Impacts of episodic acidification on in-stream survival and physiological impairment of Atlantic salmon (*Salmo salar*) smolts

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Abstract: We conducted field studies to determine the levels of acid and aluminum (Al) that affect survival, smolt development, ion homeostasis, and stress in Atlantic salmon (*Salmo salar*) smolts in restoration streams of the Connecticut River in southern Vermont, USA. Fish were held in cages in five streams encompassing a wide range of acid and Al levels for two 6-day intervals during the peak of smolt development in late April and early May. Physiological parameters were unchanged from initial sampling at the hatchery and the high water quality reference site (pH > 7.0, inorganic Al < 12 $\mu g \cdot L^{-1}$). Mortality, substantial loss of plasma chloride, and gill Na^+/K^+-ATPase activity, and elevated gill Al occurred at sites with the lowest pH (5.4–5.6) and highest inorganic Al (50–80 $\mu g \cdot L^{-1}$). Moderate loss of plasma chloride, increased plasma cortisol and glucose, and moderately elevated gill Al occurred at less severely impacted sites. Gill Al was a better predictor of integrated physiological impacts than water chemistry alone. The results indicate that Al and low pH under field conditions in some New England streams can cause mortality and impair smolt development in juvenile Atlantic salmon and provide direct evidence that episodic acidification is impacting conservation and recovery of Atlantic salmon in the northeastern USA.

Résumé : Nous avons mené des études de terrain afin de déterminer les concentrations d'acide et d'aluminium (Al) qui affectent la survie, le développement des saumoneaux, l'homéostasie ionique et le stress chez des saumoneaux du saumon atlantique (*Salmo salar*) dans des cours d'eau en restauration reliés au fleuve Connecticut dans le sud du Vermont, É.-U. Nous avons confiné des poissons dans des cages dans cinq cours d'eau représentant une gamme étendue de concentrations d'acide et d'Al pendant deux intervalles de 6 jours durant la période de développement maximal des saumoneaux à la fin d'avril et au début de mai. Les paramètres physiologiques n'ont pas varié à partir de l'échantillonnage initial, ni à la pisciculture, ni au site de qualité élevée de l'eau (pH > 7,0, Al inorganique < 12 $\mu g \cdot L^{-1}$). Aux sites de pH le plus bas (5,4–5,6) et de concentrations d'Al les plus élevées (50–80 $\mu g \cdot L^{-1}$), il se produit de la mortalité, une perte considérable de chlorures plasmatiques, une baisse de l'activité de la Na^+/K^+-ATPase de la branchie et une augmentation des concentrations d'Al dans la branchie. Dans les sites moins fortement affectés, il y a une perte modérée de chlorures plasmatiques, un accroissement du cortisol et du glucose du plasma et une augmentation modérée de l'Al des branchies. L'Al de la branchie est une meilleure variable indicatrice des impacts physiologiques intégrés que la seule chimie de l'eau. Nos résultats montrent que l'Al et le pH bas dans les conditions de terrain dans certaines cours d'eau de Nouvelle-Angleterre peuvent causer de la mortalité et nuire au développement des saumoneaux chez les jeunes saumons atlantiques; ils fournissent des preuves directes que l'acidification épisodique affecte la conservation et le rétablissement du saumon atlantique dans le nord-est des É.-U.

[Traduit par la Rédaction]

Introduction

Atlantic salmon (*Salmo salar*) populations are in decline in many areas of their natural range and are known to have been impacted by a variety of anthropogenic factors, including dams, habitat alteration, and pollution (Parrish et al. 1998). Because of their anadromous life history, Atlantic salmon may be impacted by human activity in many hydrographical regions, including small rearing streams, migratory corridors (rivers and estuaries), coastal regions, and the open ocean. In the United States, Atlantic salmon populations of most rivers were extirpated in the 1800s because of dams

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and habitat loss. These populations have been the target of large restoration programs (Gephard and McMenemy 2004). In eastern Maine, Atlantic salmon populations were stable until recently, when population declines that began in the 1970s have continued through today and resulted in their listing as a federally endangered species. Acid rain has been suggested as an important causal or contributing factor to these declines and may be affecting Atlantic salmon recovery in other regions of their native range in New England.

Acid rain is the result of atmospheric deposition of sulfate and nitrate that is primarily generated from coal-fired power plants. It has a variety of impacts on aquatic ecosystems, including increased water acidity, decreased buffering capacity of surrounding soil and the water itself, and increased aluminum (Al) (Driscoll et al. 2001). The extent of these negative impacts is dependent on the surrounding geology and soil; areas with relatively low initial buffering capacity will suffer greater impacts of acidification. In Norway and eastern Nova Scotia, acid rain is known to be responsible for extirpation of Atlantic salmon from many rivers (Clair and Hindar 2005).

The transition from fresh water to seawater as juveniles is a critical period for anadromous salmonids. Atlantic and Pacific salmon undergo a parr–smolt transformation (smolting) that is preparatory for downstream migration and ocean entry (Hoar 1988). Smolting is a size-dependent, endocrine-driven developmental event that includes a number of physiological, morphological, and behavioral changes, including the development of salinity tolerance, which is adaptive for rapid seawater entry (McCormick et al. 1998). Increased salinity tolerance is brought about by changes in the major osmoregulatory organs (gill, gut, and kidney) and includes upregulation of ion transporters, such as gill Na^+/K^+-ATPase activity.

The major toxic site of acid and Al impacts on fish is the gill, with effects on both osmoregulation and respiration (Gensemer and Playle 1999). Although salmonids are relatively tolerant of acid and Al, sensitivity changes as a function of life history stage, with smolts being more sensitive than parr or fry (e.g., Saunders et al. 1983; Lacroix 1989; Rosseland et al. 2001). It is thought that these osmoregulatory changes make smolts more sensitive to the impacts of acid and Al. In addition to the effects that may occur in fresh water, acid and Al exposure can result in loss of salinity tolerance, resulting in increased marine mortality (Saunders et al. 1983; Kroglund and Finstad 2003; Kroglund et al. 2007).

