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Evidence for episodic acidification effects on migrating Atlantic salmon *Salmo salar* smolts

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Field studies were conducted to determine levels of gill aluminium as an index of acidification effects on migrating Atlantic salmon *Salmo salar* smolts in the north-eastern U.S.A. along mainstem river migration corridors in several major river basins. Smolts emigrating from the Connecticut River, where most (but not all) tributaries were well buffered, had low or undetectable levels of gill aluminium and high gill Na^+/K^+-ATPase (NKA) activity. In contrast, smolts emigrating from the upper Merrimack River basin where most tributaries are characterized by low pH and high inorganic aluminium had consistently elevated gill aluminium and lower gill NKA activity, which may explain the low adult return rates of *S. salar* stocked into the upper Merrimack catchment. In the Sheepscot, Narraguagus and Penobscot Rivers in Maine, river and year-specific effects on gill aluminium were detected that appeared to be driven by underlying geology and high spring discharge. The results indicate that episodic acidification is affecting *S. salar* smolts in poorly buffered streams in New England and may help explain variation in *S. salar* survival and abundance among rivers and among years, with implications for the conservation and recovery of *S. salar* in the north-eastern U.S.A. These results suggest that the physiological condition of outmigrating smolts may serve as a large-scale sentinel of landscape-level recovery of atmospheric pollution in this and other parts of the North Atlantic region.

Key words: aluminium; Maine; north-eastern U.S.A.; osmoregulation; pH; stress.

INTRODUCTION

Acid rain or acidification is the result of atmospheric deposition of nitrate and sulphates from coal-fired power plants and other industrial activity. The resulting base cation

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depletion of soils and water, increased water acidity and mobilization of aluminium has been the focus of considerable research (Driscoll *et al.*, 2001). These ecosystem-level changes have in turn had a major effect on critical elements of regional biodiversity that have not been fully explored. Atlantic salmon *Salmo salar* L. 1758 in the New England region of the U.S.A. provide a particularly important case study. Extirpated from most of its historic range in New England, which originally encompassed all major rivers in the region that drain into the Atlantic Ocean south to Long Island Sound (Parrish *et al.*, 1998), *S. salar* are the subject of intense restoration and conservation efforts. These efforts have not succeeded in re-establishing self-sustaining populations, and the few existing wild runs in eastern coastal Maine are continuing to decline, resulting in the species being listed as endangered in 2000 under the U.S. Federal Endangered Species Act. Acidity of surface waters may prevent the recovery and re-establishment of *S. salar* in rivers where migratory passage and physical habitat appear to be adequate. While regulation of emissions in response to the U.S. Clean Air Act (http://www.epa.gov/air/caa/index.html) has lowered (but not removed) atmospheric concentrations of acid pollutants, base cation concentrations in soils and surface waters throughout the north-east have not recovered, resulting in continued widespread episodes of low pH, generally associated with runoff events (Driscoll *et al.*, 2001).

Acidification can affect salmonids *via* several different mechanisms, but the largest effect appears to be associated with the increased bioavailability of aluminium (Al). Aluminium is a common, naturally occurring element in surface soils. It is present in small amounts in surface waters, but under normal pH conditions is not highly soluble and rapidly complexes with colloidal and organic materials. Under these conditions, Al has minimal effect on fish physiology or stream ecosystems. Low pH conditions, however, increase the solubility, and hence the bioavailability, of Al by permitting the formation of inorganic monomeric species (Al_i). Al_i, even in minute amounts and short-term exposures, affects salmonids primarily through damage to the gills leading to impaired osmoregulatory and respiratory capacity. This is particularly critical for migratory *S. salar*, which undergo major changes in gill function associated with the development of seawater tolerance during the outmigration (smolt) phase of their life cycle. Smolts of *S. salar* are extremely sensitive to acid and aluminium (Saunders *et al.*, 1983; Lacroix, 1989; Rosseland *et al.*, 2001; Monette & McCormick, 2008), resulting in direct mortality if conditions are severe and loss of salinity tolerance in less severe cases. The loss of salinity tolerance after acid and aluminium exposure is associated with reduced marine survival (Kroglund *et al.*, 2007). Smolting occurs in the spring, when high levels of runoff frequently result in acid and aluminium levels in many rivers well above the threshold where negative effects on smolts are known to occur. Owing to their sensitivity, conservation status and wide distribution, *S. salar* are an important sentinel species for the effects of acid–aluminium on aquatic ecosystems in the New England region.

In areas with particularly poor buffering capacity, acidification effects on water chemistry are chronic and evenly distributed and have relatively predictable effects on biological productivity and diversity (Driscoll *et al.*, 2001). In many catchments, however, the effects of acidification are episodic (associated with snowmelt and high spring flows), resulting in spatial heterogeneity both among basins and within basins at relatively small spatial scales (kms), and temporally variable as a function of precipitation and flow regimes. For these reasons, estimating the overall effect of episodic acidification on *S. salar* populations presents a major challenge. Gill aluminium represents a suitable tool for monitoring *S. salar* populations for the effect of acidification. As noted above, the major effects of acidification on fishes, in general, and salmonids, in particular, are the mobilization of aluminium and its transformation to toxic ionic forms. The accumulation of aluminium in the gill has been proposed to be the major pathway for the toxic actions of acid and aluminium toxicity (Teien *et al.*, 2006), and many studies have shown a strong correlation between gill aluminium levels and both lethal and sublethal effects on *S. salar* (Staurnes *et al.*, 1993, 1996; Gensemer & Playle, 1999; Kroglund *et al.*, 2001*a*, *b,* 2007; McCormick *et al.*, 2009*a*; Monette *et al.*, 2010). The latter includes loss of salinity tolerance and gill Na^+/K^+-ATPase (NKA) activity, an enzyme critical to the development of salinity tolerance.

The goal of this study was to examine the spatial and temporal effects of acidification on *S. salar* in the north-eastern U.S.A. This was accomplished by measuring the levels of gill aluminium and NKA activity in wild *S. salar* smolts in river systems in the New England region. The results of this study indicate that effects of acidification on *S. salar* are variable from year to year and may be sufficiently severe in some rivers where population sustainability is threatened.

