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Net Sodium Loss and Mortality of Three Salmonid Species Exposed to a Stream Acidified by Atmospheric Deposition

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Toxicological studies indicate that aluminum which is organically bound is not toxic, whereas, exposure of brook trout (Salvelinus fontinalis) to inorganic complexes (principally with fluoride, hydroxide, and sulfate) and free aluminum ions can result in measurable reductions in growth and survival at concentrations of 0.2 mg Al/L (Baker and Schofield 1980; 1982). Aluminum appears to be most toxic at a pH around 5.0 to both brown (Salmo trutta) and brook trout (Muniz and Leivestad 1980; Baker and Schofield 1980, respectively). The mode of aluminum toxicity to fish was first investigated by Muniz and Leivestad (1980). Brown trout exhibited depressions in plasma sodium and chloride concentrations when exposed to stream water with high aluminum concentrations. Muniz and Leivestad (1980) hypothesized that stress due to aluminum involved the same disturbance in ion exchange mechanisms as low pH stress. Witters and colleagues (1984) demonstrated that aluminum reduced sodium influx at low pH in an aquatic insect (Corixa punctata). Havas (1985) has recently shown that mortality of the aquatic cladoceran Daphnia magna in the presence of aluminum was associated with a substantial net loss of sodium and chloride.

Death of freshwater fish exposed to low pH is primarily due to failure of sodium regulation. Low pH reduces sodium influx and greatly increases efflux resulting in a net loss of sodium (Packer and Dunson 1970; 1972). Ionoregulatory failure may trigger circulatory collapse which finally causes death (Wood and McDonald 1982). Concentrations of plasma sodium and chloride have been used to determine whether or not a particular group of fish is stressed by acidic field conditions (e.g., Leivestad and Muniz 1976; Muniz and Leivestad 1980). A fish experiencing net loss of blood salts may be able to mobilize salts from elsewhere in the body; therefore, a study of concentrations of ions in the blood provides less information than one of net ion balance with respect to pH and/or aluminum toxicity. Furthermore, survival time is related to net sodium balance (Packer and Dunson 1970; 1972). The present study was initiated to determine whether or not aluminum accelerates net sodium loss in fish exposed to pH

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5.0 soft water (calcium <5 mg/L) and if so, to discover whether or not the magnitude of loss might account for mortality of fish in waters acidified by atmospheric deposition.

MATERIALS AND METHODS

Salts (NaCl, $MgCl_2$, and $CaCl_2$) were added to deionized distilled water, producing a stock solution of artificial soft water (ASW) which was similar in composition to water from the field site (40°9'N-79°12'W) Linn Run (Table 1). ASW solutions were adjusted to either pH 7.0 with potassium hydroxide or pH 5.0 with sulfuric acid. Anhydrous aluminum chloride ($AlCl_3$) was added to the pH 5.0 ASW producing two aluminum concentrations of 0.15 and 0.38 mg/L. Stream water obtained from Linn Run (LRW Table 1) was adjusted from pH 4.8 to 5.0 by addition of potassium hydroxide. In no case did added potassium exceed 0.5 mg/L. Aquarium pH was measured and adjusted every 2 h and monitored continuously (meters from Analytical Measurements; model 30 WP) in two randomly chosen aquaria. Samples for aluminum analysis (discussed later) were collected from aquaria containing aluminum before and after testing periods.

Table 1. Concentrations of sodium, calcium, and magnesium in stock solutions utilized in laboratory sodium balance experiments. Fish were held in tap water (TW) prior to experimental exposure in artificial soft water (ASW) or Linn Run water (LRW).

	Stock Solution		
Cation (mg/L)[a]	TW	ASW	LRW
Na	4.60	0.46	0.39
Ca	34.09	3.01	2.69
Mg	16.28	0.66	0.73

[a]Concentrations were determined using a Varian Techtron (model 1280) atomic absorption spectrophotometer.

