Effects of Water Acidity, Calcium, and Aluminum on Whole Body Ions of Brook Trout (*Salvelinus fontinalis*) Continuously Exposed from Fertilization to Swim-Up: A Study by Instrumental Neutron Activation Analysis

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Water Ca, rather than pH or Al, was the most important factor affecting whole body electrolyte levels in fry exposed from fertilization to swim-up (91 d) to 84 combinations of pH (6.5, 5.2, 4.8, 4.4, 4.0), Ca (0.5, 1, 2, 8 mg/L), and Al (0, 12, 37, 111, 333, 1000 μg/L) in flowing soft water. Aluminum accumulation occurred only at water Al levels > 111 μg/L; Al accumulation was inhibited both by increasing Ca and decreasing pH. Under control conditions (pH = 6.5, Ca = 2 mg/L, Al = 0 μg/L), whole body Na, Cl, K, and Ca levels all increased greatly during development, while Mg decreased. Body Ca levels were elevated up to 3-fold, and Na, Cl, and K up to 2-fold by increasing water Ca at the same pH and Al. Low pH had a small negative influence, intermediate levels of Al (37, 111) a slight positive influence, and higher levels of Al a negative influence on Na, Cl, K, and Ca levels. Whole body Mg showed opposite trends, reflecting delayed development under adverse conditions. At pH = 6.5, the positive influence of increasing water Ca on most whole body ions showed a clear threshold between 0.5 and 1 mg/L. At lower pH, this threshold was shifted to between 2 and 8 mg/L, indicating that Ca levels sufficient to support healthy development at circumneutral pH may prove inadequate under acidified conditions.

Le calcium dans l'eau, plutôt que le pH ou l'Al, constitue le plus important facteur à agir sur l'équilibre des électrolytes chez l'alevin exposé depuis la fécondation jusqu'à la nage (91 jours) à 84 combinaisons de pH (6,5, 5,2, 4,8, 4,4, 4,0), de conc. de Ca (0,5, 1, 2, 8 mg/L) et d'Al (0, 12, 37, 111, 333, 1000 μg/L) dans une eau douce en circulation. L'accumulation d'Al s'est produite seulement à des concentrations d'Al dans l'eau sup. à 111 μg/L; l'accumulation d'Al était inhibée à la fois par l'augmentation de la conc. en Ca et l'abaissement du pH. Dans les conditions de référence (pH = 6,5, Ca = 2 mg/L, Al = 0 μg/L;), la conc. dans l'organisme de Na, Cl, K et Ca a considérablement augmenté au cours du développement alors que celle du Mg s'est abaissée. La conc. de Ca a augmenté de trois fois alors que celles des Na, Cl et K ont augmenté de deux fois lorsque la teneur en Ca de l'eau a été accrue et que le pH et la conc. en Al ont été gardés constants. Les pH acides avaient un léger effet négatif, les conc. intermédiaires d'Al (37, 111) avaient un léger effet positif et les conc. élevées d'Al avaient un effet négatif sur la conc. de Na, Cl, K et Ca. La conc. en Mg dans l'organisme variait en sens inverse, ce qui traduisait un développement retardé par des conditions adverses. À pH = 6,5, on voyait apparaître nettement un seuil entre 0,5 et 1 mg/L dans l'influence positive de l'augmentation de conc. en Ca dans l'eau sur la plupart des électrolytes. À pH acide, ce seuil passait entre 2 et 8 mg/L, ce qui indique que des conc. en Ca suffisantes pour assurer un développement sain des organismes à pH situé dans la page de neutralité, peuvent se révéler insuffisantes en milieu acide.

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In juvenile and adult salmonids, ionoregulatory disturbance is the key toxic mechanism causing death in acute acid stress. This is further exacerbated by the presence of aluminum (Al) but ameliorated by calcium (Ca) (Leivestad 1982; Wood 1989; McDonald et al. 1989). Ionoregulatory disturbance may also occur during chronic sublethal exposures, but here the situation may be complicated by acclimation and/or recovery, and by protective rather than toxic effects of Al (Wood et al. 1988).

Early life stages are more sensitive to acid/aluminum exposure than juveniles and adults; mortality over the fertilization through swim-up period may be an important cause of recruitment failure (Haines 1981; Howells 1984; Gunn 1986). A number of studies have indicated that intense ionic uptake from the water must occur at this time to achieve the rapid mineralization of the body which accompanies early development (McCayet al. 1936; Hayes et al. 1946; Zeitoun et al. 1976; Shen and Leatherland, 1978a; Talbot et al. 1982; Rombough and Garside, 1984). It is therefore surprising that the effects of acid/Al stress on ionoregulation in these early life stages have received little experimental attention. One notable exception is the work of Peterson et al. (1982) and Peterson and Martin–Robichaud (1986) on larval Atlantic salmon (*Salmo salar*) chronically exposed to acid stress alone. Pronounced reductions in Na, Ca, and K accumulation during development were documented. The effects of water Al or Ca, of exposure at different developmental stages, and of recovery were not tested. However, very recently, Gunn and Noakes (1987) have examined some of these topics after recovery from pulse acid/Al exposures in alevins of lake trout (*Salvelinus namaycush*).

The toxicological experiments described in the preceding paper (Ingersoll et al. 1990) on early life stages of brook trout (*Salvelinus fontinalis*) offered a unique opportunity to evaluate these problems in an extensive pH/Ca/Al matrix. We have employed instrumental neutron activation analysis (INAA) to measure whole body levels of Na, Cl, K, Ca, Mg, and Al in surviving fry from these experiments. INAA, which has recently been employed in other acidification studies (Fraser and Harvey 1984; Cunningham and Shuter 1986; Gunn and Noakes 1987), was a particularly suitable method for these very small samples (~5 – 15 mg dry weight). The technique provides sensitive, simultaneous, and non-destructive multi-element analysis without extensive sample preparation (Heydorn 1984).

The present paper reports the ionoregulatory effects seen in surviving brook trout after a continuous 91 d exposure (from fertilization through swim-up) to an 84 cell matrix of pH, Ca, and Al. This matrix covers the total range of occurrence of these three parameters in acid-sensitive soft waters of eastern North America. The accompanying paper (Wood et al. 1990) documents the effects on previously unexposed yolk-sac fry and swim-up fry of shorter term challenges and recoveries in this same matrix.

Materials and Methods

Exposures

Details of the fish and the exposure regimes are given by Ingersoll et al. (1990). In brief, all fish came from a single batch of 26 000 freshly fertilized eggs (from 40 females and 40 males) spawned in soft water (pH ≈6.5, Ca ≈ 2 mg/L). The exposure matrix consisted of 84 combinations (nominal values) of pH (6.5, 5.2, 4.8, 4.4, 4.0), Ca (0.5, 1, 2, 8 mg/L), and Al (0, 12, 37, 111, 333, 1000 μg/L) in flowing artificial softwater (Na ≈ 4, Cl ≈ 8 mg/L) acidified with H_2SO_4. Temperature was 10.9°C, close to the reported optimum (12.4°C) for the growth and survival of brook trout alevins (McCormick et al. 1972), and photoperiod 12-h light/12-h darkness. Fry were offered Rangens Trout Starter (Rangens Inc., Buhl, Idaho), and exposure chambers were cleaned daily; no substrate was employed in the chambers. Exogenous feeding started between days 55 and 60 post-fertilization; it was not possible to estimate the amount of food actually eaten.

