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Effect of Mercuric Chloride on Fertilization and Larval Development in the River Frog, *Rana heckscheri* (Wright) (Anura: Ranidae)

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Previous investigations have indicated that heavy metals such as copper, cadmium, lead and mercury can act as systemic toxicants in many species of wildlife (Goyer 1986). Although numerous studies have emphasized the effects of metals and pesticides on metabolism, growth, survivorship, neural processes and reproduction in a number of taxa (see Clarkson et al. 1983 ; Punzo and Kirk 1992), little information is available on the effects of sublethal concentrations of metals on the reproductive physiology of amphibians (Punzo et al. 1979; Power et al.1989; Punzo 1993). Industrial processes and mining activities can release substantial concentrations of heavy metals such as mercury into aquatic habitats (Goyer 1986). Since most amphibians have obligate aquatic larval stages, they are exposed to pollutants discharged into the aquatic environment. Amphibians can act as accumulators of heavy metals (Hall and Mulhern 1984) and their larval stages are useful indicators of pollution levels in the field (Cooke 1981 ; Punzo 1993). What little data are available, indicate that metals can significantly reduce viability in amphibians through their actions on metabolism, development and gametogenesis (Byrne et al. 1975 ; Clarkson et al. 1983 ; Goyer 1986 ; Kanamadi and Saidapur 1991 ; Punzo 1993).

The recent concerns over worldwide declines in amphibian populations (Baringa 1990) and the susceptibility of amphibian populations to environmental toxicants (Punzo 1983 ; Power et al. 1989), led me to assess the effect of mercuric chloride, one of the most common and persistent toxicants in aquatic environments , on fertilization and larval development in the river frog, Rana heckscheri (Wright). Although there is some information on fish (Khan and Weis 1987), very little data are available on the effects of mercury on fertilization in amphibians generally (Kanamadi and Saidapur 1991), and no published data exist for R. heckscheri . This species is a conspicuous component of the aquatic fauna of parts of the southeastern United States (Punzo 1992) where mercury levels have increased significantly over the last two decades (Goyer 1986).

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MATERIALS AND METHODS

All animals used in this study were laboratory - reared offspring of frogs originally collected along the Aucilla River, in Lamont, Florida (Jefferson County), during the summer and fall of 1987. They were maintained in the laboratory as previously described by Punzo (1992). Adult males and females (36.4 g ± 2.1 SD ; 8.6 ± 1.3 cm SVL, N = 84) used in these experiments were kept in separate aquaria (40 L) to prevent mating and spawning as suggested by Dustman et al. (1970). Frogs were placed in a Percival Model I - 35 VL environmental chamber (Boone, Iowa) and maintained at 21° C on a 14L : 10D photoperiod regime. Guppies and earthworms were provided ad libitum as a food source. Mercuric chloride (98% purity) was obtained from Sigma Chemical Co. (St. Louis, Missouri). Dechlorinated tap water (DTW) was used as exposure / control water for all experiments. Exposure water was examined at 24-hr intervals using appropriate chemical tests (Hach Chemical Company, St. Louis, Missouri) and atomic absorption analysis to determine and monitor water quality, mercuric chloride concentrations and pH as previously described by Punzo (1993). Water quality parameters of exposure water were as follows (Mean ± SD of the measurements, N = 15) : pH 7.23 ± 0.16 ; conductivity, 721 ± 30.4 umhos ; total hardness, 351.3 ± 14.7 mg / L as $CaCO_3$; total alkalinity , 280.3 ± 20.1 mg / L as $CaCO_3$; nitrate, 0.77 ± 0.12 mg / L ; nitrite , 0.009 ± 0.001 mg / L ; ammonia , 0.37 ± 0.02 mg / L ; calcium , 84.8 ± 4.8 mg / L ; magnesium , 31.2 ± 1.8 mg / L ; and copper , 0.003 ± 0.0001 mg / L . Mercuric chloride concentrations were determined and regularly monitored following each experiment by comparison with aqueous standards using a Perkin - Elmer 5000 atomic absorption spectrophotomter with a fuel-lean air acetylene flame as described by Punzo (1993). Acidity was recorded with a Beckman 110L pH meter.