Fingerling brook trout weighing from 1.5 to 4.0 g were obtained from the Pennsylvania Fish Commission at its Reynoldsdale hatchery on October 29, 1984. The laboratory experiments were conducted during the last week of November 1984 using these fish. Following transport from the hatchery the fish were held in pH 7.5 dechlorinated (by aeration) tap water at 10 $\pm$ 0.5°C (TW, Table 1). Each fish was rinsed with deionized distilled water prior to solitary exposure in a 4 L aquarium. Four individuals were exposed to each treatment water. The aquaria were covered with plastic film and aerated continuously during the exposure period. Net sodium flux was determined by measuring sodium concentrations of water samples (5 ml) collected from each aquarium before introducing the fish, after exposure for predetermined time periods (2, 8, 16 and 24 h), and after removal. Following exposure, fish were removed and immediately frozen (-20°C) in individual plastic bags. Subsequently, fish were digested in reagent grade nitric acid after determination of wet and dry (12 h at 100°C) mass ($\pm$0.001 g). Sodium concentration of water samples, aquarium volume, and final total body sodium content were used to calculate total body sodium concentration for each fish at each sampling period. Net flux rates were calculated as the differences between body sodium concentrations at successive sampling times divided by dry mass and time elapsed since exposure began.

A second order mountain stream, Linn Run, was selected as a site for field experiments since it has been shown to be acidified by atmospheric deposition, and because fish kills, especially during spring snow melt, have been observed (Sharpe et al. 1984). Experimental sites were at the mouth of Linn Run (at the eastern boundary of Linn Run State Park) and about 1 km upstream at a more acidic site. Water temperature was similar among sites and ranged from 8.0 to 12.5°C.

Fingerling brook, brown, and rainbow trout (Salmo gairdneri) were used in these experiments (see Table 2 for exposure times, origins, and size ranges). Brook trout were raised in water of higher ionic content than were the rainbow and brown trout (384 versus 77 µS/cm and $CaCO_3$ hardness was 163 versus 26 mg/L, respectively). Following transport from the hatchery and prior to exposure to stream water, 20 fish of each species were individually frozen (-20°C) in plastic bags to provide base line values for body sodium concentration. Remaining fish were placed in polyethylene buckets containing a 50% mixture of rearing water and stream water (from respective experimental sites) for 1 h. The fish were then placed in cages constructed of wood and fiberglass screen (2 mm mesh) measuring 60x30x30 cm for rainbow and brook trout, whereas the smaller brown trout were kept in 50x30x20 cm cages. A sample of 10 fish was removed from each cage at each site after exposure to stream water for predetermined time periods (2, 8, 16, and 24 h) and frozen in individual plastic bags. Total body sodium concentration was determined as before. Net loss (24 h) was computed based on average body sodium values from fish exposed for 24 h and from fish of each species which were transported to Linn Run but not exposed to stream water. The base line values were 4.2, 3.6, and 5.2 mg Na/g dry mass for brook, rainbow, and brown trout, respectively.

Table 2. Initial exposure times, origins, and sizes of fingerling trout utilized in field sodium balance and survival experiments.

Species Tested	Initial Exposure	Hatchery of Origin	Wet Mass(g)[a] Min.	Max.
Brook	05/29/84	Reynoldsdale	1.2 -	14.8
Rainbow	05/31/84	Rolling Rock	3.0 -	6.0
Brown	05/19/84	Rolling Rock	0.20 -	0.92

[a]Only the fish used in sodium balance experiments were weighed.

Survival time was determined (up to 168 h) for 8 to 20 fish remaining in their respective cages following the sodium balance experiments. The criteria of lethality were loss of equilibrium (turnover) or loss of all movement.

Water samples were collected at each exposure site. Suction filtration of 25-ml aliquots through 0.10 μm pore-size membrane filters (Millipore) was performed at a trailer on site at stream temperature within 24 h of collection. Immediately following filtration the aliquots were preserved by acidification to pH approximately 2.0 using ultra pure concentrated nitric acid (Ultrex). Aluminum concentrations of these aliquots and identically treated samples from the laboratory study were determined using a Perkin-Elmer (model HGA 500) atomic absorption graphite furnace operated by the staff of the Water Laboratory of the Environmental Resources Research Institute at The Pennsylvania State University. Procedure 200.2 of U.S. Environmental Protection Agency (1983) was followed including both minimum and optional requirements for internal and external quality control (listed on page 19 of METALS section). The pH of the unfiltered stream samples was determined at the same water laboratory within 72 h of collection; analysis followed procedure 150.1 of U.S. Environmental Protection Agency (1983).

Statistical analyses were performed using the Wilcoxon-Mann-Whitney test in the two-sample case and the Kruskal-Wallis test in the k-sample case. Multiple comparisons of main effects were accomplished by the Tukey test applied to rank transformed data (Conover and Iman 1981).

