

THE INFLUENCE OF THE ZONA RADIATA ON THE TOXICITIES OF ZINC, LEAD, MERCURY, COPPER AND SILVER IONS TO EMBRYOS OF STEELHEAD TROUT *SALMO GAIRDNERI*

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(Received 22 January 1985)

Abstract—1. Eyed embryos of the steelhead trout (*Salmo gairdneri*) were significantly more resistant to zinc and lead but significantly less resistant to mercury, copper and silver if the zona radiata (egg capsule) was removed than if it was intact.

2. The zona radiata appears to act as a cation exchanger and inhibits metals with high binding constants (Hg^{2+} , Cu^{2+} , Ag^+) from entering the perivitelline fluid.

3. Metals with low binding constants (Zn^{2+} , Pb^{2+} , Cd^{2+}) rapidly penetrate the zona radiata and accumulate in the perivitelline fluid according to the Donnan equilibrium.

INTRODUCTION

The zona radiata (capsule) of fish eggs has the potential of modulating the toxicities of metals since the metal ions must penetrate the zona radiata before they can directly affect the embryo. Skidmore (1966) reported that the embryo of the zebrafish (*Brachydanio rerio*) was significantly more resistant to zinc if the zona radiata was ruptured or removed than if it was intact. Rombough and Garside (1983) demonstrated that embryos of the Atlantic salmon (*Salmo salar*), similarly, were significantly more resistant to cadmium with the zona radiata removed than with the zona radiata intact. However, the zona radiata may not have the same effect on all metals. Ozoh (1980) suggested that decapsulated zebrafish embryos were more sensitive to copper than were encapsulated embryos. In the current study, I examined the influence of the zona radiata on the toxicities of zinc, lead, copper, mercury and silver ions to embryos of the steelhead trout (*Salmo gairdneri*).

MATERIALS AND METHODS

Newly fertilized eggs of the steelhead trout *Salmo gairdneri* were obtained from the Alsea River Hatchery, Oregon. Eggs with a mean (\pm SD) water hardened wet wt of 163 ± 9 mg ($N = 20$) were incubated in vertically stacked trays at 5°C until about 3 weeks prior to hatch.

A series of 7-day bioassays were conducted to assess the toxicity of Zn, Pb, Cu, Hg and Ag ions to encapsulated and decapsulated embryos at the late eyed stage (Stage 18; Garside, 1959). Static exposures to the various metal solutions were conducted in virtual darkness at 5°C in a series of 1-l. polyurethane containers. Test animals were placed in fiberglass screens suspended at mid-depth in the containers and a circulation of water past the embryos was maintained by constant aeration. Stock solutions of 1 g/l of Zn, Pb, Cu, Hg and Ag were prepared using reagent grade $\text{Zn}(\text{NO}_3)_2$, $\text{Pb}(\text{NO}_3)_2$, CuCl_2 , HgCl_2 and AgCl and distilled water. Appropriate dilutions were made using dechlorinated water from the Newport, Oregon domestic supply. Total

hardness of the dilution water was 30 mg/l (as CaCO_3) and pH levels ranged from 6.8 to 7.2.

Eggs were divided into two groups and the zona radiata of each egg in one group was carefully removed with iridectomy scissors and fine forceps. The decapsulated embryos were held overnight and any embryos showing injury caused by the procedure were removed. Ten embryos with the zona radiata intact and 10 with the zona radiata removed were placed in each toxicant concentration. Embryos were inspected after 12 and 24 hr and then once a day for the remainder of the experiment. Dead individuals were removed at each inspection and the time of death recorded. The criterion of death was cessation of heart beat. Median resistance times were estimated by inspection of plots of probit percentage mortality vs log time (Bliss, 1937). Significant differences in median resistance times of encapsulated and decapsulated embryos were assessed by analysis of variance.

RESULTS AND DISCUSSION

Metals were ranked $\text{Zn} < \text{Pb} < (\text{Cu}, \text{Hg}) < \text{Ag}$ in order of increasing toxicity to steelhead embryos on a molar concentration basis (Table 1). This is essentially the same order that Jones (1939) observed for the juvenile stickleback (*Gasterosteus aculeatus*). Jones (1939) related the acute toxicity of the various metals to their solution pressures. Danelli (1944) suggested that electronegativity was a more appropriate correlate. The theoretical basis for this relationship has been questioned (Hewitt and Nicholas, 1963; Kaiser, 1980) but a significantly better correlation between toxicity in fish and physico-chemical parameters remains to be advanced.

Steelhead embryos were significantly ($P < 0.01$) more resistant to zinc and lead but significantly ($P < 0.01$) less resistant to mercury, copper and silver with the zona radiata removed than with the zona radiata intact (Table 1). Previous experiments have demonstrated that removal of the zona radiata reduced the toxicity of zinc (Skidmore, 1966) and cadmium (Rombough and Garside, 1983) to fish

Table 1. Median resistance times (hr) for steelhead embryos exposed to various concentrations of zinc, lead, mercury, copper and silver with their zonae radiatae intact (+ZR) and with their zonae radiatae removed (-ZR)