Because most Atlantic salmon rivers in New England (northeastern USA) are not as chronically acidified as they are in affected regions of Nova Scotia and Norway, it was thought that Atlantic salmon populations were unlikely to be impacted by acid rain. However, many streams and rivers in New England do suffer from episodic acidification associated with snowmelt and rain events (Driscoll et al. 2001). It has recently become clear that Atlantic salmon are affected by relatively short-term exposure (days) to environmentally relevant levels of acid (pH 5.0–5.6) and inorganic Al (Al_i, 20–80 $\mu g \cdot L^{-1}$) (e.g., Staurnes et al. 1996; Magee et al. 2003; Monette and McCormick 2008). The present study was undertaken to determine whether the natural water chemistry of New England streams that are susceptible to

Fig. 1. Map showing location of cage sites on the Winhall River (Winhall), middle area of Ball Mountain Brook (Middle Ball), upper area of Ball Mountain Brook (Upper Ball), Wardsboro Brook (Wardsboro), and Rock River (Rock), streams of the West River system, a major tributary of the Connecticut River. Inset map shows location in northeastern USA.
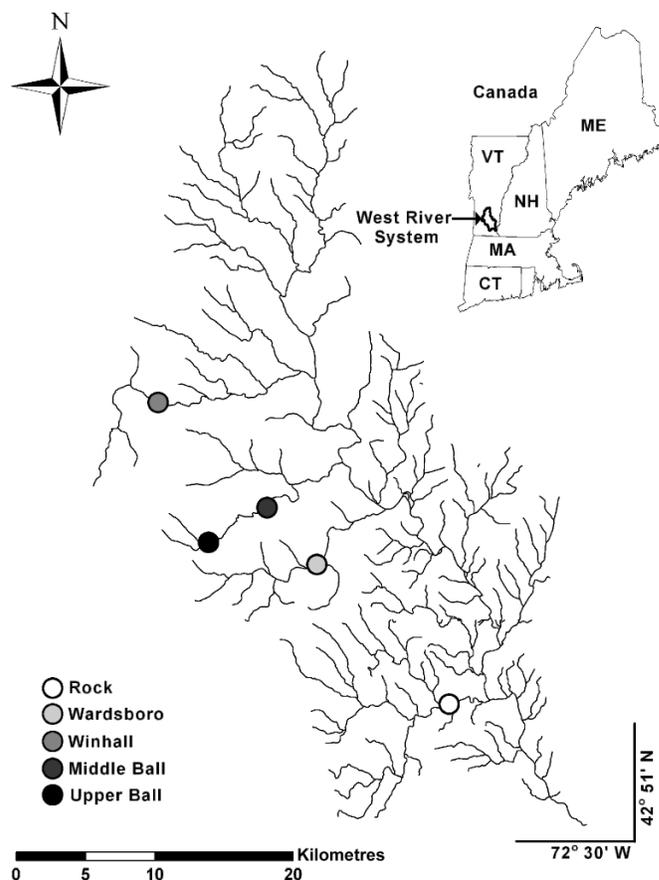
acid rain affects Atlantic salmon smolt survival and physiology. We used short-term field bioassays involving caged fish placed in five streams with varying levels of acid and Al. During these assays, we monitored water chemistry, survival, and the physiological condition of caged fish. Assessment of physiological condition included changes in plasma chloride, cortisol, glucose, gill Na^+/K^+-ATPase activity, and gill Al levels. Our goal was to relate physiologic response under natural conditions that incorporate the combined effects of multiple aspects of water chemistry.

Materials and methods

Study site and field methods

The study was conducted in five tributaries of the West River, a major tributary of the Connecticut River basin draining 496 km^2 in southeastern Vermont, USA (Fig. 1). The West River is an important site for the Connecticut River restoration program, with ∼1 million salmon fry stocked annually. The West River basin is underlain by Precambrian granitic bedrock and is generally poor in calcium carbonate and buffering capacity. However, because of a complex glacial history, there is considerable spatial variation in bedrock and surficial geology at relatively small (kil-

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Table 1. Water chemistry characteristics of the hatchery and five study sites.

Site	Trial	Temp. (°C)	pH	Na (mg·L^{-1})	Ca (mg·L^{-1})	Mg (mg·L^{-1})	Al$_i$ (µg·L^{-1})
Hatchery	1	7.5	7.3	8.04	13.0	1.59	1.6
	2	7.4	7.3	8.29	13.6	1.61	2.9
Rock	1	8.7 (0.38)	7.1 (0.04)	4.86 (0.04)	1.82 (0.13)	1.19 (0.01)	4.6 (0.4)
	2	10.2 (1.04)	7.4 (0.05)	5.62 (0.20)	2.11 (0.10)	1.57 (0.11)	11.2 (4.2)
Wardsboro	1	8.6 (0.41)	5.8 (0.01)	5.98 (0.31)	0.77 (0.07)	0.35 (0.05)	25.6 (0.8)
	2	9.4 (0.82)	6.2 (0.10)	7.79 (0.44)	0.83 (0.06)	0.37 (0.03)	38.0 (10.1)
Winhall	1	7.5 (0.54)	5.8 (0.04)	0.61 (0.01)	0.36 (0.03)	0.28 (0.00)	22.9 (22.5)
	2	8.7 (0.98)	6.2 (0.10)	0.69 (0.02)	0.41 (0.02)	0.32 (0.01)	38.3 (16.7)
Middle Ball	1	7.8 (0.29)	5.7 (0.07)	4.75 (0.13)	0.63 (0.03)	0.29 (0.01)	74.3 (18.8)
	2	9.2 (0.97)	6.0 (0.09)	5.62 (0.26)	0.64 (0.05)	0.31 (0.01)	59.1 (7.7)
Upper Ball	1	7.3 (0.90)	5.5 (0.40)	6.12 (0.53)	0.53 (0.00)	0.28 (0.02)	48.2 (9.0)
	2	9.1 (1.30)	5.7 (0.02)	7.35 (0.07)	0.71 (0.04)	0.34 (0.01)	66.7 (6.1)

Note: Values for hatchery are single measurements taken at the time of fish collection. Values from the streams are the mean (standard error in parentheses) of at least three measurements taken during each trial. Al$_i$, inorganic aluminum.

ometres to hundreds of kilometres) scales. As a result, streams in the basin differ greatly in their vulnerability to episodic acidification. We took advantage of this among-stream variation to select five sites (Table 1) representing a gradient of acid–Al impact, based on existing water quality data (generally pH and conductivity) and after consultation with local natural resource personnel. Sites selected were generally similar in overall physical habitat and were all third- to fourth-order coldwater upland streams between 10 and 15 m bankful width, draining heavily forested (>90% forest cover, northern hardwoods forest community type) catchments < 300 m in elevation, with moderate (<2%) gradients.

