Effects of episodic acidification on Atlantic salmon (*Salmo salar*) smolts

J.A. Magee, M. Obedzinski, S.D. McCormick, and J.F. Kocik

Abstract: The effect of episodic acidification on Atlantic salmon (*Salmo salar*) smolt physiology and survival in fresh water (FW) and seawater (SW) was investigated. Smolts were held in either ambient (control, pH 6.0–6.6), acidified (chronic, pH 4.4–6.1), or episodically acidified (episodic, pH reduction from control levels to pH ~5.2 for 48 h once weekly) river water for 31 days and then transferred to 34‰ SW. Smolts fed little while in acidified conditions and chronic smolts did not grow in length or weight. In FW, chronic smolts experienced increases in hematocrit and plasma potassium and reductions in plasma sodium and chloride. Upon transfer to SW, chronic and episodic smolts experienced reductions in hematocrit, increases in plasma sodium, chloride, and potassium levels, and suffered mortalities. Gill Na^+,K^+-ATPase and citrate synthase activities were reduced by exposure to acid. For most parameters, the effect of episodic acid exposure was less than that of chronic acidification. Exposure to acidic conditions, even when short in duration and followed by a 30-h recovery period in suitable water (pH 6.5), led to a 35% mortality of smolts upon transfer to SW. This study highlights the importance of measuring and assessing sublethal stresses in FW and their ultimate effects in marine ecosystems.

Résumé : Nous avons étudié les effets de l'acidification épisodique sur la physiologie des saumoneaux du saumon de l'Atlantique (*Salmo salar*) et sur leur survie en eaux douce (FW) et salée (SW). Les saumoneaux ont été gardés en eau de rivière pendant 31 jours, dans des conditions ambiantes (témoin, pH 6,0–6,6), d'acidification chronique (pH 4,4–6,1) ou d'acidification épisodique (réduction des niveaux témoins à pH ~5,2 pendant 48 h, une fois la semaine) et ensuite mis en eau de mer à 34 ‰. Dans les conditions d'acidification, les saumoneaux se nourrissent peu et ceux qui sont placées en acidification chronique ne croissent ni en longueur, ni en masse. En eau douce, les saumoneaux en acidification chronique subissent des augmentation de leur hématocrite et de leur potassium plasmatique et des réductions du sodium et des chlorures de leur plasma. Après un transfert en eau salée, les saumoneaux exposés aux conditions chroniques et épisodiques d'acidification subissent des réduction de leur hématocrite, des augmentations du sodium, des chlorures et du potassium de leur plasma et ils ont une mortalité accrue. Les activités de la Na^+,K^+-ATPase et de la citrate synthase sont réduites par l'exposition à l'acidité. Les effets d'une exposition périodique à l'acidité sont moins dommageables pour la plupart des paramètres que ceux d'une exposition chronique. L'exposition aux conditions acides, même de courte durée et suivie d'une période de récupération de 30 h en eau appropriée (pH 6,5), entraîne une mortalité de 35 % des saumoneaux lors de leur transfert en eau salée. Notre étude met en lumière l'importance de mesurer et d'évaluer les stress sublétaux en eau douce et leurs effets éventuels dans l'écosystème marin.

[Traduit par la Rédaction]

Introduction

Acidification of surface waters has led to the loss of fish species from lakes and rivers, with well-known losses of Atlantic salmon (*Salmo salar*) populations in southwestern Nova Scotia and Norway (Watt et al. 1983; Hesthagen and Hansen 1991). Severely acidified rivers have received much attention, but recent studies suggest that acidic episodes of short duration may have effects on Atlantic salmon populations in less acidified watersheds (Lacroix 1985; Buckler et al. 1995; Magee et al. 2001).

Upon exposure to acidic conditions in fresh water (FW), particularly with elevated inorganic aluminum, Atlantic salmon experience reduced feeding and growth (Saunders et al. 1983; Farmer et al. 1989), altered behavior (Pauwels 1990; Jagoe and Haines 1997), gill damage (Jagoe and Haines

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1997), and endocrine (Haya et al. 1985; Brown et al. 1990) and osmoregulatory disruption and death (Saunders et al. 1983; Lacroix and Townsend 1987; Staurnes et al. 1996). Even more severe is the inability of smolts to remain hypoosmotic in seawater (SW) after exposure to acidic conditions in FW (Farmer et al. 1989; Kroglund and Staurnes 1999; Kroglund et al. 2001*b*). This disruption of osmoregulatory capability may lead to reduced feeding and growth (Pauwels 1990; Damsgard and Arnesen 1998) and higher predation rates (Jarvi 1990; Handeland et al. 1996). Reduced growth has been implicated in lower probability of marine survival of Atlantic salmon smolts (Friedland et al. 1993).

