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Variable Sensitivity of Rainbow Trout (*Salmo gairdneri*) Eggs and Alevins to Heavy Metals

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It is well known that mammalian, including human, embryos have periods of particular sensitivity known as critical periods when their susceptibility to foreign compounds is maximal (Wilson 1972). In the case of fish, data from an extensive series of life cycle toxicity tests (McKim 1977) have indicated that the embryo-larval and early juvenile stages are generally more susceptible to toxicants than later stages and most workers agree that, of these early stages, the egg itself is more resistant than hatched sac-fry or alevins (Eaton *et al* 1978; Rombough and Garside 1982; Servisi and Martens 1978; Skidmore 1965). For example eggs of king salmon *Oncorhynchus tshawytscha* hatched normally in 0.08 mg Cu L^{-1} although fry died when exposed to 0.04 mg Cu L^{-1} (Hazel and Meith 1970). In contrast Grande (1967) observed that eggs were more sensitive to zinc than the fry of trout *Salmo gairdneri* and salmon *Salmo salar*. Blaylock and Frank (1979) made similar observations for the response of carp, *Cyprinus carpio* eggs and larvae to nickel. However, since most work on fish eggs has involved continuous exposure to the pollutant until hatching either from fertilization (Rombough and Garside 1982; Westernhagen *et al* 1975) or from the eyed stage (Beattie and Pascoe 1978; Giles and Klaverkamp 1982; Rombough and Garside 1982; Woodworth and Pascoe 1982), it has not been possible to record accurately the actual stages of development at which the egg is most sensitive. In an attempt to overcome this problem and to detect any critical periods, the toxicity of three common heavy metal pollutants was examined at six different embryonic and two alevin stages of development.

MATERIALS AND METHODS

Freshly fertilized rainbow trout eggs were incubated in egg baskets in a 300-L tank in total darkness at 7.5-9.5°C. The tank was constantly supplied with dechlorinated aerated Cardiff tap water. Cadmium stock solutions were prepared with reagent grade cadmium chloride ($CdCl_2$ $2\cdot5\,H_2O$), copper with cupric sulphate ($CuSO_4.\,5H_2O$) and zinc with zinc sulphate ($ZnSO_4.\,7H_2O$). The metal concentration range used in tests with eggs and alevins was based on preliminary tests and chosen so as to cause a range of mortalities from 0-100%

Table 1 Stage of development reached by rainbow trout eggs incubated at 7.5-9.5°C for various lengths of time.

Number of days post-fertilization	Stage of Development
5	Blastodisc
10	Enlarged embryonic shield
15	Tip of embryonic tail formed; optic lobes are small; heart tube not visible at start of test but heart beat seen within 2 days.
22	Vigorous movements of tail and body; slight eye pigmentation; pectoral fin buds visible.
29	Well developed embryo; beating pectoral fins.
36	Fully developed embryos.

within 96 hours. One control tank was included for each metal in all tests, which were performed in 4-L tanks. Nominal metal concentrations (Table 2) and water quality throughout the tests were monitored using standard analytical procedures (Beattie and Pascoe 1978). Total hardness was 87.7 $\pm$ 15.3 mg L^{-1} as $CaCO_3$; temperature 8.6 $\pm$ 0.7°C; pH 7.8 $\pm$ 0.2; dissolved oxygen 99.8 $\pm$ 0.7% air saturation value and conductivity 137 $\pm$ 24 μmhos cm^{-1}. Concentrations of cadmium, copper and zinc in the control tanks were 0.002 $\pm$ 0.001, 0.006 $\pm$ 0.002 and 0.013 $\pm$ 0.005 mg L^{-1} respectively. These represent the mean and standard deviation of >110 determinations. Toxicity tests were carried out in total darkness, with batches of 20 eggs at 5, 10, 15, 22, 29 and 36 days post-fertilization and 20 alevins at 2 and 7 days post hatch. The main characteristics (Table 1) of egg development at each stage were examined microscopically on further representative samples of 20 eggs randomly removed from the stock tank at the same times as the test eggs. During the test, dead (opaque) eggs were recorded and removed. In some cases, particularly with zinc, only the embryo body itself became opaque and death could only be confirmed, at the later stages of development, by microscopic observation for lack of heart beat. A similar confirmatory examination was made in the case of alevins. Each test was terminated after 96 hours and the median lethal time (LT50) and slope function (S) calculated by the method of Litchfield (1949). Results are shown in Table 2: confidence limits were calculated but are not included.