Zinc			Lead			Mercury			Copper			Silver		
[Zn ²⁺] (mg/l)	Median resistance time (hr)		[Pb ²⁺] (mg/l)	Median resistance time (hr)		[Hg ²⁺] (mg/l)	Median resistance time (hr)		[Cu ²⁺] (mg/l)	Median resistance time (hr)		[Ag ⁺] (mg/l)	Median resistance time (hr)	
	+ZR	-ZR		+ZR	-ZR		+ZR	-ZR		+ZR	-ZR		+ZR	-ZR
14	10	14	20	20	20	10	11	< 6	1.0	18	< 5	0.5	35	12
13	9	18	18	11	36	8	11	< 6	1.0	13	< 5	0.4	44	12
12	20	36	17.5	21	41	6	20	< 6	0.75	13	< 5	0.3	35	14
11	18	30	17	24	47	4	36	< 6	0.5	14	11.5	0.2	48	12
10	11	42	16	20	36	2	36	11	0.25	35	11.5	0.1	56	26
10	25	32	15	35	50	1	113	17	0.2	57	12	0.1	66	20
9	18	58	14	20	48	0.5	> 168	17	0.1	56	11.5	0.05	> 168	58
8	20	70	12.5	35	62	0.1	> 168	> 168	0.05	> 168	> 168	0.01	> 168	> 168
6	36	> 168	10	56	> 168									
2	> 168	> 168												

embryos. Ozoh (1980) reported that removal of the zona radiata increased copper toxicity but provided no supporting evidence. The current study confirms that copper is indeed more toxic to decapsulated embryos. The influence of the zona radiata on the toxicity of lead, mercury and silver has not been examined explicitly in previous studies. However, Davies *et al.* (1976) reported that rainbow trout (*Salmo gairdneri*) embryos were more sensitive to lead than were newly hatched alevins. On the other hand, *Oryzias latipes* was more sensitive to organic mercuric compounds immediately after hatch than immediately before hatch (Akiyama, 1970). Rainbow trout alevins died soon after hatch when incubated in silver solutions continuously from fertilization (Davies *et al.*, 1978). These comparisons of sensitivities of embryos and larvae do not provide sufficient evidence in themselves to assess whether the zona radiata enhances or reduces toxicity because of the variability in exposure times and differences in developmental stages. However, they are consistent with, and tend to confirm, my observations that the zona radiata enhances the toxicity of lead but reduces the toxicity of mercury and silver.

Rombough and Garside (1983) attributed the greater sensitivity to cadmium and zinc of encapsulated embryos to enhanced uptake. They observed that encapsulated Atlantic salmon embryos accumulated cadmium more rapidly and had a significantly greater body burden at death than did embryos with the zona radiata removed. Rombough and Garside (1983) hypothesized that cadmium concentrations in the perivitelline fluid were greater than those in the ambient environment because of the Donnan effect and that this resulted in increased uptake by the encapsulated embryo. Support for this hypothesis was provided by Wedemeyer's (1968) observation that zinc concentration in the perivitelline fluid of coho (*Oncorhynchus kisutch*) eggs was almost an order of magnitude greater than that in the ambient environment. Peterson (1984) has shown recently that H⁺, Ca²⁺ and Mg²⁺ also accumulate in the perivitelline fluid in accordance with the Donnan equilibrium. The Donnan effect provides a reasonable explanation of why Zn²⁺, Cd²⁺, and Pb²⁺ are more toxic to encapsulated embryos than to naked embryos. However, according to the Donnan equilibrium Hg²⁺, Cu²⁺ and Ag⁺ also should accumulate in the perivitelline fluid to concentrations greater

than those in the ambient environment. The fact that encapsulated embryos were more resistant to Hg²⁺, Cu²⁺ and Ag⁺ than decapsulated embryos suggests that the zona radiata somehow inhibits entry of these ions into the perivitelline fluid.

The zona radiata appears to act like a nonspecific ion exchanger (Dabrowski *et al.*, 1975; von Westernhagen and Dethlefsen, 1975; Kunze *et al.*, 1978). Radiotracer studies have shown that metal cations bind firmly to the zona radiata and cannot be leached by distilled water but will exchange readily with their nonradioactive counterparts (Wedemeyer, 1968; Dabrowski *et al.*, 1975). The zona radiata is rich in negatively charged glutamic acid (Ohzu and Kura, 1981; Kobayashi, 1982). Cations can be expected to bind selectively to such anionic sites and, according to ion exchange theory (Samuelson, 1956), those cations that bind most tightly will tend to move most slowly through the zona radiata. Binding coefficients for various cations with the zona radiata have not been determined. However, Danelli (1944) proposed that the standard electrode potential could be regarded as a measure of the equilibrium constant for the reaction between a metal and surface anions. According to Danelli (1944), the more positive the potential the more tightly a cation will bind to surface anions. Hg²⁺, Cu²⁺ and Ag⁺ all have positive standard electrode potentials (0.796, 0.340 and 0.800 V, respectively) and can be expected to bind relatively firmly to the zona radiata. This will tend to inhibit their movement through the capsule and may be the reason why encapsulated embryos were more resistant to these metals than were decapsulated embryos. Zn²⁺, Cd²⁺ and Pb²⁺, on the other hand, all have negative standard electrode potentials (-0.763, -0.403 and -0.126 V, respectively) and can be expected to penetrate the zona radiata rapidly.

Preferential binding to the zona radiata similarly can be invoked to explain the complex uptake patterns observed when eggs are exposed to metal mixtures (von Westernhagen *et al.*, 1979). However, standard electrode potentials are probably only rough approximations of actual binding constants and the interactions between the zona radiata and metals are undoubtedly more complicated than I have suggested. Determination of actual metal binding constants for the zona radiata appears to be a prerequisite for a better understanding of its role in the mineral economy of the egg.

Acknowledgement—This work was supported by a grant from the U.S. National Marine Fisheries Services.

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