MATERIALS AND METHODS

STUDY SITE AND FIELD METHODS

Salmo salar smolts were captured from 18 April to 26 May during the peak of their downstream migration in five river basins in the north-eastern U.S.A., comprising three distinct sets of physiographic and geologic settings (Fig. 1). Smolts passing the collection sites on each river would be expected to enter saline water within days. The Connecticut River is the most southerly of the study rivers, and is the largest river in New England (>26 000 km^2 drainage area) with headwaters at the U.S. and Canadian border. At the location where smolt sampling was conducted, the river drains an area of *c.* 18 000 km^2. The Connecticut River basin is geologically diverse. While a few tributaries are underlain by poorly buffered surficial and underlying geology, most of the basin, including most of the mainstem, is well buffered from acid precipitation (MassDEP, 2013). In contrast, the Pemigewasset River in the Merrimack River basin drains a substantially smaller basin area (1935 km^2). While a few tributaries have some buffering capacity associated with local glaciation effects, the combination of thin soils, extremely poorly buffered surficial and bedrock geology, and high elevation (increasing atmospheric exposure) results in most of the basin being highly exposed to episodic acidification (Driscoll *et al.*, 2001).

Sampling sites in Maine rivers encompassed a range of basin conditions. The Penobscot is the second largest river in New England and was represented by two sites, one near the mouth of the river (drainage area 21 106 km^2) and the other on the Pleasant River, which empties into the Piscataquis River, a major tributary of the Penobscot Rivera (drainage area of 822 km^2). The remaining three sites were on two smaller coastal rivers: the Sheepscot (drainage area of 424 km^2) and two sites on the Narraguagus (drainage areas of 403 and 422 km^2). While Maine sites are generally underlain by poorly buffered bedrock and surficial geology, their lower elevation and more easterly locations reduce exposure to atmospheric deposition to some extent, and mainstems also benefit from buffering capacity provided by relict marine sediments.

Smolt production in the Connecticut and Merrimack Rivers is primarily the result of fry-stocking from the progeny of sea-run adults and F1 domestic fish, with a small number produced by natural reproduction (Gephard & McMenemy, 2004). Smolts were collected in both rivers from 2006 to 2009. Collections were made on the Connecticut River at the Cabot Dam in Turners Falls, MA (river km 198; 44·5873° N; 72·5788° W; Fig. 1), utilizing a bypass structure that guided fish away from turbine intakes and through a collection facility. Smolts were collected from the Merrimack River at the Ayers Island Dam in Bristol, NH (river km 208·2; 43·5981° N; 71·7182° W; Fig. 1), utilizing a bypass structure that guided fish away from turbine intakes and into a collection tank from which fish were sampled.

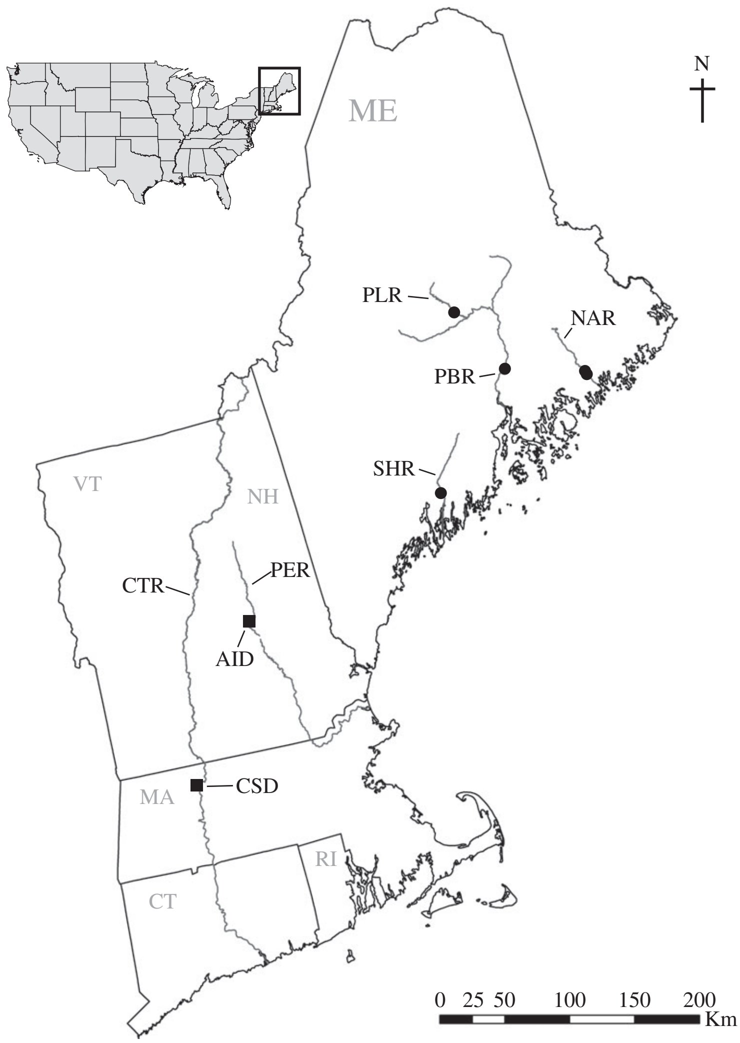

FIG. 1. Map of the New England study region including Connecticut (CT), Massachusetts (MA), Vermont (VT), Maine (ME) and Rhode Island (RI). *Salmo salar* smolts were collected at dam bypass structures (■) on the Connecticut River (CTR) at Cabot Station Dam (CSD), the Pemigewasset River (PER) at Ayers Island Dam (AID) and at rotary screw traps (●) on the Sheepscot River (SHR), Pleasant River (PLR), Penobscot River (PBR) and Narraguagus River (NAR).

Smolts were collected in the Penobscot River in 2005 on 8 May using three rotary screw traps at the head of tide (44·8212° N; 68·7041° W; Fig. 1). These fish comprised a mix of hatchery-stocked smolts (age 1 year) stocked between 18 and 26 April, autumn parr-stocked (autumn fingerlings) and naturally reared fish (product of wild spawning or fry-stocking). In 2006 and 2007, autumn parr and naturally reared smolts were collected in the Pleasant River using two (2006) or three (2007) rotary screw traps *c.* 100 m above the most upstream influence of the tide (45·2994° N; 69·0236° W; Fig. 1).