RESULTS AND DISCUSSION

Eggs and alevins died during the tests with the LT50 decreasing with increasing concentration for all three toxicants. In order to compare the sensitivity of each developmental stage an estimate of the 48 hr LC50 (Table 3) was obtained from toxicity curves relating LT50 and test concentration. In some cases it was necessary to extrapolate the curve in order to obtain an approximate value.

Table 2 The median lethal time (LT50) and slope function (S) for rainbow trout at various stages of egg and alevin development, when exposed to solutions of cadmium, copper or zinc. Where mortality was insufficient to allow an LT50 calculation the actual percentage mortality recorded in the test is given.

EGGS - number of days post-fertilization		5			10			15			22		
	Nom. Conc. mg L^{-1}	Recorded Conc. mg L^{-1}	LT50 mins	S	Recorded Conc. mg L^{-1}	LT50 mins	S	Recorded Conc. mg L^{-1}	LT50 mins	S	Recorded Conc. mg L^{-1}	LT50 mins	S
CADMIUM													
	1.8	1.79	9600	1.1	1.88	3740	1.3	1.82	No mortality		1.83	40% mortality	
	3.2												
	5.6	5.61	9600	1.3	5.75	2330	1.2	5.32	3675	1.2	5.73	3310	1.4
	10.0	9.62	6900	1.2	11.2	1910	1.3	9.49	2110	1.4	9.98	2670	1.3
	18.0	17.7	5600	1.6	18.5	1530	1.2	17.8	1320	1.6	18.7	2260	1.5
COPPER													
	0.32	0.28	45% mortality		0.31	10% mortality		0.26	25% mortality		0.29	7800	1.3
	1.0	0.96	5850	1.0	0.92	35% mortality		0.76	1250	1.3	0.97	1250	1.8
	1.8	1.44	5320	1.1	1.75	3200	1.2	1.10	840	1.3	1.55	780	1.5
	3.2	1.81	4850	1.1	2.25	2630	1.2	1.20	560	1.3	2.94	390	2.3
ZINC													
	3.2							2.08	No mortality		4.41	No mortality	
	10.0	10.1	5130	1.8	11.1	690	1.9	10.5	2350	4.0	11.7	2450	1.7
	18.0	19.3	3300	1.5	18.2	670	1.6	18.1	1100	1.9	20.1	1810	1.3
	24.0	24.6	2750	1.3	26.5	640	2.1	24.5	1610	2.0	24.4	1500	1.2
	32.0	32.5	2560	1.3	35.5	500	2.3	32.9	820	2.1	32.4	1370	1.3

Table 2 (Cont'd.)

EGGS - number of days post-fertilization		29			36			Alevins (2d post hatch)			Alevins (7d post hatch)		
	Nom. Conc. mg L^{-1}	Recorded Conc. mg L^{-1}	LT50 mins	S	Recorded Conc. mg L^{-1}	LT50 mins	S	Recorded Conc. mg L^{-1}	LT50 mins	S	Recorded Conc. mg L^{-1}	LT50 mins	S
CADMIUM													
	1.0	0.98	No mortality		0.88	No mortality		0.97	No mortality		0.99	No mortality	
	1.8	1.97	No mortality		1.77	No mortality		1.75	7800	1.5	1.97	No mortality	
	3.2							3.15	4450	1.4			
	5.6	5.96	6800	1.2	5.35	10% mortality		5.85	3150	1.4	5.96	3600	1.4
	10.0	9.52	3450	1.3	9.76	4280	1.2	11.2	1910	1.2	9.52	2900	1.5
	18.0	18.2	2040	1.3	18.2	2750	1.2				18.2	1020	1.4
COPPER													
	0.01							0.02	No mortality				
	0.032							0.04	No mortality				
	0.1	0.09	No mortality		0.08	No mortality		0.09	2650	1.5	0.09	3150	1.6
	0.32	0.31	4000	1.5	0.27	2010	1.3	0.29	660	1.2	0.31	675	1.4
	1.0	0.93	1220	1.3	0.92	1050	1.1	0.93	295	1.2	0.92	339	1.1
	1.8	1.33	850	1.2	1.81	805	1.2				1.33	260	1.1
	3.2	1.76	845	1.1	3.16	745	1.3				1.76	100% mortality in 220 minutes	
ZINC													
	0.32							0.49	No mortality				
	1.0	1.04	No mortality					0.98	No mortality		1.04	5% mortality	
	1.8							1.95	7600	1.5			
	3.2	3.90	No mortality		3.05	No mortality		3.35	1860	1.2	3.90	4150	2.3
	10.0	10.6	1775	1.4	10.5	3100	1.3	11.2	635	1.3	10.6	710	1.6
	18.0	21.8	970	1.2	19.7	1060	1.2				21.8	560	1.2
	24.0	27.7	840	1.2	29.4	895	1.3				27.7	550	1.3
	32.0	34.5	745	1.3	35.2	662	1.2				34.5	375	1.1