The water chemistry data from these exposures have been reported in detail (see table 1 of Ingersoll et al. 1990). In brief, pH and Ca levels were recorded daily and other parameters weekly or more frequently on water samples taken directly from the exposure chambers. Concentrations were generally very stable, with coefficients of variation typically <20%. On an absolute basis, measured Ca averaged about 25% less than nominal concentrations, while measured pH and Al (both total Al and monomeric Al) were in close agreement with nominal values. Nominal values have been used throughout for data analysis and tabulation.

The longest exposure, which is the focus of the present paper, started on day 0 with freshly fertilized eggs and continued for 91 d through swim-up. On day 91, every cell was sampled for INAA except where this was impossible due to mortality (see below). In addition, two 21 d age-specific challenge and 20 d recovery experiments were performed with yolk-sac fry (over days 49–90) and swim-up fry (over days 70–111; see Wood et al. 1990). In both, the fish had been raised at pH = 6.5, Ca = 2 mg/L, Al = 0 μg/L prior to challenge in the matrix, and were returned to this condition for recovery. These experiments provided some of the baseline data on temporal changes in body composition, summarized in Fig. 1.

Analyses

Typically, 8–10 survivors out of the original 50 fish in each cell were sacrificed for INAA, though this was reduced in cases where survival was low. The smallest number ever taken for INAA was 4. Cells with fewer survivors were not analysed. At sacrifice, fish were individually blotted dry, weighed (0.1 mg accuracy) and then dried to a constant weight at 65°C in an INAA vial. While both wet and dry weights were employed in data analysis (see Results), elemental concentrations have been routinely expressed in terms of wet weight. This is in accord with the recommendations of Shearer (1984) and the fact that wet weight measurements were intrinsically more accurate in these very small fish. For example, a 1% error in water content would be associated with a 5–10% error in dry weight.

Instrumental neutron activation analysis was carried out at the McMaster University Nuclear Reactor. Samples were irradiated with thermal neutrons (flux density = $5 \times 10^{12} n cm^{-2} \cdot sec^{-1}$), and after a 1 min delay, were counted for 10 min using a hyper-pure germanium detector coupled to a Canberra multichannel analyzer (Series 40 or 90). The peaks of interest and their energies (keV) were ^{24}Na (1368.4), ^{38}Cl (1642.2), ^{42}K (1524.6), ^{49}Ca (3084.4), ^{27}Mg (1014.5), and ^{28}Al (1778.9). Concentrations were calculated by comparison to known standards using routine equations (Desote et al. 1972).

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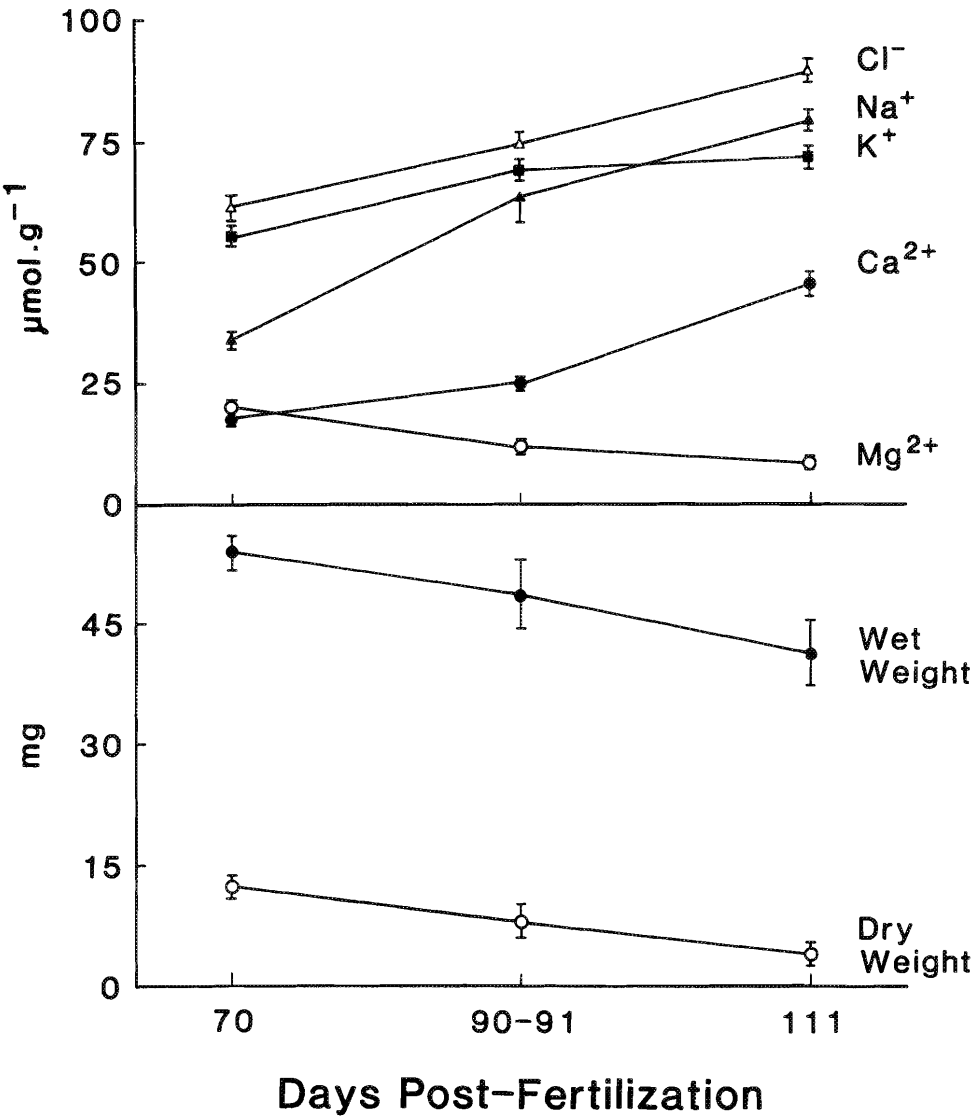

FIG. 1. Temporal changes in wet weight, dry weight, and the whole body concentrations (wet weight basis) of Cl, Na, K, Ca, and Mg in brook trout fry during early development under control conditions (pH = 6.5, Ca = 2 mg/L, Al = 0 µg/L). Means ± 1SEM for $n = 20$ at day 70, $n = 27$ at days 90–91, and $n = 9$ at day 111.

The NBS citrus leaf standard #1572 was used as a primary standard; the NRC marine fish tissue reference standard gave essentially identical results. As a check on the reproducibility of the analyses, a homogeneous pool of dried trout tissue was prepared, and 6–9 separate samples analysed. The coefficients of variation were: Na = 3.6%; Cl = 9.7%; K = 7.8%; Ca = 11.9%; and Mg = 9.8%. As a check on absolute accuracy, the samples were spiked with known amounts of atomic absorption standards (BDH, Harleco) sufficient to approximately double the native concentrations. Standard recoveries ± 1 SD (by subtraction) were: Na = 100.7 ± 10.0%; Cl = 103.7 ± 3.0%; K = 92.0 ± 6.5%; Ca = 92.6 ± 9.4%; and Mg = 113.0 ± 11.4%.