Eggs were obtained by the injection of pituitary extracts according to the method described by Johnson and Volpe (1973). Male frogs were pithed and their testes removed as described by Wilt and Wessells (1967) to obtain sperm. Testes were gently crushed in a chilled mortar and pestle containing 10 ml of amphibian Ringer's solution. Sperm become active within 4 min after their release from testicular tissue.

A series of experiments were conducted to determine the effect of various concentrations of mercuric chloride on fertilization. All experiments were conducted at 21 ± 0.5 ° C . Each experimental condition used the sperm and eggs from a single male and female, respectively, and replicated five times as described by Khan and Weis (1987). The eggs and sperm were placed in glass finger bowls containing 1.0 L of dechlorinated tap water (DTW) with mercuric chloride concentrations of : 0 , 0.5 , 1.0 , 2.5 , or 5.0 mg / L (ppm) (98% purity). Sperm and egg suspensions were allowed to interact for three hr. Following this period, eggs were washed with DTW and observed under a Unitron dissecting

scope. Cleavage was used as the criterion that fertilization had taken place (Johnson and Volpe 1973). Percent fertilization was determined for each experiment at all test conditions (Table 1). Data were analyzed with Chi Square contingency analysis procedures utilizing Yate's correction for discontinuity (Sokal and Rohlf 1981).

Additional experiments were conducted on fertilized eggs which were allowed to develop for 2 - 3 wk exposed to the various concentrations of mercuric chloride previously described. During this exposure period, embryos at various stages (Table 2) were examined for developmental abnormalities as described by Cooke (1981) : DCT / LCT (downward / lateral curvature of tail) ; LDBT (lateral deflection at base of tail) ; LKBT (lateral kink at base of tail) ; UCT (upward curvature of tail) ; VKTT (ventral kink at tip of tail) . Stage 27 tadpoles (Gosner 1960) were tested for acute responses to mercuric chloride. For these experiments a total of 70 - 90 tadpoles were exposed to 0 , 0.025 , 0.05 . 0.075 , 0.20 , 0.25 , 0.50 or 1.0 mg / L of mercuric chloride for 96 hr. Toxicity tests were run on groups of five tadpoles placed in finger bowls containing 1.0 L of the test solution according to the procedure of Khan and Weis (1987). Animals were not fed during toxicity tests, but they were checked daily and dead animals were removed. LC_{50} values were determined using probit analysis (Litchfield and Wilcoxin 1949) and chi square (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

Probit analysis yielded an LC_{50} value (3 hr) of 1.43 mg / L (X^2 = 12.4, 95% confidence limits of 1.24 - 1.52) for R. heckscheri eggs , and a 96 - hr LC_{50} value of 0.68 mg / L (X^2 = 14.2 , 95% confidence limits of 0.47 - 0.71) for stage 27 tadpoles. Mercuric chloride concentrations found in water samples taken from the original collection sites ranged from 0.45 - 0.57 mg / L over a two - year period. However, several areas in Florida have reported water levels with concentrations as high as 1.12 mg / L (Punzo 1993).

Exposure of gametes to 0.5 mg / L mercuric chloride for 3 hr had no adverse effect on fertilization success (90% , Table 1). At 1.0 mg / L , fertilization success (69%) decreased significantly (X^2 = 11.7 , $P < 0.01$). A further significant decrease (27%) was observed at 2.5 mg / L (X^2 = 19.6 , $P < 0.01$). At 5.0 mg / L , fertilization was completely blocked in this species. Mercury impairs the development of oocytes in oviduct tissues in adult R. heckscheri (Punzo 1993) and R. cyanophlyctis (Kanamadi and Saidapur 1991), as well as mammals (Clarkson et al. 1983). Thus, it is clear that the reproductive processes of various developmental stages of R. heckscheri as well as its overall fitness can be significantly impaired by the presence of mercury in the environment. The effects of exposure to mercuric chloride during the first three weeks of development are shown in Table 2. Exposure to 0.5 mg / L had no observable adverse effect on development as compared to control frogs.