RESULTS AND DISCUSSION

Brook trout exposed to high aluminum concentrations in ASW showed loss rates which were similar to those of fish exposed to LRW (Table 3). Sodium loss rates were also similar between the two groups of fish exposed in ASW solutions without aluminum (at pH's 5.0 and 7.0). Significant differences ($\alpha \leq 0.05$) were observed at the 16- and 24-h samples, where net loss rates were twice as great for fish exposed to LRW and high aluminum ASW, compared with those in waters without aluminum (Table 3). The ASW solution with low aluminum concentration resulted in net sodium loss rates which were intermediate between those previously mentioned. Hydrogen ion concentration was maintained at ± 0.2 pH units; however, aluminum concentration decreased during exposure of brook trout in the laboratory experiment (Table 3).

Packer and Dunson (1970) show a net sodium loss rate for brook trout at a pH near 5.0 corresponding to about 2% (total sodium of control)/h after 4.75-h exposure. This value is similar to median loss rates observed for brook trout after 8-h exposure to the laboratory waters with little or no dissolved aluminum 2.0 to 2.2% (initial sodium content)/h. Fish, exposed to high aluminum concentration (>0.2 mg/L) in the laboratory, experienced a 3.2 to 3.5%/h reduction in sodium content during the first 8-h exposure at pH 5.0. A pH of 4.6 resulted in sodium loss at a similar rate, approximately 3.5%/h during 4.75-h exposure in waters without aluminum (Packer and Dunson 1970). Clearly, the presence of aluminum can accelerate net sodium loss in brook trout at pH 5.0.

Table 3. Comparison of median sodium loss rate among brook trout exposed in the laboratory; n=4.

Exposure Medium				Brook Trout	
				Median Body Na Loss Rate at 16 h[1]	Body Na Loss (%) at 24 h
Water		Aluminum (mg/L)			
Type	pH	Initial	Final	($mg.g^{-1}.h^{-1}$)	Mean (SE)
LRW	5.0	0.43	0.31	0.16 a[2]	40.0(3.1)
ASW	5.0	0.25	0.11	0.16 a	36.0(4.4)
ASW	5.0	0.08	0.05	0.10 ab	33.2(1.7)
ASW	5.0	--	--	0.09 b	21.2(4.6)
ASW	7.0	--	--	0.08 b	20.0(5.5)

[1]Since one individual in LRW had expired by the 24-h sample, the statistical comparison was based on data from the 16-h sample. Medians in the same column followed by the same letter are not signficantly different ($\alpha \leq 0.05$).

Field experiments indicated that substantial net sodium loss can also occur in natural settings where aluminum concentration is high. Body sodium concentration data subjected to two-way analysis of variance showed significant ($\alpha \leq 0.01$) effects due to exposure site and time for each species tested (Figure 1).

Sodium concentration of brook trout confined in Linn Run was inversely correlated with exposure duration ($\alpha \leq 0.001; r=0.66$) where ambient dissolved aluminum concentration was near 0.45 mg/L and pH was approximately 5.1 (Figure 1). Those exposed to circumneutral pH and less than 0.05 mg Al/L at the mouth of Linn Run had significantly greater sodium concentrations at the 2-, 16-, and 24-h samples than those from the upstream site. Brook trout experienced 34% net loss of sodium when exposed for one day to ambient aluminum concentrations exceeding 0.4 mg/L, whereas at concentrations below 0.1 mg Al/L, sodium loss was less than half as great.

Rainbow trout confined in Linn Run at the upstream site had significantly less sodium than controls at each time after the 2-h sample (Figure 1). Rainbow trout exposed in Linn Run for one day experienced a 35% net loss of sodium at an ambient aluminum concentration above 0.4 mg/L and pH between 5.2 and 5.4, whereas at aluminum concentrations below 0.1 mg/L, fish exhibited approximate homeostasis with respect to sodium balance. Wood and McDonald (1982) reported only slight reduction in rainbow trout body sodium (approximately 8%) at pH 4.8 in waters without aluminum; whereas, severe depletion (approximately 32%) was observed at pH 4.6.