In rivers and associated tributaries supporting Atlantic salmon in eastern Maine, episodes of low pH and high aluminum are more common than long-term acidic conditions (Haines et al. 1990). Episodes of increased acid and aluminum in low-calcium water have been shown to increase mortality and decrease feeding and growth in brook trout (*Salvelinus fontinalis*) fry even when episodes are moderate (decreased pH from 7.2 to 6.5) in severity (Cleveland et al. 1991). It is not known what effect episodes of increased acidity and aluminum have on long-term smolt physiology and survival. However, acute stress has been shown to reduce growth and alter endocrine function of Atlantic salmon parr (McCormick et al. 1998); both processes are important for smolting to proceed normally (Farmer et al. 1989; McCormick et al. 1995). Kroglund and Staurnes (1999) demonstrated that a concurrent decrease in pH (6.52 to 5.95) with a decrease in labile Al (LAl; 22 ± 16 to 15 ± 5 μg $LAl \cdot L^{-1}$) for 24 h was sufficient to disrupt ionoregulation (plasma chloride levels) of smolts in FW. These smolts were able to recover to previous chloride levels after 3 days in limed (pH 6.28) water.

Data collected from several eastern Maine watersheds indicate that some tributaries and mainstem areas experience elevated acidity and aluminum that could impair ionoregulatory capabilities and survival of juvenile Atlantic salmon young-of-the-year, parr, and smolts (Haines et al. 1990).

Because episodes of increased acidity and aluminum associated with acid deposition are more common than chronically low pH in many river systems, we were interested in the effect of these episodes on smolt development and SW tolerance of Atlantic salmon. We hypothesized that episodes of increased acidity and aluminum would lead to osmoregulatory disruption in both FW and after transfer to SW. We tested this by examining the osmoregulatory capability of smolts exposed to periodic episodes of increased acidity and aluminum in FW and after their transfer to SW.

Materials and methods

Experimental animals

Smolts used in these experiments were 1-year-old Atlantic salmon smolts (F1 progeny of Penobscot River sea-run adults) reared at Green Lake National Fish Hatchery in Ellsworth, Maine. On 3 April 2000, 189 smolts (mean fork length ± standard error of the mean (SEM) = 172.2 ± 3.1 mm; mean weight ± SEM = 57.6 ± 3.3 g; n = 38) were taken to the Wild Salmon Resource Center in Columbia Falls, Maine. Sixty-three smolts were placed into each of three 1700-L square rearing tanks, which received ambient Pleasant River water (at 2.2 $L \cdot min^{-1}$). This water first flowed into a 60-L header tank above each rearing tank. The residence time was approximately 13 h for the rearing tanks. The long residence time in the rearing tanks likely avoided the problem of unstable forms of aluminum (Kroglund and Staurnes 1999; Kroglund et al. 2001*a*). We used two air pumps with four air stones in each rearing tank to supply the smolts with abundant oxygen. The orientation of the incoming water created a slight, circular current in the tanks. A commercial pelleted food (50 g) was placed into each tank daily until 3 May.

Experimental design

Our goal was to keep the control tank at pH >6.2, the chronic tank at pH 5.0, and the episodic tank at pH 5.0 for 2 days and pH >6.2 for 5 days weekly. We slightly increased the total aluminum in the chronic and episodic tanks. On 8 April, we started the manipulation of pH and aluminum concentrations in the chronic and episodic tanks. We lowered the pH and increased the total aluminum by adding a stock solution (0.2% HCl and 0.981 g $AlCl_3 \cdot 6H_2O \cdot L^{-1}$) to the header tank at a rate of 2 $mL \cdot min^{-1}$ for the chronic and episodic experimental tanks. This generally lowered the pH by 0.75 $units \cdot day^{-1}$ and increased the total aluminum concentration by approximately 100 μg $Al \cdot L^{-1}$. The control tank received unaltered river water for the duration of the experiment.

To assess the duration of osmoregulatory disruption, we sampled smolts during the third acid episode (sample 1) and 48 h after the episodic tank pH was allowed to increase to that of the control pH (a 48-h recovery period; sample 2). We sampled smolts after a 30-h recovery period from the fifth acid episode (sample 3). The physiological status at this time represents the status the smolts had before being SW-challenged. To assess the effect of prior FW acid exposure on hypoosmoregulatory ability in SW, we placed the remaining smolts into 34‰ SW (collected near Beals Island, Maine, and filtered to 5 μm) and sampled them after 24 h, 72 h, and 7 days.