Table 1 . Effect of mercuric chloride on percent fertilization success of Rana heckscheri egs .

Mercuric Chloride (mg / L)	Total Number of Eggs	Number Fertilized	Percent Fertilized
0	1073	990	92 a
0.5	718	647	90 a
1.0	924	639	69 b
2.5	816	222	27 c
5.0	857	0	0

Values followed by different letters are significantly different ($P < 0.01$) , using a chi square analysis (Sokal and Rohl , 1981) .

At concentrations $\geq$ 1.0 mg / L , a range known to occur in sediments associated with ephemeral ponds adjacent to incinerators and landfills (Goyer 1986), significant developmental abnormalities were observed (X^2 , $P < 0.05$). The most commonly observed developmental defects were LKBT and UCT. This agrees with known effects of various heavy metals on embryonic development in anurans (Cooke 1981). The mortality rates for R. heckscheri embryos exhibiting deformities of any kind were generally high (47 - 83%). Only 1 of the 50 embryos showing teratogenic effects (2 %) exhibited more than one type of abnormality. It should also be pointed out that the snout deformities reported for some anuran embryos (Bufo bufo , Rana pipiens , R. temporaria , Xenopus laevis) exposed to lead, chlorinated hydrocarbon pesticides and tetrazine herbicides (see Cooke 1981) , were not observed in the present study. In addition, later developmental stages (27 - 35) showed significantly higher incidences of DCT, LCT , LKBT and VKTT deformities (X^2 , $P < 0.05$) when exposed to 2.5 mg / L than did the younger stages (22 - 26, Table 2). There is some evidence to support the notion that tolerance to metals is higher in earlier life cycle stages. Khan and Weis (1987) showed that eggs of the killifish, Fundulus heteroclitus , were more tolerant of methymercury than were juvenile fish. This is most interesting in light of the fact that the younger developmental stages of anurans, fish and some aquatic insect larvae have been shown to be less tolerant to other types of pollutants such as acid rain (Punzo 1983 ; Punzo and Thompson 1990) and some pesticides (Cooke 1981 ; Matsumura 1985). The higher tolerance toward exposure to mercury exhibited by the eggs of R. heckscheri (see LC_{50} values reported above), as well the eggs of a few other anuran species exposed to mercury and other heavy metals (Dustman et al . 1970 ; Clarkson et al . 1983 ; Goyer 1986 ; Power et al . 1989), suggests that the thick, jelly envelope which encloses the egg acts to impede the penetration of metal

Table 2. Developmental abnormalities in Rana heckscheri as a result of exposure to mercuric chloride. N = 200 for each test condition. DCT / LCT (downward / lateral tail curvature); LDBT (lateral deflection at base of tail); LKBT (lateral kink at base of tail); UCT (upward curvature of tail); VKTT (ventral kink at tip of tail).

Mercuric Chloride (mg / L)	Developmental Stage	Type of Abnormality	Percent Occurrence
0	22 - 26	L K B T	0.5 a
	27 - 35	----------	0
0.5	22 - 26	L K B T	1.5 a
		U C T	2.0 a
	27 - 35	L C T	2.5 a
		U C T	1.5 a
1.0	22 - 26	L C T	4.5 b
		L K B T	5.5 b
		L D B T	1.5 a
		V K T T	4.5 b
	27 - 35	D C T	4.5 b
		L C T	4.5 b
		L K B T	6.5 b
		U C T	5.0 b
2.5	22 - 26	D C T	4.5 b
		L C T	12.5 c
		L D B T	9.5 c
		L K B T	20.0 d
		U C T	12.5 c
		V K T T	5.0 b
	27 - 35	D C T	12.5 c
		L C T	5.0 b
		L D B T	4.5 b
		L K B T	34.0 d
		U C T	13.5 c
		V K T T	12.5 c
5.0	All Dead		

toxicants into the egg. Future studies could focus on the rate of penetration of various toxicants into the egg as compared to other developmental stages.

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