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Water chemistry and its effects on the physiology and survival of Atlantic salmon *Salmo salar* smolts

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The physiological effects of episodic pH fluctuations on Atlantic salmon *Salmo salar* smolts in eastern Maine, U.S.A., were investigated. During this study, *S. salar* smolts were exposed to ambient stream-water chemistry conditions at nine sites in four catchments for 3 and 6 day intervals during the spring *S. salar* smolt migration period. Plasma chloride, plasma glucose, gill aluminium and gill Na^+- and K^+-ATPase levels in *S. salar* smolts were assessed in relation to ambient stream-water chemistry during this migration period. Changes in both plasma chloride and plasma glucose levels of *S. salar* smolts were strongly correlated with stream pH, and *S. salar* smolt mortality occurred in one study site with ambient stream pH between 5·6 and 5·8 during the study period. The findings from this study suggest that physiological effects on *S. salar* smolts are strongly correlated with stream pH and that in rivers and streams with low dissolved organic carbon (DOC) concentrations the threshold for physiological effects and mortality probably occurs at a higher pH and shorter exposure period than in rivers with higher DOC. Additionally, whenever an acidification event in which pH drops below 5·9 coincides with *S. salar* smolt migration in eastern Maine rivers, there is potential for a significant reduction in plasma ions of *S. salar* smolts.

Key words: aluminium; ATPase; chloride; DOC; glucose; pH.

INTRODUCTION

During the past century, Atlantic salmon *Salmo salar* L. 1758 populations have experienced decline on a global scale. In the U.S.A., *S. salar* have been extirpated from much of their historic range (Parrish *et al*., 1998) and the few remnant wild populations have declined in abundance to the extent that they have been federally listed as endangered and major restoration efforts are currently underway (Baum,

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1997; Fay *et al*., 2006). Over the past two centuries, *S. salar* populations in eastern Maine were reduced to a fraction of historic abundances, but remained relatively constant throughout the early to mid-1900s. In the 1990s, these populations experienced additional substantial declines, and have yet to recover. Acidification has since been identified as a possible contributory factor to *S. salar* declines in eastern Maine and in other areas of the north-eastern U.S.A. (Clegg *et al*., 2004). Acidic conditions are common in aquatic ecosystems throughout the north-eastern U.S.A. and frequently occur as episodic acidification events that reduce stream pH for days to weeks (Haines *et al*., 1990; Kahl *et al*., 1992; Johnson & Kahl, 2005).

Decreases in stream pH, as a result of acidification, can harm aquatic organisms (Haines, 1981; Schindler, 1988) and all life stages of *S. salar* are known to be negatively affected by acidification (Haines, 1981; Staurnes *et al*., 1993; Kroglund & Staurnes, 1999). In addition to the effects of reduced pH, the negative effects to aquatic biota owing to acidic deposition are often compounded by the enhanced mobilization of aluminium (Haines, 1981). Between pH 6·0 and 8·0 aluminium is relatively insoluble and generally not toxic to aquatic species. Below pH 6·0, aluminium exists in a number of charged forms (*e.g*. Al^{3+}) that increase in toxicity to aquatic organisms as pH decreases (Haines, 1981; Leivestad *et al*., 1987; Gensemer & Playle, 1999). These charged forms of aluminium may exist in bound (organic) or unbound (inorganic) forms, with the latter generally thought to be the more toxic form in aquatic ecosystems.

The effects of low pH and inorganic aluminium on *S. salar* have been studied in detail in northern Europe where acidification results in chronic (year-round) low pH (Nyberg *et al*., 1995; Staurnes *et al*., 1995, 1996; Kroglund & Staurnes, 1999; Kroglund *et al*., 2001; Ytrestoyl *et al*., 2001; Bjerknes *et al*., 2003) which has resulted in extirpation of *S. salar* in >25 Norwegian rivers (Hesthagen & Hansen, 1991). Research has also found that *S. salar* smolts are more sensitive to acidic conditions than other life stages, and this sensitivity appears to increase as the parr-smolt transformation progresses (Staurnes *et al*., 1993, 1996; Monette & McCormick, 2008).