Sampling and analytical methods

We randomly sampled 10 smolts from each treatment on 22 April (sample 1), 26 April (sample 2), and 8 May (sample 3) and sacrificed them by overdose of $NaHCO_3$-buffered tricaine methanosulfate. Length (to the nearest mm) and weight (nearest 0.1 g) were recorded. Six to 10 gill filaments were removed, placed into 100 μL ice-cold SEI buffer (150 mM sucrose, 10 mM ethylene diamine tetraacetic acid (EDTA), 50 mM imidazole, pH 7.3), and immediately frozen for determination of gill Na^+,K^+-ATPase and citrate synthase (CS) activity using the methods of McCormick (1993) and Leonard and McCormick (1999). Smolts were bled from the caudal vein and hematocrit was immediately read from centrifuged blood. The remaining blood was centrifuged at 3000 × g for 3 min, and the supernatant was removed and frozen at –20°C. Serum plasma sodium, potassium, and chloride concentrations were determined using an AVL 9810 electrolyte analyzer. We stopped the addition of acid and aluminum to the episodic tank on 5 May, and the pH slowly increased to 6.5 by 7 May. Thirty-six hours later (8 May), we conducted a SW challenge test (SWCT) using modified methods of Clarke (1982). We moved remaining smolts into 375 L of 5-μm-filtered 34‰ SW. We randomly sampled smolts after

24 h, 72 h, and 7 days (on 9, 11, and 15 May) and analyzed the parameters described above. On 11 May, approximately 75% of the SW was replaced with SW of the same salinity collected and filtered that day.

We monitored temperature and pH hourly using automatic recording units (Hydrolab Datasonde 2, Hydrolab-Hach Co., Loveland, Colo., U.S.A.) and once daily with an Orion pH meter (Model 250 SA, Thermo Orion, Beverly, Mass., U.S.A.) and PerpHect electrode (Model 8256). The salinity of the SW was measured with a calibrated conductivity meter (YSI 600 XLM Sonde, YSI Inc., Yellow Springs, Ohio, U.S.A.). We analyzed cations, anions, dissolved organic carbon (DOC), acid-neutralizing capacity, closed-cell pH, and aluminum (total, organic, inorganic) of water in each tank on 5 May (during the fifth acid episode exposure) and 8 May (immediately before the SWCT) by standard methods (U.S. Environmental Protection Agency (USEPA) 1987). The sample was not filtered before passing it through the DOWEX column; therefore, this fraction contained both organically bound and particulate Al. We calculated the particulate Al (total (unfiltered) Al minus total dissolved Al) and subtracted this amount from the analyzed organic Al to obtain the "true" organic Al. We assumed the fraction of dissolved aluminum not bound to organics, termed LAl, to be the toxic form. LAl was calculated by subtracting the true organic from the inorganic fraction. The detection limit for all analyzed forms of aluminum was 10 $\mu g \cdot L^{-1}$. All water samples were taken and pH was measured near the tank outlet.

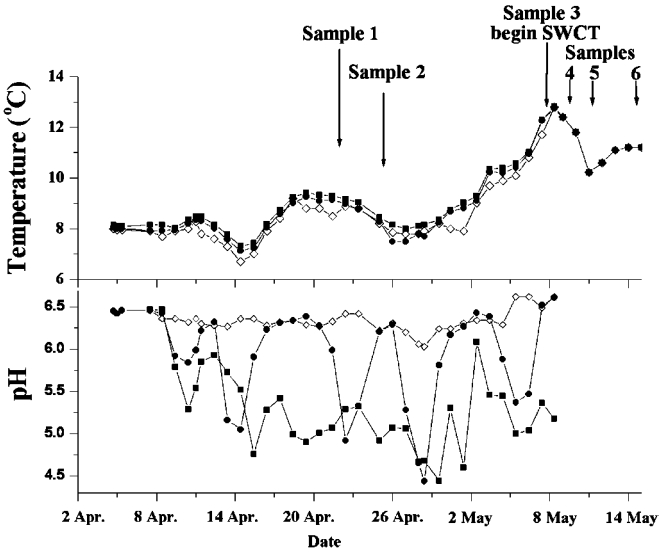
Statistical analyses

Data are presented as means ± SEM. A two-way analysis of variance (ANOVA) was conducted for each parameter using treatment and sample date as the independent variables. Differences over time were determined using Dunnett's test. We compared FW exposure means on 26 April and 8 May to 22 April and SW exposure means on 11 May and 15 May to 9 May. Differences between treatments within a sampling date were determined using Tukey's honestly significant difference test. Differences at $p < 0.05$ were considered significant.

