Whole Body Ions of Brook Trout (*Salvelinus fontinalis*) Alevins: Responses of Yolk-Sac and Swim-Up Stages to Water Acidity, Calcium, and Aluminum, and Recovery Effects

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Water pH, rather than Ca or Al, was the most important factor affecting whole body ions in yolk-sac or swim-up fry exposed to a matrix of pH (6.5–4.0), Ca (0.5–8 mg/L), and Al (0–1000 µg/L). Fry were raised from fertilization (day 0) in flowing soft water (pH = 6.5, Ca = 2 mg/L, Al = 0 µg/L), exposed to pH/Ca/Al on day 49 (yolk-sac, 2 d post-hatch) or day 70 (swim-up) for 21 d, and then allowed to recover a further 20 d. Yolk-sac fry were extremely resistant at pH≥4.4; developmental effects, as indicated by body weight and Mg, were negligible. However whole body Na, Cl, K, and Ca were depressed by low pH, while water Ca was protective. Aluminum (37–111 µg/L) raised most ions above control values, while higher Al lowered them. Swim-up fry were more sensitive, showing pronounced developmental inhibition (lower weight, higher Mg) under adverse conditions; mortality continued during recovery. Low pH was again the dominant influence on body ions, water Ca was protective, while Al (12–111 µg/L) was only protective and not stimulatory. These effects persisted significantly; indeed responses in body Ca were larger after recovery than after the exposure itself. In the field, emergence from the redd into ambient acidic water is probably the critical stage. Water pH will be the principal determinant of whole body ions in alevins surviving this emergence, in contrast to fry exposed continuously from fertilization.

Le pH de l'eau plutôt que la conc. en Ca ou en Al, a constitué le plus important facteur à agir sur l'équilibre électrolytique d'alevins au stade du sac vitellin et au stade de la remontée à la nage et qui étaient exposés à des combinaisons de pH (6,5–4,0), de conc. en Ca (0,5–8 mg/L) et d'Al (0–1000 µg/L). Les alevins ont été gardés dès la fécondation (jour 0) dans une eau douce en circulation (pH = 6,5, conc. Ca = 2 mg/L, conc. Al = 0 µg/L), et exposés aux combinaisons de pH/Ca/Al au jour 49 (sac vitellin, 2 jours après l'éclosion) ou au jour 70 (remontée à la nage) pendant 21 jours; une période de récupération de 20 jours a été accordée. Les alevins au stade du sac vitellin étaient extrêmement résistants à pH 4,4 et plus; les effets sur le développement mesurés par la masse corporelle et la teneur en Mg étaient négligeables. Cependant, l'équilibre électrolytique en Na, Cl, K et Ca a été déprimé à pH acide alors que la concentration de Ca dans l'eau conférait une protection. La conc. en Al à 37–111 µg/L a fait passer la conc. de la plupart des ions à des valeurs supérieures aux valeurs de référence alors que les conc. élevées en Al ont eu l'effet contraire. Les alevins qui remontaient à la nage étaient plus sensibles que les autres et manifestaient une profonde inhibition de leur développement (poids inférieur, conc. en Mg supérieure) dans des conditions adverses; il y a eu de la mortalité pendant la période de récupération. Les bas pH sont demeurés l'influence dominante sur l'équilibre électrolytique, la conc. en Ca dans l'eau assurait une protection alors que les conc. en Al (12–111 µg/L) avaient seulement un effet de protection, mais pas de stimulation. Ces effets ont persisté de manière significative; en effet, la réaction des organismes mesurée en termes de Ca était plus importante après la période de récupération qu'après l'exposition elle-même. Sur le terrain, le

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passage du nid de fraie à l'eau acide ambiante constitue probablement l'étape critique. Le pH de l'eau constitue le principal déterminant de l'équilibre électrolytique chez les alevins qui survivent à cette émergence, contrairement aux alevins exposés continuellement depuis la fécondation.

In the preceding study, water calcium concentration (Ca) was the principal determinant of whole body ions in surviving brook trout fry which had been continuously exposed to a pH/Ca/Al matrix for 91 d from fertilization through swim-up (Wood et al. 1990). Water pH had a negative effect on most whole body ions, but its influence was small relative to that of Ca while Al had a slight positive influence at intermediate concentrations. While these findings are relevant to the field situation of salmonid species such as the lake trout (*Salvelinus namaycush*; Gunn and Keller 1984a) and Atlantic salmon (*Salmo salar*; Lacroix 1985) where the earliest life stages may be exposed to such conditions, there is some question as to their relevance to the natural reproduction of brook trout (*Salvelinus fontinalis*). Adult brook trout may select regions of groundwater seepage, especially alkaline upwellings, in which to build their redds, and actively avoid areas of low pH when spawning (Webster and Eiriksdottir 1976; Johnson and Webster 1977). As Trojnar (1977) and Gunn (1986) have pointed out, this strategy may effectively insulate the earliest developmental stages from adverse conditions. Instead, toxicity may occur primarily when the older alevins (yolk-sac or swim-up fry) emerge from the gravel into overlying softer, more acidic, Al-contaminated water, especially during episodic acid surges accompanying snow-melt in early spring.

The principal goal of the present study was to determine whether similar or different effects on whole body ions occurred when brook trout were exposed to pH/Ca/Al conditions only in the yolk-sac or swim-up fry stages, rather than continuously from fertilization to swim-up. The exposures were performed as part of the toxicological studies reported by Ingersoll et al. (1990a). To facilitate comparison with the 91 d continuous exposure study (Wood et al. 1990), the experiments were performed simultaneously, and the fry stocks, exposure matrix, and analytical techniques were all identical. A second objective was to compare the sensitivity and responses of yolk-sac vs. swim-up fry. The relative importance of the gills as an ionoregulatory and respiratory site should be greater in swim-up fry, and the importance of the vitelline membrane and skin for these functions correspondingly reduced. A final goal was to examine recovery effects on whole body ions and Al accumulation in survivors, for by definition episodic acid surge events are short-lived, generally lasting a few days to a few weeks (Christophersen et al. 1984; Marmorek et al. 1985).

Materials and Methods

Ingersoll et al. (1990a) and Wood et al. (1990) describe details of the fish, the experimental regimes, the water chemistry, the sampling procedures, the instrumental neutron activation analysis (INAA) technique for whole body ions and Al, and the statistical procedures used in analyzing the data. The 84 cell matrix 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) was the same as that used in the 91 d exposures. In the yolk-sac fry experiments, cells at Ca = 1 and 8 mg/L and Al = 12 μg/L were

not sampled for INAA. In the swim-up fry experiments, cells at Ca = 1 mg/L were not sampled.

In brief, for the studies which constitute the focus of the present paper, brook trout were raised from fertilization under control conditions (pH = 6.5, Ca = 2 mg/L, Al = 0 μg/L) in flowing artificial soft water (Na≈4, Cl≈8 mg/L, 10.9°C). Control conditions were maintained until the fish were exposed to the pH/Al/Ca matrix. After exposure, fish were returned to control conditions for the recovery period. Typically, 8–10 survivors were preserved for INAA from each cell which was sampled, though this was reduced in cases where mortality had rendered this number unavailable. The smallest numbers taken were four per cell at the end of the challenges, and three per cell at the end of the recoveries. Each fish was analyzed by INAA for whole body concentrations of Na, Cl, K, Ca, Mg, and aluminum accumulation (ΔAl) as described by Wood et al. (1990). In both yolk-sac and swim-up series, several cells were sacrificed for other purposes, and therefore could not be used for INAA.

The yolk-sac fry challenge started on day 49 post-fertilization, which was about 2 d after the median time of hatch. Twenty-five yolk-sac fry were transferred into each cell of the matrix for 21 d. After sampling on day 70, the remaining alevins were returned to the control condition for a further 20 d. Recovery samples were taken on day 90. In these yolk-sac fry experiments, only the surviving fish challenged at Ca levels of 0.5 and 2 mg/L were sampled for INAA, and fish at Al = 12 μg/L were omitted.