On the Sheepscot River, naturally reared and autumn parr-stocked smolts were collected from 2005 to 2007 with two rotary screw traps (44·1152° N; 69·6238° W; Fig. 1). In the Narraguagus River, naturally reared smolts were collected from 2005 to 2007 in two rotary screw traps at each of the two trapping sites located at 7·7 and 11·7 km (44·6310° N; 67·9540° W; Fig. 1). In both rivers, the traps were located above the most upstream influence of the tide.

SMOLT SAMPLING

At both dam collection sites, smolts were collected at night between 1900 and 2200 hours and sampled within 1 h of capture. At rotary screw traps, smolts were captured overnight and sampled in the morning between 0600 and 1000 hours. *Salmo salar* are listed as an endangered species in Maine, so all sampling was conducted using non-lethal biopsies (McCormick, 1993) and permitted under by the U.S. Endangered Species Act (http://www.fws.gov/endangered/index.html; Section 10 Permit #TE 697823–1). Fish were anaesthetized (200 mg l^{-1} MS-222 neutralized to pH 7·0) and fork length (L_S) to the nearest mm and mass to the nearest 0·1 g were recorded. Four to six gill filaments from the first gill arch on the left side were severed above the septum, placed in 100 μl of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA and 50 mM imidazole, pH 7·3), frozen immediately on dry ice and then stored at −80° C for subsequent measurement of gill NKA activity. Another six to eight gill filaments from the first gill arch on the right side 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 aluminium levels. All work was conducted under U.S. Geological Survey Institutional Animal Care and Use Committee protocol SP9066.

LABORATORY ANALYSES

NKA 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 the study of McCormick (1993). Gill tissue was homogenized in 125 μl of sucrose-EDTA-imidazole-deoxycholic acid (SEI buffer and 0·1% deoxycholic acid) and centrifuged at 5000 *g* 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 mM ouabain. The resulting ouabain-sensitive ATPase activity is expressed as μmoles adenosine diphosphate (ADP) mg protein^{-1} h^{-1}. Protein concentrations are determined using bicinchoninic acid (BCA) protein assay (Pierce; www.piercenet.com). Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices; www.moleculardevices.com).

Gill aluminium accumulation was analysed by modification of the method described by Teien *et al.* (2006). 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; www.thermoscientific.com). 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 (*c.* 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 analysed using graphite furnace atomic absorption spectrophotometry (GFAAS; HGA-800/AAnalyst 100, Perkin Elmer; www.perkinelmer.com). Samples were read in replicates of two, and calibration was checked every 10 samples with a reference standard created by diluting atomic spectroscopy calibration standard (aluminium pure single-element standard, Perkin Elmer) with deionized water and 100% trace metal grade HNO_3 to a concentration of 40 μg Al l^{-1}. A background correction was made for gill biopsy samples by subtracting the Al present in digestion blanks. Gill Al measurements were expressed as μg Al g^{-1} gill dry mass.

WATER CHEMISTRY

Water chemistry measurements from several different sources including point samples, *in situ* samples and data logging sondes were taken from sites near the smolt traps. It should be noted that these rivers are heterogeneous in their water chemistry and that these sites only partially characterize the river; upstream locations may have more severe acid and aluminium conditions.

Point measurements of water pH were made *in situ* using a portable pH meter (3 Star, Thermo-Orion; www.thermoscientific.com) and a low-ion pH electrode (ROSS, Thermo-Orion). Prior to each measurement, the meter was calibrated using two low-ionic strength buffers (Pure Water pH 6·97 and pH 4·10, Thermo-Orion) that had been allowed to equilibrate to ambient stream temperature. The electrode was suspended directly in the stream in an area where there was some water movement but out of the direct current, and pH was recorded after 2 min. After each measurement, the electrode was returned to the pH 6·97 buffer to check the calibration, and the calibration and measurement process was repeated if the results deviated from the expected pH.

Seasonal pH in Maine was also recorded hourly by water quality sondes (600 XLM, YSI; www.ysi.com) deployed on the Penobscot (2005), Narraguagus (2005, 2006), and Sheepscot (2006, 2007) Rivers. Sondes in the Penobscot and Sheepscot Rivers were deployed during the smolt migration window (April to June) and downloaded and calibrated at the end of that time period, whereas the Narraguagus River sonde was deployed year-round and tended every 2 months. Each time a sonde was tended or retrieved, a two-point buffer check was performed (4·00 and 7·00 pH). If the sonde performed as expected, it was re-calibrated and re-deployed, if not, the sonde was replaced.

Total Al was analysed by GFAAS as described above in filtered (0·45 μm, nitrocellulose) and acidified (0·2% trace metal grade HNO_3) water samples. Inorganic Al (Al_i) was determined by passing a filtered water sample at 30 ml min^{-1} 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 analysed for Al as described above. This Al fraction was called organically bound Al. Inorganic Al was determined by calculating the difference between the filtered total and organic Al fractions.

Additional water samples were collected in high-density polyethylene bottles and stored on wet ice in the dark prior to analysis. Acid neutralizing capacity (ANC) was measured with an automatic titrator system (ABU 90, Radiometer; www.radiometer-analytical.com) and dissolved organic carbon (DOC) was measured with a total organic carbon analyser (model 1010, OI analytical; www.oico.com) at the Senator George J. Mitchell Center for Environmental and Watershed Research, University of Maine, Orono, ME, U.S.A.

DATA ANALYSES

Values are reported as mean ± S.E. All statistical analyses were conducted using NCSS 2007 (v.07.1.21, Hintze 2007; www.ncss.com) and significance was accepted when $P < 0·05$. The effect of river of origin and year was examined using a two-way ANOVA. If river was a significant variable, then individual rivers were compared by Tukey's *post hoc* test. The Connecticut and Pemigewasset Rivers and the rivers in Maine (Sheepscot, Narraguagus and Penobscot–Pleasant) were grouped together because of geographic proximity and because the years of sampling were the same. For gill aluminium, the results were not normally distributed, so two-way ANOVA was performed on ranks. In order to further explore the effect of year on gill Al levels, a regression analysis of mean gill Al *v.* flow during the period of smolt migration (25 April to 15 May) was analysed for each year. Discharge data for each river were obtained from the U.S. Geological Survey (USGS) gauging station nearest to the area of smolt collection: Connecticut River at Montague City, MA, (station 01170500; USGS, 2014*a*), Pemigewasset River at Plymouth, NH (station 01076500; USGS, 2014*e*), Sheepscot River at North Whitefield (station 01038000; USGS, 2014*f*), Narraguagus River at Cherryfield (station 01022500; USGS, 2014*b*), Penobscot River at West Enfield (station ID 01034500; USGS, 2014*c*) and Piscataquis River at Medway (station 01034000; USGS, 2014*d*).