A pH below 6·0 has been shown to impair osmoregulatory abilities and seawater tolerance of *S. salar* smolts (Staurnes *et al*., 1993, 1996; Kroglund & Staurnes, 1999). When low pH (*i.e*. <6·0) is coupled with inorganic exchangeable aluminium (Al^{3+}), the resulting interaction is a synergistic effect that produces even greater physiological effects in *S. salar* than that of pH alone (Skogheim *et al*., 1986). Declines in growth and feeding behaviour (Magee *et al*., 2003), physical damage to the gill tissue (Hamilton & Haines, 1995; Jagoe & Haines, 1997), impaired physiological functions (Staurnes *et al*., 1996; Monette & McCormick, 2008) and direct mortality (Hamilton & Haines, 1995; Staurnes *et al*., 1996; Monette & McCormick, 2008) have all been associated with the synergistic effects of low pH and Al^{3+} exposure.

In eastern Maine, experimental modifications of naturally occurring pH and aluminium concentrations in rivers have demonstrated negative physiological effects such as decreased plasma chloride in *S. salar* smolts in fresh water (Haines *et al*., 1990; Magee *et al*., 2001). Magee *et al*. (2003) found that wild *S. salar* smolts exposed to artificially acidified, aluminium-enriched, natural river water in eastern Maine's Pleasant River experienced decreased osmoregulatory capabilities in both fresh and sea water, and up to 35% mortality upon transfer to sea water. Although experimental modifications of ambient pH and aluminium in eastern Maine

rivers have occurred, little is known about the extent of effects from natural water chemistries on *S. salar* physiology in North American rivers and streams.

In order to evaluate the magnitude of effects from natural variations in stream pH on *S. salar* smolts, a streamside rearing study was conducted in several rivers in eastern Maine, from 2004 to 2006. In this study, only natural water chemistry was used to examine regional trends in acid-related water chemistry and the associated physiological effects in smolting *S. salar* in eastern Maine. The goal of the study was to further the scientific understanding of the effects of natural water chemistry on *S. salar* populations and to determine if there are detectable thresholds for the effect of pH on *S. salar* smolts in eastern Maine.

MATERIALS AND METHODS

Nine study sites in four catchments (Fig. 1) were selected. These sites represented a broad range of stream pH and water chemistry conditions typical of *S. salar* rivers in the U.S.A., and in particular, eastern Maine. Catchments with study sites included the Dennys River (two sites), Cathance Stream (one site), Pleasant River (two sites), Narraguagus River (one site), West Branch Narraguagus (one site), East Bear Brook (one site) and Kenduskeag Stream (one site).

At each study site, two gravity-fed 245 l circular fibreglass aquaculture tanks similar to those used in the satellite rearing studies conducted by the Atlantic Salmon Federation (Dupuis

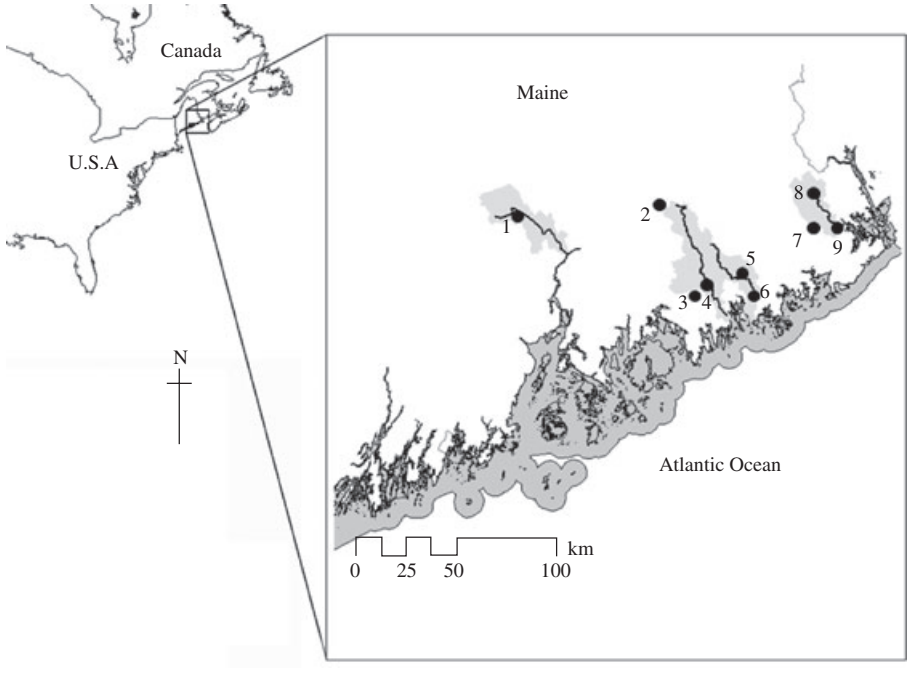

FIG. 1. Locations of catchments (□) and specific sites (●) used during the streamside study in eastern Maine, U.S.A., from 2004 to 2006. ___, selected historic *Salmo salar* rivers; 1, Kenduskeag; 2, East Bear Brook; 3, Sprague Falls; 4, Little Falls; 5, Saco Falls; 6, Columbia Falls; 7, Cathance; 8, Meddybemps; 9, Dennysville.