Field bioassays were conducted using 1-year-old juvenile Atlantic salmon between 15 and 22 cm fork length (normal size range for wild, migrating Atlantic salmon smolts in New England) obtained from the White River National Fish Hatchery (WRNFH, Bethel, Vermont, USA). Ten fish were sampled directly from the rearing tank (see details of sampling procedure below), and then 120 fish were placed in an insulated 1 m × 1 m transport tank supplied with constant aeration and transported to five sites on tributaries of the West River. In two trials, one beginning on 26 April and another on 6 May 2005, fish were transported, based on the minimum overall transport time, first to the Winhall River (abbreviated as Winhall), followed by the middle area of Ball Mountain Brook (Middle Ball), the upper area of Ball Mountain Brook (Upper Ball), Wardsboro Brook (Wardsboro), and the Rock River (Rock). At each stream, 12 fish were placed in each of two cages approximately 50 m apart. Cages were 76 cm × 46 cm × 31 cm and constructed of 3 cm wooden supports with 1 cm plastic mesh on the outside, with small rocks added to keep them submerged. They were placed behind large boulders or inside scour pools so that the cages had adequate flow but were protected from both high flow events and reduced water levels.

On day 3, six fish were removed from each cage and sampled immediately, and on day 6 all the remaining fish from each cage were sampled. Fish were anesthetized (200 mg·L^{-1} tricaine methanesulfonate (MS-222) neutralized to pH 7.0), and fork length (to the nearest mm) and weight (to the nearest 0.1 g) were recorded. All fish were sampled

within 5 min of removing them from cages. Blood was drawn from the caudal blood vessels into a 1 mL ammonium heparinized syringe and spun at 8000g for 5 min at 4 °C. Plasma was aliquoted, frozen immediately on dry ice, and then stored at –80 °C. Four to six gill filaments were severed above the septum, placed in 100 µL of ice-cold SEI buffer (150 mmol·L^{-1} sucrose, 10 mmol·L^{-1} EDTA, 50 mmol·L^{-1} imidazole, pH 7.3), frozen immediately on dry ice, and then stored at –80 °C for subsequent measurement of gill Na$^+$/K$^+$-ATPase activity. Another six to eight gill filaments were severed above the septum, placed in an acid-washed 1.5 mL microcentrifuge tube, frozen immediately on dry ice, and then stored at –80 °C for subsequent measurement of gill Al levels.

Laboratory analyses

Na$^+$/K$^+$-ATPase activity was determined with a kinetic assay run in 96-well microplates at 25 °C and read at a wavelength of 340 nm for 10 min as described in McCormick (1993). Gill tissue was homogenized in 125 µL of SEID (SEI buffer and 0.1% deoxycholic acid) and centrifuged at 5000g for 30 s. Two sets of duplicate 10 µL samples were run: one set containing assay mixture and the other assay mixture and 0.5 mmol·L^{-1} ouabain. The resulting ouabain-sensitive ATPase activity is expressed as µmol ADP·mg protein^{-1}·h^{-1}. Protein concentrations are determined using BCA (bicinchoninic acid) Protein Assay (Pierce, Rockford, Illinois, USA). Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, California, USA).

Plasma cortisol levels were measured by a validated direct competitive enzyme immunoassay as outlined in Carey and McCormick (1998). Sensitivity as defined by the dose-response curve was 1–400 ng·mL^{-1}. The lower detection limit was 0.3 ng·mL^{-1}. Using a pooled plasma sample, the average intra-assay variation was 5.5% (n = 10), and the average inter-assay variation was 8.8% (n = 10).

Plasma chloride was analyzed by the silver titration method using a Buchler–Cotlove digital chloridometer and external standards. Plasma glucose was measured by the hexokinase and glucose-6-phosphate dehydrogenase enzymatic method (Stein 1963).

Gill Al accumulation was analyzed by modification of

Teien et al. (2006*b*). Gill biopsies were thawed, dried at 60 °C for 24 h, and weighed to the nearest 0.1 µg using a Series 30 microbalance (Cahn Instruments, Cerritos, California). For acid digestions, 98 µL of 100% trace-metal-grade HNO_3 and 2 µL of H_2O_2 were added to tubes with biopsies and heated at 100 °C until completely evaporated (~ 3 h). The same amounts of HNO_3 and H_2O_2 were again added to biopsy tubes and heated with tube caps on at 60 °C for 1 h. Samples were diluted (9:1) by the addition of 900 µL of deionized water, and Al concentration was analyzed using graphite furnace (HGA-800/AAnalyst 100, Perkin Elmer, Wellesley, Massachusetts) atomic absorption spectrophotometry (GFAAS). Samples were read in replicates of two, and calibration was checked every 10 samples with a reference standard. A background correction was made for gill biopsy samples by subtracting the Al present in digestion blanks. Gill Al measurements were expressed as $\mu g\ Al \cdot g^{-1}$ gill dry weight. In preliminary field studies, we found that gill Al did not differ between fish sampled with and without anesthetization and that values for whole gill arches and gill biopsies were not detectably different (M.Y. Monette and S.D. McCormick, unpublished data).