It is clear that the blastodisc stage, 5 days post-fertilization (at this test temperature) was markedly more resistant to all three metals than any other developmental stage. This was followed by an increase in sensitivity. In the case of cadmium and zinc, the stage at which the embryonic axis becomes clear (10 days) was the most sensitive but with copper sensitivity increased throughout embryonic development. The next major change occurred after hatching when, with both cadmium and zinc, alevins were more sensitive than eggs immediately prior to hatch. With copper there was no such change.

The results with cadmium and zinc, though not copper, confirm the observations of most workers (Eaton *et al.* 1978; Rombough and Garside 1982; Skidmore 1965) that the egg is typically more resistant to pollutants than the hatched alevin. This is probably due to a protective effect of the chorion preventing free passage of pollutant to the embryo (Beattie and Pascoe 1978; Rosenthal and Sperling 1974; Westernhagen and Dethlefsen 1975) rather than to a change in sensitivity of the animal before and after hatching.

However, since the structure and physiology of the egg itself does constantly change as development progresses it is to be expected that the response to environmental changes, including pollutants, will also change. Evidence relating to this is somewhat conflicting. Skidmore(1965) demonstrated that newly laid zebra fish,*Brachydanio rerio* eggs were highly resistant to zinc but became more sensitive as development progressed while Benoit and Holcombe quoted by McKim (1977), in contrast, found that the effects of zinc were greatest when fathead minnow (*Pimephales promelas*) eggs were exposed before water hardening had occurred.

One of the most comprehensive investigations was carried out by Stoss and Haines (1979) who examined the effects of toluene on medaka, *Oryzias latipes* eggs. Exposure during water hardening and cleavage was lethal while eggs tested during the onset of gastrulation and organogenesis suffered from severe structural abnormalities. As organogenesis advanced, the teratogenic affect declined. A second period of high mortality was correlated with the increasing metabolic demands in the fully developed medaka embryo.

The present investigation confirms that embryo sensitivity is not uniform throughout development, being dependent on the stage of development and the metal to which it is exposed. The blastodisc stage was far more resistant to cadmium, copper and zinc than other stages. This was also noted by Rombough and Garside (1982) in their studies of cadmium toxicity to Atlantic salmon although Mori (1979) found that eggs of *Carassius auratus* were more sensitive to cadmium and mercury at the blastodisc stage than at the eyed stage. In the current work,the stage most sensitive to cadmium and zinc was ten days post fertilization during development of the embryonic axis. Rombough and Garside (1982) similarly observed peak mortality during gastrulation and axiation as well as during development of the vitelline circulation and shortly before hatching.

Table 3 Estimated 48 hr LC50 (mg L^{-1}) for eggs and alevins exposed to cadmium, zinc or copper at various stages of development

Stage of egg development: days post-fertilization	48-hr LC50 Cadmium	48-hr LC50 Zinc	48-hr LC50 Copper
5	>100.0*	24	8.0*
10	3.3	<1.0*	2.0
15	7.2	9.1	0.4
22	8.0	7.0	0.6
29	12.5	4.3	0.4
36	16.5	9.2	0.1
Alevins			
Days post-hatch			
2	5.8	3.2	<0.1
7	8.3	3.4	0.1

* values obtained by extrapolation of toxicity curves.

The pattern of egg sensitivity to copper was somewhat different and although (as with zinc and cadmium) eggs at the blastodisc stage were most resistant, sensitivity then increased gradually with embryonic development until hatching. This difference in sensitivity of eggs to copper, compared with cadmium and zinc, suggests that copper may have a different target tissue or mode of action as has been proposed by some workers for adult fish (Bilinski and Jonas 1973). In the present investigation trout eggs were exposed to metals at specific stages of development and thus the recorded toxicity reflects the actual sensitivity at these stages of embryonic development. This contrasts with continuous exposure experiments, as many previous investigations have been, which may not reflect the sensitivity of each stage since a toxic effect could have been initiated early in development only to be detected later.

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