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CHRONIC EFFECTS OF LOW pH ON SOME PHYSIOLOGICAL ASPECTS OF SMOLTIFICATION IN ATLANTIC SALMON (SALMO SALAR)

by

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ABSTRACT

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Atlantic salmon (Salmo salar) were chronically exposed for several months to acidified water to determine if mean pH levels of 4.7 and 4.9 prevent smoltification from proceeding normally. Physiological changes associated with smoltification developed normally when pH was above 4.9. When pH fell below 4.7, there was impairment of ionic regulatory mechanisms leading to excessive loss of electrolytes in fresh water. Failure of ionic regulation appeared to follow an inhibition of (Mg^{++})-ATPase and (Na^+, K^+)-ATPase enzyme systems. Prolonged exposure to acid water allowed salmon to develop compensatory branchial mechanisms, including increased ATPase activity, that led to improved ionic regulation.

RÉSUMÉ

Johnston, C. E., R. L. Saunders, E. B. Henderson, P. R. Harmon, and K. Davidson. 1984. Chronic effects of low pH on some physiological aspects of smoltification in Atlantic salmon (Salmo salar). Can. Tech. Rep. Fish. Aquat. Sci. 1294: iii + 7 p.

Des saumons atlantiques (Salmo salar) ont été soumis à une exposition chronique de plusieurs mois à de l'eau acidifiée dans le but de déterminer si le processus normal de la smoltification était entravé à des pH moyens de 4,7 et 4,9. Les changements physiologiques associés à la smoltification se produisent normalement quand le pH est au-dessus de 4,9. Quand ce dernier baisse au-dessous de 4,7, il y a altération des mécanismes de régulation ionique, qui conduit à une perte excessive d'électrolytes en eau douce. Le manque de régulation ionique semble être la conséquence d'une inhibition des systèmes enzymatiques (Mg^{++})-ATPase et (Na^+, K^+)-ATPase. Une exposition prolongée à de l'eau acide permet au saumon de développer des mécanismes branchiaux compensatoires, y compris une activité accrue de l'ATPase, qui résultent en une régulation ionique améliorée.

INTRODUCTION

Exposure of salmonids to pH conditions below 4.5 increases gill membrane permeability, mucus secretion from gills and skin, hydrogen ion uptake, blood acidosis, hematocrit, hemoglobin concentration, hemopoietic activity, respiratory and metabolic rates and loss of sodium, chloride and calcium ions from the plasma and body tissue (Plonka and Neff 1969; Packer and Dunson 1970; Vaala and Mitchell 1970; Hargis 1975; Daye and Garside 1976; Dively et al. 1977; Neville 1979a, b; Packer and Sunkin 1979; McDonald et al. 1980; Booth et al. 1981; Milligan and Wood 1982; Saunders et al. 1983). Other physiological consequences of acidification in salmonids are reduced active transport processes in chloride cells of the gill, inhibition of (Na^+,K^+)-ATPase activity and a decreased oxygen carrying capacity of the blood (Packer 1979; McDonald et al. 1983; Saunders et al. 1983).

Tolerance to low pH varies with species, developmental stage and length of exposure. Grande et al. (1979) reported that, of four salmonid species, brook trout (*Salvelinus fontinalis*) are more acid tolerant than brown trout (*Salmo trutta*), Atlantic salmon or rainbow trout (*Salmo gairdneri*). Eggs, alevins and fry of Atlantic salmon are the most sensitive developmental stages and many physiological and pathological alterations occur in the presence of high hydrogen ion concentrations (Daye and Garside 1977, 1979, 1980; Peterson et al. 1980; Haya and Waiwood 1981). Most insight into the toxic effects of low pH on salmonids has been gained through experiments involving acute exposure to acid, usually lasting less than 7 d. Only a few experiments have employed chronic acid exposures of longer duration, and most have dealt with egg or alevin stages (Spry et al. 1981; Wood and McDonald 1982). Although Atlantic salmon are one of the most pH sensitive and economically important species in Atlantic Canada, few chronic laboratory or field experiments have been conducted on juveniles (fry, parr and smolts) or adults returning from the sea. Knowledge of the toxic levels and of the impact of prolonged exposure to hydrogen ions on seasonally determined endogenous rhythms and on physiological and biochemical mechanisms still remains poorly elucidated.