Water samples were taken on days 0, 3, and 6 of each trial. Total Al was analyzed by GFAAS (as described above) in filtered (45 µm, nitrocellulose) and acidified (0.2% trace-metal-grade HNO_3) water samples. Al_i was determined by passing a water sample at 30 $mL \cdot min^{-1}$ via peristaltic pump through a strong acid cation-exchange column with a 9.5 mL resin volume (Amberlite 120) immediately upon collection (Driscoll 1984). Column-processed samples were then acidified (0.2% trace-metal-grade HNO_3) and subsequently analyzed for Al as described above. This Al fraction was called organically bound Al. Al_i was determined by calculating the difference between total and organic Al fractions. Calcium and sodium were measured by flame atomic absorption spectrophotometry (AAnalyst 100, Perkin Elmer, Wellesley, Massachusetts).

Data analyses

For each physiological parameter, a three-way analysis of variance (ANOVA) was used to determine the significance of stream (5 sites), trial (1 or 2), and day of sampling (3 or 6). All parameters were log-transformed prior to ANOVA to achieve homogeneity of variance. Potential cage effect was tested by nesting cage within site, and no significant cage effect was found for any parameter ($p > 0.2$). A significant site effect was found for each parameter, and the significance of site was further examined by one-way ANOVA followed by a Student–Neuman–Keuls test ($p < 0.05$) at each time point. Initial sampling at the hatchery was compared with sampling at the reference site (Rock River) by two-way ANOVA followed by a Student–Neuman–Keuls test ($p < 0.05$). All statistics were run using the STATISTICA (StatSoft Inc., Tulsa, Oklahoma) software package.

Individual physiologic responses to acid–Al are generally strongly intercorrelated. In addition to assessing the effects of water chemistry on individual physiological variables, we wanted to derive a measure of integrated physiological response and to determine which water chemistry parameters were most closely related to this overall measure of physiological response. To do this, we first used principal compo-

nents analysis (PCA) to extract major axes of variation accounted for by the physiologic response variables (plasma chloride, cortisol, glucose, and gill Na^+/K^+-ATPase activity). We then used stepwise multiple regression to test the relationship of these principal component axes with water chemistry variables and gill Al measured in the biopsies as predictors and selected the best predictive model using Akaike's information criteria (AIC) values. This analysis was restricted to day 3 samples for both trials and by necessity excluded fish that died before this sampling event.

Results

Water chemistry

Water chemistry results indicated that the sites were best classified into three general levels of acid–Al severity (Table 1). As expected, hatchery water was well buffered, with high pH, high base cation levels, and essentially no Al_i in water samples (Table 1). Our reference site, the Rock River, while exhibiting slightly lower pH and base cations and slightly higher Al_i than did the hatchery, maintained relatively well-buffered, low Al conditions throughout the study period. In contrast, the Upper and Mid Ball Mountain sites had consistently low pH and high Al_i values for both trials. The Winhall and Wardsboro sites were intermediate with respect to pH and Al_i. However, the Winhall site had much lower Ca and Na concentrations than did the Wardsboro or Ball Mountain sites. Total Al in the streams ranged from a low of 5–16 $\mu g \cdot L^{-1}$ in the Rock to between 90 and 120 $\mu g \cdot L^{-1}$ for Winhall and Upper Ball.

Fish survival and physiological responses

There were no mortalities in Rock or Wardsboro (Fig. 2). In trial one, two fish died by day 3 in Winhall, but there were no mortalities in Winhall thereafter. One of the fish that died in Winhall had external abrasion and some scale loss indicative of a physical injury.

In Upper Ball, all but two fish had died by day 3 in trial 1, and all but one fish died by day 3 in trial 2. In Middle Ball, 4 of 12 fish died between days 3 and 6 in trial 1, and there were no mortalities in trial 2. There were no signs of external injury in mortalities at Middle and Upper Ball. There was no significant difference in gill Na^+/K^+-ATPase activity and plasma cortisol between the initial sampling at the hatchery (time 0) and subsequent levels at Rock (reference site) at days 3 and 6 ($p > 0.05$; Figs. 2 and 3). There were slight but significant elevations in gill Al and plasma glucose and decreases in plasma chloride in the Rock at days 3 and 6 in both trials compared with initial hatchery levels ($p > 0.05$; Figs. 2 and 3).

After 3 days in trial 1, plasma chloride was substantially lower in Upper and Middle Ball (–26%) and Winhall (–21%) compared with Rock and Wardsboro (Fig. 2). At day 6, plasma chloride was still low in Middle Ball and Winhall. After 3 days in trial 2, plasma chloride of the one surviving fish in Upper Ball was 23% lower than that of fish at Rock. There was no significant change in plasma chloride at days 3 or 6 in Middle Ball in trial 2. Plasma chloride in Winhall decreased by 12% on day 3 but recovered by day 6. As with trial 1, plasma chloride at Rock and Wardsboro remained constant and similar to initial levels in the hatchery.