Aluminum analyses by INAA are complicated by the conversion of ^{31}P to ^{28}Al during irradiation. While there is approximately 2000 times more P than Al in uncontaminated fish (Shearer 1984), INAA is fortunately over 1000 times more sensitive to Al than to P because of the correspondingly higher conversion efficiency. We therefore adopted an empirical approach, calibrating the Al/P radioactivity of experimental samples against known Al standards. The mean "Al/P concentrations" of all fish in a particular treatment from Al = 0 cells (i.e. no Al exposure) were subtracted from the Al-exposed fish to give mean "ΔAl concentrations." The assumption here was that the P concentration was relatively constant across treatments. This assumption appears valid as variation in "Al/P concentrations" between groups over wide ranges of water pH and Ca (at Al = 0) was generally less than 10%. Based on these considerations, the detection limit for Al accumulation was a ΔAl of approximately 0.3 µmol/g, which corresponded to a 20–30% elevation in radioactivity for "Al/P concentration" above that present in fish from the relevant Al = 0 cells. The reproducibility for Δ Al was better than ± 10% above 2 µmol/g, based on repeated irradiations.

Statistical Analyses

Within each cell, data were expressed as means ± 1 SEM for $n = 8 - 10$ fish generally, though for a few cells (<10%) the n was as low as 4. Over the complete matrix, the relative

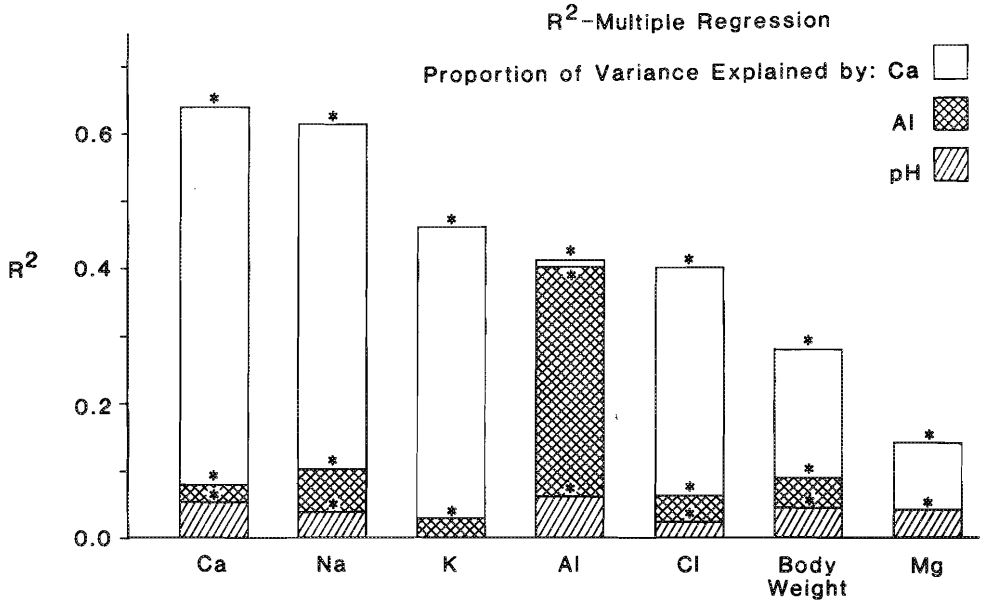

FIG. 2. Total variance explained (R^2) and proportion attributable to each factor (Ca, Al, pH), as determined by stepwise multiple regression analysis, for various whole body parameters in brook trout fry after 91 d continuous exposure to the matrix of pH, Ca, and Al. Asterisks indicate relationships which were also significant by factorial analysis of variance.

effects of pH, and Ca on whole body ion status and weight were first assessed using a factorial analysis of variance. The relative effects of the significant factors were then modelled, using stepwise multiple linear regression over the entire matrix. While relationships with pH appeared linear, marginal plots of the data suggested that some relationships with Ca and Al were not necessarily linear, and might be logarithmic. In addition, relationships with Al often appeared parabolic ($y = ax^2 + bx + c$), the greatest effects being seen at intermediate Al levels, with lesser effects at lower and higher levels. Therefore in these analyses both linear and logarithmic relationships were evaluated, and Al^2 and $(\log Al)^2$ terms were included in the regression program to permit Al to assume parabolic relationships. Factors were included in a regression only if their value of F to enter was significant ($p<0.05$). In some instances, significant factors as determined by analysis of variance did not enter the regression equations. This would occur when their interaction effect(s) had entered with a more dominant factor. The opposite, a non-significant factor by analysis of variance entering the regression equation, occurred less often than predictable by chance alone. The final relationship accepted for each ion was the one which accounted for the maximum amount of variance. The total explained variance (R^2) and the proportion of that variance contributed by each factor (factor R^2) are summarized for each whole body parameter in Fig. 2. Asterisks indicate that the factor was also significant by factorial analysis of variance. Statistical analysis of the survival data has been reported by Ingersoll et al. (1990).

Results

Temporal Changes

As a background to this and the following study (Wood et al. 1990), Fig. 1 summarizes time-dependent changes in body composition of trout raised continuously under control conditions (pH = 6.5, Ca = 2mg/L, Al = 0 μg/L). This summary combines data sets taken at day 91 post-fertilization in the present study with those taken at days 70, 90 – 91, and 111 in several experiments described by Wood et al. (1990). All fish were raised simultaneously, and at any one time there were no significant differences among the different experiments. Exogenous feeding started between days 55 and 60; by day 70 the yolk-sac appeared to be about half resorbed, and by day 111 only a small remnant persisted.

From days 70 through 111, there was a small decline (~20%) in wet weight and a large decline (~60%) in dry weight as the alevins converted yolk into somatic tissue (Fig. 1). Thus water content increased considerably. During this same period, the concentrations of all measured whole body ions except Mg increased significantly (Fig. 1). The relative increases ranged from ~1.3-fold for K to ~3-fold for Ca. Only in the case of K could the reduction of wet body weight possibly explain the increase in concentration. Thus for Na, Cl, and Ca, there were real increases on a per individual basis as well as a body weight basis. In contrast, the concentration of Mg fell by ~60% between days 70 and 111.

Relative Influence of pH, Ca, and Al after 91 D

Fig. 2 summarizes the results of the multiple regression analyses for the major measured parameters. In every case, a factor significant by this analysis was also significant by factorial analysis of variance. The proportion of overall variance explained by the three measured variables Ca, Al, and pH ranged from over 60% for body Ca and Na concentrations down to about 14% for Mg concentration. For all parameters except ΔAl, water Ca was clearly the dominant influence after 91 d exposure, with water Al and pH playing relatively minor roles.