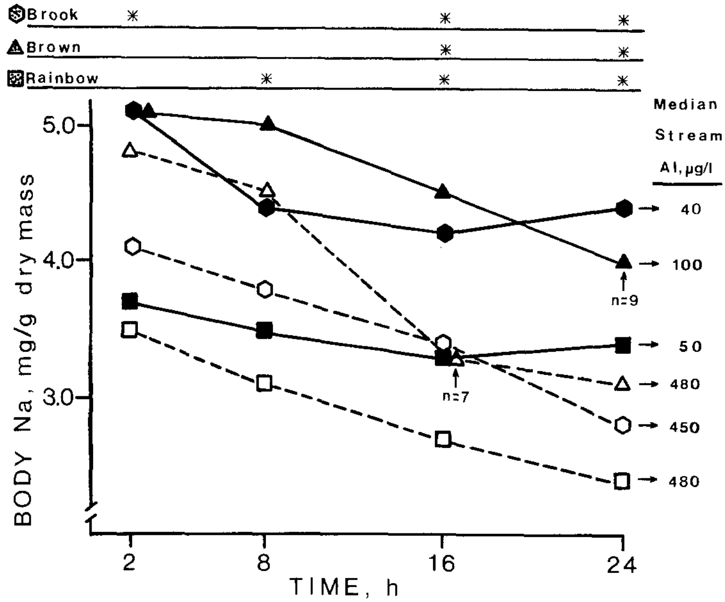

Figure 1. Body sodium concentration versus time for three salmonid species confined at the mouth of Linn Run (solid symbols) and at an upstream site (open symbols). * Indicates signficant difference ($\alpha \leq 0.05$) within a species between median sodium concentration of fish from the two sites. Median values are plotted and n=10 except where noted.

Net sodium loss by brown trout at the upstream site was 40% in 24-h and body sodium was significantly less than controls at the 16- and 24-h sample (Figure 1). Even though pH at the mouth of Linn Run was above 6.0 and aluminum concentration averaged only 0.1 mg/L, the rate of net sodium loss was still substantial, almost 1% (total)/h. McWilliams and colleagues (1980) reported net loss rate of sodium by brown trout of about 1% (total)/h at a pH of 4.0 with no aluminum present, whereas sodium influx and efflux were approximately equal at pH 6.0.

Body sodium depletion in excess of 30% in a single day was observed for each species at the upstream site. Aquatic organisms cannot survive net loss of sodium indefinitely. Wood and McDonald (1982) found that rainbow trout which died from acid exposure showed a 60-70% depression of body sodium and chloride.

Survival of fingerling brook, brown, and rainbow trout at the mouth of Linn Run was 100% for 168 h. Mortality of each species was observed between the first and second day of exposure to Linn Run water at the upstream site (Figure 2). Daye and Garside (1975) found 100% survival of fingling brook trout at pH 4.2 in a laboratory study with no aluminum (168 h test). While the lowest pH observed during the brook trout bioassay in the present study was 4.7, LT_{50} was only 53 h. Baker and Schofield (1980) reported

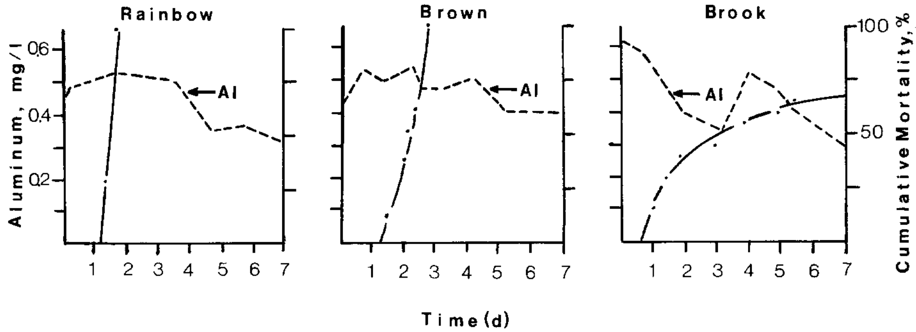

Figure 2. Cumulative mortality (solid line) and dissolved aluminum concentration for three bioassays in Linn Run. Median pH was 5.28, 4.80 and 4.94 for bioassays of rainbow, brown, and brook trout, respectively.

brook trout fry LT_{50} of less than 4 d at pH 5.2 with 0.5 mg Al/L added. The lowest recorded pH during the rainbow trout toxicity test was 5.2 at the upstream site and LT_{50} was 44 h. Median survival time for rainbow trout exceeded 168 h at pH 4.4 in soft water without aluminum (Graham and Wood 1981). The same investigators showed an LT_{50} of approximately two days at pH 4.0. While the lowest pH observed during the brown trout toxicity test was 4.8 at the upstream site and LT_{50} was 50 h, Brown (1982) has shown that brown trout LT_{50} exceeds 200 h at pH 4.5 in waters without aluminum. Median survival time of about two days was observed at pH 4.1 (Brown 1982). Each species showed rapid mortality in Linn Run at pH's which should not be harmful.