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Fig. 2. Survival (*a*, *b*), plasma chloride (*c*, *d*) and gill Na$^+$/K$^+$-ATPase activity (*e*, *f*) in Atlantic salmon (*Salmo salar*) smolt held for 3 and 6 days in five southern Vermont streams beginning on 26 April (trial 1: *a*, *c*, *e*) and 6 May (trial 2: *b*, *d*, *f*). White River National Fish Hatchery (WRNFH) fish were sampled in the hatchery just prior to transport to stream sites. Values are mean + standard error (*n* = 12, except in cases where mortality occurred). Asterisks indicate significant differences from Rock River (reference site; *p* < 0.05, Student–Neuman–Keuls test). Three-way analysis of variance (ANOVA) indicated a significant effect of location, trial, and day on plasma chloride, Na$^+$/K$^+$-ATPase activity, and gill aluminum (*p* < 0.02).
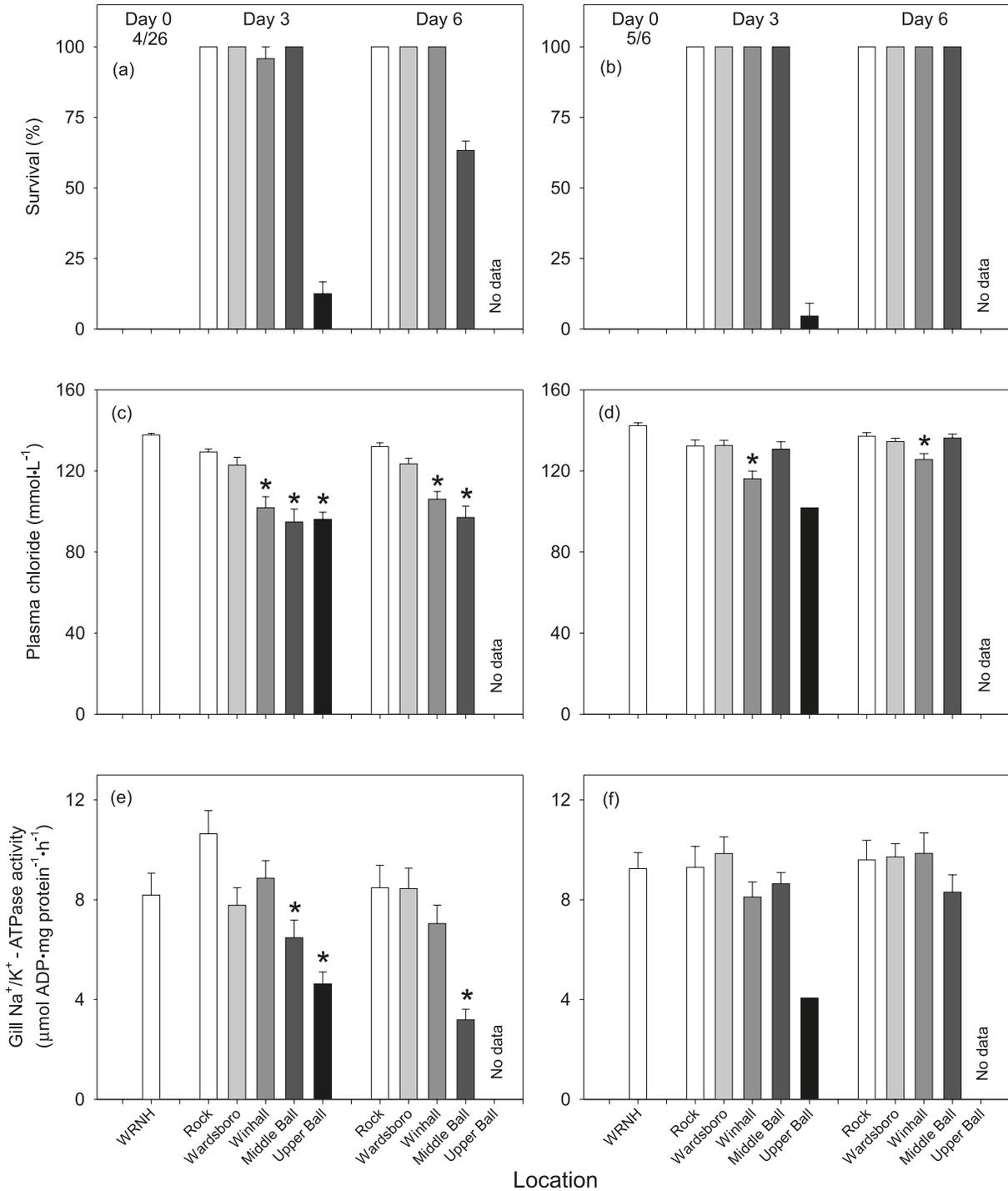

In trial 1, gill Na$^+$/K$^+$-ATPase activity at day 3 was substantially lower at Upper (–57%) and Middle Ball (–39%) than those at Rock (Fig. 2). Gill Na$^+$/K$^+$-ATPase activity continued to decline at Middle Ball through day 6. In trial 2, gill Na$^+$/K$^+$-ATPase activity of the one surviving fish in

Upper Ball was less than half of that of fish at Rock. Gill Na$^+$/K$^+$-ATPase activity at the other sites was not significantly impacted in trial 2.

After 3 days in trial 1, plasma cortisol was substantially higher at Upper and Middle Ball (10-fold), Winhall (6-