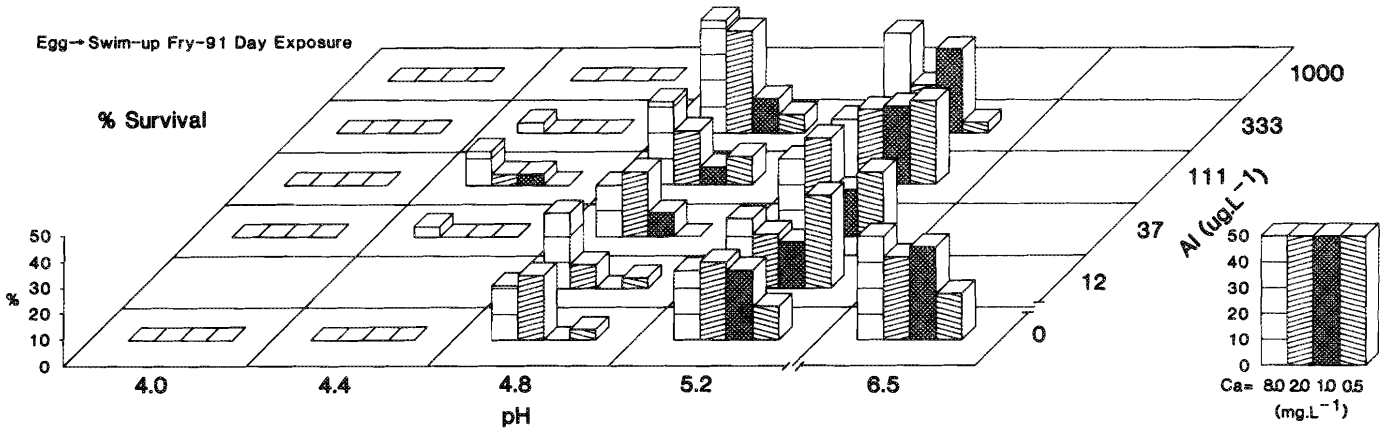

FIG. 3. Percentage survival of brook trout fry after 91 d continuous exposure to the designated matrix of pH, Ca, and Al from fertilization through swim-up. Each cell originally contained 50 eggs.

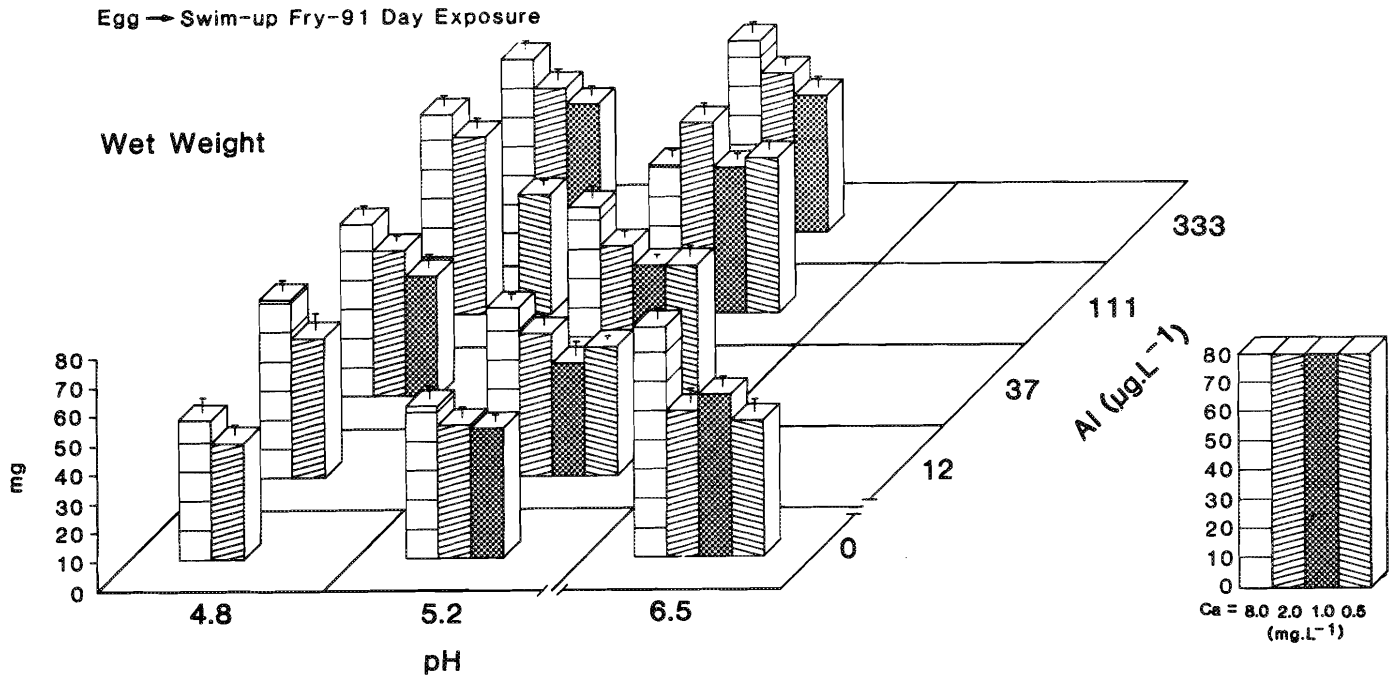

FIG. 4. Wet body weight of brook trout fry after 91 d continuous exposure to the designated matrix of pH, Ca, and Al. In cells not shown, data were not available due to low survival (cf. Fig. 3). Means ± 1 SEM for $n = 8 - 10$ fish in most cells, falling as low as four in cells with high mortality.

Mortality and Body Weights after 91 D

The relatively low survival (<50%; Fig. 3) even under control conditions was probably due to incomplete fertilization (see Ingersoll et al. 1990). At pH = 6.5 without Al, survival was strongly dependent on water Ca (Fig. 3). A similar protective effect of Ca was seen throughout the matrix, especially at pH = 4.8. Survival was negatively correlated with acidity; mortality was virtually complete at pH = 4.0 and 4.4. Al had no significant effect, though intermediate levels (Al = 37 – 111 μg/L) may have slightly ameliorated acid toxicity.

Wet body weight was also positively correlated with Ca (strongest with log Ca) and negatively correlated with acidity throughout the matrix (Fig. 2, 4). Al had a small influence in raising wet weight under acidic conditions. Dry body weight (not shown) was unaffected by Ca or Al, but was influenced in a biphasic fashion by pH, being greater at pH = 4.8 and 6.5 than at 5.2.

Whole Body Electrolytes after 91 D

Because of heavy mortality, fish were not available for analysis from some of the lower Ca cells at pH = 4.8 and 5.2, and from all cells at pH = 4.0 and 4.4. As some mortality occurred in every cell, the data are representative of the most resistant individuals in each group — i.e. after the process of selection has occurred.

In fish which had survived 91 d in the matrix, water Ca was clearly the dominant influence on the concentrations of all five whole body ions (Fig. 2), an effect which was pronounced even at pH = 6.5. Increasing Ca raised whole body levels of Na (Fig. 5) and Cl (Fig. 6), the two major extracellular electrolytes, in a concentration-dependent fashion in virtually every cell of the matrix (Fig. 2). The effect of Ca = 8 mg/L was particularly pronounced, increasing Na and Cl levels up to 2-fold above those at Ca = 0.5 mg/L. In addition, low pH had a very