In a recent long-term experiment with Atlantic salmon we reared parr in low pH environments (4.2-4.7) at constant 10°C and rising temperature to the smolt stage (Saunders et al. 1983). Salmon exposed to low pH during the period when they should have been undergoing parr-smolt transformation failed to develop tolerance to high salinity, failed to elevate branchial (Na^+,K^+)-ATPase activity, failed to maintain normal plasma Na^+, Cl^- and Ca^{++} concentrations and failed to maintain normal tissue moisture content and growth. Based on these results, the process of smoltification did not appear to proceed normally in the pH range 4.2-4.7 even though the fish survived. The present laboratory study was undertaken to define the threshold pH above which smoltification proceeds normally and to examine further the physiological changes that accompany chronic exposure to acid water during parr-smolt transformation.

MATERIALS AND METHODS

In mid-February 1982, 1500 yearling Atlantic salmon parr (12-15 cm fork length) were randomly distributed among six 1-m^2 Swedish style rearing tanks (250/tank) at the St. Andrews Biological Station. Water in these tanks was gradually heated from an ambient temperature of ca. 2°C in mid-February to 10°C by March 4, from which time it was maintained at this temperature for the remainder of the experiment. Flow rates were ca. 12 L/min. Initial sampling was conducted on March 5, after which three pH regimes were established in the six experimental tanks. Two control tanks used normal laboratory water with a mean ambient pH of 6.78 (range 6.55-6.99) and Na, Cl, Ca, Mg, K and SO_4 levels of 100, 100, 38, 15, 10 and 20 µmol/L, respectively. Three groups were held under low pH (mean pH 4.66, range 4.33-5.00) and the remaining group was at intermediate pH (mean pH 4.94, range 4.46-5.40). Experimental conditions were maintained by using the methodology outlined by Saunders et al. (1983). Although pH conditions were relatively constant, some fluctuations occurred during the study period; these fluctuations (expressed as mean pH over 5-d periods) are shown in Fig. 1.

The methodology involved in feeding, photoperiod regimes, length-weight measurements, moisture determinations, measurement of ATPase activity, salinity tolerance tests, and ion measurements follows those of Saunders et al. (1983) with the following changes: fish were fed to satiety at a rate of 2% body weight/d; blood samples were collected from the caudal artery, using 1-cc heparinized syringes; a Perkin-Elmer 303 atomic absorption spectrophotometer was used for analysis of Na^+ and Ca^{++} levels.

Statistical comparisons between means were made by using a Duncan's Multiple Range Test or a Student's *t*-test.

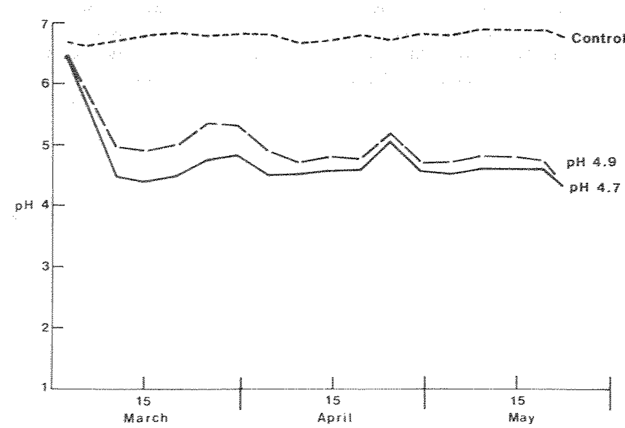

Fig. 1. Three pH regimes maintained during the experiment which ran from March 5-May 25, 1982.

RESULTS AND DISCUSSION

SURVIVAL AND GROWTH

Salmon exposed to the low pH treatment exhibited higher mortality (56.1%) during the experiment than those exposed to the intermediate and control pH regimes where mortality was 2.4% and 0%, respectively. Mortality in the low pH group followed a modal pattern that was directly related to excursions in pH level (Fig. 2). Initiation of the modes occurred when pH levels (calculated over 5-d period) dropped to less than 4.5. If the pH level was increased above this value, there was recovery, but the ameliorative effect of this increase was not immediate, creating a lag effect.

In the intermediate pH regime, there were six mortalities during the period May 14-16. The timing of this mortality coincided with late stages of smoltification in the control fish and may represent a lowered resistance to high concentrations of hydrogen ions and a progressive deterioration in health and condition owing to long-term exposure to acid water.