RESULTS

CONNECTICUT AND PEMIGEWASSET RIVERS

Gill biopsies for both gill Al levels and gill NKA activity were collected from 185 smolts migrating in the Connecticut and 101 smolts migrating in the Pemigewasset

Table I. Summary of number of *Salmo salar* smolts sampled and river discharge during smolt migratory period (18 April to 26 May) from 2005 to 2009

Catchment	2005	2006	2007	2008	2009
Number of smolts sampled (gill Na^+/K^+-ATPase/Al)					
Connecticut River	–	18/18	57/56	20/20	90/59
Pemigewasset River	–	15/15	26/24	16/16	44/44
Sheepscot River	59/11	109/40	137/49	–	–
Narraguagus River	60/9	71/40	45/27	–	–
Penobscot River	52/10	–	–	–	–
Pleasant River	–	34/31	46/46	–	–
Mean river discharge during smolt migration ($m^3 s^{-1}$)					
Connecticut River		516	898	941	591
Pemigewasset River		123	206	243	131
Sheepscot River	32	6	14		
Narraguagus River	52	15	22		
Penobscot River	1486	457	1219		
Piscataquis River	344	71	272		

River from 2006 to 2009 (Table I). The number of samples collected each year ranged from 15 to 90 due to the stochasticity of the migratory movements of the smolts. There was a highly significant difference in the levels of gill Al between rivers (two-way ANOVA, $F_{1,242} = 244{\cdot}24$, $P < 0{\cdot}001$) and years (two-way ANOVA, $F_{3,242} = 11{\cdot}26$, $P < 0{\cdot}001$), but no interaction effect was found between those factors (two-way ANOVA, $F_{3,242} = 0{\cdot}46$, $P > 0{\cdot}05$). In all years, smolts migrating in the Connecticut River had low levels of gill Al; in contrast, gill Al was always significantly higher in smolts migrating in the Pemigewasset River during the same year [Fig. 2(a)].

The difference in levels of gill NKA was also highly significant between rivers (two-way ANOVA, $F_{3,278} = 55{\cdot}06$, $P < 0{\cdot}001$) and years (two-way ANOVA, $F_{3,278} = 72{\cdot}10$, $P < 0{\cdot}001$), with no interaction effect between those factors (two-way ANOVA, $F_{3,278} = 0{\cdot}43$, $P > 0{\cdot}05$). Significantly higher levels of gill NKA activity were observed in smolts migrating in the Connecticut River compared with the Pemigewasset River in all years except in 2008 [Fig. 2(b)]. There was a significant negative correlation in individual measurements of gill Al and NKA activity in the Pemigewasset River ($r^2 = 0{\cdot}23$, $t_2 = -5{\cdot}4026$, $P < 0{\cdot}01$) but not in the Connecticut River ($r^2 < 0{\cdot}01$, $t_2 = -0{\cdot}3606$, $P > 0{\cdot}05$).

Previous research has indicated that decreases in pH and increases in inorganic aluminium are related to rainfall events and increased flow. Absolute river flow differed between the two rivers, but yearly variations had the same trend in each river (Table I). Mean daily discharge at the gauging station nearest to each smolt collection site was approximately four-fold higher in the Connecticut River than in the Pemigewasset River in all years. The rank order of discharge between years was the same for both rivers, with 2008 being the year of highest flow and 2006 the lowest flow (Table I). There was no significant correlation between mean river flow during the smolt migration period and mean gill Al in the Connecticut River ($r^2 = 0{\cdot}03$, $t_2 = 0{\cdot}2456$, $P > 0{\cdot}05$) or the Pemigewasset River ($r^2 = 0{\cdot}07$, $t_2 = 0{\cdot}3755$, $P > 0{\cdot}05$).

Water samples taken from mainstem sites adjacent to the smolt collection sites indicate marked differences in water chemistry. In all years, pH in the Pemigewasset River

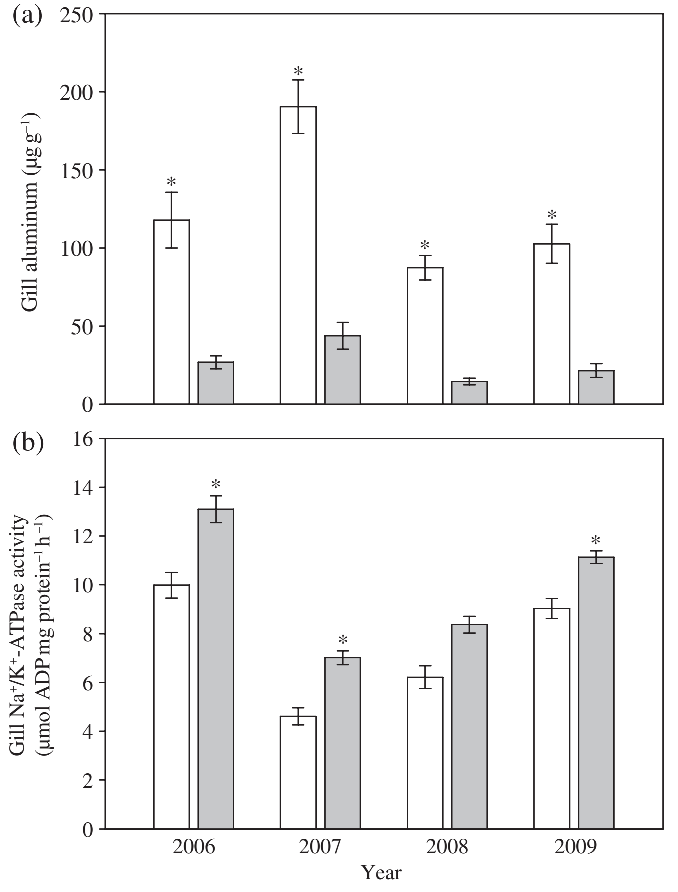

FIG. 2. (a) Gill aluminium (river and year $P < 0{\cdot}001$, river × year $P > 0{\cdot}05$) and (b) gill Na^+/K^+-ATPase activity (river and year $P < 0{\cdot}001$, river × year $P > 0{\cdot}05$) in *Salmo salar* smolts in the Connecticut (▩) and Pemigewasset (□) Rivers. Values are mean ± S.E. (sample sizes are listed in Table I). *, Significant difference between the two rivers ($P \leq 0{\cdot}05$, two-way ANOVA, Tukey's HSD).

