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Toxicity of Potash Brines to Early Developmental Stages of Atlantic Salmon (*Salmo salar*)

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The mining of potash in southeastern New Brunswick emerged as a major industry for the area in the early 1980's. Concentrated brines of the potash solutes are used as part of the mining process and excess quantities have to be transported via pipeline to dumping areas in the Bay of Fundy. These brines consist primarily of the chlorides of potassium, sodium and calcium. Pipeline ruptures have resulted in two spills of concentrated brine into Fowler's Brook (45°33'N, 66°34'W), a tributary of the North Hammond River, with resultant peak conductivities in excess of 15,000 µS, and in excess of 3000 µS for 11 h. Peak conductivity in the North Hammond was near 4000 µS with values near 2000 µS measured over a 9-h span. Dead fish were observed for a distance of 1.5 km downstream of the spill site. As a consequence of these events, we tested the toxicity of this brine on various aspects of early salmonid development to determine whether fish mortality could be attributed to the spill.

There is no published information on the toxicity of potassium chloride (the principal component of potash brine) to salmonids. The toxicities of chlorides of K^+, Na^+ and Ca^{2+} to other kinds of fishes have been determined several times. Potassium chloride is the most toxic of these salts with threshold toxicities ranging from 750 mg/L (10 mM) for pickerel (Stizostedion v. vitreum) to 10,368 mg/L (141 mM) for whitefish (Coregonus sp.) fry (Edmister and Gray 1948). The corresponding values for NaCl or $CaCl_2$ were 3859–16,500 mg/L (66–282 mM) and 12,060–22,080 mg/L (110–200 mM), respectively. Trama (1954) determined the 96 h TL_m's of KCl and NaCl to bluegills to be 2054 mg/L (28 mM), and ca. 12,946 mg/L (221 mM), respectively, and Dowden and Bennett (1965) reported the 25 h Tl_m of KCl to the same species to be 5,500 mg/L (75 mM) while that of NaCl was 14,125 mg/L (241 mM). In general, KCl appears to be slightly more toxic than NaCl or $CaCl_2$ on a molar basis.

In this paper we investigate the toxicity of various dilutions of potash brine on Atlantic salmon (Salmo salar L.) eyed eggs, newly hatched alevins and newly feeding fry. We examine the influence of various dilutions on water hardening of salmon eggs and on early blastodisc formation. We also determine to what extent the toxicity of pure KCl, NaCl and $CaCl_2$ solutions can account for the toxicity of the mine effluent.

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MATERIALS AND METHODS

All Atlantic salmon used in these experiments were of Saint John River stock, supplied courtesy of the Mactaquac Hatchery, Freshwater and Anadromous Division of the Department of Fisheries and Oceans, Canada. Eggs and alevins were reared under standard hatchery conditions at 4.1±0.3°C in Chamcook Lake water. All dilutions of the potash brine and of pure KCl, NaCl and $CaCl_2$ were made up with laboratory tap water (dechlorinated, filtered Chamcook Lake water), which contained 105, 7, 78, 20, 155 and 24 µM of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl and SO_4^{2-}, respectively. The tap water's conductivity was ca. 50 µS. Na^+, K^+ and Ca^{2+} concentrations were determined by atomic absorption spectrometry (Fishman and Downs 1966) and by ion chromatography, and chloride was measured by the latter method (see also Charmantier et al. 1985).

A saturated potash brine was prepared by dissolving crushed potash ore (from the Denison Mine, Sussex, N.B.) in boiling tap water. This solution (cooled to 20°C) was filtered through a Whatman #2 filter and the supernatant was mixed with water to the appropriate assay concentration.

Ten eyed-eggs (276 degree-days from fertilization) and 10 newly hatched alevins (446 degree-days) were placed in 200 mL of each test solution at 4°C (including a tap-water control). Egg and alevin mortalities (determined by microscopically observing cessation of heart beat) were counted after 15 min, 30 min, 60 min, 4 h, 8 h, 16 h, 24 h and daily until the completion of 7 d of exposure. Ten newly feeding fry were placed in 1 L of each test solution at 4°C. Mortalities (determined by cessation of opercular movement) were counted at the time intervals given above.

Tests were also conducted with newly hatched alevins reared at 8°C to determine the lethality of the major cation constituents, individually and combined. Stock solutions of K^+ (1790 mM), Na^+ (2175 mM) and Ca^{2+} (6.24 mM) were prepared and diluted with lab water to concentrations approximating LC50 potash brine levels for alevins. Various combinations of K^+, Na^+ and Ca^{2+} were prepared to simulate potash brine, and tested.

Water uptake by newly fertilized eggs after 6 h in the various test solutions was measured by subtracting the estimated water content of newly shed eggs from that of the water-hardened eggs. Six hours was considered an adequate time period, based upon previous experiments (e.g. Peterson and Martin-Robichaud 1982).

Blastodisc formation was assessed after 7- and 17-d exposure to each of the test solutions by measuring disc diameter with an ocular micrometer and by blastodisc appearance.