Growth of salmon exposed to control and intermediate pH regimes was similar and greater than those exposed to low pH (Fig. 3). Slower growth and the apparent decrease in size of salmon exposed to the low pH was probably caused by size-related mortality and a poor feeding response.

MOISTURE CONTENT AND CONDITION FACTOR

Total moisture content for salmon from the low pH regime, although slightly higher in April and early May and lower in late May than values for the other regimes, was not significantly different from those of the other pH regimes (Table 1).

The condition factor for salmon at low pH was significantly lower ($P < 0.05$) throughout the study period than those for the other regimes. Extremely low values were reached in late May. Such low condition factors suggest that salmon exposed to low pH lose body weight and body energy reserves at a rate greater than salmon at higher pH levels.

SALINITY TOLERANCE

Survival of salmon in initial salinity tolerance tests (conducted at 35 o/oo salinity) in March was 50% after 96 h for control pH conditions (Fig. 4); in subsequent tests, control fish had 80-100% survival. Survival of salmon in intermediate and low pH conditions was 60-70% and 0-30%, respectively, between mid-April and late May. These data clearly indicate that salinity tolerance of juvenile salmon was greatly impaired at low pH and to a lesser extent at pH 4.9.

BRANCHIAL ATPASE ACTIVITY

Branchial (Mg^{++})-ATPase activity was significantly reduced ($P < 0.05$) in salmon exposed to pH 4.7 (Fig. 5). In mid-April and early May (Mg^{++})-ATPase levels of the low pH group were significantly lower ($P < 0.05$) than those for either of the other regimes. By late May, however, (Mg^{++})-ATPase activity of the low pH group increased and was not significantly different from the other regimes. The pattern of (Mg^{++})-ATPase activity followed that of the plasma ions (Fig. 6, 7, 8).

Branchial (Na^+, K^+)-ATPase activity was significantly lower during April and May ($P < 0.01$) in salmon exposed to the low pH regime than to the intermediate or control regimes (Fig. 5). Salmon exposed to the intermediate pH regime also had significantly lower ($P < 0.05$) ATPase levels than the controls during May. These results are in agreement with those of the salinity tolerance tests, indicating marked physiological impairment of osmoregulatory function in acid-stressed juvenile salmon in salt water. The marked decrease in ATPase activity and salinity tolerance in the controls between May 6th and 21st suggests that the smoltification process may have peaked in early May and that desmoltification commenced thereafter.

PLASMA ION CONCENTRATIONS

Mean plasma Na^+, Cl^- and Ca^{++} ion concentrations varied seasonally and in magnitude according

Table 1. Mean moisture content (expressed as a percentage of total wet body weight) and condition factor (CF) for Atlantic salmon parr-smolts exposed to three different pH regimes.

Sampling date	pH regime: Low (%)	Low (CF)	Intermediate (%)	Intermediate (CF)	Control (%)	Control (CF)
1982						
March 3	-		-		72.4	1.02
April 19	75.5	0.91	74.7	0.97	74.6	0.94
May 10	75.6	0.88	73.9	0.92	74.8	0.92
May 25	73.6	0.81	73.1	0.90	75.1	0.92

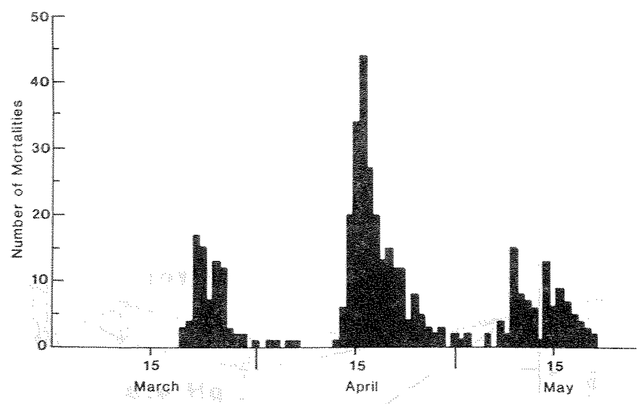

Fig. 2. Mortalities that occurred in Atlantic salmon populations maintained at low (4.66) pH levels during the period March 5–May 26, 1982.