Fig. 3. Plasma cortisol (*a, b*), plasma glucose (*c, d*), and gill aluminum (*e, f*) in Atlantic salmon (*Salmo salar*) smolts held for 3 and 6 days in five southern Vermont streams beginning on 26 April (trial 1: *a, c, e*) and 6 May (trial 2: *b, d, f*). White River National Fish Hatchery (WRNFH) fish were sampled in the hatchery just prior to transport to stream sites. Values are mean + standard error (*n* = 12, except in cases where mortality occurred). Asterisks indicate significant differences from Rock River (reference site; *p* ≤ 0.05, Student–Neuman–Keuls test). Three-way analysis of variance (ANOVA) indicated a significant effect of location and trial but not day on plasma cortisol and glucose (*p* < 0.03).
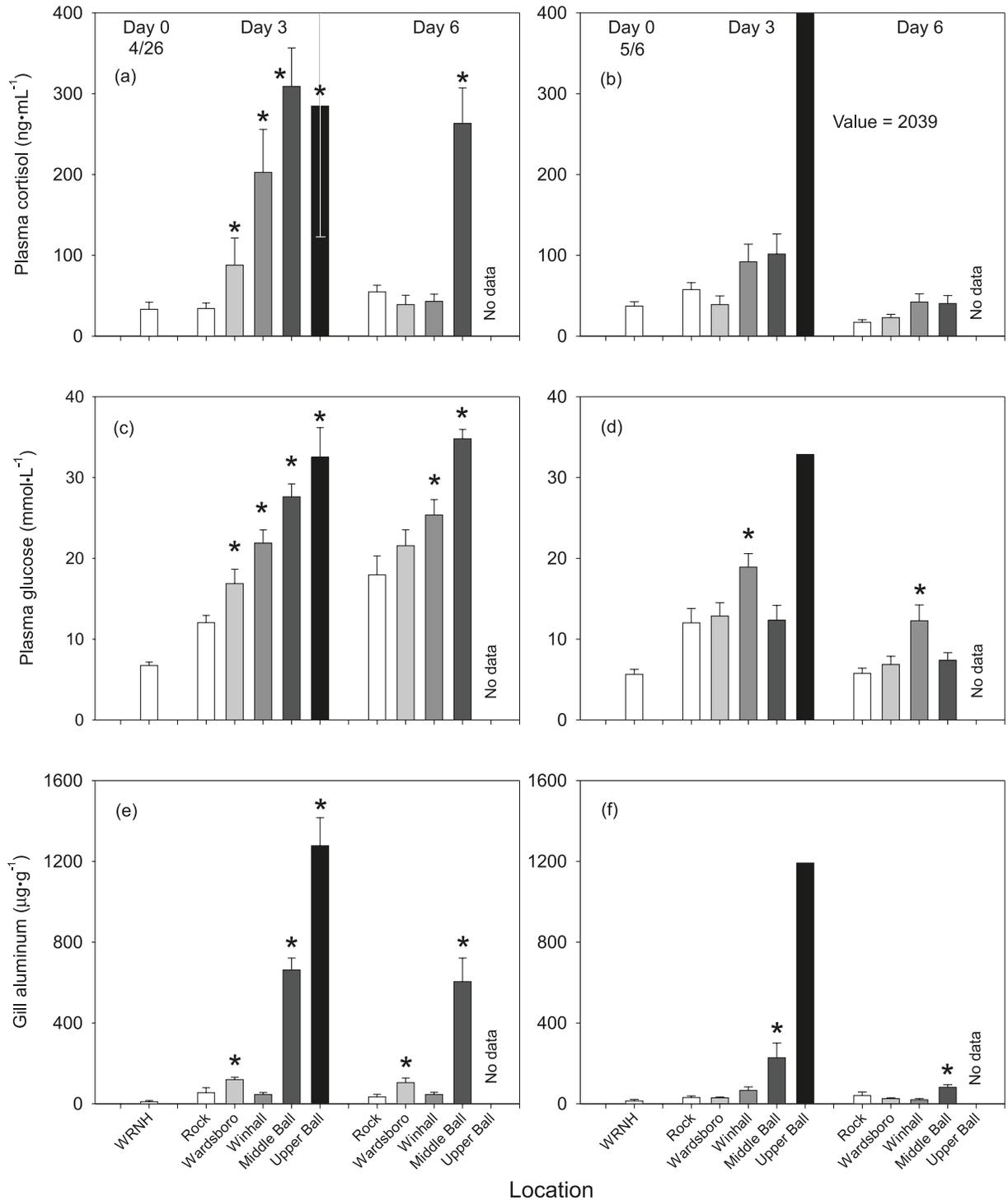
fold), and Wardsboro (2.5-fold) compared with Rock (Fig. 3). At day 6, plasma cortisol was still highly elevated at Middle Ball, whereas Winhall, Wardsboro, and Rock were all relatively low. After 3 days in trial 2, plasma corti-

sol of the one surviving fish in Upper Ball was extremely high. There was no significant difference in plasma cortisol at either days 3 or 6 among the other sites in trial 2.

After 3 days in trial 1, plasma glucose was substantially

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Table 2. Principal component analysis (PCA) table for four physiological response parameters measured on Atlantic salmon smolts exposed to episodic acidification in cage bioassays.

Parameter	Loadings
Cortisol	0.917
Glucose	0.991
Chloride	–0.913
Na$^+$/K$^+$-ATPase	–0.955
Variance explained by component	3.57
Percentage of total variance explained	89.2

Fig. 4. Relationship between pH (*a*) or gill aluminum (*b*) and physiological response factor (PC1) of Atlantic salmon (*Salmo salar*) smolts in the five study sites and two trials. Physiological response factor includes plasma chloride, cortisol, glucose, and gill Na$^+$/K$^+$-ATPase activity. Line in panel (*a*) is a third-order regression ($r^2 = 0.80$) and in panel (*b*) is a linear regression ($r^2 = 0.81$).

higher at all sites relative to Rock, with Upper Ball (2.7-fold) > Middle Ball > Winhall > Wardsboro (Fig. 3). At day 6, plasma glucose was still elevated in Middle Ball and Winhall, whereas Wardsboro and Rock were similar. After 3 days in trial 2, plasma glucose of the one surviving fish in Upper Ball was again 2.7-fold higher than that at Rock. At both days 3 and 6 in trial 2, plasma glucose was elevated at Winhall (66% and 2.1-fold, respectively), whereas there was no significant difference among fish in Middle Ball, Wardsboro, and Rock.
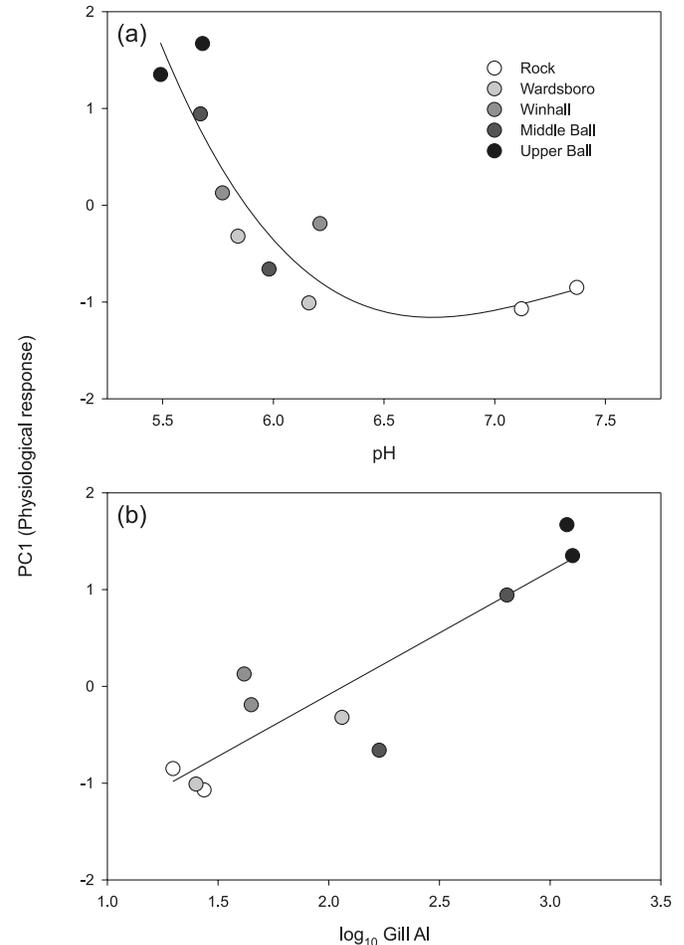
In both trials 1 and 2, gill Al levels were highest at Upper Ball (>20-fold higher than Rock). Gill Al levels were also substantially elevated at Middle Ball in both trials, with levels in trial 1 higher (10-fold) than those in trial 2 (2- to 4-fold). In trial 1, gill Al was 2-fold higher in Wardsboro at days 3 and 6 than at Rock or Winhall, whereas in trial 2 the levels were similar.

