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Uptake of Cadmium by Eggs and Alevins of Atlantic Salmon (*Salmo salar*) as Influenced by Acidic Conditions

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The long range transport of air pollutants (LRTAP) has become an important environmental issue within the last 10 to 15 yr (Cowling 1982; Last et al. 1980). Studies of the impacts of LRTAP on fish physiology have been focussed on two problems - that of increased hydrogen ion concentration, and that of increased metal solubility at low pH. Metals which may be affecting fish health and reproduction may be derived from soils and bedrock of the drainage system, through leaching by the acidic precipitation, or may be codeposited as a component of LRTAP. Cadmium is an example of a metal in the latter group. Cd distribution in the ecosystem and its availability to fish may also be altered as the result of acidification.

The toxicity of Cd to salmonid eggs and alevins, its uptake by them and distribution in them have been the subject of several studies (Beattie and Pascoe 1978; Woodworth and Pascoe 1982; Rombough and Garside 1982; Peterson et al. 1983), which indicate that the chorion is the primary site of Cd accumulation in the salmonid egg. The chorion thereby affords some protection to the embryo by acting as a shield and reducing embryonic Cd uptake. Over 90% of the Cd accumulated by the egg may be shed with the chorion at hatching. Accordingly, it has been found that newly hatched alevins are less tolerant to Cd exposure than are unhatched embryos (Beattie and Pascoe 1978). Conversely, Rombough and Garside (1982) found unhatched embryos to be more sensitive and attributed this to precipitation of $CdCO_3$ in the perivitelline space. This precipitate was thought to interfere with gas exchange.

In a study of Zn absorption onto the salmonid chorion (Wedemeyer 1968), it was suggested that the Zn^{++} ions were bound to anionic sites of the chorion. Lowering the incubation pH could affect heavy metal binding to the chorion by neutralization of these anionic sites. It is important, in view of the acidification problem, to know the pH range at which chorionic binding of metals is reduced and whether metal uptake by the embryo is affected. In this study we have examined the uptake of Cd by chorion, embryo and yolk of Atlantic salmon eggs and of alevins resulting from three levels of Cd exposure, at each of four incubation pH's.

MATERIALS AND METHODS

Eggs were stripped from a female salmon on November 23, 1978 and fertilized with the milt from two males of the same stock. The eggs were allowed to water harden for 2 h before introduction to the experimental treatments. One hundred, twenty-five eggs were placed in each of 20 cylindrical plastic containers with 1 L of test solution. A second plastic container with a 1-mm plastic mesh floor was nested within each of the above containers to hold the eggs. The containers were covered and the test solutions were aerated, using glass pipets passed through a hole in the lid of each container. During each daily change of test solutions, the interior containers were raised gently from the old solution then slowly lowered into a container with fresh solution maintained at the same temperature. The interior containers were discarded after hatching was completed. Alevins were transferred to new solutions by decanting the old solution, then slipping the alevins into containers with fresh solution. Temperature of the test solutions was maintained at that of the ambient water supply in a running water bath. Experiments were terminated after completion of hatching in all containers at 99 d post-fertilization.

Sixteen treatments were tested consisting of all possible combinations of four pH levels (4.5, 5.0, 5.5 and 6.5) and four Cd concentrations (0, 1, 2, and 5 ng/mL). Stock solutions of pH 5.5, 5.0, and 4.5 were prepared by adding 1% nitric acid (Aristar grade) to filtered, dechlorinated tapwater, while unacidified tapwater at pH 6.5 served as the control stock solution. The acidified solutions were aerated 24 h prior to use to drive off CO_2 evolved through acidification. Concentrations of Na, Cl, Ca, Mg, K, and SO_4 in the water supply were 100, 100, 38, 15, 10, and 20 μ Mol, respectively. The bicarbonate level in unacidified water was 100 μ Mol. A Cd stock solution of 2.0 μgCd/mL (as $CdCl_2$) was prepared in deionized water. Concentrations of 1, 2 or 5 ngCd/mL were obtained by adding 0.5, 1.0 or 2.5 mL of Cd stock to 1 L of the appropriate pH stock.