The swim-up fry challenge started on day 70, by which time exogenous feeding had been occurring for 10–15 d (though unquantifiable) and the yolk-sac appeared to be about 50% resorbed. Again, 25 fish were transferred into each cell of the matrix for 21 d. After sampling on day 91, the remaining fry were returned to the control condition for 20 d. Recovery samples were taken on day 111. In these swim-up fry experiments, the surviving fish challenged at Ca levels of 0.5, 2, and 8 mg/L were sampled for INAA.

Results

Yolk-Sac Fry — Relative Influence of pH, Ca, and Al

Figure 1 summarizes the results of the multiple regression analyses for the major measured parameters at the end of the 21 d exposure, and after a further 20 d recovery under control conditions. Except for the very small effect of water pH on whole body Mg after recovery (Fig. 1B), all factors significant by this analysis were also significant by factorial analysis of variance. The proportion of overall variance explained by the three measured parameters (Ca, Al, and pH) was much greater immediately after exposure (Fig. 1A) than after 20 d recovery (Fig. 1B). Nevertheless, small effects, particularly of pH, persisted. After exposure, pH was the dominant influence on whole body Na and Cl, followed by Ca and then Al, the three together accounting for approximately 60% of the variance
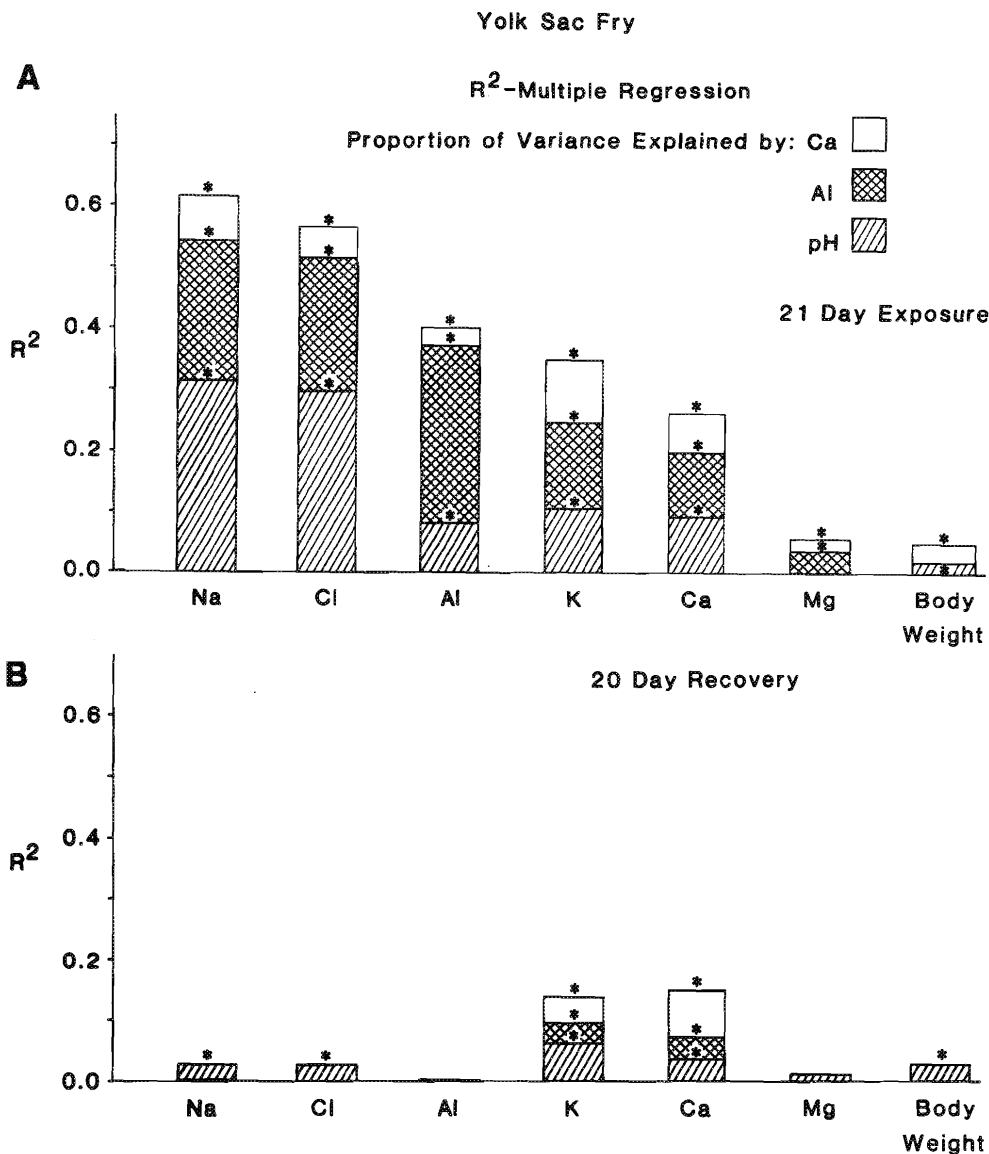
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Yolk Sac Fry

A

R^2-Multiple Regression

Proportion of Variance Explained by: Ca ☐

Al ▨

pH ▨

21 Day Exposure

B
20 Day Recovery

FIG. 1. Total variance explained (R^2) and proportion attributable to each factor (Ca, Al, pH of the exposure matrix), as determined by stepwise multiple regression analysis, for various whole body parameters in yolk-sac fry of brook trout. Results are shown for fry after 21 d exposure and following 20 d recovery under control conditions (pH = 6.5, Ca = 2 mg/L, Al = 0 μg/L). Asterisks indicate relationships which were also significant by factorial analysis of variance.

(Fig. 1A). For whole body K and Ca, the three factors accounted for approximately equal, though much smaller, percentages of the variance. Not surprisingly, water Al was the dominant influence on whole body ΔAl, an effect which completely disappeared after 20 d recovery (Fig. 1B).

Yolk-Sac Fry — Mortality and Body Weights

In terms of survival, yolk-sac fry were extremely resistant to low pH and Al, except at pH = 4.0, where mortality was virtually complete (cf. Ingersoll et al. 1990a). At the two Ca levels sampled for INAA (0.5 and 2 mg/L), survival was 80–100% in most cells at pH = 4.4 and above. The only exceptions were pH = 4.4 at Al = 0 or 1000 μg/L), where survival was 30–45%; intermediate levels of Al (37–333 μg/L) were protective at pH = 4.4. Ca had no significant effect. During recovery, sur-

vival was again high (80–100%), and there were no significant effects of the previous pH, Ca, or Al levels during challenge.

Body weight, either wet or dry, was similarly unresponsive to pH, Ca, or Al, during both challenge and recovery. Multiple regression analyses detected significant positive effects on wet weight of pH and Ca during the challenge and of pH alone during recovery. However the R^2 values were so small (<0.04) as to be of doubtful biological importance. There were no effects on dry weight.

Yolk-Sac Fry — Whole Body Electrolytes after Challenge

Despite the insensitivity of mortality and body weight to the experimental conditions in yolk-sac fry, INAA revealed marked sublethal effects on all whole body ions except Mg.