Median mortality times were estimated by standard probit analysis. The probit analysis method of Finney (1952) was used to determine a 7-d median lethal concentration (LC50) and confidence limits (Davies 1971).

Conductivity values (μS) are reported to make results comparable to measurements which used conductivity to assess the time course of spills. An analysis of variance and Duncan's a posteriori test was used to evaluate the egg water contents data from the water hardening experiment.

RESULTS AND DISCUSSION

The potash brine consisted primarily of sodium and potassium chlorides in a nearly equimolar ratio (Table 1).

Of the stages tested, newly feeding fry were most sensitive to potash brine (Table 2), with a 7-d LC50 at a dilution corresponding to a conductivity of 3600 μS. The K^+ concentration at this dilution strength is 16 mM.

Newly hatched alevins were less sensitive than fry with a 7-d LC50 of a brine dilution corresponding to a conductivity of 10,740 μS. The K^+ concentration at this dilution strength was 48.2 mM. The toxicity of the brine solution to salmon alevins could be accounted for by the K^+ present in it; because the 7-d LC50 for pure KCl solutions of 49.4 mM was not significantly different from the K^+ concentration in brine solutions corresponding to the LC50. The 7-d LC50 for pure NaCl was more than four times that in the brine solution corresponding to the LC50.

Eyed eggs were more tolerant of potash brine than either fry or alevins with a 7-d LC50 at a dilution corresponding to conductivities between 20,500 and 26,300 μS (containing 92-118 mM K^+). Insufficient partial mortality was obtained to calculate the LC50 more accurately.

Water uptake during the hardening process was significantly reduced at potash solutions with conductivities equal to or exceeding 1950 μS, with maximum reduction at conductivities exceeding 3500 μS (Figure 1). A potash brine dilution with a conductivity of 15,000 μS reduced the water uptake during hardening by about 60%. With potash brine of 30,000 μS conductivity, the situation was complicated by the apparent entry of a measurable amount (about 2 mg) of potash salts into the perivitelline fluids. This salt is osmotically active, and some water uptake into the perivitelline fluid would be associated with the dissolved salts -- resulting in greater water uptake at 30,800 μS than at 15,000. This is indicated in Figure 1 by the dotted lines. The estimated "initial" egg water content is higher after hardening in a potash brine of 30,800 μS, because the weight of the salt was not subtracted from the total dry weight prior to estimation of the water content. The influence of the potash solutions on water uptake by the egg would not be due to the K^+ concentration alone, but to the total osmolality of the solution. At 30,800 μS, the total cation concentration is 390 mM (180 mM K^+, 210 mM K^+).

Cell division during early development of the salmon eggs was also affected by exposure to potash solutions. At the end of 7-d

Table 1. Concentrations of the three major cations and chloride (mM) in potash solutions, as related to dilution factor and to conductivity.

	Stock sol'n	Dilution					Control (tap water)
		0.1	0.05	0.01	0.005	0.001	
Conductivity (µS)	-	30,800	15,000	3,500	1,950	395	47
K^+	1800	180	90	18	9	1.8	0.006
Na^+	2100	210	105	21	10.5	2.1	0.1
Ca^{2+}	6.6	0.66	0.33	0.07	0.03	0.007	0.06
Cl^-	4118	412	206	41	20.5	4.1	0.12

Table 2. The 7-d median lethal concentrations of potash brine dilutions and of pure chloride solutions of K^+ and Na^+ to three developmental stages of Atlantic salmon. The 95% confidence limits are in parentheses. Brine dilutions are given in terms of conductivity and K^+ concentrations (conductivity = 222.86 [K], r = 0.998).

Developmental stage	Potash solution	K^+	Na^+
Eyed eggs	20,500-26,300 µS 92.0-118.0 mM K^+	-	
Alevins	10,740 µS (6753-17078) 48.2(30.3-76.6) mM K^+	- 49.4(40.3-60.5)	>217
Fry	3600 µS (3465-3740) 16.0(15.5-16.8) mM K^+	-	-

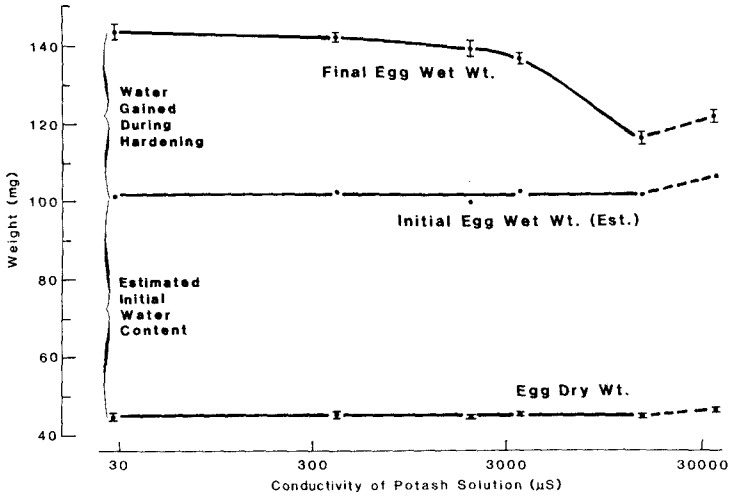

Figure 1. Water balance of newly fertilized Atlantic salmon eggs during the hardening process as affected by various potash solutions (as given by conductivity along the abscissa). Bars indicate ± 1 SE. Since initial egg wet weight has been derived from dry weights, no error estimates were made.