The pH was measured daily for both the fresh and old solutions in all treatments just prior to changing the solutions. Concentrations of Cd in the test solutions were monitored regularly. Weekly samples of 2 eggs (pooled for analysis) were removed from each treatment, except for weeks 5 and 6. On week 10, samples of 4 eggs were removed and dissected into chorion, embryo and yolk components, and each component pooled for analysis. On week 13, 2 eggs and 2 newly hatched alevins were removed from each treatment for analysis. On week 6, all unfertilized eggs were removed from each test container and discarded. The five ng/mL treatment was accidentally terminated at 74 d. Eggs, alevins and dissected components were rinsed in deionized water and frozen for analysis. Water and tissue samples were analyzed for Cd as reported earlier (Peterson et al. 1983). Cd concentrations in tissues are given on a dry weight basis.

RESULTS AND DISCUSSION

The test temperature decreased linearly from 10 to 4°C during the experiment. The pH levels were not constant over the 24-h period between solution changes. The pH of the unacidified solutions rose from about 6.5 to 6.9 over the 24-h period. This increase could be effected by aeration in containers with no eggs, and is attributable to the initial water being slightly supersaturated with CO_2. The pH rise in the acidified solutions is not attributable to CO_2 supersaturation, but is probably due to substances (such as HCO_3^-) released by the eggs (Peterson et al. 1980). The pH 4.5, 5.0 and 5.5 solutions rose, on the average, to pH 4.7, 5.4, and 6.2, respectively, over 24 h. Since pH is a logarithmic scale, the actual reduction in H^+ concentration was greatest at pH 4.5. For reasons of simplicity only the initial pH levels are referred to in the text.

The Cd concentrations in undosed solutions were undetectable (Table 1). Since Cd adsorbs to the surface of the containers in non-acidic media, Cd concentrations in the 1.0 and 2.0 ng/mL treatments were more stable at pH 4.5 than at pH 6.8 as indicated by comparing concentrations in fresh and 24-h-old solutions at the two pH levels.

The percent mortalities of fertilized eggs over the course of the experiment varied from 0 to 14.4 (Table 2). Mortalities tended to be highest at low pH and high Cd concentrations. Some mortality may be attributable to the daily changes of solution, particularly in the early stages of development which are sensitive to shock. Precipitates in the perivitelline fluid, as observed by Rombough and Garside (1982), were not observed in these experiments.

Cd accumulation by salmon eggs was strongly influenced by the pH of the incubation medium (Table 3). The Cd levels in eggs exposed to various pH levels with no added Cd varied from 0.04 to 0.10 µg/g with no significant difference (ANOVA). For eggs exposed to Cd, however, Cd accumulation was strongly influenced by the ambient pH. The multiple regression of $[Cd]_e$ in eggs as a function of ambient $[Cd]_a$ and pH is: $[Cd]_e = -1.27 - 0.53\ [CD]_a + 0.29\ pH + 0.13\ [Cd]_a \times pH$ [$r^2 = 0.92$; $p < 0.001$]. The regression coefficient for ambient [Cd] is negative, because the dominant influence is the positive sign of the interaction term. The concentration factors vary from 916 at an exposure level of 1 ng/mL Cd to 424 at 5 ng/mL Cd for eggs incubated at pH 6.5 (Table 4). The concentration factors at pH 4.5 are reduced to 15 to 39. The pH range from 5.5 to 5.0 seems critical as 70% of the decrease in concentration factor occurred over this range.