& Dominy, 1994) were set up at the streamside. The tank inflow was *via* a 3·2 cm diameter polyethylene pipe with a nylon screened intake with an average flow rate of 5 l min^{-1}. Effluent was discharged through a screened overflow pipe into a naturally occurring basin within the flood plain in an effort to minimize any possibility of escapes.

During this study, there were two trials per year within a 21 day period between 22 April and 12 May in 2004, 2005 and 2006. This period corresponds with the normal spring *S. salar* smolt migrations in eastern Maine rivers (Kocik *et al*. 2009). *Salmo salar* smolts used in this study were of Penobscot River origin and obtained from the Green Lake National Fish Hatchery in Ellsworth, ME, U.S.A. One week in advance of each study period, *S. salar* smolts were isolated in a pool at the hatchery. At the start of each study period, 12 *S. salar* smolts were randomly removed from the hatchery pool and anaesthetized in 100 mg l^{-1} tricaine methanesulphonate (MS-222) buffered with 100 mg l^{-1} $NaHCO_3$ and mixed in hatchery pool water. Once anaesthetized, 0·7 ml blood was withdrawn from the caudal vasculature into heparinized syringes in order to measure plasma chloride and plasma glucose. Blood samples were placed immediately on wet ice. Within 10 min of collection, blood samples were centrifuged at 8000 *g* for 5 min and plasma was removed and placed in pre-labelled 0·5 ml microcentrifuge tubes and placed immediately in dry ice. On each *S. salar* smolt, four to six gill filaments were collected from the first gill arch, just above the septum, on the left side of the fish for the measurement of Na^+- and K^+-ATPase activity. Samples were placed immediately into 100 µl of ice cold sucrose–EDTA–imidazole (SEI) buffer (250 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7·3) in a pre-labelled 0·5 ml microcentrifuge tube and transferred directly to dry ice. A second gill biopsy of four to six gill filaments was collected for the measurement of gill aluminium. This gill biopsy was removed, placed into an acid-washed 1·5 ml microcentrifuge tube and transferred directly to dry ice. Upon returning to the laboratory, all physiological samples were stored at −80° C until analysis. All *S. salar* smolts were lethally sampled and removed from the study population.

Salmo salar smolts for use in the field studies were transported in 113 l coolers fitted with a recirculation aeration system and filled with water from their pool of origin at Green Lake National Fish Hatchery. Once at the study site, *S. salar* smolts were then transferred directly from the transport coolers to the streamside rearing tanks. Exposure periods were considered to begin upon transfer to the streamside rearing tanks, which were already flowing with ambient stream water. Each streamside rearing tank housed 12 *S. salar* smolts. These *S. salar* smolts were continuously exposed to ambient water conditions and unfed while housed in the rearing tanks. After 3 days' exposure, six of the 12 *S. salar* smolts within each tank were randomly removed for sampling following the same protocol as used during the hatchery sampling. The remaining six *S. salar* smolts in each streamside rearing tank were sampled on day 6 using the same protocols. Due to high mortality rates at the East Bear Brook study site, additional rearing tanks were added at this location and *S. salar* smolts housed in the rearing tanks at this site were sampled at 24, 48, 72 and 96 h intervals after initial exposure to ambient water conditions began. With the exception of more frequent time intervals, sampling at East Bear Brook was conducted following the same protocols as all other sites. Any smolt mortalities occurring within the streamside rearing tanks during the treatment periods were documented and deducted from that sampling period.

Water chemistry samples were collected at the hatchery and each study site during each sampling period and analysed to determine pH, acid neutralizing capacity, calcium, sodium, magnesium, potassium, dissolved organic carbon (DOC) and total aluminium.