The laboratory experiment showed that in the absence of added chelating agents, ambient aluminum could account for severe body sodium depletion at pH 5.0. No direct method was used in the present study to determine the amount of dissolved aluminum which was in a nontoxic form due to chelation with organic ligands. However, Andelman and Miller (1986) have recently shown that less than 11% of the total (nonfiltered) aluminum concentration, of samples collected near the upstream site (their Boot Hollow station), was in a slowly exchangeable or inert form. These fractions were considered to include aluminum complexed with organic constituents, while those forms which were more reactive apparently included inorganic complexes (Andelman and Miller 1986). Results of the present study are consistent with the hypothesis that elevated aluminum concentrations due to acidification can cause fish mortality by interfering with ionoregulation.

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REFERENCES

Andelman JB, Miller JR (1986) Impact of acid rain on aluminum species in streams. Water Quality Bull 11(1):19-25

Baker JP, Schofield CL (1980) Aluminum toxicity to fish as related to acid precipitation and Adirondack surface water quality. In: Drablos D, Tollan A (eds) Ecological impact of acid precipitation. (SNSF) Project, Aas, Norway, pp 292-293

Baker JP, Schofield CL (1982) Aluminum toxicity to fish in acidic waters. Water, Air, and Soil Pollut 18(1-3):289-309

Brown DJA (1982) The effect of pH and calcium on fish and fisheries. Water, Air, and Soil Pollut 18(1-3):343-351

Conover WJ, Iman RL (1981) Rank transformations as a bridge between parametric and nonparametric statistics. Am Statistician 35(3):124-129

Daye PG, Garside ET (1975) Lethal levels of pH for brook trout, Salvelinus fontinalis (Mitchill). Can J Zool 53:639-641

Graham MS, Wood CM (1981) Toxicity of environmental acid to the rainbow trout: interactions of water hardness, acid type, and exercise. Can J Zool 59:1518-1526

Havas M (1985) Aluminum bioaccumulation and toxicity to Daphnia magna in soft water at low pH. Can J Fish Aquat Sci 42:1741-1748

Leivestad H, Muniz IP (1976) Fish kill at low pH in a Norwegian river. Nature 259:391-392

McWilliams PG, Brown DJA, Howells GD, Potts WTW (1980) Physiology of fish in acid waters. In: Drablos D, Tollan A (eds) Ecological impact of acid precipitation. (SNSF) Project, Aas, Norway, pp 282-283

Muniz IP, Leivestad H (1980) Toxic effects of aluminum on the brown trout, Salmo trutta L. In: Drablos D, Tollan A (eds) Ecological impact of acid precipitation. (SNSF) Project, Aas, Norway, pp 320-321

Packer RK, Dunson WA (1970) Effects of low environmental pH on blood pH and sodium balance of brook trout. J Exp Zool 174:65-72

Packer RK, Dunson WA (1972) Anoxia and sodium loss associated with the death of brook trout at low pH. Comp Biochem Physiol 41(1): 17-26

Sharpe WE, DeWalle DR, Leibfried RT, Dinicola RS, Kimmel WG, Sherwin LS (1984) Causes of acidification of four streams on Laurel Hill in southwestern Pennsylvania. J Environ Qual 13(4): 619-631

U.S. Environmental Protection Agency (1983) Methods for chemical analysis of water and wastes. Report EPA-600/4-79-020. Cincinnati, OH

Witters H, Vangenechten JHD, Puymbroeck S Van, Vanderborght OLJ (1984) Interference of aluminum and pH on the Na-influx in an aquatic insect Corixa punctata (Illig.). Bull Environ Contam Toxicol 32:575-579

Wood CM, McDonald DG (1982) Physiological mechanisms of acid toxicity to fish. In: Johnson, RE (ed) Acid rain/fisheries, American Fish Soc, Bethesda, MD, pp 197-226

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