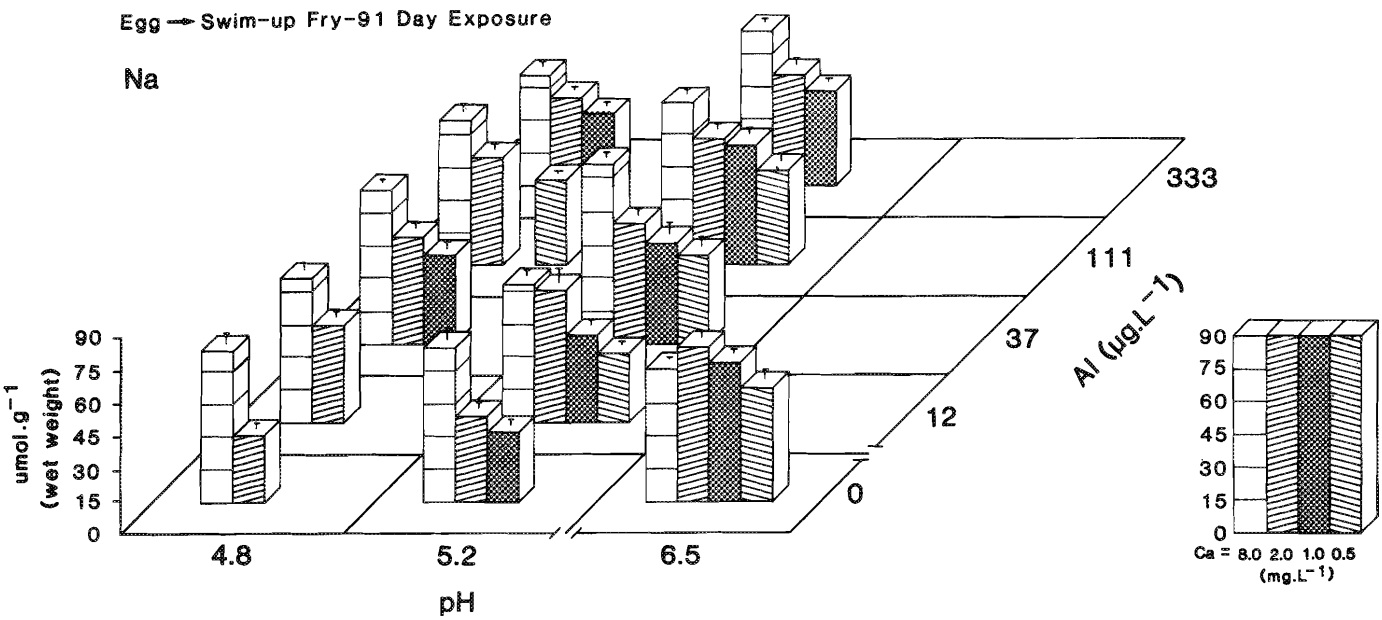

FIG. 5. Whole body concentrations of Na in brook trout fry after 91 d continuous exposure to the designated matrix of pH, Ca, and Al. Other details as in legend of Fig. 4.

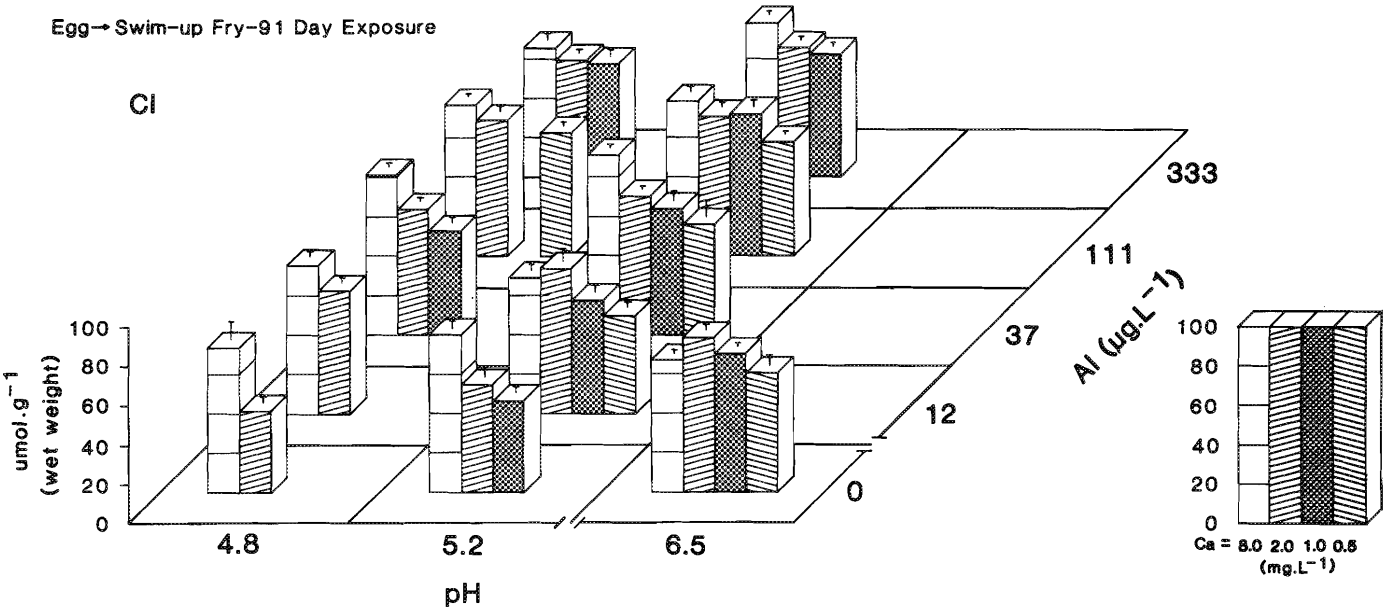

FIG. 6. Whole body concentrations of Cl in brook trout fry after 91 d continuous exposure to the designated matrix of pH, Ca, and Al. Other details as in legend of Fig. 4.

slight inhibitory effect, and Al a slight supportive effect (parabolic with log Al) which was greatest at 37 and 111 µg/L.

The positive influence of Ca was equally dominant for K (Fig. 7), the major intracellular electrolyte; here the relationship was strongest with log Ca. Again intermediate Al levels were slightly supportive, but pH had no significant effect (Fig. 2).

Not surprisingly, the greatest positive influence of Ca was on whole body Ca concentrations (Fig. 2, 8). Levels increased up to 3-fold in a step-wise fashion for any one cell over the range tested. Low pH had a small negative influence, and intermediate Al levels a small supportive effect.

The threshold for the supportive effect of water Ca on whole body Ca concentration at pH = 6.5 was between Ca = 0.5 and 1 mg/L, whereas under acidic conditions it lay between Ca = 2 and 8 mg/L (Fig. 8). Similar trends were seen for whole body Na (Fig. 5), Cl (Fig. 6), and K (Fig. 7), suggesting that the difference in threshold under acid conditions was a general phenomenon. None of the positive effects of Ca were due to expressing the data on a body weight basis, because Ca also positively influenced wet weight (Fig. 2, 4). Thus on a per fish basis, the effects were even greater.

In contrast to the other ions, whole body Mg was decreased by increasing water Ca (strongest with log Ca), an effect seen clearly in every cell of the matrix (Fig. 9). Acidity had a slight positive influence on Mg levels, while Al was without effect. Thus adverse conditions which tended to reduce the other ions and wet body weight also tended to raise whole body Mg concentrations.