After 21 d exposure, pH was the dominant influence on the concentrations of the two major extracellular electrolytes, Na
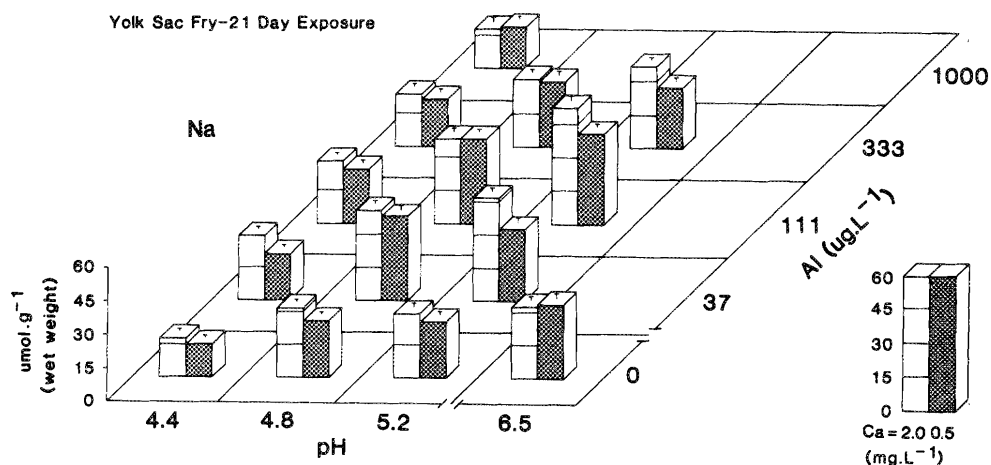
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Yolk Sac Fry-21 Day Exposure

Na

FIG. 2. Whole body concentrations of Na in yolk-sac fry of brook trout after 21 d exposure to a matrix of pH, Ca, and Al. Means ± 1 SEM for $n = 8$–10 fish in most cells, falling as low as four in cells with high mortality.

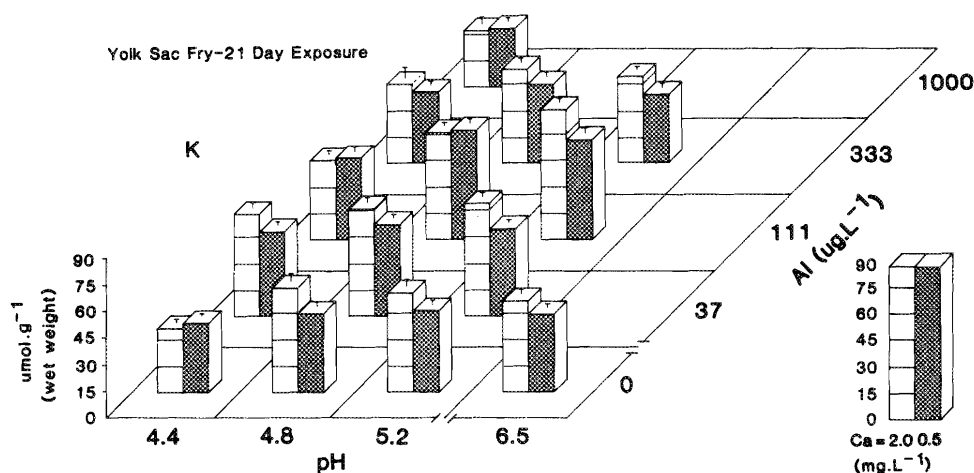Yolk Sac Fry-21 Day Exposure

K

FIG. 3. Whole body concentrations of K in yolk-sac fry of brook trout after 21 d exposure to a matrix of pH, Ca, and Al. Other details as in legend of Fig. 2.

and Cl, accounting for about 30% of the overall variance (Fig. 1A). Only the Na data are shown (Fig. 2), for all trends with Cl were very similar, though absolute concentrations were almost twice as high at this stage, day 70 (cf. figure 1 of Wood et al. 1990). In the absence of Al, whole body Na and Cl were depressed in a linear fashion by low pH, reaching only half the control levels at pH = 4.4 (Fig. 2). At all acidic pH's, intermediate levels of Al exerted pronounced protective/stimulatory effects on Na and Cl concentrations. These effects were greatest at Al = 37 and 111 μg/L, and were best described by parabolic relationships with log Al, which explained a further 20% of the overall variance (Fig. 1A). Al not only protected the fish against the inhibitory effects of low pH, but actually raised whole body Na and Cl levels up to 65% *above* the control values. These stimulatory effects were seen most clearly at pH = 4.8 and 5.2 with Al = 37 and 111 μg/L (Fig. 2). Exposure to higher levels of water Ca (2 vs. 0.5 mg/L) also exerted a small positive influence in most cells.

Whole body K was also strongly affected by the exposures (Fig. 3), being lowered by acidity, raised by Ca and interme-

diate Al levels (37–111 μg/L), and slightly depressed by the highest Al levels (333–1000 μg/L). Each factor accounted for about 10% of the total variance (Fig. 1A). As for Na and Cl, K was significantly elevated *above* control levels by the presence of Al in several cells at pH 4.8 and 5.2; the absolute increases were approximately 30%. The overall effect was best described by a parabolic relationship with Al.

Whole body concentrations of Ca were much lower than those of K, but responded in a generally similar fashion after 21 d challenge (Fig. 1A, 4). Thus Ca was depressed in a linear fashion by low pH, increased by water Ca, and affected by Al in a parabolic manner. The highest Al levels tended to decrease whole body Ca, while intermediate levels were clearly protective. However, the presence of Al raised Ca above the control level in only one cell, a less pronounced effect than seen for Na, Cl, and K.

In contrast to the other ions, Mg was largely unaffected by the exposure conditions. There were weak negative influences of Al and Ca, but together these accounted for less than 6% of the overall variance; pH had no significant effect (Fig. 1A).
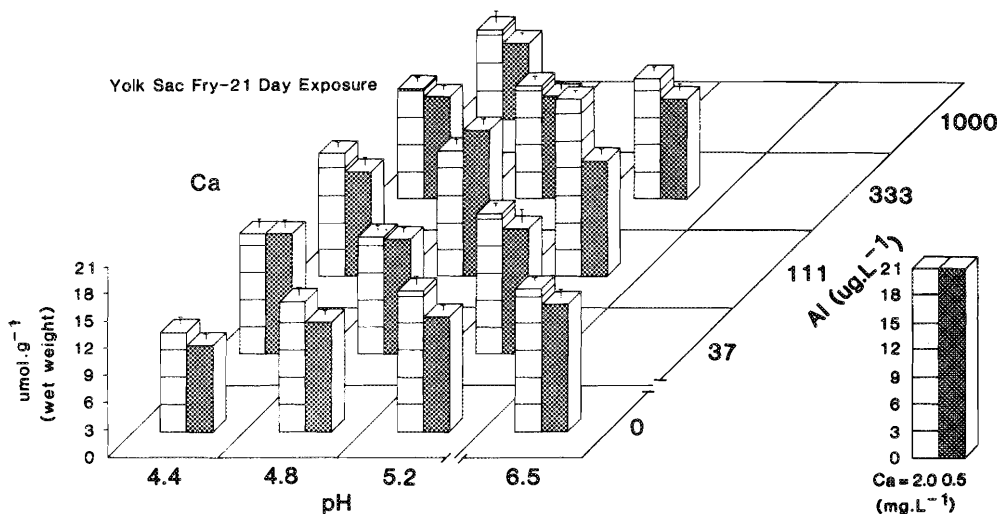
Fig. 4. Whole body concentrations of Ca in yolk-sac fry of brook trout after 21 d exposure to a matrix of pH, Ca, and Al. Other details as in legend of Fig. 2.

TABLE 1. A comparison of whole body ion levels (μmol/g wet weight) in selected cells (Ca = 2 mg/L) of the yolk-sac fry challenge experiment at the end of 21 d exposure, and after 20 d of recovery under control conditions (pH = 6.5, Ca = 2 mg/L, Al = 0 μg/L). Means $\pm$ 1 SEM (N). * = $p \leqslant$ 0.05 relative to comparable control value (pH = 6.5, Al = 0 μg/L) at this time; $\dagger$ = $p \leqslant$ 0.05 relative to comparable value for this same treatment at end of 21 d exposure.

| pH | Al | 21 d exposure (day 70) | | | 20 d recovery (day 90) | | |
		Na	K	Ca	Na	K	Ca
6.5	0	32.7	52.5	16.0	51.0†	72.3†	24.1†
		$\pm$1.6	$\pm$3.2	$\pm$0.7	$\pm$4.1	$\pm$6.1	$\pm$1.5
		(10)	(10)	(10)	(9)	(9)	(9)
5.2	111	54.4*	73.9*	20.1*	52.5	69.7	25.8†
		$\pm$2.6	$\pm$2.7	$\pm$0.3	$\pm$7.1	$\pm$2.4	$\pm$1.7
		(9)	(9)	(9)	(5)	(5)	(5)
4.4	0	17.6*	36.9*	10.9†	34.0†,*	45.1†,*	17.3†,*
		$\pm$0.4	$\pm$1.6	$\pm$0.8	$\pm$6.8	$\pm$4.0	$\pm$1.7
		(10)	(10)	(10)	(6)	(6)	(6)

Yolk-Sac Fry — Whole Body Electrolytes after Recovery

Twenty days of recovery under control conditions (by which time the fry were in the swim-up stage) removed most of the effects of pH, Al, and Ca seen at the end of 21 d challenge in the matrix (Fig. 1B). Small negative influences of low pH exposure persisted in recovery for most ions, a trend particularly prominent in cells at Al = 0 μg/L. Small positive effects of water Ca and a slight parabolic influence of Al on whole body K and Ca also persisted.