Results

In FW, the temperature increased gradually from 8 to 13°C, and SW temperature varied between 10 and 13°C (Fig. 1). The pH in the control tank averaged 6.32 (range 6.2 to 6.5). Our planned additions of concentrated acid and aluminum solution and the variable river water composition led to designed and natural variability of pH in both the chronic and episodic tanks. Chronic tank pH averaged 5.25 from 10 April to 8 May. The pH ranged from 5.93 to 4.44 from 9 to 29 April and from 4.6 to 6.26 from 30 April to 8 May. The sharp increase in pH beginning on 1 May was due to an accidental decrease in the dosing rate on that day and was corrected on 2 May. Acidification episodes were initiated weekly in the episodic tank and effectively lowered pH as in the chronic tank. The average pH for all days during acid addition ($n = 15$) was 5.93 (includes those times when pH was increasing or decreasing). The average pH for days with no acid addition ($n = 21$) was 6.34. It was generally 1–2 days after the acid addition was terminated that the episodic treatment returned to the pH of the controls. The temperature and

Fig. 1. Temperature and pH of rearing tanks: control (open diamonds), chronic (solid squares), and episodic (solid circles) treatments. The temperature in all seawater (SW) rearing tanks was the same.



pH of the control tank was always within 1°C and 0.2 pH units of ambient Pleasant River water measured at Columbia Falls, Maine (data not shown), effectively mimicking the natural environment. The calculated LAl concentration was 23, 66, and 59 $\mu g\ LAl \cdot L^{-1}$ on 5 May for control, chronic, and episodic tanks, respectively. On 8 May, the calculated LAl concentration was 8, 67, and 15 $\mu g\ LAl \cdot L^{-1}$ for control, chronic, and episodic tanks, respectively. LAl on 8 May was low in the control and episodic tanks as a result of nearly complete complexation of Al with organics (Table 1). Calcium in all three treatments on these dates was 1.78–2.14 $mg \cdot L^{-1}$, and DOC was 6.8–7.3 $mg \cdot L^{-1}$.

Control smolts were active, fed eagerly, and used all areas of the tank, whereas chronic smolts fed little and often remained at the edges of the tank. During acid exposure, episodic smolts displayed behavior similar to that of chronic smolts but were active and fed eagerly when in ambient water. No smolts died while in FW; however, chronic and episodic smolts experienced mortalities in SW (Fig. 2). Only seven chronic smolts were alive after 72 h in SW, and these were sacrificed for analysis of physiological parameters. Control and episodic smolts grew slightly while in FW, whereas chronic smolts were significantly shorter and lighter at the end of FW exposure (Fig. 3). After 72 h in SW, chronic smolts were shorter and lighter than control and episodic smolts. After 7 days in SW, episodic smolts weighed 40% less than controls, despite being only 5% smaller in length. The fork length of survivors did not differ from that of smolts that died (t test, $p = 0.38$).

Exposure to acidified water had clear negative effects on gill Na^+,K^+-ATPase activity, with chronic smolts having the lowest activity on all sampling dates (Fig. 4). The gill Na^+,K^+-ATPase activity of episodic and chronic smolts did not increase from 22 April to 8 May and was lower than that of control smolts in both FW (8 May, after 5 weeks of pulse exposure) and after 24 h SW exposure. The Na^+,K^+-ATPase

Table 1. Water quality parameters on 5 and 8 May and exposure groups.

Date collected	Treatment	Ca^{2+} ($mg \cdot L^{-1}$)	ANC ($\mu eq \cdot L^{-1}$)	TAl ($\mu g \cdot L^{-1}$)	DAl ($\mu g \cdot L^{-1}$)	PAl ($\mu g \cdot L^{-1}$)	OAl ($\mu g \cdot L^{-1}$)	TOAl ($\mu g \cdot L^{-1}$)	LAl ($\mu g \cdot L^{-1}$)	ClpH (pH units)	DOC ($mg \cdot L^{-1}$)
5 May	Ambient	1.85	92.4	148	109	39	125	86	23	6.18	7.3
	Episodic	1.78	2.5	257	216	41	198	157	59	4.97	7.1
	Chronic	1.81	–3.8	268	236	32	202	170	66	4.97	7.3
8 May	Ambient	2.01	118	144	103	41	136	95	8	6.36	7.1
	Episodic	2.14	117	184	107	77	169	92	15	6.25	6.8
	Chronic	2.09	9.8	300	228	72	233	161	67	5.25	6.8

Note: TAl, unfiltered aluminum (total dissolved and particulate); DAl, total dissolved aluminum; PAl, particulate aluminum (TAl minus DAl); OAl, analyzed organic aluminum (organic and particulate); TOAl, OAl minus PAl; LAl, labile aluminum (DAl minus TOAl); ClpH, closed cell pH; DOC, dissolved organic carbon. TAl, unfiltered aluminum (total dissolved and particulate); DAl, total dissolved aluminum; PAl, particulate aluminum (TAl minus DAl); OAl, analyzed organic aluminum (organic and particulate); TOAl, OAl minus PAl; LAl, labile aluminum (DAl minus TOAl); ClpH, closed cell pH; DOC, dissolved organic carbon.

Fig. 2. Cumulative mortality (%) of smolts in exposure groups: control (open diamonds), chronic (solid squares), and episodic (solid circles) treatments. No chronic smolts remained after 72 h in seawater (SW) because of mortality and removal for analysis of each physiological parameter.