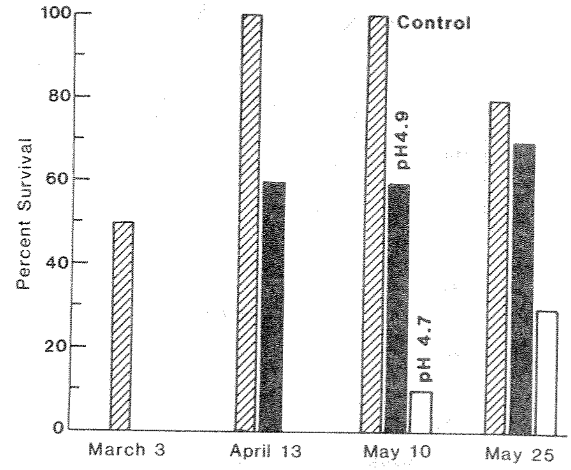

Fig. 4. Salinity tolerance of Atlantic salmon reared at control (6.78), intermediate (4.94), and low (4.66) pH levels. Salinity tolerance expressed as percent survival after 96 h in 35 o/oo seawater.

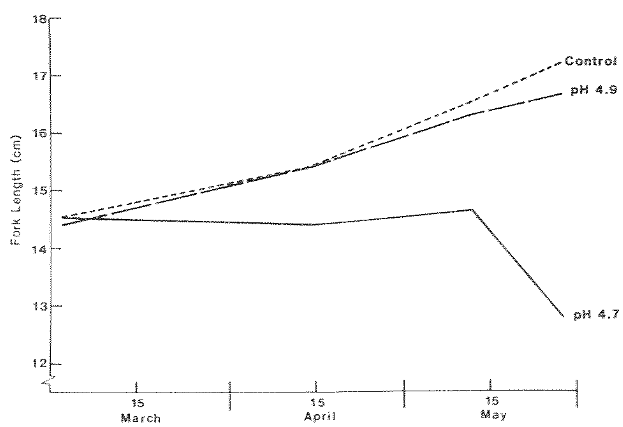

Fig. 3. Fork lengths of Atlantic salmon reared at control (6.78), intermediate (4.94), and low (4.66) pH levels.

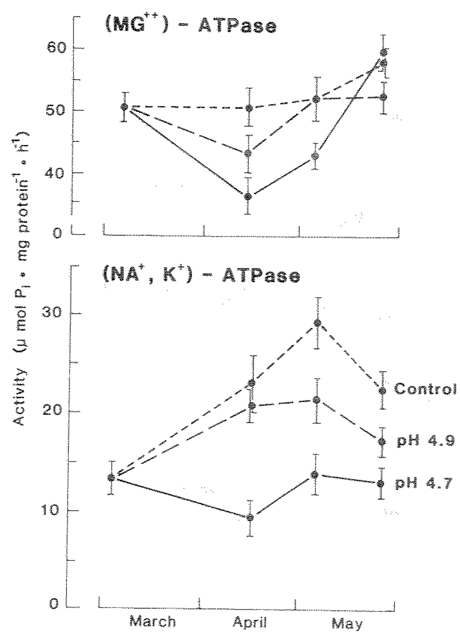

Fig. 5. Mean (Mg^{++})- and (Na^{+},K^{+})-ATPase activity levels (± SE in Atlantic salmon reared at control (6.78), intermediate (4.94), and low (4.66) pH levels. ATPase activity expressed as µmole P_i·mg protein^{-1}·h^{-1}.

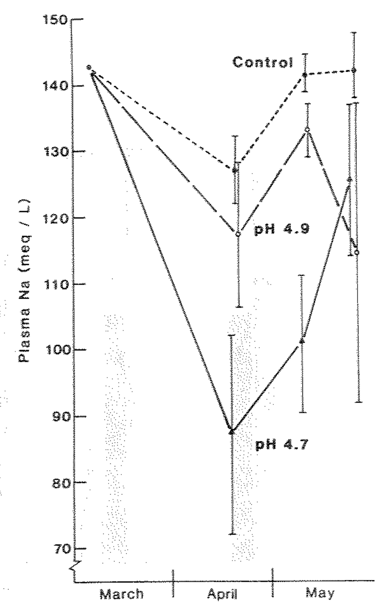

Fig. 6. Mean plasma Na^{+} levels ($\pm$ SE) in Atlantic salmon reared at control (6.78), intermediate (4.94), and low (4.66) pH levels.