was low (pH = 5·20–6·22) and both total and inorganic aluminium were high, up to 190·2 and 65·5 µg Al l^{-1}, respectively (Table II). In multiple samples taken from the Connecticut River in 2008 and 2009, pH was always near neutral and total and inorganic aluminium levels were low, <50·2 and <23·1 µg Al l^{-1}, respectively (Table II).

MAINE RIVERS

Gill biopsies were collected from smolts migrating in the Sheepscot (NKA: $n = 305$, gill Al: $n = 100$) and Narraguagus (NKA: $n = 176$, gill Al: $n = 76$) Rivers from 2005 to 2007, the Penobscot River (NKA: $n = 52$, gill Al: $n = 10$) in 2005 and the Pleasant River (NKA: $n = 80$, gill Al: $n = 75$) in 2006 and 2007 (Table I). The number of samples collected each year ranged from 34 to 137 for NKA and nine to 49 for gill Al due to the stochasticity of the migratory movements of smolts. There was no significant difference in the levels of gill Al between rivers (two-way ANOVA, $F_{2,258} = 0{\cdot}23$, $P > 0{\cdot}05$), but

TABLE II. Water quality variables recorded near *Salmo salar* smolt collection sites in the Pemigewasset River (Merrimack catchment) and Connecticut River. Ayers Island Reservoir measurements were taken where the Pemigewasset River enters the reservoir, 5·5 river km from Ayers Island Dam, Rt 116 Bridge is located 15 river km downstream of Cabot Station Dam and Rt 10 Bridge is located 21 river km downstream of Cabot Station Dam

Sampling location	Date	pH	Al_t	Al_i
PEMIGEWASSET RIVER				
2006				
Ayers Island Reservoir[a]	10 May	5·72	–	–
Ayers Island Reservoir[a]	24 May	5·31	–	–
2007				
Ayers Island Reservoir[a]	25 April	5·20	–	–
Ayers Island Dam	25 April	6·00	190·2	65·5
Ayers Island Reservoir[a]	7 May	5·49	–	–
2008				
Ayers Island Dam	17 April	5·51	116·4	58·0
Ayers Island Reservoir[a]	30 April	5·25	–	–
Ayers Island Reservoir[a]	14 May	5·60	–	–
Ayers Island Reservoir[a]	28 May	5·81	–	–
2009				
Ayers Island Reservoir[a]	22 Apr	5·48	–	–
Ayers Island Reservoir[a]	12 May	5·47	–	–
Ayers Island Reservoir[a]	27 May	6·22	–	–
CONNECTICUT RIVER				
2008				
Cabot Station Dam	6 May	7·00	–	–
Rt 116 Bridge, Deerfield, MA[b]	6 May	7·00	–	–
Rt 10 Bridge, Northfield, MA[b]	30 May	7·40	–	–
2009				
Cabot Station Dam	1 May	7·14	45·3	19·4
Cabot Station Dam	6 May	7·31	39·5	23·1
Cabot Station Dam	12 May	6·73	50·2	23·1

Al_t, total aluminium after filtration ($\mu g\ l^{-1}$); Al_i, inorganic aluminium ($\mu g\ l^{-1}$).
[a]USEPA (2014).
[b]MassDEP (2013).

there was a difference between years (two-way ANOVA, $F_{2,258} = 8{\cdot}31$, $P < 0{\cdot}001$) and a significant interaction effect between river and year (two-way ANOVA, $F_{4,258} = 5{\cdot}98$, $P < 0{\cdot}001$) [Fig. 3(a)]. Mean gill Al was higher, but not significantly ($P > 0{\cdot}05$), in smolts migrating in the Sheepscot and Narraguagus Rivers in 2005 than those from the Penobscot–Pleasant system. In 2006, mean gill Al was significantly higher in fish from the Penobscot–Pleasant system than from either the Sheepscot or Narraguagus Rivers, although the levels were low in all three rivers [Fig. 3(a)]. There was no significant difference in mean gill Al in 2007, with similarly low levels observed in all three rivers.

There were significant differences in gill NKA activity between rivers (two-way ANOVA, $F_{2,739} = 42{\cdot}43$, $P < 0{\cdot}001$) and years (two-way ANOVA, $F_{2,739} = 90{\cdot}96$, $P < 0{\cdot}001$), and a significant interaction between river and year (two-way ANOVA, $F_{4,739} = 26{\cdot}81$, $P < 0{\cdot}001$) [Fig. 3(b)]. In 2005, smolts migrating in the Sheepscot River