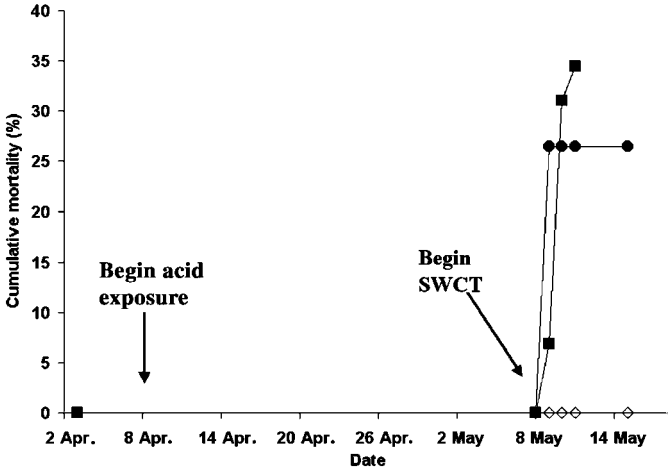
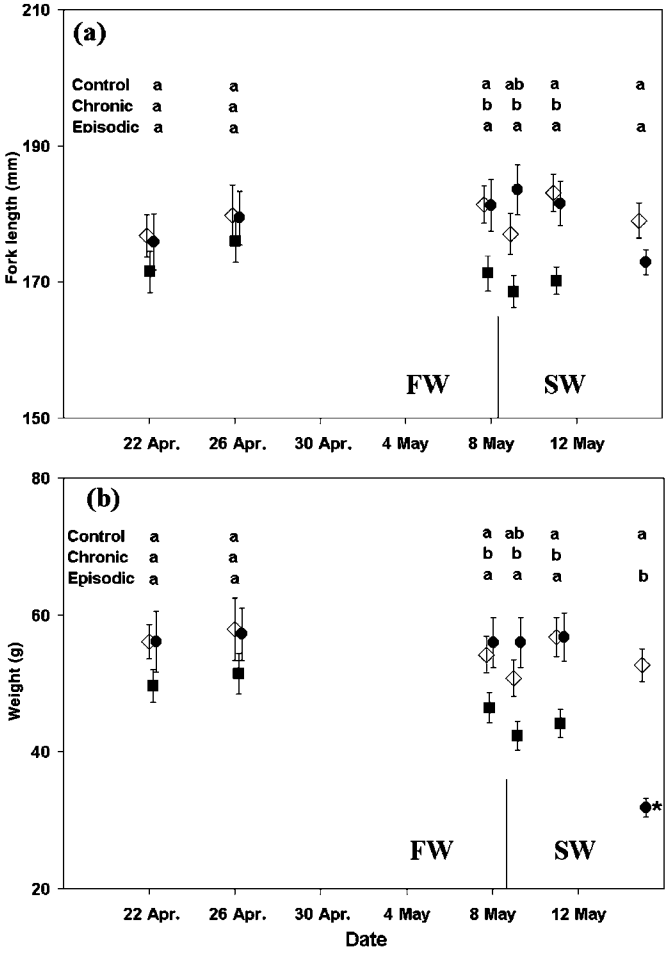


Fig. 3. (*a*) Fork length and (*b*) weight of exposure groups: control (open diamonds), chronic (solid squares), and episodic (solid circles) treatments. Asterisks (*) denote significant difference between 22 April and other freshwater (FW) dates and between 9 May and other seawater (SW) dates. Treatments with the same letter within a date are not significantly different. For all comparisons, $p < 0.05$ was used.



activity of surviving episodic smolts was similar to that of control smolts after 72 h in SW.

The effect of acid exposure on gill CS activity was similar to that on Na^+,K^+-ATPase activity, although not as pronounced (Fig. 4). Chronic and control smolts always had the lowest and highest activity, except after 72 h in SW when control smolts had the lowest and episodic smolts the highest.

Exposure to acidic water elicited similar effects on plasma sodium and chloride levels throughout the experiment (Fig. 5). During sample 1, chronic smolts had lower levels than the other treatments. Four days later (sample 2), plasma chloride of chronic smolts was lower than that of control but not that of episodic smolts. By this time, episodic smolts had been allowed to recover in ambient river water for 48 h, and sodium and chloride levels were nearly identical to those of control smolts. Chronic smolts had lower levels of sodium than those of the other treatments. During sample 3, when episodic smolts had recovered in control water for 30 h, the sodium and chloride levels of chronic smolts were lower than those of episodic smolts, whereas those of control smolts were intermediate. After 24 h SW exposure, chronic and episodic smolts had significantly elevated levels of both ions, and both groups suffered mortalities. Chronic smolts suffered additional mortality after 48 h in SW. After 72 h in SW, the surviving episodic smolts appeared to recover their ionoregulatory capabilities, whereas chronic smolts still had elevated plasma sodium and chloride. Episodic smolts had lower plasma sodium and chloride levels than controls after

Fig. 4. Gill (*a*) Na^+,K^+-ATPase and (*b*) citrate synthase activity of exposure groups: control (open diamonds), chronic (solid squares), and episodic (solid circles) treatments. Asterisks (*) denote significant difference between 22 April and other freshwater (FW) dates and between 9 May and other seawater (SW) dates. Treatments with the same letter within a date are not significantly different. For all comparisons, $p < 0.05$ was used.