Individual physiological responses (plasma ions, glucose, cortisol, and gill Na$^+$/K$^+$-ATPase activity and cortisol) were strongly intercorrelated, with a single principal component axis (PC1) accounting for 90% of the total variation in the data set (Table 2). Fish in sites with high PC 1 scores had high plasma cortisol and plasma glucose, low plasma Cl$^-$, and low gill Na$^+$/K$^+$-ATPase activity, demonstrating that PC1 was a clear indicator of overall physiological impairment. In a stepwise linear regression model using water chemistry variables only, PC1 scores were best explained by low pH ($r^2 = 0.53$, AIC = 26.116, $p = 0.02$) with Al$_i$ and cation concentrations contributing no additional explanatory power. The relationship between pH and PC1 was clearly nonlinear (Fig. 4*a*), and a third-order polynomial had an $r^2 = 0.80$. Gill Al was also a strong predictor of overall physiological impairment (AIC = 16.37, $p < 0.001$), explaining 81% of the variation in PC1 scores across sites (Fig. 4*b*).

Discussion

Our results indicate that variation in stream chemistry was strongly associated with physiological impairment in Atlantic salmon smolts under field conditions. Compared with the well-buffered reference stream, in the four streams where pH dropped below 6.0 salmon smolts showed significant impairment of osmoregulatory function, increased stress response, and an accumulation of Al in gill tissue. In the two streams with the lowest pH and highest levels of inorganic and gill Al, salmon smolts suffered direct mortality. Our results demonstrate that Atlantic salmon are negatively im-

pacted by episodic acidification that occurs in eastern North America; this is consistent with previous findings in Norway and Nova Scotia (Lacroix 1989; Staurnes et al. 1996), where conditions are generally more extreme. These results are also consistent with episodic acid–Al impacts found for other species, such as brook trout (*Salvelinus fontinalis*) in the Adirondack and Catskill regions of upstate New York (Baker et al. 1996). The presence of Al$_i$ in stream water has been suggested to be direct evidence for anthropogenic acidification (Lawrence et al. 2007), and our results therefore indicate a direct link between atmospheric deposition, cation depletion, and negative impacts on Atlantic salmon. Further, our results reinforce the findings of previous work indicating that the presence of Al in nonlethal gill biopsies is a reliable indicator of the presence of Al$_i$ in stream water and of physiological impairment to salmon smolts (Monette and McCormick 2008).

Our results suggest that anthropogenic acidification is impacting conservation and recovery of Atlantic salmon in this region. Episodic acidification events that drive stream pH below 6.0 occur in many northeastern streams (Driscoll et

al. 2001). These episodic events, which are generated by runoff and high stream discharge in response to precipitation and (or) snowmelt, frequently occur during the time of smolt transition in spring (McCormick et al. 1998). Our results clearly show the sensitivity of this life-history stage in Atlantic salmon. Exposure to acid–Al resulted in impairment to osmoregulatory capacity, which has been shown to result in decreases in seawater tolerance and survival after smolts outmigrate to the ocean (Kroglund and Finstad 2003). Salinity tolerance of smolts is strongly correlated with gill Na^+/K^+-ATPase activity, and the loss of gill Na^+/K^+-ATPase activity indicates that salinity tolerance is also lost under the short-term and moderate pH and Al conditions of the present study.

Smolt impairment and subsequent risk of mortality is likely to have important population consequences (Korman et al. 1994). In contrast with earlier life-history stages, it is unlikely that losses at the smolt stage can be compensated by density-dependent increases in adult survival (Jonsson et al. 1998). Further, there is some evidence that low smolt survival is contributing to population declines in the last remaining USA wild salmon rivers in northeastern Maine, several of which are subject to episodic acidification events and where smolt survival has been consistently lower than expected over the last decade (Clegg et al. 2004). The temporal correspondence of declines of Atlantic salmon populations in eastern Maine that began in the 1970s with historic lows in rainfall pH (Driscoll et al. 2001) may not be a coincidence.