Table 1 compares selected whole body ion levels after 21 d exposure and after 20 d recovery for three representative exposure combinations at Ca = 2 mg/L: control (pH = 6.5, Al = 0 μg/L), a cell (pH = 5.2, Al = 111 μg/L) where the stimulatory effect of Al had been greatest, and a cell (pH = 4.4, Al = 0 μg/L) where the depressant effect of acidity had been greatest. During the 20 d period of "recovery", whole body Na, Cl, K, and Ca levels all increased significantly in the control group as part of normal development (cf. figure 1 of Wood et al. 1990). In the group which had been exposed to severe acid-

ity alone, electrolyte levels also increased substantially, but did not "catch up" to the levels of the controls. In contrast, the group which had been exposed to Al showed virtually no change during 20 d of recovery. Their electrolyte levels at the end of 21 d exposure were already at values attained 20 d later in the controls, and remained generally static over this period, except for Ca which increased slightly.

Swim-Up Fry — Relative Influence of pH, Ca, and Al

Figure 5 summarizes the results of the multiple regression analyses for the effects of pH, Ca, and Al on the various measured parameters in swim-up fry. Apart from the influence of water Al on whole body Na and ΔAl levels after 20 d recovery (Fig. 5B), all factors significant by multiple regression were also significant by factorial analysis of variance. At the end of 21 d exposure (Fig. 5A), overall patterns in swim-up fry were similar to those seen at this point with yolk-sac fry (cf. Fig. 1A), although the relative influence of water pH on whole body Na, Cl, and K was even greater, while that of water Al was reduced. In addition, the three experimental variables exerted a much greater effect on body weight and Mg content than seen with yolk-sac fry. After 20 d recovery (Fig. 5B), influences on whole body Na, Cl, and Ca levels persisted much more strongly than in yolk-sac fry (cf. Fig. 1B), a phenomenon which was most marked for the effects of both water Ca and pH on whole body Ca content.

Swim-Up Fry — Mortality and Body Weights

In terms of survival, swim-up fry were more sensitive than yolk-sac fry to low pH and Al (cf. Ingersoll et al. 1990a). Mortality was complete in all cells at pH = 4.0, in all cells at pH = 4.4 with Al = 1000 μg/L, and at pH = 5.2 with Al = 333 μg/L, Ca = 0.5 mg/L. Survival was also depressed from normal levels of 80–100% to 20–70% in some of the other more acidic cells, especially at lower Ca and higher Al levels. A further difference from the yolk-sac pattern was seen in recovery, for mortality continued during the recovery period at equal or higher levels than during exposure itself. Survival during the recovery period was strongly positively correlated with the water Ca of the exposure period.
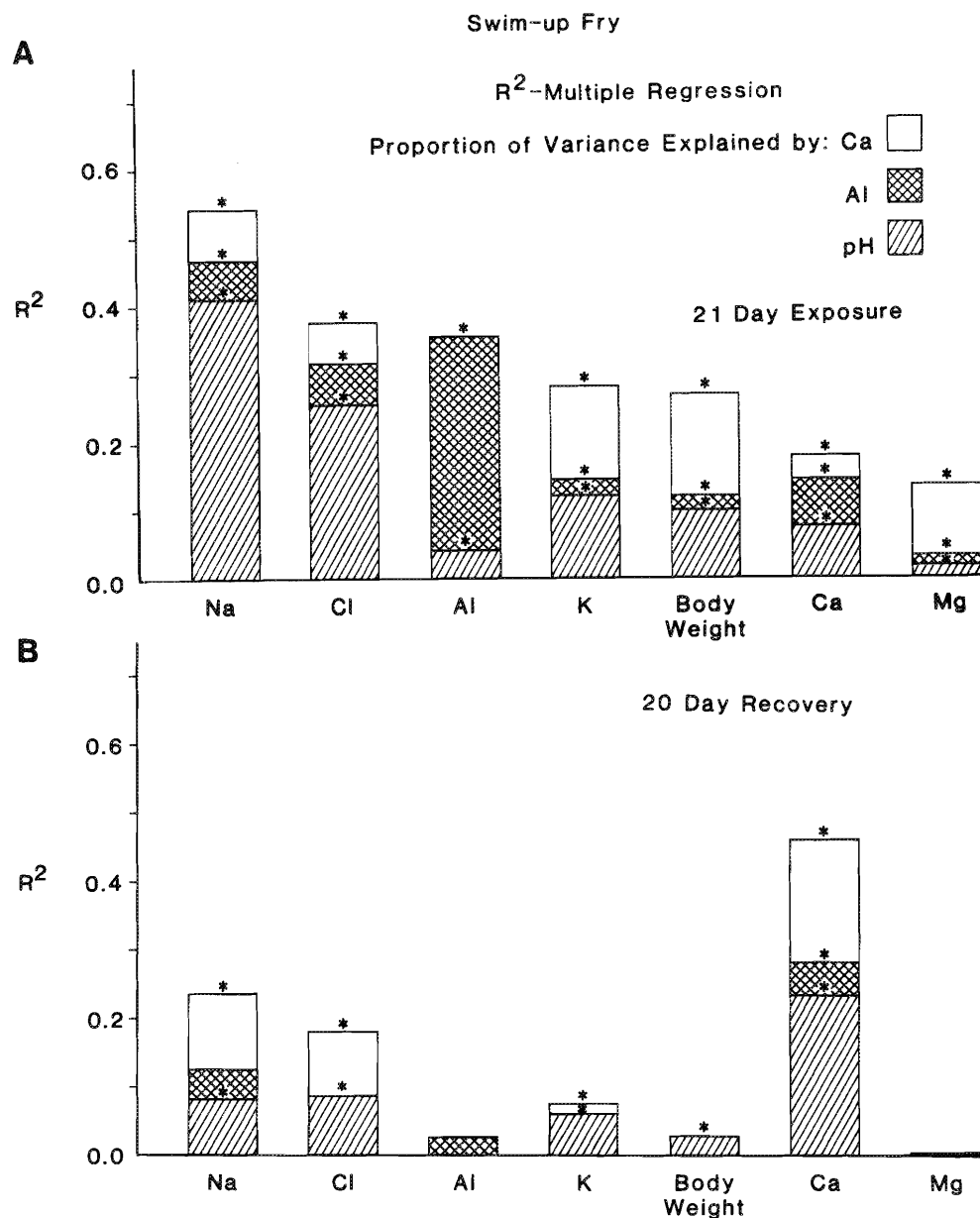
Swim-up Fry

A

B

FIG. 5. Total variance explained (R^2) and proportion attributable to each factor (pH, Ca, Al of exposure matrix) as determined by stepwise multiple regression analysis, for various whole body parameters in swim-up fry of brook trout. Results are shown for fry at the end of 21 d exposure and following 20 d recovery under control conditions (pH = 6.5, Ca = 2 mg/L, Al = 0 μg/L). Asterisks indicate relationships which were also significant by factorial analysis of variance.