PHYSIOLOGICAL MEASUREMENTS

The Na^+- and K^+-ATPase activity was assessed following microplate assay procedures described by McCormick (1993). Gill biopsies were thawed immediately prior to assay, and 25 µl of SEID (0·05 g sodium deoxycholate ml^{-1} SEI) added to the microcentrifuge tube with tissue and homogenized for 10–15 s using a Kontes pellet pestle motor. The homogenate was then centrifuged at 3200 *g* for 30 s, and the supernatant assayed both for Na^+- and K^+-ATPase activity and total protein (BCA protein assay; www.piercenet.com). This kinetic assay was run at 25° C for 10 min in a temperature-controlled plate reader (www.moleculardevices.com) and read at a wavelength of 340 nm. Gill Na^+- and K^+-ATPase activity was calculated as

the difference in the production of adenosine diphosphate (ADP) in the absence and presence of 0·5 mM ouabain and expressed as μmoles ADP mg protein^{-1} h^{-1}.

Gill aluminium content was assessed following the method outlined in Teien *et al*. (2006) as modified by Monette & McCormick (2008). Gill biopsies were thawed, dried at 60° C for 24 h and weighed to the nearest 0·0001 mg using a Series 30 microbalance (Cahn Instruments, Cerritos, CA, U.S.A.). Gill biopsies were then digested by adding 98 μl of 100% trace metal grade HNO_3 and 2 μl of H_2O_2 to biopsy tubes and heating 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 ultrapure water, and aluminium concentration was analysed by graphite furnace atomic absorption spectrophotometer (GFAAS) as described by Monette & McCormick (2008). A background correction was made for gill biopsy samples by subtracting the aluminium present in digestion blanks. Digestion blanks were prepared in the same way as gill biopsy tubes including transport and opening in the field, but no tissue was added. Gill aluminium was expressed as μg Al g^{-1} gill dry mass.

Plasma chloride was measured using a digital Buchler-Cotlove Chloridometer (Model 442-5000; www.labconco.com). Plasma glucose was measured by enzymatic coupling with hexokinase and glucose-6-phosphate dehydrogenase (Stein, 1963).

WATER CHEMISTRY

Water chemistry samples were collected from tanks concurrently with each physiological sampling event. Samples were collected in high-density polyethylene bottles prepared according to Method 1669 (USEPA, 1996) and stored on wet ice in the dark prior to analysis. After aluminium speciation, the samples were acidified with 50% trace metal grade HNO_3 to a 2% concentration. Closed cell analysis of pH was performed according to methods outlined by USEPA 600/4-87-026 (1987) and Hillman *et al*. (1986) using a Thermo Scientific Orion 720Aplus ISE meter and a Ross 8104BN pH probe which was calibrated with U.S. National Institute of Standards and Technology (NIST) traceable low ionic strength Thermo Scientific Orion Pure Water pH buffers in pH 4·10 and 6·97 (www.thermoscientific.com). Acid neutralizing capacity (ANC) was measured with an ABU 90 Radiometer titrator system (USEPA 600/4-87-026, 1987; Hillman *et al*., 1986). A two-channel ion chromatograph was used to determine calcium (Ca), sodium (Na), magnesium (Mg) and potassium (K) content (ASTM, 2003). An OI analytical 1010 carbon analyser was used to determine DOC content by heated persulphate oxidation (USEPA 600/4-79-020, 1983) and a Perkin Elmer 600 GFAAS (www.perkinelmer.com) was used for aluminium measurements (USEPA 600/R-93-100, 1993). All water chemistry samples were processed at the Senator George J. Mitchell Center for Environmental and Watershed Research, University of Maine, Orono, ME, U.S.A.

STATISTICS

In order to determine if the different stream chemistries affected the physiology of *S. salar* smolts, a three-way ANOVA of trial (two trials in each of 3 years), day of sampling (3 or 6) and location (site) were conducted. If location was significant, the data were further analysed for a relationship between pH and the physiological response of *S. salar* smolts using a non-linear mixed model (SAS procedure NLMIXED; www.sas.com) to identify break points in correlations of *S. salar* smolt physiology and water chemistry. The NLMIXED procedure was chosen because it included an estimated parameter for the break point, or changepoint, of the segmented fit to the data that cannot be written as a general linear model and because the mixed models capabilities allowed for the inclusion of random sampling location effects in the model. NLMIXED analyses in SAS use a dual quasi-Newton optimization technique with an adaptive Gaussian quadrature integration method. Breakpoint estimates identified through the NLMIXED procedure are assumed to represent thresholds at which changes in physiological response to ambient conditions were most significant and therefore indicate the point at which ambient conditions may have the potential for negative physiological effects. Data from East Bear Brook were excluded from the non-linear mixed models due to the extreme difference in both water chemistry and physiological response of *S. salar* smolts

exposed to this system. Separate analyses were conducted to assess the study site at East Bear Brook relative to other study locations in eastern Maine.