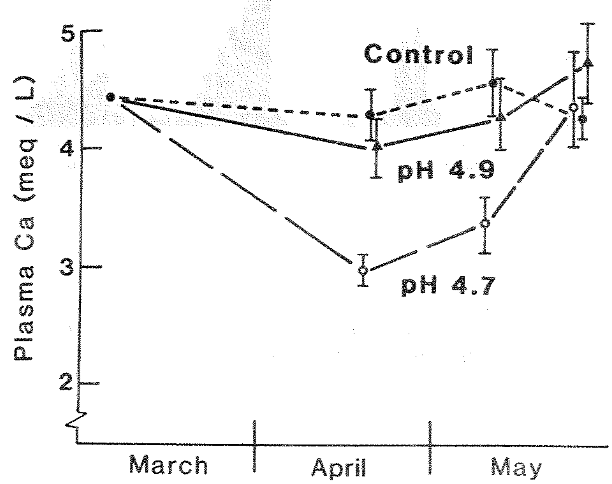

Fig. 8. Mean plasma Ca^{++} levels ($\pm$ SE) in Atlantic salmon reared at control (6.78), intermediate (4.94), and low (4.66) pH levels.

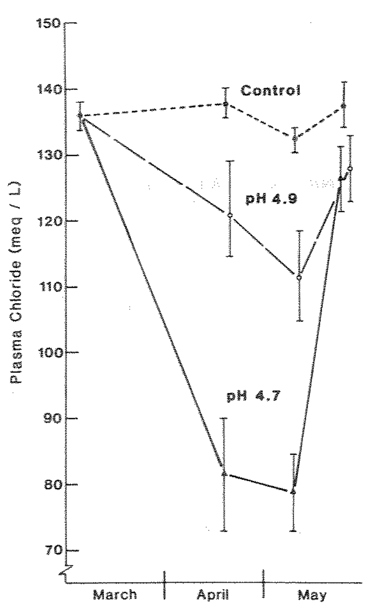

Fig. 7. Mean plasma Cl^{-} levels ($\pm$ SE) in Atlantic salmon reared at control (6.78), intermediate (4.94), and low (4.66) pH levels.

to the pH regime (Fig. 6, 7, 8). Except for the control salmon, the pattern of seasonal change in plasma ion concentrations for each of the other regimes was similar, consisting of an initial decline followed by a rise in ion concentrations. The greatest decline in ion concentrations occurred in salmon from the low pH regime. Levels of all ions in April and early May were significantly lower ($P < 0.05$) in salmon reared at low pH than in those at intermediate and control pH. By late May, those surviving in the low pH regime had plasma ion concentrations not significantly different from those of the other regimes. This rise in ion levels to near normal values in the low pH regime may reflect the slightly higher pH conditions at the time of sampling in early and late May (pH 4.53-4.63) compared with mid-April (pH 4.49-4.51), or it may reflect some form of branchial compensation in which plasma ion losses are reduced and active uptake of ions from the water is increased.

GENERAL DISCUSSION

The effect of long-term acid exposure on smoltification of Atlantic salmon has been described to some extent already by Saunders et al. (1983). However, in that experiment, it was not possible to demonstrate the level of pH above which smoltification would proceed normally. In this experiment, we have compared the effect of three pH regimes considered to be above and below the threshold level for smoltification.

Although pH below 4.7 did not appear to influence body silvering, the development of marginal fin pigmentation or the process of scale loss in smolts, it did have a marked effect on growth and condition factor. Growth data for the low pH regime are not suitable for statistical comparisons because of the high mortality. However, the data do suggest poorer growth in the low pH condition. Poor feeding response and a heightened level of swimming activity appear to be the main causes of this poor growth.

The rate of decline in condition factor for salmon exposed to low pH was much greater than rates for salmon in either of the other regimes. These changes in condition are probably associated with weight loss. The early depletion of lipid reserves and lower condition factor were probably caused by increased energy demands for muscle contraction, increased cardiovascular activity, and increases in other body functions affected by exposure to low pH.

These data also clearly demonstrate that physiological rhythms and mechanisms associated with ionic regulation are impaired when salmon are chronically exposed to pH conditions below 4.7. Smoltification appears to proceed when pH is at or above 4.9, since loss of plasma ions, reduced condition, impairment of ATPase activity and salinity tolerance are greatly attenuated.

The physiological mechanism of acid toxicity for chronically exposed juvenile Atlantic salmon during the time when they should be smolting appears to be associated with failure of ionic regulation in fresh water and sea water. Death in fresh water coincided with low plasma Na^+, Cl^- and Ca^{++} levels, and loss of body condition. Death in sea water of 35 o/oo salinity coincided with the high plasma Na^+ and Cl^- concentrations, increased hematocrit and low branchial (Na^+,K^+)-ATPase activity (Saunders levels and ATPase activity are much higher and close et al. 1983). When pH is 4.9 or higher, plasma ion to controls. Few salmon died in these conditions.