Our results are consistent with a variety of other laboratory and field studies indicating that Atlantic salmon smolts are susceptible to acidification impacts when pH is below 6.0. Our major contribution is in demonstrating that these effects can occur rapidly (within days) under "natural" conditions that exist in USA rivers. In Norwegian rivers impacted by acidification, there is generally lower buffering capacity and greater susceptibility to chronic acidification than in most rivers in the USA. Studies that demonstrate that short-term (Staurnes et al. 1996; Monette and McCormick 2008; present study) or multiple acid pulses (Magee et al. 2003) can affect smolt survival and development indicate that the episodic acidification more characteristic of streams in the USA may be affecting Atlantic salmon populations in these rivers. Also, there are more Atlantic salmon rivers in the USA with moderate to high levels of dissolved organic carbon (DOC), which is low in acid-impacted Norwegian rivers. DOC can protect salmon from acidification impacts by binding Al and reducing the amount of the biologically reactive Al_i (Gensemer and Playle 1999). However, DOC can also make streams more acidic, and the acidification-related declines in Atlantic salmon in Nova Scotia have occurred in spite of high DOC in these rivers (Clair et al. 2004). Lacroix (1989) has suggested that when pH is below 5.0, smolts will be impacted even when Al_i is not detectable. The present and previous studies (Saunders et al. 1983; Kroglund et al. 2007) indicate that when pH is between 5.0 and 6.0, the impact will be dependent on the concentration of Al_i and an interaction with pH (i.e., greater impact with lower pH and higher Al_i). Mixing zones and other sources of Al instability, reduced calcium, and possibly other ions can make the impact more severe (Teien et al. 2006*a*), leading to recommendations of pH > 6.5 for safeguarding smolts in Norwegian rivers (Kroglund and Staurnes 1999). Mitigation through liming that has achieved these thresholds had lead to substantial recovery in a large number of Norwegian rivers (Hesthagen and Larsen 2003).

In the present study, gill Al was a strong predictor of mortality and physiological impact. Gill Al was a better predictor of overall physiological impairment than was any single measure of stream chemistry. This result suggests that Al accumulation in salmon gills may better reflect the integrated impact of acid, Al, and other stream conditions than do environmental measurements, likely resulting from both measurement challenges and complex, short-term chemical dynamics (Driscoll and Schecher 1990). Newer techniques, which measure Al exposure at temporal and spatial scales directly relevant to fish, may provide a better assessment of acid–Al impact and physiological risk (Royset et al. 2005). Gill Al has also been shown to be predictive of reduced salinity tolerance and marine survival of smolts exposed to acid and Al (Kroglund et al. 2007). Previous studies have shown that as pH decreases, Al becomes more reactive and thus more capable of binding to the fish gill and causing physiological impairment (Gensemer and Playle 1999). The much greater impact at Upper Ball compared with other sites in spite of only slight elevations in Al_i suggests that relatively small decreases in pH (0.2) can have dramatic effects. We should also point out that our measurements of water chemistry occurred only at 3-day intervals, and it is possible that changes in pH or Al_i occurred that we did not capture by these spot measurements, but were nonetheless experienced by the fish. This is perhaps an additional explanation as to why gill Al is a better predictor of physiological impacts compared with water chemistry, as it integrates the entire exposure period of the fish. Based on previous research in a number of fish species, the elevated gill Al found is likely to be directly responsible for the loss of plasma ions and gill Na^+/K^+-ATPase activity (Gensemer and Playle 1999). In turn, the observed stress response (increased plasma cortisol and glucose) is likely to be a response to the loss of plasma ions caused by acid and Al exposure (Wendelaar Bonga 1997), though other routes such as a direct response to Al accumulation cannot be ruled out. Since cortisol is involved in regulating ion uptake (McCormick 2001), the observed increase in plasma cortisol may promote recovery from reduced loss of plasma ions caused by acid and Al exposure.

While an interaction between Al_i and pH in stream water appears to be a primary driver of mortality and physiological impairment due to episodic acidification, our results suggest that several complicating factors need to be considered. Specifically, in the two streams where smolts were physiologically impaired but did not suffer direct mortality, we found that Winhall smolts had higher levels of impairment than did Wardsboro smolts, particularly with respect to plasma chloride and plasma glucose. This result may be explained by the low concentration of Ca and particularly Na in Winhall compared with the other sites. Low Na is likely to increase the osmoregulatory burden in fresh water, where fish are hyperosmotic relative to the environment and must expend energy to prevent ion loss. This finding suggests that impacts on episodic acidification at low and intermedi-

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ate levels of Al_i availability might be influenced by other aspects of water chemistry. Laboratory experiments that decouple these effects, as well as examining interactions, will help in understanding the overall influence of water chemistry variation on smolt performance and production.

While our results suggest important consequences for Atlantic salmon conservation and recovery in New England, some knowledge gaps remain. Because of the complexities and spatial and temporal variation of Al chemistry in surface waters (Driscoll and Schecher 1990), it is difficult to estimate the proportion of smolts that are likely to experience acid–Al conditions in New England. Based on their reduced alkalinity, Haines (1980) suggested that areas of the West River drainage of the Connecticut River (including the present study area), the upper, upland reaches of the Merrimack River, and rivers in eastern Maine are the most acidification-susceptible Atlantic salmon rivers in the USA. In the Connecticut and Merrimack rivers, acid–Al conditions are most likely to occur in higher elevation upland streams, while mainstem rivers tend to be relatively well-buffered (Douglas et al. 2002). As a result, smolts that experience acid–Al conditions in their rearing streams may have the chance to recover in less-impacted mainstem waters before entering the ocean. While some laboratory studies have found that smolts can recover osmoregulatory function after exposure to acid–Al (Kroglund et al. 2001), Magee et al. (2003) found that negative impacts can be persistent. In laboratory experiments, we have found that loss of salinity tolerance due to acid–Al exposure persists for at least a week even if fish are maintained in highly buffered, neutral recovery water (S.D. McCormick, unpublished data). Individual Atlantic salmon smolts appear to outmigrate quickly (several days to a week; McCormick et al. 1998), making it more likely that negative impacts in freshwater rearing streams would influence the seawater performance of postsmolts, though the influence of acid–Al exposure on smolt behavior has not been examined. Smolts produced in short rivers with limited buffering capacity, such as those in eastern Maine, may be at the greatest risk of exposure to episodic acidification. Integrating studies of acid–Al impacts with movement and life-history analyses in the field will increase our understanding of overall impacts of acidification on conservation and restoration of wild Atlantic salmon stocks.

Acknowledgements

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