Comparisons of *S. salar* smolt physiology at the East Bear Brook site were conducted using a one-way ANOVA followed by least significant difference (LSD) multiple range tests (Statgraphics Centurion v15; www.statgraphics.com). These methods allowed for the identification of significant difference in physiological response among exposure times as well as the capability to formulate relative comparisons within and among study sites. Pearson's product-moment (linear) correlation (r) was used to examine the relationship between individual water chemistry variables and physiological responses.

RESULTS

STREAM CHEMISTRY AND PHYSIOLOGICAL RESPONSE

Study site pH ranged from 5·0 to 7·5 (Fig. 2), total water aluminium content from 11 to 222 μg l^{-1} and DOC content from 4·3 to 15·5 mg l^{-1}. In 2004 and 2006, pH typically remained >6·0, but in 2005, coincident with increased rainfall, pH dropped to <6·0 at several sites. The mean water chemistry variables from each site are shown in Table I. There was a significant correlation of most water chemistry variables with one another (Table II). There were no mortalities in any of the streamside tanks, except for East Bear Brook which was analysed separately because of its distinct water chemistry. Fish in two locations with low pH in 2005, however, were unresponsive to external stimuli after 6 days of exposure.

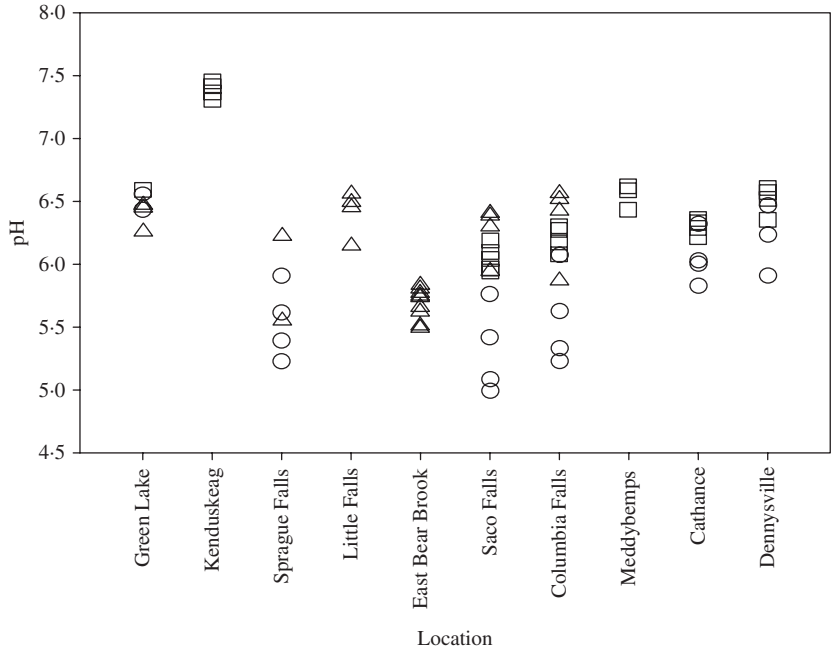

FIG. 2. Stream pH measurement collected at eastern Maine, study sites at which *Salmo salar* smolts were held in 2004 (□), 2005 (○) and 2006 (△).

TABLE I. Summary of water chemistry data collected at 10 locations in eastern Maine at which *Salmo salar* smolts were held between 2004 and 2006. Data are presented as mean ± S.E.