Again in contrast to yolk-sac fry, wet body weight of swim-up fry was strongly influenced by the exposure conditions, which accounted for almost 30% of the total variance (Fig. 5A). Wet weight was significantly decreased by low pH and raised by Ca, while Al exerted a weak parabolic effect in raising weight at intermediate levels. Dry weight was unaffected by Ca or Al, but was influenced in a biphasic fashion by pH, being slightly higher at pH = 4.4 and 6.5 than at 4.8 and 5.2. These weight trends were similar to those seen during the 91 d exposures (Wood et al. 1990; Ingersoll et al. 1990a). During recovery, the only significant weight effect was the persistence of a small negative influence of acidity on wet weight (Fig. 5B).

Swim-Up Fry — Whole Body Electrolytes after Challenge

The pH/Ca/Al exposures exerted major sublethal effects on whole body Na and Cl levels in swim-up fry; only the Na data are shown (Fig. 6), for Cl exhibited similar trends, though with greater variability. In the absence of Al, low pH alone lowered these extracellular electrolytes by as much as 60% in a linear fashion. In the matrix as a whole, pH was the major determinant of Na and Cl concentrations, accounting for 26% (Cl) to 41% (Na) of the variance (Fig. 5A). Calcium exerted a definite protective effect (proportional to log Ca), though this occurred only at acidic pH, and was indefinite at Al levels ≥37 μg/L (Fig. 6). Aluminum itself showed a weak tendency (parabolic with log

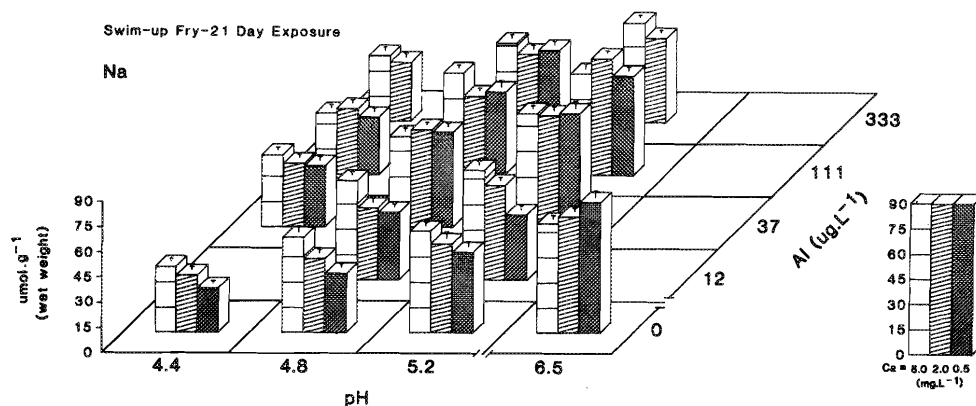Can. J. Fish. Aquat. Sci., Vol. 47, 1990

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FIG. 6. Whole body concentrations of Na in swim-up fry of brook trout after 21 d exposure to a matrix of pH, Ca, and Al. Other details as in legend of Fig. 2.
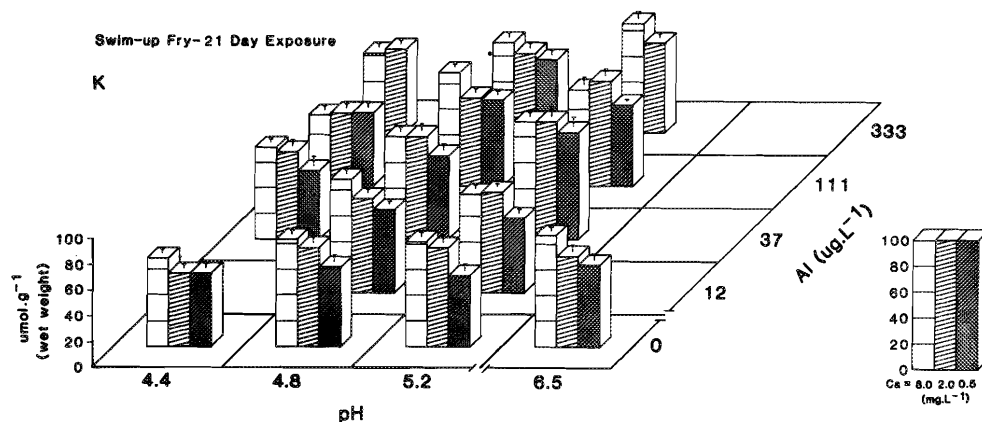

FIG. 7. Whole body concentrations of K in swim-up fry of brook trout after 21 d exposure to a matrix of pH, Ca, and Al. Other details as in legend of Fig. 2.

Al) to raise Na and Cl. While these trends were similar to those for yolk-sac fry (Fig. 2), the relative contribution of pH was greater and that of Al reduced. Furthermore, the presence of intermediate Al levels did not raise Na and Cl above the control values (Fig. 6).

The concentration of K, the major intracellular electrolyte, was also strongly affected by the 21 d exposure (Fig. 7). Potassium was lowered by acidity and raised by log Ca, the two factors contributing almost equally to the 30% of variance explained (Fig. 5A). Aluminum had only a very small parabolic effect, and did not elevate K above control values. Apart from the reduced influence of Al, these trends were similar to those seen in yolk-sac fry (Fig. 3).

Whole body levels of Ca (Fig. 8) were less affected by the 21 d exposure than those of Na, Cl, and K, although the effects of all three variables (pH, Ca, and Al) were still significant (Fig. 5A). These effects were also less than those seen in yolk-sac fry (Fig. 4). Whole body Ca concentrations were depressed by low pH, affected in a parabolic manner by Al, and very slightly raised by increasing Ca (Fig. 8).

Magnesium responded in an opposite fashion, being elevated by factors which had a deleterious effect on the other ions (Table 2). This was seen most clearly in the negative influence of log Ca, as well as in small negative effects of increasing pH and Al (Fig. 5A).

TABLE 2. A comparison of whole body Mg levels (μmol/g wet weight) in selected cells (Al = 0 μg/L) of the swim-up fry challenge experiment at the end of 21 d exposure. Means $\pm$ 1 SEM (N). * = $p \leq 0.05$ relative to comparable value at Ca = 2 mg/L; † = $p \leq 0.05$ relative to comparable value at pH = 4.4.

pH	Ca = 2 mg/L	Ca = 8 mg/L
4.4	16.6 ± 0.9	13.0 ± 1.0*
	(10)	(9)
6.5	14.3 ± 1.6	10.5 ± 0.6*,†
	(10)	(10)

Swim-Up Fry — Whole Body Electrolytes after Recovery

The effects of 21 d exposure in the matrix persisted to a much greater extent in swim-up fry (Fig. 5B) than in yolk-sac fry (Fig. 1B). Indeed, for whole body Ca, the effects of pH and water Ca were far greater after recovery than at the end of the exposure itself (compare data in Fig. 8 and 9), despite the fact that the fish had been returned to constant control conditions for almost 3 wk. This comparison emphasizes the much higher absolute Ca concentrations at the end of 20 d recovery, reflecting the fact that the period from 91–111 d post-fertilization was a time of rapid Ca accumulation (cf. figure 1 of Wood et al. 1990). The delayed effects of water Ca on whole body Na and
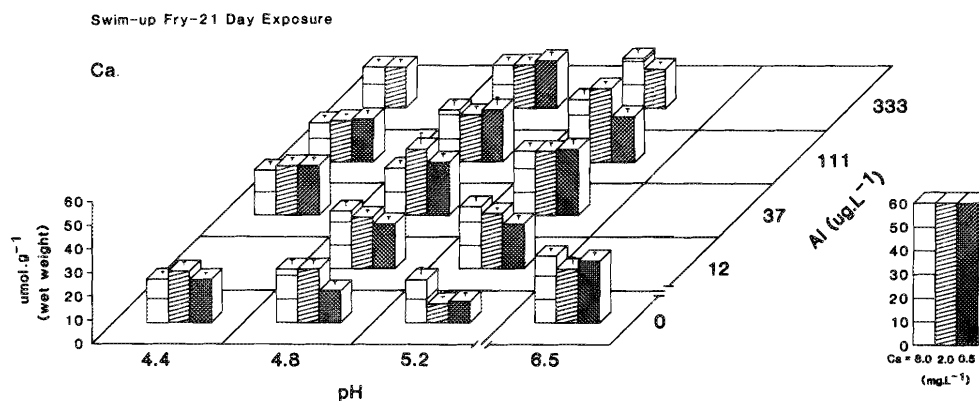
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Swim-up Fry-21 Day Exposure

