be much less toxic to euryhaline clams, *Rangia cuneata*, acclimated in 2 ppt salinity than those acclimated in 15 ppt salinity while the reason for being less toxic in low salinity is not well understood. Dillon and Neff (1978) suggested that a decrease of toxicity in brackish water is due to the enhanced ability to depurate mercury in the body.

The relationship between probit of mortality and log concentration at various exposure times in different salinities and life stages for hard clams was calculated and is listed in Table 2. All linear regression lines estimated are significant from the chi-square test with a high correlation coefficient, suggesting that they are highly satisfactory, as expressed by Trevors and Lusty (1985). There is a great difference in slopes between both juvenile and adult groups, regardless of different salinities used and time of exposure to mercury. This finding confirms the fact that adults are more tolerant of mercury poisoning than juveniles. When concentration of mercury increases, it has a more serious effect on survival time of juveniles than of adults (Fig. 2).

Resistance to mercury toxicity with low salinity in *Meretrix lusoria* and *Rangia cuneata* (Dillon, 1977)

Table 1. The LC_{50} and their 95% confidence limits of mercury (as $HgCl_2$) for the hard calm, *Meretrix lusoria*, in different salinities at 25 ± 1°C

	Concentration (mg/l Hg) (ppt)					
	10		20		30	
Time (hr)	LC_{50}	95% CL	LC_{50}	95% CL	LC_{50}	95% CL
Juvenile						
24					0.991	0.760–1.290
48			0.799	0.663–0.964	0.400	0.329–0.488
72			0.435	0.378–0.500	0.248	0.210–0.294
96	0.328	0.218–0.493	0.392	0.344–0.447	0.194	0.169–0.223
120	0.108	0.075–0.156	0.351	0.305–0.404	0.175	0.151–0.202
144			0.275	0.231–0.328	0.136	0.113–0.163
168			0.213	0.174–0.259	0.115	0.090–0.147
Adult						
48					0.783	0.572–0.952
60			1.121	0.738–1.703	0.428	0.311–0.590
72			0.701	0.496–0.989	0.209	0.128–0.340
96			0.341	0.244–0.476	0.140	0.078–0.235
120	5.071	2.078–12.370	0.135	0.072–0.235		
144	2.993	1.517–5.928	0.099	0.054–0.184		

Table 2. Relationship between probit of mortality (Y) and log concentration of mercury as mg/l Hg (X) at various exposure time, salinities and sizes for the hard clam, *Meretrix lusoria*

Salinity	Time (hr)	$Y = a + bX$	N	R^2	χ^2
D-larvae					
15 ppt					
	30	$Y = 3.3910 + 0.2437X$	3	0.9847	1.1517
Juvenile					
10 ppt					
	96	$Y = 5.7536 + 1.5556X$	3	0.9583	0.3834
	120	$Y = 7.3300 + 2.4080X$	4	0.9268	1.9546
20 ppt					
	48	$Y = 5.3612 + 3.7136X$	3	0.8772	2.6191
	72	$Y = 7.1828 + 6.0331X$	3	0.9583	2.1197
	96	$Y = 7.6588 + 6.5366X$	3	0.9987	0.2634
	120	$Y = 7.7267 + 5.9949X$	3	0.9847	0.6431
	144	$Y = 7.5338 + 4.5222X$	3	0.9635	1.0086
	168	$Y = 7.3181 + 3.4462X$	3	0.9051	1.9533
30 ppt					
	24	$Y = 5.0109 + 2.5234X$	4	0.9513	1.3658
	48	$Y = 6.3383 + 3.3653X$	4	0.9693	1.9153
	72	$Y = 7.7184 + 4.4937X$	4	0.8847	3.1381
	168	$Y = 8.2069 + 3.4120X$	3	0.7864	4.2277
Adult					
10 ppt					
	120	$Y = 4.1685 + 1.1794X$	3	0.8146	1.7492
	144	$Y = 4.4233 + 1.2093X$	3	0.7589	2.2663
20 ppt					
	60	$Y = 4.9217 + 1.5807X$	5	0.9912	0.2488
	72	$Y = 5.2511 + 1.6257X$	5	0.9693	1.1038
	96	$Y = 5.8592 + 1.8393X$	6	0.9161	4.4780
	120	$Y = 6.3195 + 1.5983X$	5	0.9125	2.3626
	144	$Y = 6.8872 + 1.8810X$	4	0.8967	1.2960
30 ppt					
	48	$Y = 5.3092 + 2.3443X$	5	0.9625	2.0751
	60	$Y = 5.6275 + 1.7041X$	5	0.9978	0.0678
	72	$Y = 5.9755 + 1.4334X$	5	0.9074	1.9803
	84	$Y = 6.2250 + 1.5230X$	5	0.9579	1.1651
	96	$Y = 6.2436 + 1.4560X$	5	0.9749	0.4610