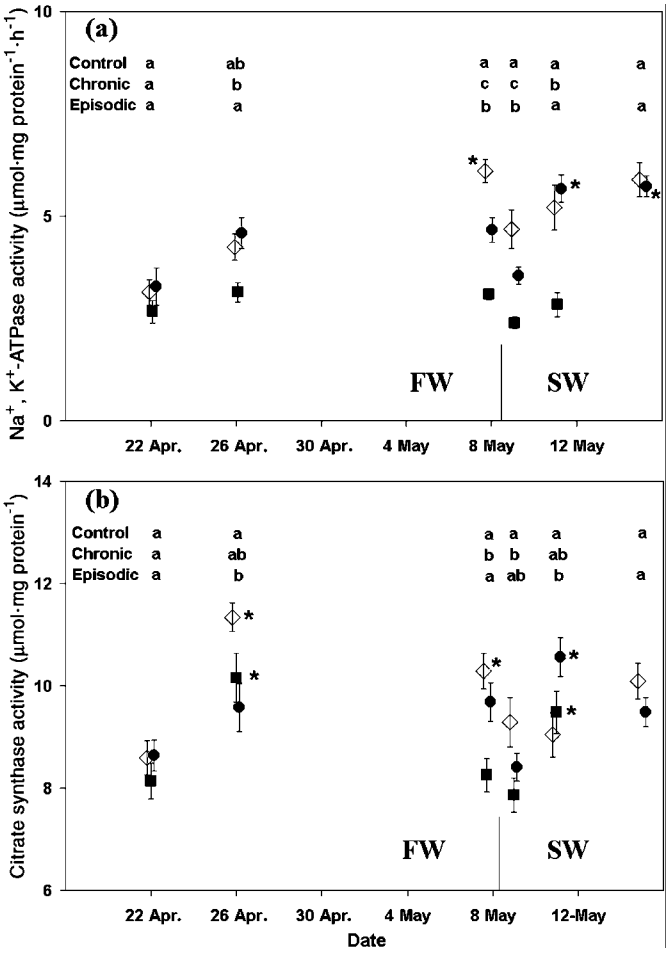
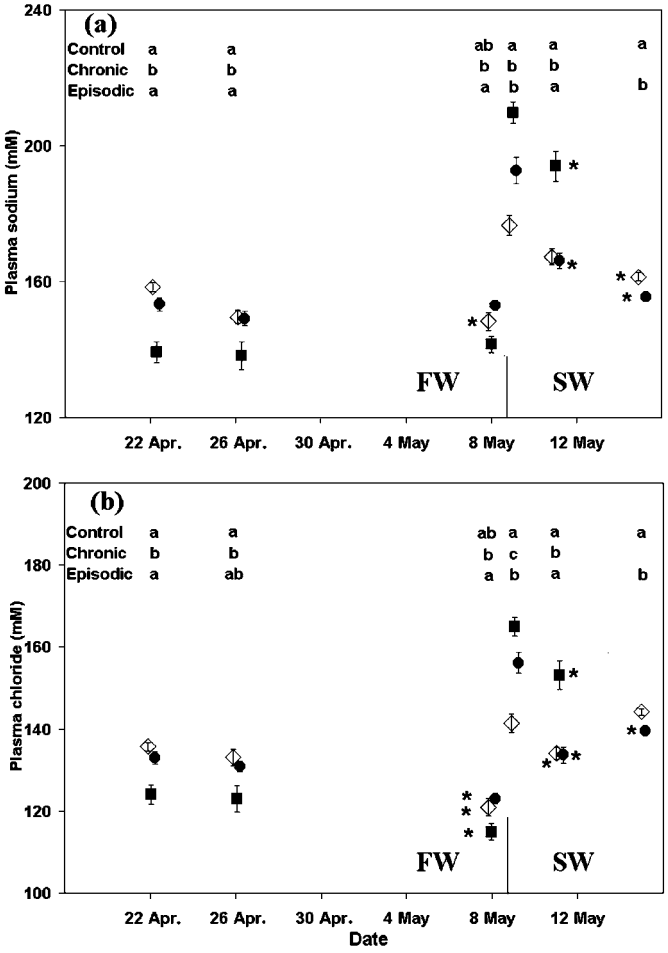


Fig. 5. Plasma (*a*) sodium and (*b*) chloride of exposure groups: control (open diamonds), chronic (solid squares), and episodic (solid circles) treatments. Asterisks (*) denote significant difference between 22 April and other freshwater (FW) dates and between 9 May and other seawater (SW) dates. Treatments with the same letter within a date are not significantly different. For all comparisons, $p < 0.05$ was used.