The Cd accumulation by the salmon egg is rapid with the concentrations apparently equilibrating within the first 24 h of exposure, compared with 48 h for rainbow trout eggs (Beattie and Pascoe 1978). Wedemeyer (1968) found Zn uptake by the chorion to equilibrate in about 40 min. Analysis of the three components of the salmon egg (embryo, yolk, chorion) shows that usually most of the Cd is taken up by the chorion, and the yolk accumulates the least (Table 5). Chorionic Cd concentrations were greatly dependent on incubation pH with levels much lower at lower pH levels for a given Cd treatment. For example at 2.0 ng/mL treatment level, chorionic Cd concentration was 26.7 µg/g at pH 6.5, but only 0.5 µg/g at pH 4.5. Embryonic Cd concentrations were also pH-dependent with high concentrations occurring at pH 6.5, decreasing at ambient pH levels of 5.5 to 5.0, then increasing again at pH 4.5. The percentage of the total Cd burden residing in the chorion varied from 24 to 98.2% (Table 6), although the chorion represents only 7% of the total egg dry weight. (The yolk comprised 85% of the total egg dry weight and the embryo 8%). The ratio of chorionic Cd to embryonic Cd was maximal at a pH of about 5.5 for eggs exposed to Cd (Fig. 1). At pH levels less than 5.5 the ratios declined and were near control ratios at pH 4.5.

The distribution of Cd in the Atlantic salmon egg, after exposure to 1-5 µg/L Cd in ambient water at pH 6.8, is similar to that described for rainbow trout eggs by Beattie and Pascoe (1978), and for herring eggs by Rosenthal and Sperling (1974). All these studies were done at fairly high pH levels and approximately 90% of the Cd accumulated is associated with the chorion. It has been suggested that the Cd associated with the chorion is bound to anionic sites, such as sulfhydryl groups, of either the protein or mucopolysaccharide components of the chorion (Wedemeyer 1968; Rosenthal and Sperling 1974). In support of this idea, chorionic zinc uptake was inhibited by iodoacetate which blocks sulfhydryl groups (Wedemeyer 1968). It has also been shown that hatched alevins are more sensitive to Cd than unhatched embryos (Beattie and Pascoe 1978; Woodworth and Pascoe 1982). It is tempting to suggest a causal relationship between the high chorionic Cd binding and the greater tolerance of unhatched embryos. It has not yet been shown, however, that the chorion shields the embryo to Cd penetration. Levels of Cd in the perivitelline fluid have not been measured and such measurements would be required to demonstrate the effectiveness of chorionic shielding.

Michibata (1981) found that Cd could be removed from Oryzias chorions by rinsing in a glycerin buffer at pH 2. Wedemeyer (1968) found that Zn binding to chorion of coho salmon eggs could be reduced if the pH were lowered from 4 to 2. Rosenthal and Sperling (1974) suggested that low pH would probably reduce Cd binding and observed that binding was reduced at higher salinities perhaps because Ca^{++} competes with Cd^{++} for the anionic binding sites. Our results indicate that Cd binding is reduced at pH levels as high as 5.5. The relationship between embryonic and chorionic Cd uptake at various pH levels is complex. Embryonic Cd is reduced proportionately more than chorionic Cd as the pH is lowered from 6.8 to 5.5. Chorionic Cd uptake is further reduced as the pH is lowered to 4.5, while embryonic Cd stabilizes or increases. Probably several mechanisms are responsible for the relationship described here. Cd may be more available for embryonic uptake at low pH due to lowered chorionic binding, while the embryonic binding sites may have different pk's than those of the chorion.

Table 1. Cd concentrations (ng/mL) of test solutions.

Time analyzed (days post-fertilization)	Age of solution	Treatment ($pH/[Cd^{++}]$)							
		6.8/0	6.8/1	6.8/2	6.8/5	4.5/0	4.5/1	4.5/2	4.5/5
1	fresh	ND*	0.95	1.96	4.60	ND	1.02	2.07	4.50
7	24 h	ND	0.45	1.48	3.65	ND	1.04	2.13	4.60
22	fresh	ND	1.28	2.04	5.34	ND	1.07	1.99	4.94
23	24 h	ND	0.97	1.91	5.00	ND	1.01	2.03	4.98
56	fresh	ND	0.90	1.92	-	ND	1.03	2.15	-
57	24 h	ND	0.65	1.13	-	ND	0.87	2.15	-
Mean concentration		ND	0.87	1.74	4.65	ND	1.01	2.09	4.76

*ND = not detectable; <.02 ppb.