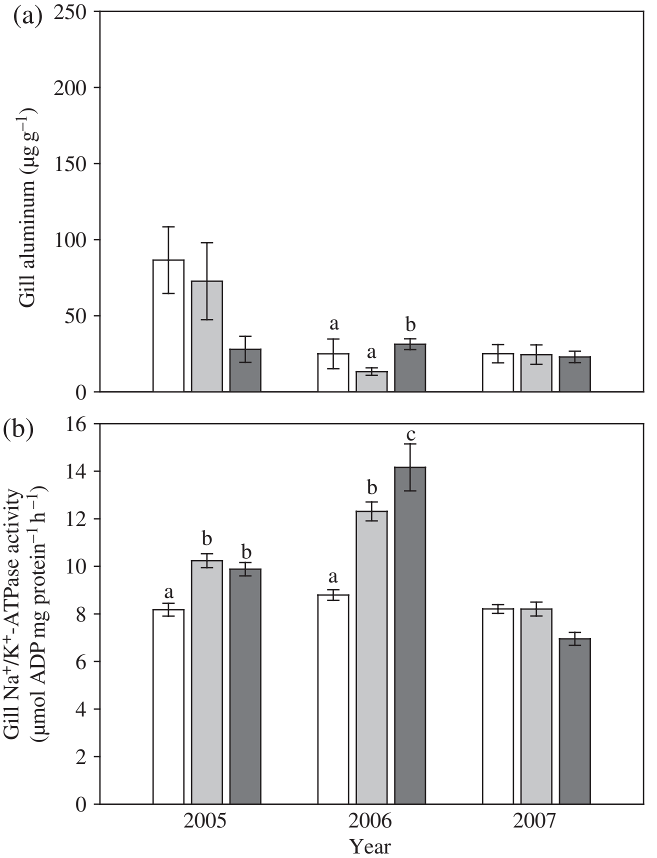

FIG. 3. (a) Gill aluminium (river $P > 0{\cdot}05$, year $P < 0{\cdot}001$, river × year $P < 0{\cdot}001$) and (b) gill Na^+/K^+-ATPase activity (river, year and river × year $P < 0{\cdot}001$) in *Salmo salar* smolts in Maine Rivers: Sheepscot (□), Narraguagus (▢) and Penobscot–Pleasant (■). Values are mean ± S.E. (sample sizes are listed in Table I. Lower-case letters indicates significant difference between rivers ($P \leq 0{\cdot}05$, two-way ANOVA, Tukey's HSD).

had significantly lower levels of NKA activity ($8{\cdot}2 \pm 0{\cdot}3$ μmol ADP mg^{-1} protein h^{-1}) than smolts in either the Narraguagus River ($10{\cdot}3 \pm 0{\cdot}3$ μmol ADP mg^{-1} protein h^{-1}) or the Penobscot–Pleasant system ($9{\cdot}9 \pm 0{\cdot}3$ μmol ADP mg^{-1} protein h^{-1}). In 2006, samples from all three rivers were significantly different from each other; however, fish migrating in the Penobscot–Pleasant River had the highest gill NKA activity levels ($14{\cdot}2 \pm 1{\cdot}0$ μmol ADP mg^{-1} protein h^{-1}) while the lowest levels were again found in the Sheepscot ($8{\cdot}8 \pm 0{\cdot}2$ μmol ADP mg^{-1} protein h^{-1}). No significant differences were found between rivers in 2007, with values ranging from a high of $8{\cdot}2 \pm 0{\cdot}2$ μmol ADP mg^{-1} protein h^{-1} in the Sheepscot and a low of $7{\cdot}0 \pm 0{\cdot}3$ μmol ADP mg^{-1} protein h^{-1} in the Penobscot–Pleasant.

Absolute river flow differed considerably in the three systems during the period of the study. Mean daily discharge at the gauging station nearest to each smolt collection site was always highest in the Penobscot–Pleasant system whereas the Sheepscot and Narraguagus were more closely matched (Table I). As in the Connecticut and

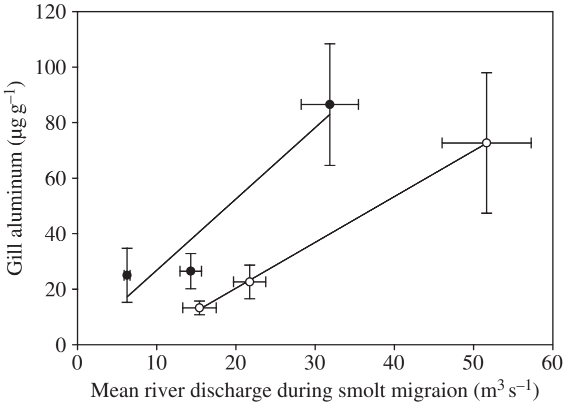

FIG. 4. Gill aluminium of *Salmo salar* smolts as a function of river flow during the smolt migratory period in Narraguagus (○; $y = 1·6503x - 12·657$, $r^2 = 0·9997$) and Sheepscot (●; $y = 2·5685x + 1·1318$, $r^2 = 0·9178$) Rivers. Values are mean ± S.E. (sample sizes are listed in Table I).

Pemigewasset Rivers, the rank order of discharge between years was the same for all three rivers. The highest flows were recorded in 2005 and the lowest in 2006. There was a strong correlation between river flow and gill Al in the Sheepscot ($r^2 = 0·92$, $t_1 = 3·3404$) and Narraguagus Rivers ($r^2 = 0·99$, $t_1 = 57·1366$) (Fig. 4); however, this relationship was only statistically significant in the Narraguagus River ($P < 0·01$) but not in the Sheepscot ($P > 0·05$). No significant relationship between flow and gill Al was noted in the Penobscot–Pleasant system ($r^2 = 0·39$, $t_1 = -0·8061$, $P > 0·05$).

Levels of pH from continuous data logging sondes (Fig. 5) and point samples taken adjacent to the rotary screw traps where smolts were collected (Table III) show considerable variation among rivers as well as interannual variation. In most years, pH at all three sampling locations was near neutral, with the exception of 2005 when low pH was recorded in the Narraguagus River. Total aluminium levels were high in all rivers at all times with the highest levels recorded (135·8 μg Al l^{-1}) in the Pleasant River in 2007.

DISCUSSION

In this study, the levels of gill aluminium and NKA activity of migrating *S. salar* smolts were examined in five river basins in the north-eastern U.S.A. over a 5 year span. The most distinct finding was that gill aluminium levels are consistently elevated in smolts emigrating from the Pemigewasset River, and that this was associated with lower levels of gill NKA activity. There was no evidence of elevated gill aluminium in smolts migrating in the Connecticut River. The elevated levels of gill Al observed in the Pemigewasset River were associated with reduced river pH and elevated Al_i relative to the Connecticut River. Prior studies have shown that elevated gill aluminium occurs primarily when pH levels are below 6·0, which alters the stoichiometry of any aluminium that is present to make it both more soluble and more toxic to fishes and other aquatic animals (Gensemer & Playle, 1999). Measurements of pH and aluminium were taken at the location of trapping in the mainstem of each river, but the fish were in