N: number of nominal time for calculation.
R^2: coefficient of correlation.

differs from that in other bivalves. Nelson *et al.* (1977) demonstrated that the survival of juvenile bay scallops, *Argopecten irradians*, was significantly affected by mercury, as well as salinity. In that study, juvenile bay scallops, considered a stenohaline and cold water species, were reared at 20 ± 2°C and 25 ± 2 ppt salinity for about 3 months prior to testing. Results showed that low salinity alone enhanced the toxicity of low concentrations of mercury. High temperature and low salinity also acted synergistically with mercury to increase mortality. Therefore, the lowest concentration of mercury that caused 96-hr LC_{50} occurred at the highest temperature (25°C) and lowest salinity (15 ppt) tested (Table 3). It is generally accepted that, under optimal conditions, the toxicity of a pollutant to aquatic animals could be reduced to a minimum. Bryan (1971) suggested that the increased toxicity of heavy metals to marine organisms under unfavorable environmental conditions be related to changing rates of absorption. Furthermore, this rate can be varied with experimental conditions, species tested, and life stage of the test animal (Fowler *et al.*, 1978; Langston, 1982).

Toxic effects of mercury chloride on other bivalve species are summarized in Table 3. As mentioned previously, Nelson *et al.* (1976) reported that at 20 ± 1°C in natural seawater(25 ± 2 ppt salinity), the 96-hr LC_{50} value and their 95% confidence limits were 89 and 54–147 μg/l Hg, respectively, for juvenile bay scallops, *Argopecten irradians*. For *Mytilus edulis*, Strömgren (1982) recorded that acute lethal effects were observed at 25 μg/l Hg within 24 hr, and EC_{50} for growth at about 0.3–0.4 μg/l Hg. However, Martin *et al.* (1975) recorded a relatively high tolerance of *Mytilus edulis* to inorganic mercury, with 7 d-LC_{50} at 150 μg/l Hg. The 96-hr LC_{50} value for the hooked mussel, *Brachiodontes recurvus*, calculated from actual exposure mercury concentrations, was 27 μg/l (Green *et al.*, 1975). Mohan *et al.* (1986) reported that the 96-hr LC_{50} values, and their 95% confidence limits, of the tropical green mussel, *Perna viridis*, exposed to mercury were 230 and 210–250 μg/l Hg, respectively. A concentration of 29–38 μg/l Hg reduced the byssal-thread production by 50% during both light and dark phases. Oxygen uptake in *Perna viridis* decreased with increasing mercury levels, concentrations between 40 and 50 μg/l reducing uptake by 50–60%. The 120-hr LC_{50} for freshwater mussels, *Lamellidens marginalis*, was 2754–3311 μg/l Hg (Sivaramakishna *et al.*, 1991). Adult oysters, *Ostrea edulis*, were exposed to mercury and 50% died with 48 hr at 4200 μg/l (Conner, 1972). The 6–10 day LC_{50} for short-necked clams, *Venus japonica*, was 100–500 μg/l Hg (Irukayama, 1962). The 60-d LC_{50} for three species of bivalve molluscs of the Caspian Sea, *Mytilaster lineatus*, *Didacna trigonoides* and *Monodacna caspia*, was 5–10 μg/l Hg. Therefore, in this case, no significant differences between species were noted (Patin, 1982). Olson and Harrel (1973) found that 8700 μg/l mercury was needed to attain 96-hr LC_{50} of the estuarine clam, *Rangia cuneata*, a value considerably higher than that affecting other bivalve species. Therefore, in the present study, the hard clam, *Meretrix lusoria*, may be considered as an organism which is very sensitive to mercury poisoning.

The earlier developmental stages of marine biota have repeatedly been found to be more sensitive to environmental pollutants than their adult counterparts. The toxicity of mercuric chloride to the hard clam D-stage larvae at 28°C in 15 ppt salinity is shown in Fig. 3. Statistical analysis indicated that the average percentages of D-stage larvae had a decaying exponential relationship with mercury concentrations. The percentages of the clam embryos developing into D-stage larvae after 30 hr in controls, 2.5, 5.0, 10.0 and 20 μg/l Hg were 44 ± 6, 32 ± 3, 23 ± 4, 19 ± 6 and 3 ± 2%, respectively. All embryos exposed to 40 μg/l Hg and above died within 30 hr. In the control, 44% of hatched embryos developed into D-stage larvae within 30 hr, while those exposed to 20 μg/l Hg were still in the trochophore stage. Furthermore, most of the retarded larvae developed into abnormal forms. The 30-hr EC_{50} and their 95% confidence limits for the clam embryos were obtained as low as 4.6 and 3.9–5.3 μg/l Hg, respectively.