FIG. 8. Whole body concentrations of Ca in swim-up fry of brook trout after 21 d exposure to a matrix of pH, Ca, and Al. Other details as in legend of Fig. 2.
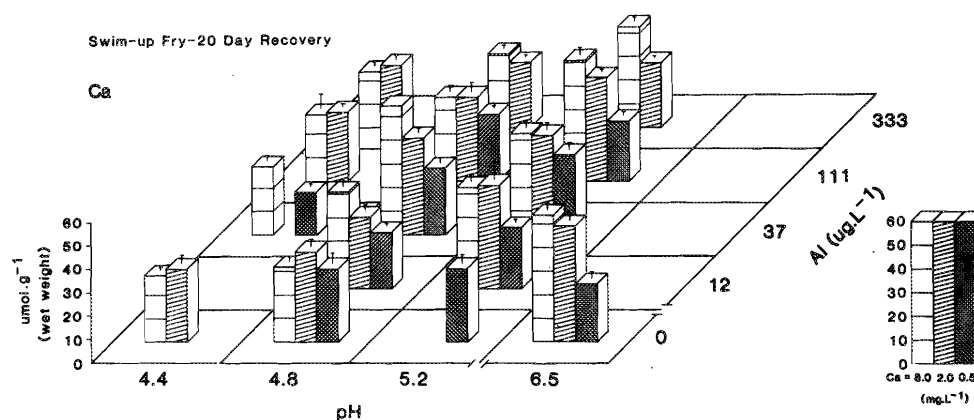

Swim-up Fry-20 Day Recovery

FIG. 9. Whole body concentrations of Ca in brook trout fry after 20 d recovery under control conditions (pH = 6.5, Ca = 2 mg/L, Al = 0 μg/L) following 21 d exposure in the swim-up stage to a matrix of pH, Ca, and Al. Means ± 1 SEM for 8–10 fish in most cells, falling as low as three in cells with high mortality.

TABLE 3. A comparison of whole body ion levels (μmol/g wet weight) in selected cells (Ca = 2 mg/L) of the swim-up fry challenge experiment at the end of 21 d exposure, and after 20 d of recovery under control conditions (pH = 6.5, Ca = 2 mg/L, Al = 0 μg/L). Means ± 1 SEM (N). * = $p \leq 0.05$ relative to comparable control value (pH = 6.5, Al = 0 μg/L) at this time; † = $p \leq 0.05$ relative to comparable value for this treatment at end of 21 d exposure.

		21 d exposure (day 70)			20 d recovery (day 90)		
pH	Al	Na	K	Ca	Na	K	Ca
6.5	0	69.2	70.1	22.7	80.8†	72.8	48.8†
		±2.8	±4.3	±2.5	±4.4	±2.7	±2.9
		(10)	(10)	(10)	(10)	(10)	(10)
5.2	37	66.4	69.5	26.6	74.3†	74.6	41.8†
		±2.4	±7.7	±1.2	±2.9	±2.4	±3.0
		(10)	(10)	(10)	(9)	(9)	(9)
4.4	0	33.7*	56.9*	21.6	50.3†,*	62.2	31.0†,*
		±1.5	±3.7	±1.6	±3.3	±7.4	±3.5
		(10)	(10)	(10)	(5)	(5)	(5)

Cl were also greater than those seen at the end of the challenge (Fig. 5B). The inhibitory effects of acidity on Na, Cl, and K, while attenuated, remained highly significant at the end of recovery (Fig. 5B). As in yolk-sac fry, this reflected a failure

of the acid-exposed fish to "catch-up" to the controls during the recovery period (Table 3). There were no persistent effects on Mg (Fig. 5B), which continued to decline during the recovery period, in contrast to the other ions.

Aluminum Accumulation and Loss

Significant ΔAl was detected only at water Al≥111 μg/L in both yolk-sac and swim-up fry after 21 d exposure (Fig. 10). Over the matrix as a whole, water Al was the major determinant of accumulation (Fig. 1A, 5A), but in cells at Al≥111 μg/L, both Ca and acidity had strong negative influences on ΔAl. These trends and the values of ΔAl in comparable cells were similar in the two stages (Fig. 10), as well as in the alevins exposed for 91 d (cf. figure 10 of Wood et al. 1990). During 20 d recovery in Al-free water at pH = 6.5, the accumulated load was lost almost completely, suggesting surficial adsorption onto, rather than into the fish. In the swim-up group, approximately 5% of the original ΔAl persisted after 20 d recovery in fish from several exposure combinations, giving rise to the slight positive correlation shown in Fig. 5B. However, the biological significance is questionable; these values approximated the detection limit of our analytical technique, and the effect was not significant by factorial analysis of variance (Fig. 5B).
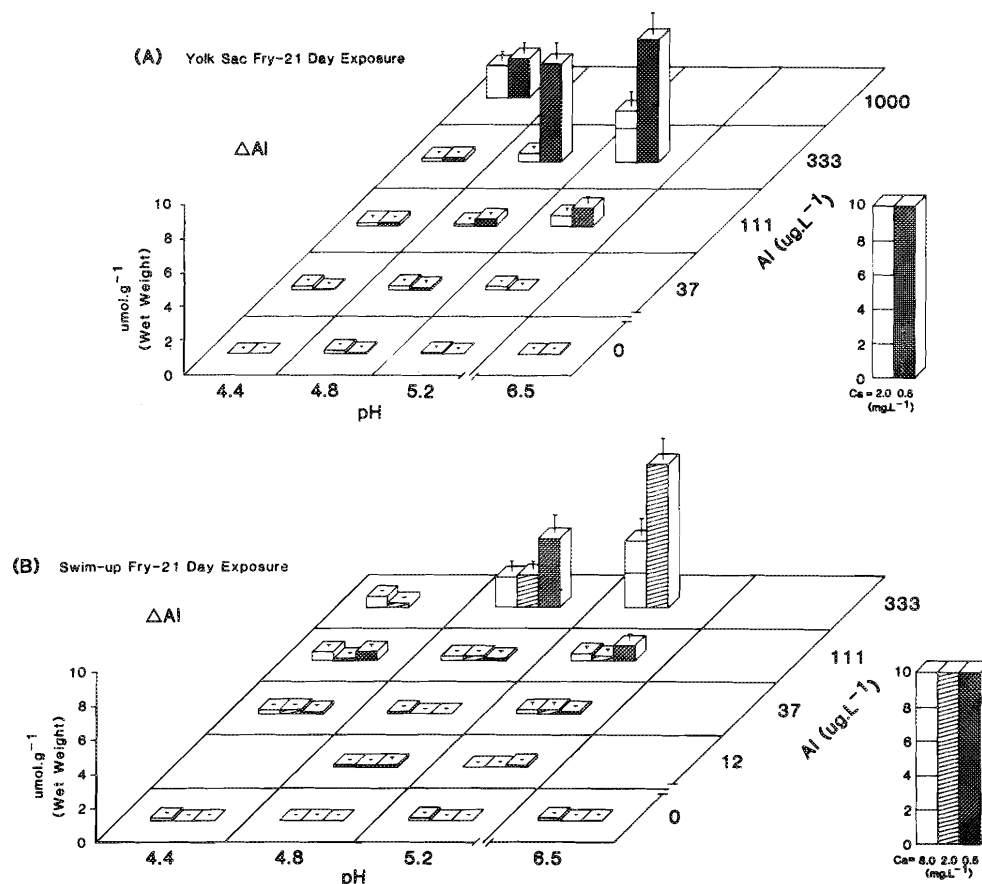
FIG. 10. Whole body accumulation of aluminum (ΔAl) in (A) yolk-sac fry and (B) swim-up fry of brook trout after 21 d exposure to a matrix of pH, Ca, and Al. Other details as in legend of Fig. 2.