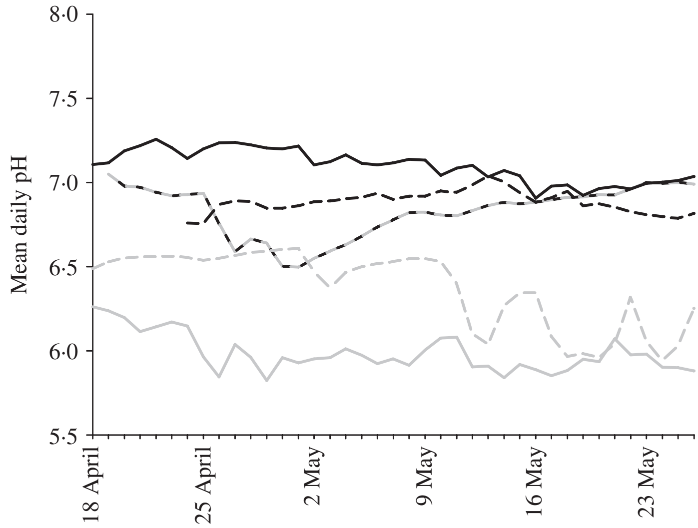

FIG. 5. Mean daily pH recorded by in-stream data sondes in rivers in Maine (, Penobscot 2005; , Narraguagus 2005; , Narraguagus 2006; , Sheepscot 2006; , Sheepscot 2007) during the smolt migration period (18 April to 26 May).

the midst of migration and had until recently been experiencing water chemistry from farther upstream. Thus, some level of caution must be exercised in associating water chemistry and gill Al in studies of actively migrating fish. Individual fish may have come from one of the many tributaries that differed in water chemistry, and smolts may have been migrating for different durations. An extensive survey of water chemistry in the region has been carried out (J. T. Kelly & S. D. McCormick, unpubl. data) and elevated Al_i and low pH conditions were found to be widespread during the spring in many streams in the Pemigewasset catchment. By comparison, the Connecticut River catchment is relatively well buffered, with elevated Al_i and low pH conditions isolated to a few tributaries (MassDEP, 2013).

Recently, emission regulations have produced increases in precipitation pH region wide including the White Mountains region of New Hampshire (Likens & Buso, 2012), which encompasses most of the Pemigewasset drainage basin. Base cation concentrations remain at very low levels, slowing the recovery of stream pH and making the rate of recovery site-specific. *Salmo salar* smolts may, therefore, serve as an ecologically relevant indicator of long-term basin-scale recovery in the Pemigewasset and similar basins.

In contrast to the basin-level consistency observed across years in the Pemigewasset and Connecticut Rivers, gill Al levels in the three rivers in Maine varied considerably between years and rivers. In almost all cases, gill Al levels observed in Maine were similar to the moderate levels found in smolts from the Connecticut River. Gill NKA varied greatly between rivers in 2005 and 2006, but it is hard to ascribe those differences to the effects of low pH and inorganic aluminium alone. In 2005, the lowest levels of gill NKA activity were observed in the Sheepscot River, coincident with the highest mean levels of gill Al; however, in the same year, fish in the Narraguagus River had the highest levels of gill NKA observed despite experiencing similar levels of gill Al. Discharge

TABLE III. Water quality variables recorded near rotary screw trap locations used to collect *Salmo salar* smolts in Maine Rivers

	Date	pH	Al_t	DOC	ANC
Sheepscot River					
2005	10 May	6·77	65·4	6·6	135·3
	19 May	6·92	50·8	6·3	167·8
	24 May	6·86	79·5	7·6	176·6
2007	26 April	7·25	70·6	–	–
Narraguagus River					
2005	3 May	6·10	131·3	9·3	51·1
2006	27 April	6·48	71·0	5·8	124·1
	7 May	6·63	66·7	6·5	124·1
2007	23 April	6·42	93·6	–	–
Penobscot–Pleasant System					
Penobscot River					
2005	5 May	6·74	85·2	8·7	149·2
Piscataquis River					
2007	25 April	6·88	135·8	–	–

Al_t, total aluminium after filtration; DOC, dissolved organic carbon (mg l^{-1}); ANC, acid neutralizing capacity (µeq l^{-1}).

from both rivers was very high during the smolt migration that year, and the Narraguagus in mid-March had low pH and had elevated levels of total Al, indicating an episodic acidification event occurred in that period. By May, pH in both rivers had returned to near neutral, although total Al remained high in the Narraguagus. The following year, there was again substantial variation in gill NKA between the three rivers, despite low levels of gill Al in all sampled fish. River discharge was low during this time, and there is no evidence of acidification at either location. This interannual variability in pH and environmental aluminium in rivers in Maine was also noted in a previous study of acid and aluminium exposure of hatchery-reared smolts exposed to natural river water from four catchments in Maine including the Narraguagus River (Liebich *et al.*, 2011). In that study, low pH conditions in 2005 were associated with high discharge and negative physiological effects on smolts (reduced plasma ions and elevated glucose), but in years without high discharge, low pH and physiological effects were absent. There is significant correspondence in these studies in that in 2005 smolts were observed to have elevated gill aluminium (this study) and a significant stress in response to river water exposure, both probably driven by reduced pH. It is apparent that the effect of acid and Al in Maine rivers is highly episodic and appears to be correlated to relative discharge. Effects on smolts in these rivers may only occur when acidified rain or snow melt events correspond with the smolt migration. This contrasts with the results for the Pemigewasset River in which elevated gill aluminium was found every year, suggesting that for the Pemigewasset River, basin scale effects occur every year.

The *in situ* sampling of gill tissue from free swimming fish affords the opportunity to sample large numbers of animals over a broad geographic range and thus allowed the effects of acidification at the basin and catchment scale to be examined. There is an inherent trade-off in this approach, in that it does not allow for a direct connection of organismal effects to water chemistry as the water-chemistry history of each fish is not known. As noted in the observations from the three rivers in Maine, this variability may in fact make it difficult to detect effects, such that only the most affected systems will be detected.

In this study, gill aluminium concentration was measured from acid digestion of a non-lethal gill biopsy as described above. It is likely that this measurement includes both Al accumulated on the surface of the gills and Al that is within the gill epithelium. Previous studies with *S. salar* smolts have demonstrated that short-term exposure to low to moderate levels of environmental Al results in impaired seawater tolerance and it was hypothesized that this was the result of a shift in the phenotype of chloride or 'salt secretory' cells in the gill epithelium (Monette *et al.*, 2009). In the same study, smolts exposed to moderate to high levels of environmental Al were unable to osmoregulate in both fresh and seawater due to extensive damage to the gill epithelium and loss of gill-ion transport machinery (Monette *et al.*, 2009).