Wisely and Blick (1967) reported that when larvae of the mussel, *Mytilus edulis lanulatus*, and the oyster,

Table 3. Toxicity of mercury (as $HgCl_2$) to other bivalve molluscs

Organism	Water quality parameters	Length of μg/l Hg exposure (95% CL)	Authors
Argopecten irradians	pH 7.0–8.0	24-hr LC_{50} 370	Nelson *et al.* (1976)
Shell height	20 ± 1°C	48-hr LC_{50} 130	
20–30 mm	25 ± 2 ppt	72-hr LC_{50} 96	
		96-hr LC_{50} 89 (54–147)	
Argopecten irradians	°C × ppt		Nelson *et al.* (1977)
Shell height	15 × 15	96-hr LC_{50} 83 (78–89)	
16–27 mm	15 × 20	96-hr LC_{50} 118 (110–126)	
	15 × 25	96-hr LC_{50} 112 (105–120)	
	20 × 15	96-hr LC_{50} 78 (72–84)	
	20 × 20	96-hr LC_{50} 128 (119–138)	
	20 × 25	96-hr LC_{50} 122 (114–131)	
	25 × 15	96-hr LC_{50} 54 (48–60)	
	25 × 20	96-hr LC_{50} 132 (120–145)	
	25 × 25	96-hr LC_{50} 115 (105–126)	
Brachiodontes recurvus		2-hr LC_{50} 180,000	Wisely and Blick (1967)
Larvae	19–20°C		
	19–20 ppt		
Crassostrea gigas	27°C	Affected 32	Okubo and Okubo (1962)
Embryos		Not affected 10	
Crassostrea gigas	pH 8.1 ± 0.2	48-hr EC_{50} 6.7 ± 1.55	Martin *et al.* (1981)
Embryos	DO 6.5–8.0 mg/l		
	20 ± 1°C		
	33.7 ± 0.1 ppt		
Crassostrea gigas	pH 8.1 ± 0.2	48-hr EC_{50} 5.7	Glickstein (1978)
Embryos	20 ± 1°C		
	33.7 ± 0.1 ppt		
	DO 6.5–8.0 mg/l		
Crassostrea gigas		Morphological abnormalities 27	Woelke (1965)
Larvae			
Crassostrea virginica	26 ± 1°C	48-hr LC_{50} 5.6 (4.2–6.8)	Calabrese *et al.* (1973)
Embryos	25 ppt		
Crassostrea virginica		48-hr LC_{50} 10.2	MacInnes and Calabrese (1978)
Embryos			
Crassostrea virginica	pH 7.0–8.5	12-d LC_{50} 12.0 (3.3–20.7)	Calabrese *et al.* (1977)
Larvae	25 ± 1°C		
2 days old	24 ± 2 ppt		
Didacna trigonoides	60-d LC_{50} 5–10		Patin (1982)
Lamellidens marginalis	pH 7.6 ± 0.2	120-hr LC_{50} 2754–3311	Sivaramakrishna *et al.* (1991)
Weight	Total hardness 100 ± 5 mg/l CaCO		
15 ± 1 g	28 ± 1°C		
	DO 5.7 ± 0.4 mg/l		
	Freshwater		
Mercenaria mercenaria	pH 7.0–8.5	42–48-hr LC_{50} 4.8 (3.8–5.6)	Calabrese and Nelson (1974)
Embryos	26 ± 1°C		
	25 ppt		
Mercenearia mercenaria	pH 7.0–8.5	8–10-d LC_{50} 14.7 (4.0–25.4)	Calabrese *et al.* (1977)
Larvae	25 ± 1°C		
2 days old	24 ± 2 ppt		
Monodacna caspia		60-d LC_{50} 5–10	Patin (1982)
Mytilaster lineatus		60-d LC_{50} 5–10	Patin (1982)
Mytilus edulis planulatus	pH 7.8–8.2	2-hr LC_{50} 13,000	Wisely and Blick (1967)
Larvae	18 ± 2°C		
14 days old	19–20 ppt		
Mytilus edulis	13–17°C	Affected 32	Okubo and Okubo (1962)
Embryos		Not affected 10	
Mytilus edulis	pH 8.1 ± 0.2	48-hr EC_{50} 5.8 ± 1	Martin *et al.* (1981)
Embryos	DO 6.5–8.0 mg/l		
	17 ± 1°C		
	33.79 ± 0.07 ppt		
Mytilus edulis	Flow rate (1 l/min)	24-hr LC_{50} 25	Strömgren (1982)
Shell length	8.5–8.7°C	5-d EC_{50} 0.3–0.4	
12–29 mm	33.8 ppt	(For growth)	
Mytilus edulis		7-d LC_{50} 150	Martin *et al.* (1975)
Ostrea edulis	15°C		Conner (1972)
Larvae 1–3 days old		48-hr LC_{50} 1–3.3	
Adults		48-hr LC_{50} 4200	
Perna viridis	pH 8.15–8.30	48-hr LC_{50} 1000	Mohan *et al.* (1986)
Shell length	28 ± 1°C	60-hr LC_{50} 520	
20–25 mm		84-hr LC_{50} 230	
	(For byssal-threaded production)	EC_{50} 29–38	
	(For oxygen uptake)	EC_{50-60} 40–50	
Rangia cunneata	5.5 ppt	72-hr LC_{50} 20,000	Olson and Harrel (1973)
		96-hr LC_{50} 10,000	
	22 ppt	72-hr LC_{50} 9500	
		96-hr LC_{50} 8700	
Rangia cuneata	2 ppt	72-hr LC_{50} 242 (224–262)	Dillon (1977)
		96-hr LC_{50} 122 (114–130)	
	15 ppt	72-hr LC_{50} 57 (37–89)	
		96-hr LC_{50} 58 (50–68)	
Venus japonica		6–10-d LC_{50} 100–500	Irukayama *et al.* (1962)