	ANC (μeq l^{-1})	pH	DOC (mg l^{-1})	Al-total (μg l^{-1})	Ca (μeq l^{-1})	Mg (μeq l^{-1})	Na (μeq l^{-1})	K (μeq l^{-1})	Number of samples
Green Lake	71·2 ± 1·0	6·5 ± 0·0	4·5 ± 0·1	50·5 ± 1·4	100·8 ± 4·7	41·0 ± 1·6	110·1 ± 2·6	10·1 ± 0·2	6·0
Kenduskeag	923·1 ± 56·1	7·4 ± >0·0	5·0 ± 0·6	17·9 ± 4·0	837·5 ± 53·1	210·5 ± 11·3	134·3 ± 5·4	19·5 ± 0·2	4·0
Sprague Falls	33·8 ± 9·0	5·7 ± 0·1	12·0 ± 0·8	167·2 ± 13·1	59·3 ± 6·3	31·8 ± 2·0	85·3 ± 5·6	12·4 ± 1·0	6·0
Little Falls	111·7 ± 16·7	6·4 ± 0·1	7·8 ± 1·2	96·3 ± 22·0	108·2 ± 8·0	44·8 ± 1·2	121·1 ± 10·2	13·9 ± 0·6	4·0
East Bear Brook	8·9 ± 1·2	5·7 ± >0·0	1·7 ± >0·0	119·0 ± 8·8	61·5 ± 1·8	24·1 ± 0·5	80·8 ± 1·8	5·9 ± 0·4	10·0
Saco Falls	50·8 ± 8·3	5·9 ± 0·1	11·2 ± 0·4	121·9 ± 8·4	68·1 ± 4·7	34·2 ± 2·1	111·4 ± 4·1	10·6 ± 0·6	12·0
Columbia Falls	63·1 ± 10·2	6·0 ± 0·1	11·4 ± 0·5	137·7 ± 9·8	79·3 ± 5·9	45·3 ± 3·4	118·8 ± 5·9	10·8 ± 0·7	12·0
Meddybemps	85·8 ± 0·9	6·6 ± >0·0	6·6 ± >0·0	40·3 ± 2·6	150·4 ± 2·0	55·1 ± 0·6	129·4 ± 2·1	6·8 ± 0·1	4·0
Cathance	55·8 ± 2·5	6·2 ± 0·1	6·0 ± 0·2	61·5 ± 3·7	98·7 ± 3·0	43·3 ± 1·5	99·4 ± 1·7	6·7 ± 0·3	8·0
Dennysville	145·6 ± 33·9	6·9 ± 0·3	8·5 ± 0·1	95·1 ± 3·9	194·9 ± 32·6	53·3 ± 3·6	130·9 ± 13·1	7·7 ± 0·4	10·0

ANC, acid neutralizing capacity; DOC, dissolved organic carbon.

TABLE II. Linear correlation matrix (r) of water chemistry variables and *Salmo salar* smolt plasma chloride, plasma glucose, Na^+- and K^+-ATPase and gill aluminium after 3 day exposures of *S. salar* smolts to natural water chemistry at eastern Maine, study sites between 2004 and 2006. Only significant ($P < 0{\cdot}05$) correlation values are shown. Correlation matrix does not include data from East Bear Brook study site

		1	2	3	4	5	6	7	8	9	10	11	12
1	Chloride	1·00											
2	Glucose	−0·92	1·00										
3	Na^+- and K^+-ATPase	0·63		1·00									
4	Gill Al				1·00								
5	PH	0·70	−0·70	0·46		1·00							
6	ANC				0·38	0·72	1·00						
7	DOC (mg l^{-1})	−0·43	0·39			−0·71	−0·56	1·00					
8	Al-total (μg l^{-1})					−0·65	−0·59	0·93	1·00				
9	Ca (μeq l^{-1})				0·40	0·71	0·99	−0·57	−0·61	1·00			
10	Mg (μeq l^{-1})				0·38	0·75	0·99	−0·56	−0·60	0·99	1·00		
11	Na (μeq l^{-1})	0·67	−0·64	0·48		0·82	0·46	−0·40	−0·42	0·44	0·50	1·00	
12	K (μeq l^{-1})					0·59	0·72			0·65	0·67	0·50	1·00

ANC, acid neutralizing capacity; DOC, dissolved organic carbon.

There was a significant effect of location (stream where fish were held) and trial (two trials during each of 3 years) on plasma chloride and glucose (three-way ANOVA, $P < 0{\cdot}001$), but no effect of day of sampling (3 *v*. 6). Gill Na^+- and K^+-ATPase activity varied substantially within and among study sites, and there was no significant effect of location, trial or sampling day. There was a significant effect of location on gill aluminium, but no effect of trial or day of sampling (three-way ANOVA, $P > 0{\cdot}05$).

There was a significant correlation between plasma chloride and plasma glucose with pH, DOC and sodium (Table II). Gill Na^+- and K^+-ATPase activity was positively correlated with pH and sodium. There was no significant correlation between gill aluminium and any of the water chemistry variables measured. Plasma chloride, glucose and gill Na^+- and K^+-ATPase activity were significantly correlated with one another, but not with gill aluminium.