Previous work has indicated that smolts are more sensitive to low acid and elevated inorganic aluminium than other life stages of *S. salar*, probably due to the changes in osmoregulatory physiology that occur during smolt development (Rosseland *et al.*, 2001). Previous work has shown that exposure of parr and smolts to the same levels of low pH and elevated Al_i results in higher levels of gill aluminium in parr but greater ion perturbations in smolts, suggesting a greater sensitivity rather than greater Al uptake (Monette & McCormick, 2008). As the smolt transition is preparatory to migration into seawater, even non-lethal effects to osmoregulatory capacity experienced while in fresh water can have significant consequences to long-term survival in the marine environment (McCormick *et al.*, 2009*b*). Even exposure to relatively moderate, sublethal levels of acid and aluminium for as short as 2 days can result in increased gill Al levels and loss of salinity tolerance (Monette *et al.*, 2008). Studies of long-term (10–40 day) exposure of smolts to moderate acid and Al conditions (pH = 5·3–5·6, <15 μg Al_i l^{-1}) results in detectable increases in gill Al, loss of gill NKA activity and salinity tolerance, and most importantly reductions in adult return rates (Kroglund *et al.*, 2007).

The elevated levels of gill Al found in smolts from the Pemigewasset River were associated with decreased levels of gill NKA activity. Although the overall level of gill NKA activity varied between years, levels were always lower in fish from the Pemigewasset River relative to Connecticut River fish from the same year. A number of previous studies have shown a strong causal connection between acid and aluminium exposure, elevated gill Al levels and reduced gill NKA activity in *S. salar* smolts (McCormick *et al.*, 2009*a*; Nilsen *et al.*, 2010). It should be noted that there are other factors that affect gill NKA activity during downstream migration of smolts. Although photoperiod is the primary determinant of the timing of smolt development and thus gill NKA activity, temperature can have a secondary influence on the rate of increase (and reversion) of gill NKA activity (Björnsson, 1997). Gill NKA activity may also be influenced by the act of migration itself, including the length of time that fish have been migrating (McCormick *et al.*, 2009*b*). Nonetheless, the distance from sampling location to the ocean is similar for the Pemigewasset and Connecticut Rivers, suggesting that the lower levels of gill NKA in smolts from the Pemigewasset River may result in an impaired capacity to osmoregulate as they enter seawater which will have negative consequences for seawater survival and adult return rates (Kroglund *et al.*, 2007).

The results presented here are indicative of differences in water chemistry that drive elevated gill aluminium and then cause physiological effects on gill NKA activity. It is unlikely that fish can reduce the amount of aluminium they accumulate on their gills, but it is possible that there may be differences in their capacity to resist or respond to this perturbation. Previous results have shown that there is heritability of resistance to acidification effects in salmonids including *S. salar* (Gjedrem & Rosseland, 2012). This might be a contributing factor for the poor relationship between gill aluminium and gill NKA, especially in the rivers in Maine where native stocks still persist (King *et al.,* 2000).

While there is substantial information on the effects of acid and aluminium on ion homeostasis in fresh water and the loss of salinity tolerance, there is less known about how these physiological changes recover following seawater exposure. Gill Al may disappear relatively quickly after exposure to high quality fresh or seawater, but physiological impairment appears to persist long after exposure. After long=term (>3 month) exposure to pH 5·9–6·1 and 27 μg Al_i l^{-1}, salinity tolerance did not recover after 9 days when fish were held in water of pH 6·1 or less, but did recover in that period if pH was 6·3 (Kroglund *et al.*, 2001*b*). In recent trials involving short-term exposure to moderate conditions (pH = 5·2, <50 μg Al_i l^{-1}) there was only partial recovery of salinity tolerance after 6 days in highly buffered water of pH 6·5 (J. Kelly, S. McCormick, M. Monette, unpubl. data). The movement from acidified waters to the ocean may take only a few days for the majority of migrating *S. salar* smolts, especially in eastern Maine rivers where rivers are relatively short (Magee *et al.*, 2001). The demonstrated persistence of decreased salinity tolerance for many days after acid and aluminium exposure indicates that recovery is unlikely to take place and that even minor decreases in salinity tolerance will have negative effects on population sustainability.

The results of this study indicate that most of the smolts leaving the upper Merrimack basin have elevated gill aluminium levels and only moderate increases in gill NKA activity. As noted above, both laboratory and field studies indicate that the presence of elevated gill aluminium is indicative of exposure to low pH and elevated inorganic aluminium, which has been shown to negatively affect gill NKA activity, salinity tolerance and adult return rates (Kroglund *et al.*, 2007). *Salmo salar* in the Merrimack River were extirpated in the 1800s due to dam construction, and similar to the Connecticut River, have been the subject of an active restoration programme for the last 40 years. This restoration has two major strategies: (1) the release of fry into the upper catchment where they rear for two or more years before migrating downstream as smolts and (2) fish reared in the hatchery for 1 year and then released as smolts. The latter are released in the lower part of the Merrimack River that is more highly buffered and less susceptible to the effects of acidification. Fry-stocking has mostly occurred in the upper catchment, and return rates from these efforts are relatively low (0·0015% adult return rate from 2004 to 2010). The results presented here suggest that a major contributing factor to these poor returns is exposure to acid and aluminium conditions in the upper Merrimack that result in accumulation of aluminium in the gill and a subsequent loss of osmoregulatory performance during downstream migration and seawater entry with consequences to long-term fitness.

We dedicate this paper in memory of our co-author, colleague and dear friend Michael F. O'Dea who passed away on 2 August, 2015; he is greatly missed. We thank J. McKeon (USFWS) and C. Mooney and colleagues of Public Service of New Hampshire for their help in conducting smolt collections at the Pemigewasset River. We thank B. Stira (First Light Power Resources) and B. Adams and students of Greenfield Community College for their help in conducting smolt collections at the Connecticut River. We would like to thank C. Lipsky, P. Ruksznis as well as all other NOAA and Maine Department of Marine Resources staff who assisted in smolt and water-quality sample collection. Thanks also to J. Magee for helpful comments on an early draft of this manuscript. This study was funded in part by a grant from the Northeast States Research Cooperative. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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