Loss of ions from the plasma of salmonids exposed to low pH conditions in these chronic experiments may be owing to several different actions. Ion losses could be caused by an increase in diffusive permeability of the branchial epithelium, inhibition of exchange diffusion mechanisms involving Na^+, H^+ and NH_4^+ or Cl^- and HCO_3^-, inhibition of active transport pumps associated with the branchial chloride cell, or to some combination of these actions (Wood and McDonald 1982). Present data suggest that ion loss may be caused by a failure of freshwater and saltwater ion transport mechanisms driven by (Mg^{++})-ATPase or (Na^+,K^+)-ATPase enzyme systems. In fresh water, a decline in (Mg^{++}) and (Na^+,K^+)-ATPase activity coincided with declines in all plasma ion concentrations. Conversely, high (Mg^{++})-ATPase activity in fresh water always coincided with elevated plasma ion concentrations. Elevated (Na^+,K^+)-ATPase activity coincided with better survival in seawater and better regulation of Na^+ and Cl^- (Saunders et. al. 1983).

It has been suggested that an increase in branchial ATPase activity is directly related to the excretion of sodium and chloride by the gill in salt water or to the reabsorption of these ions in fresh water (Epstein et al. 1967; Kamiya and Utida 1968, 1969; Pickford et al. 1970; Johnson 1973). Although the role played by (Mg^{++})-ATPase and (Na^+,K^+)-ATPase enzymes in ion transport across the epithelial tissues of the gill remains speculative, data indicate that the pattern of ATPase activity follows the pattern of plasma Na^+ and Cl^- fluctuations. McDonald et al. (1983), in an experiment with rainbow trout, suggested that losses of Na^+ and Cl^- across the gills were caused by a pronounced inhibition of active transport mechanisms. The effect of hydrogen ions on the branchial ion transport system, however, is not clearly understood. Judging from the marked decline in (Mg^{++})-ATPase and (Na^+-K^+)-ATPase activity in low pH conditions, our data suggest that failure of the energy producing component in the chloride cells or in the transport mechanism is the underlying reason for failure of the ionic regulatory system.

Failure of active transport mechanisms to replace or remove plasma ions may not be the only cause of abnormal plasma ion concentrations. Changes in the intercellular cementing agents restricting the paracellular diffusion channels, changes in the permeability of the apical membranes of chloride cells or altered exchange diffusion rates could also lead to reductions in plasma ion concentrations (McDonald 1983). It is not possible to state which of these is most affected by low pH conditions.

In late May, salmon exposed to low pH conditions showed elevated (Mg^{++})-ATPase activity and near normal plasma ion concentrations in fresh water. This change in physiological response may be associated in some way with the smoltification process or with the development of acid tolerance that is independent of smolting. Some degree of acid resistance may exist, or develop, in survivors of exposure to low pH. Several studies have shown improved resistance of eggs and alevins of brook trout and of juvenile brown trout during long-term studies (Trojnar 1977; McWilliams 1980). There is evidence also that elevated hormonal levels associated with smoltification could play an important role in stimulating branchial ATPase activity. Folmar and Dickhoff (1979) and Gallis et al. (1979) reported that gill ATPase activity was influenced by plasma thyroxine and cortisol levels. Since both of these hormones show seasonal changes, it is possible that increased activity of these hormones is responsible for this change in acid tolerance. Further investigations are needed to describe the nature of the structural and functional interactions that develop in the chloride cells of Atlantic salmon chronically exposed to low pH during smoltification.

In conclusion, we suggest that the pH level at which smoltification will proceed normally is at or above 4.9. If pH falls below this level, some impairment of ionic-regulatory mechanisms occurs, leading to excessive loss or gain of electrolytes in fresh water and sea water, respectively. The primary causes of ionic-regulatory failure in this study appear to be associated with inhibition of (Mg^{++})-ATPase and (Na^+,K^+)-ATPase systems and possibly altered membrane permeability in fresh water and salt water. Following prolonged exposure to acid media, some smolting salmon appear to develop compensatory branchial mechanisms and improved ionic regulation. The nature of the compensatory mechanisms is not understood.

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