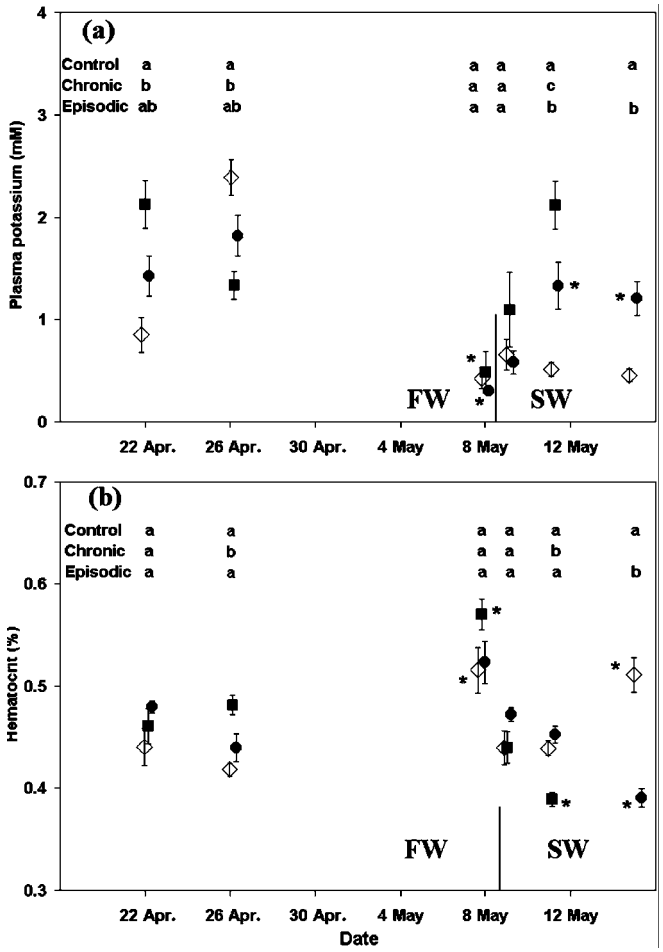


7 days in SW. Exposure to acidic water seemed to increase the plasma potassium levels of smolts, although not always significantly (Fig. 6). On most sampling dates, plasma potassium levels were lowest in control and highest in chronic smolts. Both acid-exposed groups had elevated plasma potassium levels after 72 h in SW, and plasma potassium was also elevated in the surviving episodic smolts after 7 days in SW. In general, hematocrit was inversely correlated with plasma sodium and chloride levels but was much less affected by acid exposure (Fig. 6).

Discussion

Generally, the physiological and behavioral responses to acid and aluminum stress in FW and subsequent SW exposure of control and chronic smolts in the present study were similar to those reported in other studies of Atlantic salmon (Kroglund and Staurnes 1999; Magee et al. 2001) and of brook trout in FW (Buckler et al. 1995). However, we found elevated plasma potassium, whereas others have documented reductions in or no effect on plasma potassium in response to acidified water (Lacroix and Townsend 1987; Booth et al. 1988). Smolts exposed to acid and aluminum lost plasma sodium and chloride in FW and were unable to remain hypoosmotic upon transfer to SW because of a reduction in Na^+,K^+-ATPase activity. This was likely the cause of mortality for the acid- and aluminum-exposed smolts. The present study differs from most in two important ways. First, we exposed smolts to discrete episodes of acidity. This is a more appropriate acid-exposure test relative to riverine ecosystems of Maine. This had a moderate physiological effect in FW, as inferred by plasma ion levels and hematocrit. Second, smolts were transferred to SW after they were allowed to recover in higher pH (~6.5) for 30 h. In other studies, smolts were placed into SW directly from acidified conditions. We allowed this recovery time to determine the effects of acid and aluminum on those smolts entering SW when the FW environment was more physiologically suitable. Our results indicate that prior acid and aluminum exposure significantly compromises salinity tolerance despite a 30-h recovery.

Fig. 6. Plasma (*a*) potassium and (*b*) hematocrit of exposure groups: control (open diamonds), chronic (solid squares), and episodic (solid circles) treatments. Asterisks (*) denote significant difference between 22 April and other freshwater (FW) dates and between 9 May and other seawater (SW) dates. Treatments with the same letter within a date are not significantly different. For all comparisons, $p < 0.05$ was used.