Discussion

Relative Importance of pH and Ca in Different Exposure Regimes

In both yolk-sac and swim-up fry exposed for 21 d in the matrix, water pH was the principal determinant of whole body ion status for most electrolytes (Fig. 1A, 5A). This directly contrasts with the responses of alevins raised continuously from fertilization for 91 d in the matrix, where water Ca was the principal determinant of whole body ion status (cf. figure 2 of Wood et al. 1990). To some extent, these different conclusions are a function of the different experimental designs.

The first difference relates to the fact that our experiments measured only sublethal responses; fry exhibiting lethal responses were lost prior to sampling and did not contribute their presumably more severe ionic changes to the data pool. In other words, selection eliminated the most extreme responders from the data set. The cells where the sublethal pH effects were most extreme in the 21 d challenges (at pH = 4.4, and at pH = 4.8, low Ca; eg. Fig. 2, 3, 4, 6, 7, and 8) were lost entirely in the 91 d exposures and did not contribute data to the 91 d analysis (cf. figure 5, 6, 7, 8, and 9 of Wood et al. 1990). In other low pH cells where 91 d survivors were available for analysis, these survivors were presumably those fish most resistant to the effects of acidity. The second difference is that the range of water Ca concentrations examined in the present age-specific challenges was less extensive (two

or three Ca levels) than in the 91 d exposures (four Ca levels), which could have reduced the influence of Ca in the factorial analysis. To test whether this was the case, the data from the 91 d exposures were re-analyzed using only those two or three Ca levels employed in the yolk-sac and swim-up experiments. The influence of Ca (in terms of R^2) was unaltered when three Ca levels were employed (0.5, 2, and 8 mg/L as in the swim-up exposures), but was generally reduced when only two Ca levels were employed (0.5 and 2 mg/L as in the yolk-sac exposures). Nevertheless, Ca remained the dominant factor for all five whole body ions.

These two differences notwithstanding, the results of the two sets of experiments remain fundamentally different from one another, and suggest that the responses in a field situation will depend critically on the nature of the exposure. Fry surviving a continuous 91 d exposure from fertilization will maintain an ionic status most dependent upon the water Ca during the exposure, while alevins surviving a 21 d challenge in the yolk-sac or swim-up phase will exhibit a status most dependent upon the pH of the challenge. As outlined in the Introduction and below, the latter situation (episodic exposure, especially of the swim-up stage) appears to be the more environmentally realistic.

Relative Importance of Al in Different Exposure Regimes

While never as pronounced as that of pH, the curious parabolic influence of Al was an important determinant of whole

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body ion status in these episodic exposures, especially in the yolk-sac stage (Fig. 1, 5). This influence was largely positive, and in many cells offered a greater protection against the deleterious effects of low pH than did higher levels of Ca. This protective and/or stimulatory action of Al in both the 21 d and 91 d exposures (Wood et al. 1990) was surprising, for toxic effects have usually been associated with the presence of Al at low pH (cf. Schofield and Trojnar 1980; Brown 1983; Gunn and Noakes 1987; McDonald et al. 1988). Positive effects were most pronounced with Al levels of 37 and 111 μg/L at pH = 4.8 and 5.2, especially in the yolk-sac fry. In these cells, whole body Na, Cl, K, and Ca concentrations were raised well above those in the control yolk-sac fry at pH = 6.5, Al = 0 μg/L (Fig. 2, 3, 4). Negative effects were seen only rarely in surviving alevins, and only at the highest concentrations of Al (333, 1000 μg/L) where the metal was clearly accumulated by the fish (Fig. 10). It is worth noting that water Al levels measured in the exposure chambers were always in close agreement with nominal values in the present experiments (Ingersoll et al. 1990a; Wood et al. 1990).

The mechanism of action for the negative effects of Al is probably the same as in adult salmonids — a combination of inhibition of active ion influx through the "chloride cells" and stimulation of passive efflux through paracellular channels (cf. Booth et al. 1988; McDonald and Milligan, 1988). The influx effect may result from direct inhibition of transport enzymes by Al (Staurnes et al. 1984), and the efflux effect from an inflammation and edema of the transporting epithelia in response to surficial Al build-up (Karlsson–Norrgren et al. 1986a, b). Both of these effects would be additional to the separate actions of acidity alone in inhibiting active influx and stimulating passive efflux.

The explanation for the positive effects of Al is less clear. Several previous studies have demonstrated that Al may protect against the effects of severe acidity (pH$\leq$4.5; Muniz and Leivestad 1980; Schofield and Trojnar 1980; Baker and Schofield 1982; Hunn et al. 1987), an action attributed to the ability of polyvalent cations such as Al^{3+} to stabilize membrane permeability in much the same manner as Ca^{2+}. However at pH = 4.8–5.2, where effects were most prominent in the present study, total Al = 37–111 μg/L would yield free Al^{3+} concentrations of only about 0.3–2.1 μmol/L (McDonald et al. 1989), relative to Ca^{2+} = 12.5–200 μmol/L. It seems unlikely that such low levels of free Al^{3+} could be solely responsible for complete protection against the toxic effects of low pH on ionoregulation, let alone for stimulation of whole body ions above control levels.

Nevertheless, a similar protective influence of comparable levels of Al (75–150 μg/L) was seen in adult brook trout exposed to pH = 5.2 for 70 d (Wood et al. 1988a, b). An alternative or additional explanation is the proliferation of "chloride cells" on the branchial epithelium of adult salmonids seen during comparable long-term acid/Al exposures (Karlsson–Norrgren et al. 1986a, b; Tietge et al. 1988), which is thought to explain the greater Na uptake capacity of such fish (McDonald and Milligan 1988). It is unknown whether similar "chloride cell" proliferation occurs on the transporting epithelia (vitelline membrane, developing gills) of alevins. Ingersoll et al. (1990b) could detect no significant effect of Al on mucous cells in the general body epithelia of brook trout alevins in exposures similar to those of the present study.

A final possibility is that Al in some way accelerated development, for increases in the concentrations of Na, Cl, K, and Ca occurred as part of normal ontogeny (cf. figure 1 of Wood et al. 1990). This explanation would account for whole body ions remaining almost static over the 20 d recovery period in yolk-sac fry previously exposed to stimulatory levels of Al (Table 1). However, other data in the present study suggest that developmental rates were unaffected in the yolk-sac stage where the greatest stimulatory effects of Al occurred (see below). Furthermore, in other investigations at comparable pH, Al clearly inhibited both development and mineralization of salmonid fry (Hunn et al. 1987; Gunn and Noakes, 1987). In summary, the mechanism(s) remain problematical; there is a clear need for further mechanistic studies on this unusual protective/stimulatory action of sublethal Al on ionoregulation in both alevins and adults.

Ionoregulatory Effects vs. Developmental Effects

As normal development proceeds in these early life stages, there are substantial increases in the whole body concentrations of most electrolytes, but decreases in body weight and Mg levels as the yolk is resorbed (Hayes et al. 1946; Rombough and Garside 1984; Geen et al. 1985; Peterson and Martin–Robichaud 1986; Gunn and Noakes 1987; Wood et al. 1990). In the continuous 91 d exposures (Wood et al. 1990), it was not possible to separate ionic responses caused by direct actions of the exposure conditions on ion exchange processes from those arising indirectly via changes in developmental rate. However, in the present 21 d challenges, there was evidence for the occurrence of both phenomena, in agreement with the findings of Peterson and Martin–Robichaud (1986) on *Salmo salar* alevins at low pH.