Table 2. Cumulative percent mortality of fertile eggs for each treatment for the period from fertilization to hatching.

[Cd]	pH			
	6.8	5.5	5.0	4.5
0	0	4.1	1.3	6.6
1	6.3	13.3	5.0	6.7
2	2.1	13.3	10.7	14.4
5	0	6.7	7.7	10.8

Table 3. Cd levels in salmon eggs as µg/g dry weight. Means are of 7 to 10 samples (95% confidence limits). Values are combined for eggs sampled from 7 to 91 d post-fertilization; concentrations equilibrated within the first 24 h of exposure.

Nominal [Cd^{++}] of exposure medium (ppb)	pH of exposure medium			
	6.8	5.5	5.0	4.5
0	0.07 (0.04)	0.09 (0.13)	0.10 (0.09)	.04 (0.07)
1	0.98 (0.26)	0.76 (0.30)	0.18 (0.07)	.06 (0.05)
2	1.48 (0.20)	1.08 (0.26)	0.20 (0.10)	.10 (0.12)
5	2.19 (0.36)	1.47 (0.31)	0.59 (0.24)	.24 (0.14)

Table 4. Cd concentration factors for the eggs. Concentration factor =

$$\frac{[Cd^{2+}] \text{ in treated eggs} - [Cd^{2+}] \text{ in untreated eggs}}{[Cd^{2+}] \text{ in exposure medium}}$$

Nominal [Cd^{++}] of incubation medium (µg/L)	pH of exposure medium			
	6.8	5.5	5.0	4.5
1	916	663	81	15
2	705	494	48	27
5	424	276	98	39

Table 5. Accumulation of Cd by embryo, yolk and chorion components after 10 wk of exposure. Tissue levels are in F = concentration factor. Each value is for a single measurement on 4 pooled tissues.

Nominal conc. of Cd in exposure medium (ng/mL)		pH of exposure medium											
		6.8			5.5			5.0			4.5		
		Embryo	Yolk	Chorion	Embryo	Yolk	Chorion	Embryo	Yolk	Chorion	Embryo	Yolk	Chorion
0	$[Cd^{++}]$	0.23	0.04	0.64	0.43	0.02	0.31	0.31	0.02	0.61	0.03	0.02	0
	CF	-	-	-	-	-	-	-	-	-	-	-	-
1	$[Cd^{++}]$	1.20	0.01	12.8	0.50	0.02	10.4	0.27	0.04	2.07	0.28	0.01	0.24
	CF	980	0	12,000	70	0	10,000	0	20	1,400	250	0	140
2	$[Cd^{++}]$	2.00	0.06	26.7	0.10	0.02	15.6	0.16	0.01	5.0	0.65	0.06	0.51
	CF	860	6	13,000	0	0	7,600	0	0	2,200	310	20	200
5	$[Cd^{++}]$	2.10	0.03	35.8	0.08	0.02	22.7	0.94	0.08	6.2	3.60	0.05	1.4
	CF	372	0	7,000	0	0	4,500	126	12	1,100	723	6	256

Table 6. Bioaccumulation of Cd in egg components - chorion, embryo and yolk.

pH / conc. of CD	% Cd in each component at week 10											
	6.8			5.5			5.0			4.5		
	Embryo	Yolk	Chorion	Embryo	Yolk	Chorion	Embryo	Yolk	Chorion	Embryo	Yolk	Chorion
0	19.6	42.3	38.1	44.1	30.7	25.2	37.0	22.6	40.4	9.7	60.2	30.1
1	8.5	0.7	90.8	5.6	2.5	91.9	7.8	21.6	70.6	43.9	19.4	36.7
2	10.2	3.3	86.5	1.1	2.2	96.7	3.4	1.7	94.9	40.0	36.0	24.0
5	7.4	0.7	91.9	0.6	1.2	98.2	13.8	12.1	74.1	67.4	9.3	23.3