Osmotic stress has also been shown to affect the antipredatory behavior of Atlantic salmon smolts, leading to increased mortalities (Jarvi 1990; Handeland et al. 1996). In a telemetry study, Magee et al. (2001) found that a greater number of acid-exposed hatchery smolts returned upstream to FW shortly after migrating into SW of about 10‰ than did ambient-exposed (pH > 6.3) hatchery smolts. Additionally, those acid-exposed smolts that returned upstream remained in low-saline water for multiple tidal cycles, whereas the ambient-exposed smolts stayed in this section of the river for an average of only 12 h (usually one or two tidal cycles). Ambient-exposed (pH > 6.3) smolts migrated directly into SW without making upstream movements and remained in low-saline water for only a short time. Because estuaries represent an area of high Atlantic salmon predator abundance (Hvidsten and Lund 1988), minimizing time there should increase the survival rate of smolts. If smolts are suffering from osmotic stress (e.g., resulting from acidity) leading to altered migratory behavior, it is reasonable to expect smolt survival to be reduced.

Episodic smolts lost weight after 7 days in SW. This is likely due to a combination of dehydration and increased metabolic rate (basal and active metabolism may have been affected). Both of these may be directly linked to the reduced hypoosmoregulatory ability caused by acid exposure. Salmon juveniles transferred to SW outside of their normal period of smolt development when SW preparedness is high often develop into "stunts", having very low feeding and growth rates. Therefore, one may expect that long-term growth could be negatively affected by prior exposure to acid conditions. Friedland et al. (1993) correlated early marine growth to survival at sea for Atlantic salmon from the Connecticut River, U.S.A., and argued that slow-growing postsmolts do not survive in the marine environment. Crozier and Kennedy (1999) also concluded that marine growth influences marine survival of Atlantic salmon. Other studies have shown that continuous exposure to elevated acid and aluminum affects feeding behavior and leads to reductions in growth while in FW (Haya et al. 1985; Buckler et al. 1995). Based on our results, acidic episodes may similarly affect early marine survival by indirectly reducing early marine growth.

The time needed for recovery of acid-exposed smolts has been less well studied than other aspects of acid and aluminum toxicity. Kroglund and Staurnes (1999) determined that acid-exposed (pH 6.0 and 5.6) smolts were able to reestablish their plasma chloride levels within 3 days in pH 6.28 water, but they did not sample smolts until the end of the 3 days. Kroglund et al. (2001*b*) reported that smolt recovery (normal gill morphology, blood homeostasis, and SW tolerance) from exposure to acidic conditions was achieved within 9 days at pH 6.3. For brook trout fry in FW, swimming and feeding behavior were significantly altered even after a 48-h recovery period, but ionoregulatory parameters were not evaluated (Cleveland et al. 1991). Based on these studies, ionoregulatory recovery time seems to be on the order of at least 1–3 days provided that the water quality is good. However, episodic smolts were not able to recover their SW tolerance after 30 h because the 5-week episodic exposures led to a suppression of Na^+,K^+-ATPase. This demonstrates that ionoregulatory recovery in FW is not equivalent to complete physiological recovery. Recovery time of ionoregulatory ability in SW seems to be similar to recovery time in FW after acid exposure. Control and surviving episodic smolts in the present study were able to recover their ionoregulatory balance after 72 h in SW but not after 24 h. Handeland et al. (1996) reported that smolts suffering osmotic stress after transfer from FW to SW were able to stabilize their plasma chloride levels after 48 h.

Both chronic and episodic acid exposure led to a suppression in gill Na^+,K^+-ATPase and CS activities. These changes may have been causal to the reduced plasma ions in the chronic group in FW, as well as the reduced salinity tolerance seen in chronic and episodic groups. Current models of ion regulation in fish indicate that Na^+,K^+-ATPase is used for both ion uptake and salt secretion (Marshall and Bryson 1998). A reduced number of chloride cells as a result of acid exposure would explain reductions in Na^+,K^+-ATPase and CS activities as chloride cells have much higher amounts of these enzymes than other cell types in the gill. Most previous research suggests that acid and aluminum exposure results in an increase in the number of gill chloride cells (Reid

1995; Jagoe and Haines 1997). If this occurred in the present study, then the reduced enzyme activities must have been due to reductions in the amount of these enzymes in chloride cells (or less likely, in other cell types of the gill). Just before the SW exposure, there was a significant decrease in gill Na^+/K^+ ATPase activity in the episodic group compared with the control, whereas no other change in FW physiology (plasma ions or hematocrit) was detected. After SW exposure, it was clear that the episodic group had decreased salinity tolerance. This indicates that changes in gill Na^+/K^+ ATPase activity are more sensitive than many other aspects of the animal's physiology in freshwater, providing a useful and sensitive indicator of subsequent performance in SW.

Results of the present study demonstrate that episodes of increased acidity and aluminum led to a suppression of gill Na^+,K^+-ATPase activity and subsequent reduction in hypoosmotic capability and survival in SW. The effect was less severe than for exposure to constant low pH (~5.2) for 5 weeks, and ionoregulatory disturbance was not manifest until after exposure to SW. These negative impacts on osmotic balance and growth may have substantial effects on the long-term growth and survival of smolts in the wild.

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