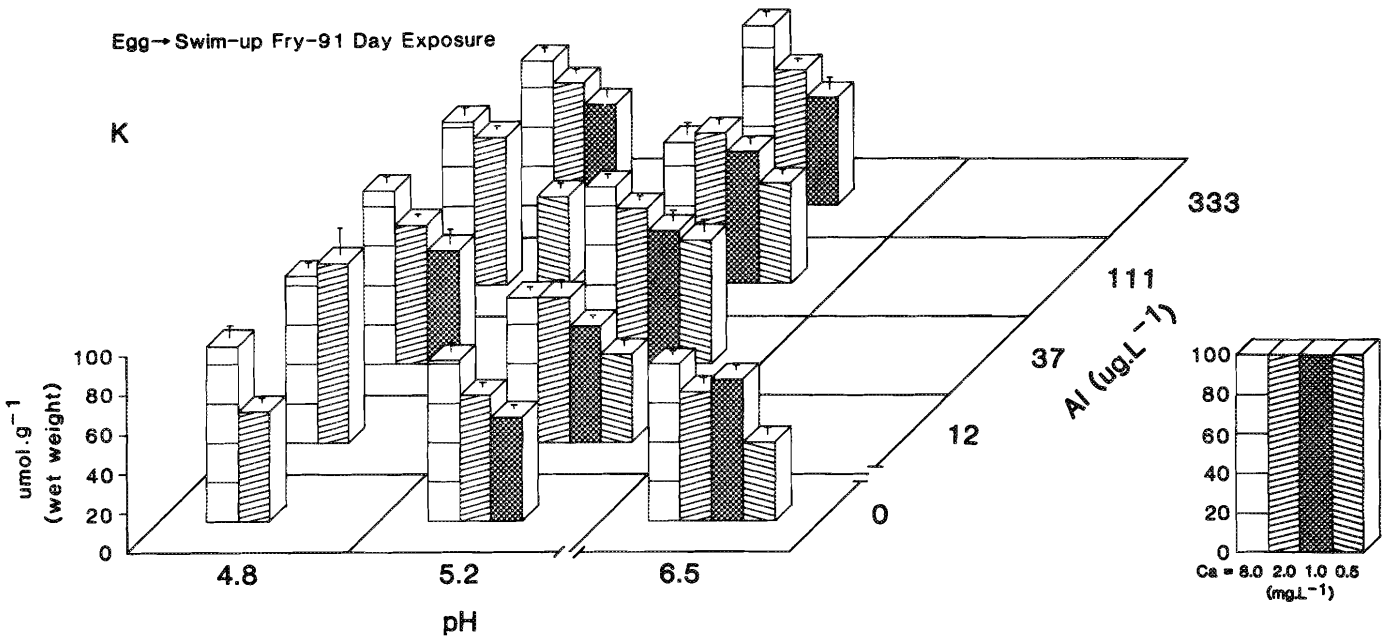

FIG. 7. Whole body concentrations of K in brook trout fry after 91 d continuous exposure to the designated matrix of pH, Ca, and Al. Other details as in legend of Fig. 4.

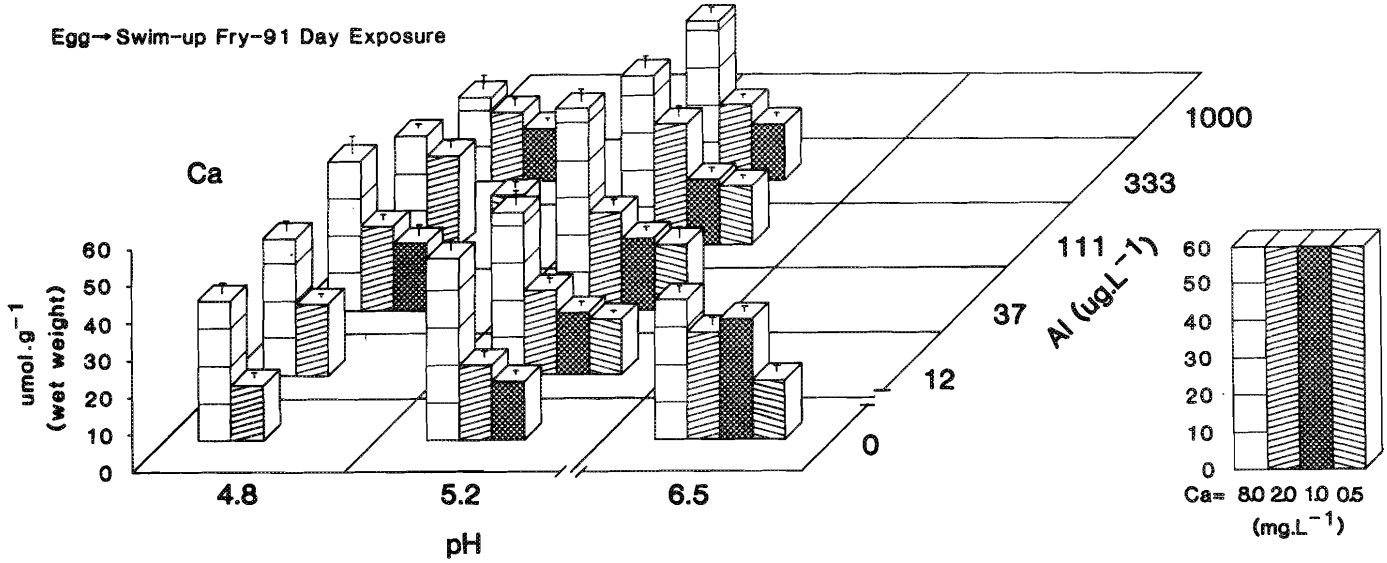

FIG. 8. Whole body concentrations of Ca in brook trout fry after 91 d continuous exposure to the designated matrix of pH, Ca, and Al. Other details as in legend of Fig. 4.

Aluminum Accumulation

Significant ΔAl was detected only at water Al = 111 and 333 μg/L, so was positively correlated with the water Al level Fig. 10). Over the matrix as a whole, the effects of Ca and acidity were small relative to those of water Al (Fig. 2). However, in those cells showing significant aluminum accumulation, both Ca and acidity had strong negative influences on ΔAl. For example, the peak accumulation of 9.4 μmol/g (at pH = 5.2, Ca = 1 mg/L, Al = 333 μg/L) was halved by either a reduction of pH to 4.8 at the same Ca, or by increasing Ca to 8 mg/L at the same pH.

Discussion

Electrolyte Concentrations and Changes during Development

Concentrations of Na, Cl, K, Ca, and Mg and their changes over time in developing brook trout embryos under control conditions, as determined by INAA in the present study (Fig. 1), were similar to those reported by Hayes et al. (1946) in developing *Salmo salar*. McCay et al. (1936) reported similar Ca concentration data in brook trout embryos. These early studies employed chemical analytical techniques. There was also good agreement with more recent atomic emission determinations of Na, K, Ca, and Mg in early life stages of other salmonid species (Zeitoun et al. 1976; Shen and Leatherland 1978a; Talbot et al. 1982). Direct comparison with the most detailed study, that of Peterson and Martin-Robichaud (1986) on Na, K, Ca, and Mg changes (by ion chromatography) in developing *Salmo salar*, is difficult because of the different basis for data expression used in their study. Nevertheless, the two studies appear in broad qualitative agreement.