incubation post-fertilization, control embryos had reached the morula stage with a morula diameter of 1.5 mm. At conductivities of 390 to 15,000 µS, the morulae appeared normal, but were slightly smaller (1.0-1.2 mm diameter). Morulae developed in a potash solution of 70,800 µS conductivity had indistinct edges, and the diameter varied from <1.0 to 1.5 mm. After 17-d incubation, control blastodiscs had attained a diameter of 3.1 mm, and a visible, thickened terminal node (ca. early stage 8 of Ballard 1973) was apparent at one end, indicating formation of the anterior end of the embryo. In potash brine of 390-3500 µS conductivity after 17 d, the blastodiscs were 1.8-2.5 mm diameter, with no recognizable terminal node. In the two most concentrated potash solutions (15,000 and 30,800 µS), the blastodiscs were of no greater diameter than at 7 d, and the cells were dead.

Atlantic salmon fry were more sensitive to potash solutions than were eyed eggs or alevins. In the case of alevins, we believe that K^+ is the most toxic component of the solutions, as the 7-d LC50 for the pure KCl solutions was nearly identical to the K^+ concentration at the 7-d LC50 for potash solutions (49 mM vs 48 mM). This may also be true for fry and eyed eggs, but was not tested. In more concentrated potash solutions (20,000-30,000 µS), Na^+ may be toxic as well. Edmister and Gray (1948) found toxicity thresholds of 19 mM and 266 mM K^+ for yellow pickerel and whitefish fry, respectively. The sensitivity of pickerel fry to K^+ was thus very similar to that determined for salmon fry in our experiments. Several studies have indicated that the toxicity of K^+ to older stages of fish varies from 2000 to 5000 ppm (50-128 mM K^+),

depending upon the species and the time of the toxicity tests (e.g. Ellis 1937; Trama 1954; Dowden and Bennett 1965). It may be that fry are more sensitive to elevated K^+ levels than are older stages.

Fish plasma normally contain slightly more than 2 mM of K^+ (Wood and McDonald 1982). Abnormally high concentrations of K^+ in the extracellular fluids depresses membrane potentials of excitable tissues, that of heart muscle being particularly critical. As a result, cause of death due to high extracellular K^+ is usually considered to be due to loss of contractility of heart muscle. The heart becomes flacid and fills with blood (Guyton 1961). Our observations on salmon alevins, whose hearts are easily observed in the transparent embryo, are in accordance with the concept of heart failure. Alevins near death had hearts which appeared enlarged, which beat sluggishly and were engorged with blood. We therefore suggest that the cause of death was heart failure due to a rise of K^+ in extracellular fluids. This rise was the result of K^+ influx into the fish from the ambient water containing an abnormally high K^+ concentration; 16 mM is about 1000 x the normal ambient concentration.

The influence of the potash solutions on water hardening is probably related to the total osmotic concentration, rather than the effect of a specific ion. Potts and Rudy (1969) demonstrated that 145 mM NaCl completely inhibited formation of perivitelline fluid, with some reduction of water uptake at concentrations as low as 0.5 mM. Formation of the perivitelline fluid is effected by release of colloid from the yolk into the sub-chorionic space which is triggered by fertilization. The colloid induces water uptake into the perivitelline space as a result of its osmotic pressure. High salt concentrations in the ambient water probably inhibits release of the colloid (Potts and Rudy 1969). Since this release can occur only at fertilization, inhibition of perivitelline fluid formation is irreversible. The perivitelline fluid is essential for normal embryonic development, as it provides a fluid-filled space in which the embryo may grow, and may serve as a cushion against physical impact.

Early cell divisions of newly fertilized salmon eggs are also extremely sensitive to potash solution, with growth of the blastodisc being slowed at the lowest dilution tested (1.8 mM K^+), and mortality occurring at 90 mM. It is unknown whether this reduction in rate of blastodisc growth during temporary exposure to a potash solution has any permanent effect.

The impact of hypersaline potash spills, of the sort described in the introduction, obviously is dependent upon the life stages of salmon in the impacted stream at the time of the spill. Such events would appear to have the most impact when newly feeding fry are present (late May-early June) or at fertilization and early cleavage (early November). Judging from the results obtained in these experiments, temporary pulses (a few hours duration) with peak conductivities no greater than 350 µS (ca. 1.6 mM K^+) would probably

have no direct lethal effects on any life stage of salmon, although some delay of early cell division might occur. Fortunately, the documented spills occurred in mid-winter, and eggs at that time should have been fairly tolerant (7-d LC50 ca. 10,000 µS).

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