There were significant correlations among most of the water chemistry variables, and it was therefore not appropriate to use stepwise regression or similar analyses to determine the relative importance of these variables on physiological responses. Because the strongest correlation of plasma chloride and plasma glucose was with pH, and that previous research has established a relationship between low pH and physiological impact on *S. salar* smolts, the relationship of pH and these variables was analysed further. Although there was a significant correlation of gill Na^+- and K^+-ATPase activity and pH, this relationship was not graphically or statistically analysed because the correlation was relatively weak and a significant effect of location on this variable had not been found.

Graphic analysis indicated a possible break or transition point in the effect of pH on both plasma chloride and plasma glucose. Coefficient estimates for the break points in physiological response at pH for *S. salar* smolts exposed to ambient conditions differed between plasma chloride and plasma glucose as well as 3 and 6 day exposure periods (Fig. 3). The breakpoint estimate using the NLMIXED model for plasma chloride after a 3 day exposure to ambient conditions was $136{\cdot}0 \pm 1{\cdot}4$ (mean $\pm$ S.E.; $P < 0{\cdot}001$) at pH $5{\cdot}9 \pm 0{\cdot}1$ (mean $\pm$ S.E.; $P < 0{\cdot}001$) with a slope estimate of 22·1 for values below this breakpoint estimate (Table III). The breakpoint estimate for plasma chloride after a 6 day exposure to ambient conditions was $136{\cdot}0 \pm 2{\cdot}5$ (mean $\pm$ S.E.; $P < 0{\cdot}001$) at pH $5{\cdot}7 \pm 0{\cdot}1$ (mean $\pm$ S.E.; $P < 0{\cdot}001$) with a slope estimate of 58·5 for values below this breakpoint estimate (Table III).

Plasma glucose had a breakpoint estimate of $8{\cdot}5 \pm 0{\cdot}7$ (mean $\pm$ S.E.; $P < 0{\cdot}001$) at pH $5{\cdot}5 \pm 0{\cdot}1$ (mean $\pm$ S.E.; $P < 0{\cdot}001$) and a slope estimate of $-18{\cdot}14$ for values below this breakpoint estimate after this same 3 day exposure (Table III). The breakpoint estimate for plasma glucose after a 6 day exposure to ambient conditions was $7{\cdot}3 \pm 0{\cdot}6$ (mean $\pm$ S.E.; $P < 0{\cdot}001$) at pH $5{\cdot}7 \pm 0{\cdot}1$ (mean $\pm$ S.E.; $P < 0{\cdot}001$) with a slope estimate of $-27{\cdot}3$ for values below this break point (Table III).

EAST BEAR BROOK

The pH in East Bear Brook ranged from 5·6 to 5·8, total water aluminium levels ranged from 81 to 173 $\mu g\ l^{-1}$ and DOC content ranged from 1·5 to 1·9 $mg\ l^{-1}$. Gill Na^+- and K^+-ATPase activity at East Bear Brook did not change over time compared to the control samples (d.f. = 3,87, $P > 0{\cdot}05$). A decline in mean values, however, was observed in fish exposed to East Bear Brook when compared with