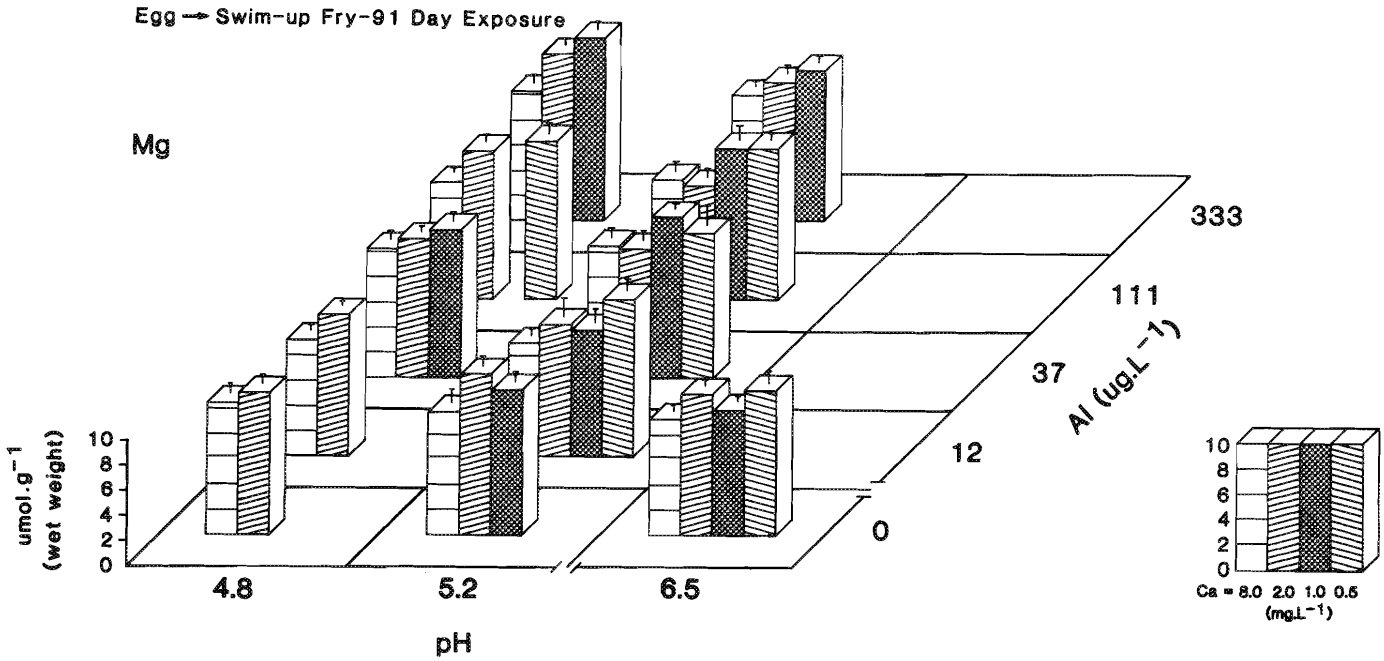

FIG. 9. Whole body concentrations of Mg in brook trout fry after 91 d continuous exposure to the designated matrix of pH, Ca, and Al. Other details as in legend of Fig. 4.

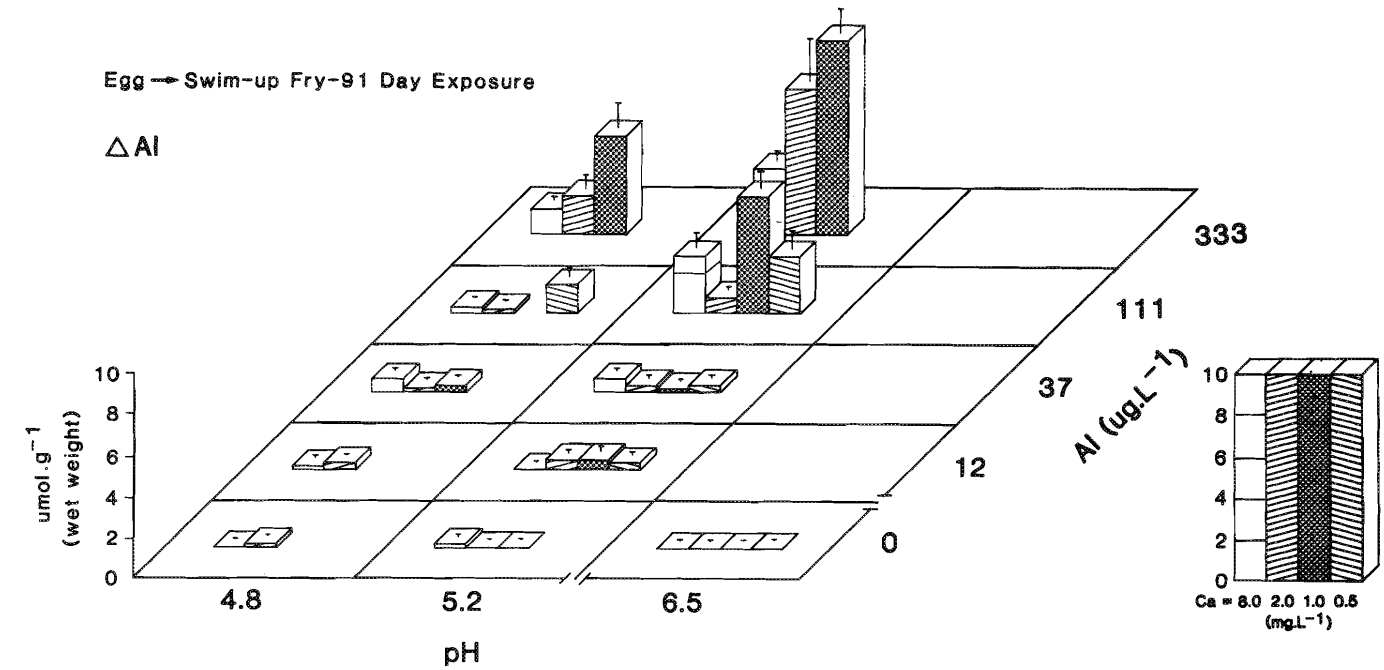

FIG. 10. Whole body accumulation of aluminum (ΔAl) in brook trout fry after 91 d continuous exposure to the designated matrix of pH, Ca, and Al. Other details as in legend of Fig. 4.

A finding common to all these studies is that development from fertilization through yolk-sac resorption involves marked net accumulation of electrolytes by the embryo (cf. Fig. 1). The detailed analyses of Hayes et al. (1946), Zeitoun et al. (1976), Rombough and Garside (1984), and Peterson and Martin-Robichaud (1986) indicate that the external environment is the source of more than half the Na and Ca uptake, and at least in three of the studies, a smaller fraction of the K uptake. Magnesium is the only ion present in the yolk in clear excess of the requirements of the embryo; it therefore decreases in concentration during development (cf. Fig. 1). The major routes of ion uptake are thought to occur via rudimentary "chloride cells" which occur on the yolk-sac epithelium and skin, as well as on the developing gills (Shen and Leatherland 1978b; Hwang and Hirano 1985). Net accumulation will represent the difference between active uptake from and diffusive loss to the dilute external environment. Exogenous feeding may also play a role, especially after swim-up (Talbot et al. 1982). However, to date it has not been possible to quantify the contribution of this route to net ion accumulation.

Effects of 91 D Exposure

The most important finding of the present study is that water Ca level, rather than pH or Al, was the major determinant of

net electrolyte accumulation in fish which have been raised continuously from fertilization through swim-up in an extensive pH/Ca/Al matrix (Fig. 2). The effects were most dramatic for Ca and Na, where water Ca explained more than 50% of the overall variance; for Cl and K, Ca accounted for more than 35% of the variance. This does not mean that acidity and Al were without toxic effect. Indeed acidity was the major determinant of mortality in this exposure prior to hatching, and Al was the major determinant after hatching (Ingersoll et al. 1990). While Ca was positively related to survival over both periods, its influence was less than those of the other two factors. In other words, the toxic effects of pH and Al eliminated all of the fish in many of the exposure cells and depleted other cells prior to the sample time of this experiment. What remained were survivors where the positive influence of Ca was clearly the dominant sublethal effect.