In the yolk-sac fry, the 21 d exposure had pronounced effects on whole body Na, Cl, K, and Ca concentrations, but negligible influence on either Mg concentrations or body weight (Fig. 1A). This suggests that developmental effects were insignificant and that changes in whole body ions resulted largely from direct actions of pH, Ca, and Al on transport and permeability processes in yolk-sac fry. However, in swim-up fry there were substantial effects of the matrix on both body weight and Mg levels (Table 2) in addition to those on the other whole body ions (Fig. 5A). Exposure conditions which tended to lower Na, Cl, K, and Ca (i.e. low pH, low Ca, high Al) simultaneously tended to raise Mg and lower wet body weight, while dry body weight varied in a biphasic fashion with pH. These patterns were very similar to those seen during the 91 d exposures (cf. Wood et al. 1990) where they reflected delayed yolk-resorption, reduced feeding, inhibited growth, and increased metabolic costs under adverse conditions. Thus the exposure conditions clearly exerted developmental actions on the swim-up stages which may have made some contribution to the overall ionic effects. Furthermore, these findings suggest that at least some of the developmental effects seen at the end of 91 d continuous exposure occurred during the swim-up phase.

Lacroix et al. (1985) followed growth and whole body ion levels in larval *Salmo salar* exposed in situ to natural acidic conditions. In terms of developmental stage, their exposures started at about the point where the present experiments terminated, so their fish undoubtedly relied more on exogenous feeding than the present fry. In agreement with the present findings, exposure to pH $\approx$5.0 in soft water (Ca$\approx$0.6 mg/L) with high Al ($\approx$200 μg/L) induced depressions in most whole body ions and produced a retardation of development. Feeding and growth were depressed, and eventual emaciation resulted. As

the ionic effects tended to precede the developmental effects, there was evidence for at least a partial separation of the two phenomena.

Recovery

Only a few studies have examined the physiology of recovery from acid stress for more than several days in juvenile or adult fish (Leivestad et al. 1976; Brown et al. 1986; Jones et al. 1987). In general, these indicated complete recovery within 7–14 days. Only one investigation has examined recovery in early life stages after acid or acid plus Al exposure. Gunn and Noakes (1987) reported significant negative effects on whole body ions, development, and skeletal calcification in lake trout alevins (*S. namaycush*) after 21–32 d recovery from a 5 d pulse exposure to Al = 100 or 200 μg/L at pH = 5.0 in the yolk-sac stage. There was no persistent effect of exposure to this single low pH (5.0) alone; the influence of water Ca was not examined. In the present study on brook trout alevins (*S. fontinalis*), deleterious effects persisted after 20 d of recovery from 21 d pulse exposures to low pH and low Ca conditions (Tables 1, 3; Fig. 1B, 5B). This persistence was especially prominent in swim-up fry, where both mortality and negative effects on whole body Ca were greater during recovery than during the original exposure (Fig. 5B, 8, 9). Interestingly, the protective effects of Al, which were directly opposite those reported by Gunn and Noakes (1987) in lake trout fry, also exhibited some persistence in recovering brook trout swim-up fry, though the marked stimulatory effects of Al largely disappeared in recovering brook trout yolk-sac fry (Table 1).

These differences from previous findings on older fish undoubtedly reflect the fact that exposure during the yolk-sac and swim-up stages coincided with a normal period of intense accumulation of ions. When this accumulation was blocked by either low pH or low Ca, the surviving fry failed to "catch-up" to controls which had accomplished a substantial net uptake over this same period. The effect was particularly pronounced when the adverse conditions of the exposure also delayed development, as during the swim-up stage. The remarkable effects on whole body Ca during the swim-up recovery (Fig. 9) presumably resulted from a combination of inhibited uptake during exposure (Fig. 8), inhibited development, and coincidence of the recovery period with a normal period of high Ca uptake (cf. figure 1 of Wood et al. 1990).

Relative Sensitivity of Yolk-Sac vs. Swim-Up Fry

In terms of relative effects on whole body ions after 21 d exposure to the matrix, the responses of yolk-sac and swim-up fry were quantitatively similar, apart from the more pronounced protective/stimulatory influence of Al in the former. This suggests that the toxic actions of H$^+$ and the protective actions of Ca are similar on vitelline and on branchial membranes, while the balance between protection and toxicity by Al is shifted slightly more towards the latter in the branchial epithelium. However, in terms of survival, body weight, development, and the persistence of deleterious ionoregulatory effects during recovery, the swim-up stage was clearly the more sensitive. The explanation probably relates to the increased importance of the gills in the swim-up stage for both ion uptake and respiratory gas exchange.

Associated with this transition from vitelline to branchial membranes as the principal exchange site will be an increasing reliance on the external environment and a decreasing reliance on yolk-sac stores for both minerals and nutrients to fuel further

development. Therefore it is not surprising that the effects of toxicants in this external environment became more severe as development progressed. Other studies have reported sensitivity patterns both in agreement and disagreement with those of the present study (reviewed by Ingersoll et al. 1990a). Such discrepancies probably result from differences in temperature which will affect developmental rates, and in the balance of Al relative to H$^+$ as the primary toxic agent. Based on mortality data, Ingersoll et al. (1990a) detected a general trend for Al toxicity to increase and H$^+$ toxicity to decrease as development proceeded in the present experiments.

Environmental Significance

As outlined in the Introduction and discussed in detail by Trojnar (1977) and Gunn (1986), the yolk-sac and swim-up stages tested in the present experiments are probably the critical life stages for brook trout in terms of susceptibility to episodic acid pulses. For example, in acid-impacted Ontario streams, fry emerging from the relatively sheltered conditions of the redd may experience sudden pH decreases of 0.5–2.0 units, increases in total Al from background levels to over 150 μg/L, and substantial decreases in Ca (Keller 1983; Gunn 1986). The present experiments are therefore representative of such conditions.

The most important conclusion of these experiments is that water pH will be the major factor affecting whole body ion levels in fry emerging into an acidic environment. For example, if this emergence coincided with the swim-up stage of the present experiments, and the ambient water was at pH = 4.8 compared to an interstitial pH = 6.5 in the redd (unchanged Ca = 2 mg/L, Al = 0 μg/L), then whole body Na and Cl would be depressed by 25–35% in survivors, and K and Ca by about 5% relative to fry maintained in the interstitial conditions. After 20 d recovery at pH = 6.5, the Na, Cl, and K depressions would persist at the same relative magnitude, though they would be absolutely larger due to the general increase in whole body ion levels over this period. At the same time, the depression in whole body Ca would reach 25%. In the unlikely event the ambient water was at Ca = 8 mg/L during this acidic pulse, these effects would be slightly attenuated. However, if, as is more probable, the acidic water was at a lower Ca (e.g. 0.5 mg/L) than in the redd, then the Na, Cl, and K depressions would be slightly exacerbated, while the Ca depression would reach almost 40%. Thus, while water Ca has a significant influence, it is certainly less important than pH in episodic exposures, in contrast to the situation of continuous exposure from fertilization through swim-up (Wood et al. 1990). Beggs and Gunn (1986) found pH alone to be a good indicator of the presence/absence (threshold pH = 5.0) of brook trout populations in Ontario lakes.

A surprising finding of these experiments is the largely positive, rather than negative, influence of Al at low pH on whole body ions in brook trout alevins. This contrasts with the recent findings of Gunn and Noakes (1987) on lake trout fry; the only obvious reasons for this discrepancy are differences in fish species and exposure regime. In the scenario outlined above, whole body Na and Cl levels of brook trout fry would be depressed by only 15–20% after the recovery period if the acidic water (pH = 4.8, Ca = 2 mg/L) into which the fry emerged also contained Al = 111 μg/L. As yet there appears to be no evidence that Al can actually protect in this manner in the field situation. There are also no field data to confirm two other interesting laboratory observations which complicate extrapolation of the present data to the field. Firstly, Gunn and Noakes (1986) have

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shown that swim-up fry of *Salvelinus fontinalis* can actively avoid water of low pH and high Al content. Secondly, the mortality data of Trojnar (1977) indicate that fry pre-exposed to acidic conditions in the redd may be more tolerant of severe acidity during emergence. There is a clear need for more field trials at brook trout spawning sites, of the type pioneered by Gunn and Keller (1984a, b) with *Salvelinus namaycush* redds and Lacroix (1985) with *Salmo salar* redds, especially in combination with physiological and behavioral measurements (e.g. Lacroix et al. 1985).

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