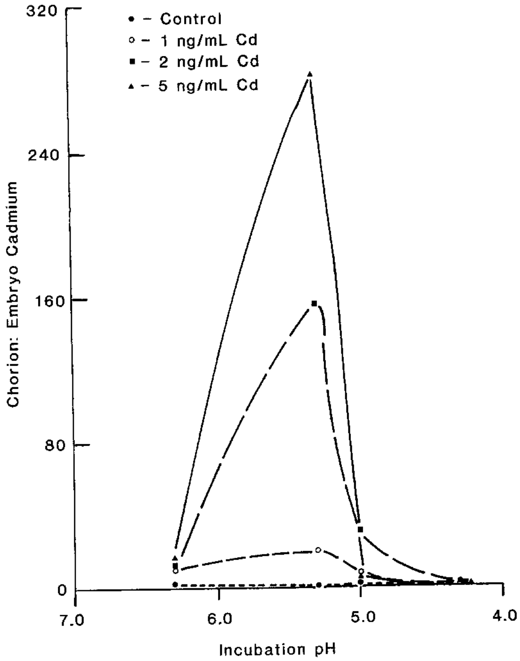

Figure 1. Ratios of chorionic to embryonic Cd concentrations are indicated as a function of incubation pH.

Cd levels in 24-h hatched alevins were considerably higher at low pH than were those of unhatched embryos (Table 7). Low pH stimulates ionic pumps across the gill surface and perhaps Cd^{++} is taken up in this manner along pathways normally accumulating Ca^{2+} (McWilliams and Potts 1978). The increased uptake by hatched alevins is a probable reason why they are less tolerant of ambient Cd than are unhatched embryos (Beattie and Pascoe 1978). An analysis of variance (Table 9) performed on mean lengths of newly hatched alevins from each experimental treatment revealed that both pH and Cd influence alevin size at hatching (Table 8). There was also a significant interaction between the two stressors. Examination of mean alevin lengths for the various treatments indicates the nature of the effects of low pH and Cd (Table 8). In the absence of Cd, alevins were progressively smaller at lower pH levels. Alevins hatching at pH 4.5 were larger at higher ambient Cd concentrations. Peterson et al. (1983) found yolk utilization and growth of alevins to be reduced by 2 ng/mL Cd. Unhatched embryos contain less Cd than the hatched alevins at the same ambient [Cd], and yolk utilization and growth are apparently normal until hatching occurs. Exposure to 2 ng/mL Cd at low pH seems to offset the reduction in size of hatched alevins at low pH with no added Cd. It is probable that yolk proteins are transported as Ca^{2+} complexes to the embryonic liver for use in growth processes. As a possible explanation for the H^{+} - Cd^{2+} interaction on alevin size, it is hypothesized that H^{+} ions may interfere with the formation of these complexes - possibly through loss of plasma Ca^{2+}. Cd^{2+}, when present, might replace Ca^{2+} in in forming these complexes, thus allowing growth to proceed.

Table 7. Concentration of Cd (µg/g dry wt) in 10-wk-old embryo compared with the levels in 13-wk-old newly hatched alevins (less than 24-h old).

Nominal conc. of Cd in exposure medium (ng/mL)	Stage	pH of exposure medium			
		Control	5.5	5.0	4.5
0	unhatched	.06	.06	.05	.02
	hatched	.05	.02	.05	.07
1	unhatched	.08	.06	.05	.03
	hatched	.07	.03	.63	.27
2	unhatched	.21	.03	.02	.10
	hatched	.23	.79	.98	.57

Table 8. Mean alevin lengths (mm) at hatching for various combinations of incubation pH and Cd concentration (ppb). Each mean is derived from 10 measurements.

[Cd]	Incubation pH			
	6.8	5.5	5.0	4.5
0	19.40 ($\bar{x}$)	18.80	17.10	15.00
	0.52 (s.d.)	0.42	0.46	0.28
1	18.40	17.90	15.40	17.10
	0.46	0.34	0.46	0.57
2	19.60	18.70	17.10	18.40
	0.66	0.48	0.57	0.64

Table 9. Results of analysis of variance test on data presented in Table 7.

Variance source	df	F
pH	3	186.42**
Cd	2	68.00**
Interaction	6	38.37**
Error	108	-

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