This large positive influence of Ca on whole body Na, Cl, K, and Ca levels, the much smaller positive influence of Al, and the negative influence of acidity could result from effects on developmental rate and/or on ion exchange rates. Body weight, either wet or dry, is not a good index of developmental rate for these early life stages because it tends to decrease as development proceeds under control conditions (Fig. 1; also Geen et al. 1985). Furthermore it may be affected in a complex fashion by adverse conditions, as indicated by the biphasic pattern in dry weight with pH, combined with the negative influence of acidity and positive influence of Ca on wet weight (Fig. 4). When taken together with the temporal changes in Fig. 1 and the observations of Ingersoll et al. (1990) that low pH delayed hatching, yolk-sac resorption, swim-up, and feeding, these data suggest that the body weight changes resulted from two opposing influences. Unfavourable conditions tended to delay development, thereby reducing the decrease in weight (especially dry weight) due to yolk-sac resorption. At the same time, these conditions tended to increase metabolic costs and reduce food intake, thereby accelerating metabolic weight loss and decreasing embryo growth. Thus, depending on their severity, adverse conditions which delay development may either retard or accelerate the normal weight loss associated with ontogeny.

However, Mg present in high concentration in the yolk but low concentration in the embryo (Hayes et al. 1946; Peterson and Martin–Robichaud 1986), appears to be a useful marker for developmental rate (Fig. 1). Whole body Mg concentrations were clearly lowered by increasing Ca and slightly raised by increasing acidity, in contrast to the trends with all other ions (Fig. 9). This suggests that development was delayed by low water Ca and low pH. Many other studies have shown that low pH, either alone or in combination with Al, inhibits development in early life stages of salmonids (eg. Geen et al 1985; Peterson and Martin–Robichaud 1986; Hunn et al 1987; Gunn and Noakes, 1987), but the marked effect of low water Ca has not been documented previously.

On the basis of the present data alone, it is not possible to conclude whether effects of Ca, Al, and pH on whole body ions were due to altered ion exchange rates or to altered developmental rates, or both. Indeed, developmental rate may either limit or be limited by the rate of net electrolyte accumulation. However, the companion study which examines age-specific challenges in the matrix (Wood et al. 1990) indicates that both effects occur and, at least in the yolk-sac stage, that pronounced influences on whole body ion accumulation can be demonstrated in the absence of developmental effects. Peterson and Martin–Robichaud (1986) also documented an inhibition of ion accumulation which was both separate from and additional to inhibited development in *Salmo salar* embryos exposed to low pH alone for 135 d.

The negative effect of low pH on body ion concentrations has been repeatedly demonstrated in adult fish, where it results from both inhibition of active uptake, probably through the "chloride cells," and stimulation of diffusive loss, probably through the paracellular channels of the gills (cf. Wood 1989). Similar explanations probably apply to the transporting epithelia (vitelline membrane, developing gills) of the embryo. In the adult, the protective effect of water Ca is largely due to its action in curtailing diffusive losses by reducing branchial permeability (Wood 1989). This is probably also the basis of its present dramatic action on whole body ion levels in embryos. The particularly marked effect of water Ca on whole body Ca levels (Fig. 8) may result from an additional factor, the pronounced concentration-dependence of branchial Ca uptake which has been documented in adult fish (Mayer–Gostan et al. 1983; Perry and Wood 1985). The small but significant "parabolic" effect of Al on most whole body ions is considered in greater detail by Wood et al. (1990). In brief, it can be interpreted as a protective action at intermediate levels due to the ability of Al to either substitute for Ca in stabilizing membrane permeability or to induce proliferation of "chloride cells." At higher levels, this protection disappears as Al itself becomes toxic to ionoregulation.

Protective effects of Al were seen at water Al levels below which significant ΔAl accumulation was detected (Fig. 10). Accumulation occurred in exposure cells (pH = 5.2, Al = 111 and 333 μg/L; pH = 4.8, Al = 333 μg/L) where Al was close to its solubility limit (Johnson et al. 1981). Some or all of the ΔAl may represent an adherence to the external surface of the embryos. This could result from precipitation in the more alkaline micro-environment of the fish and/or binding to anionic organic ligands on the surface. The inhibitory effect of lower pH and higher Ca on ΔAl accumulation could result from H^+ and Ca^{2+} competition for binding sites, the change in relative speciation from a predominance of cationic hydroxides at higher pH to free trivalent Al^{3+} at lower pH, and the greater absolute solubility of Al at lower pH. Cleveland et al. (1986) found a similar inhibition of Al accumulation by lower pH (4.5 vs. 5.5) in early life stages of brook trout exposed to Al = 300 μg/L at Ca = 3 mg/L for 30 d; absolute ΔAl levels were similar in the two studies.

Environmental Significance

The protective action of Ca against the adverse effects of low pH and/or high Al on ionoregulation in adults and on survival in early life stages is well known (eg. Howells et al. 1983; Wood 1989; McDonald et al. 1989). However, the present sublethal effects on mineralization are the largest yet documented. Furthermore, this appears to be the first demonstration that water Ca has a critical influence on net ion accumulation even at pH = 6.5, in the absence of Al. The hatching to swim-up period is a time of intense mineralization; water Ca level will have a marked effect under neutral conditions, and an even greater influence under low pH and/or high Al conditions. For example, a fry raised for the first 91 d of its life at Ca = 0.5 mg/L (at pH = 6.5) would have only about 75% of the Na and Cl concentration, and only about 50% of the K and Ca concentration of one raised at Ca = 1, 2 or 8 mg/L. If the pH were

4.8 for the first 91 d, even at Ca = 2 mg/L, these values would be reduced to about 55% for Na and Cl, and 40% for Ca. The long-term survival prospects of such fish are probably poor. However, Ca = 8 mg/L affords almost complete protection, even at pH = 4.8.

This analysis emphasizes that the threshold for the beneficial effects of Ca, which is between 0.5 and 1 mg/L at pH = 6.5, is shifted to a higher Ca level, between 2 and 8 mg/L, under low pH. Thus natural soft waters which have sufficient Ca to support healthy fish development at circumneutral pH may lose this capacity if acidified. A Ca level of 8 mg/L is at the upper end of the softwater range, and probably unlikely to occur in most natural acid stress situations. This conclusion as to the importance of Ca, based on whole body ion analysis, is in general agreement with those of Wright and Snekvik (1978) and Howells et al. (1983) based on field surveys of acidified lakes. These surveys also indicated that Al was not a major determinant of fishery status in acidified lakes, which again is in accord with the present data.

One important reservation about the environmental significance of the present study must be noted. Here, fish were exposed continuously to toxic conditions from fertilization through swim-up. However, in the wild, brook trout may select areas of groundwater seepage for spawning, and avoid areas of low pH (Johnson and Webster 1977; Gunn 1986). The water in the redd may therefore be relatively alkaline, higher in Ca, and lower in Al than that in the overlying stream. Exposure to toxic conditions may only occur when the alevins emerge from the substrate to commence feeding (Trojnar 1977). The experiments of the following paper were designed to evaluate this situation (Wood et al. 1990).

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