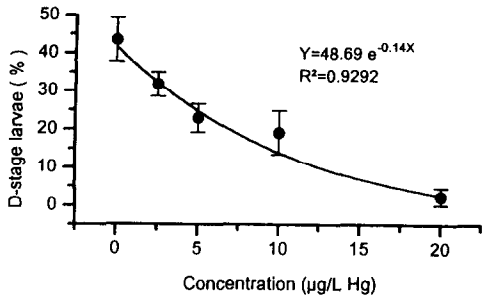

Fig. 3. The percentages of *Meretrix lusoria* embryos developing into D-stage larvae after 30 hr of exposure to mercury.

Crassostres commericalis, were exposed to mercury, 50% died within 2 hr at 13.0 and 180.5 mg/l, 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 tissues. These concentrations were considerably higher than those tested in the larval studies of *Mytilus edulis* (Okubo and Okubo, 1962; Martin *et al.*, 1981), *Mercenaria mercenaria* (Calabrese and Nelson, 1974; Calabrese *et al.*, 1977), *Crassostrea viginica* (Calabrese *et al.*, 1973; Calabrese and Nelson, 1974; MacInnes and Calabrese, 1978), *Crassostrea gigas* (Okubo and Okubo, 1962; Woelke, 1965; Glickstein, 1978; Martin *et al.*, 1981), *Ostrea edulis* (Conner, 1972) and *Merertrix lusoria* (the present study). This indicates that variation of mercury toxicity in bivalves depends on the species, developmental stage, animal size, adaptive ability, amount of accumulation, as well as environmental factors such as temperature, pH, and water hardness.

In assessing the environmental hazard of pollutants and protecting the natural aquatic resources, 'safe' concentrations have been estimated by multiplying the 96-hr LC_{50} by a factor of 0.1–0.01 as suggested by Sprague (1971) and FAO (1977). We calculated the safe level to be 1.9–3.9 μg/l Hg based on the 96-hr LC_{50} value of the juvenile, with an application factor of 0.01 (Sprague, 1971) in 30–10 ppt salinity. Environmental perturbations, which affect successful larval development, can potentially have serious detrimental effects on populations and ecosystems. Such effects would be subtle, causing decreased recruitment to exisiting populations. Bioassays, utilizing embryos and larvae, are potentially very sensitive tools for the identification of environmental stress. Goldberg (1963) reported that the average mercury concentration in unpolluted surface water was about 0.03 μg/l. FAO (1970) reported that surface seawater containing more than 0.2 μg/l Hg should be considered to be polluted with mercury. Therefore, we suggest that a more sensitive estimate of the 'safe level' be 0.046 (0.039–0.053) μg/l Hg, based on the most sensitive embryological stage, with an application factor of 0.01. This biologically safe level of mercury is similar to the levels of criterion (<0.05 μg/l Hg for freshwater organisms, <0.1 μg/l Hg for marine organisms) recommended by Klein *et al.* (1979).

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