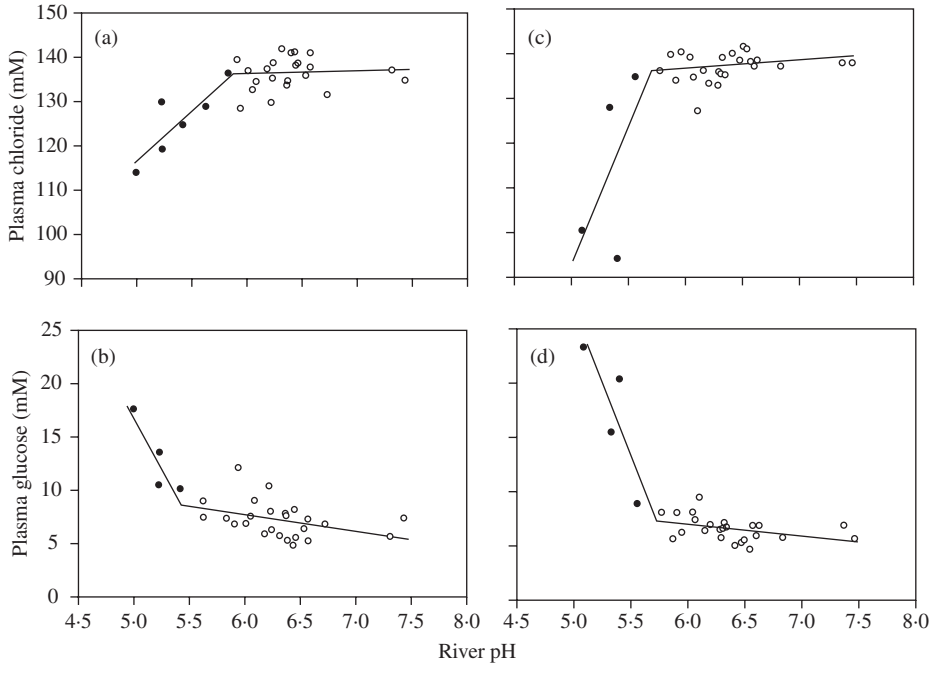

FIG. 3. Physiological response in (a), (c) plasma chloride and (b), (d) plasma glucose of *Salmo salar* smolts exposed to ambient water chemistry conditions at various study sites throughout eastern Maine, for (a), (b) physiological response of *S. salar* smolts after a 3 day and (c), (d) 6 day exposure to ambient stream pH. The estimated changepoint (○) or break point (●) in physiological response of *S. salar* smolts to stream pH are illustrated. Each point is the mean of 12 individuals sampled from two replicate tanks (see Table III).

the control site. Mean ± S.D. values for gill Na^+- and K^+-ATPase activity were 14·3 ± 3·4 at initial sampling site ($n = 35$), 13·7 ± 2·3 at 24 h ($n = 36$), 12·5 ± 2·7 at 48 h ($n = 31$), 12·7 ± 2·4 at 72 h ($n = 18$) and 12·4 ± 2·0 at 96 h ($n = 2$).

Plasma chloride samples collected at East Bear Brook were $\log_{10}$ transformed and varied over time compared to the control samples (d.f. = 3,89, $P < 0{\cdot}001$). Plasma chloride values of East Bear Brook fish were lower than control fish within 24 h and showed another decline between 24 and 48 h exposure. Longer exposure time to East Bear Brook did not cause plasma chloride to decline beyond the measurements taken after 48 h exposure (Fig. 4).

Plasma glucose at East Bear Brook increased over time compared to the control samples (d.f. = 3,89, $P < 0{\cdot}001$). Plasma glucose values of East Bear Brook fish were elevated within 24 h and continued to increase between 48 and 72 h exposure (Fig. 4).

Gill aluminium samples collected at East Bear Brook increased over time compared to the control samples (d.f. = 3,76, $P < 0{\cdot}001$). Gill aluminium values of East Bear Brook fish increased within 24 h. No additional increases occurred after 24 h exposure (Fig. 5).

Unlike other study sites, mortality of *S. salar* smolts did occur at East Bear Brook and after 3 days of exposure *S. salar* smolt mortalities reached 40% and

TABLE III. Results from non-linear mixed model analyses (NLMIXED) of pH and *Salmo salar* smolt plasma chloride and plasma glucose after 3 and 6 day exposures of *S. salar* smolts to ambient river conditions at nine eastern Maine, study sites. Model output does not include data from the East Bear Brook study site

	Variable	Breakpoint estimate ± S.E.	d.f.	*t*-value	*P*
3 day exposure	pH	5·87 ± 0·14	7	42·15	<0·001
	Chloride	135·89 ± 1·40	7	97·39	<0·001
	Slope	22·14 ± 5·06	7	4·38	<0·01
	pH	5·47 ± 0·09	7	57·57	<0·001
	Glucose	8·47 ± 0·74	7	11·41	<0·001
	Slope	−18·15 ± 5·01	7	−3·63	<0·01
6 day exposure	pH	5·71 ± 0·14	7	42·19	<0·001
	Chloride	135·82 ± 2·46	7	55·15	<0·001
	Slope	58·50 ± 18·03	7	3·25	<0·05
	pH	5·70 ± 0·07	7	85	<0·001
	Glucose	7·33 ± 0·59	7	12·37	<0·001
	Slope	−27·34 ± 4·24	7	−6·45	<0·001

continued to increase through to day 4, at which point all remaining fish were sampled (Fig. 4). A comparison of East Bear Brook to other eastern Maine study sites at 72 h exposure (Fig. 6) showed that total water aluminium levels at East Bear Brook (95·8–109·2 μg l^{-1}) were within the observed range at other study

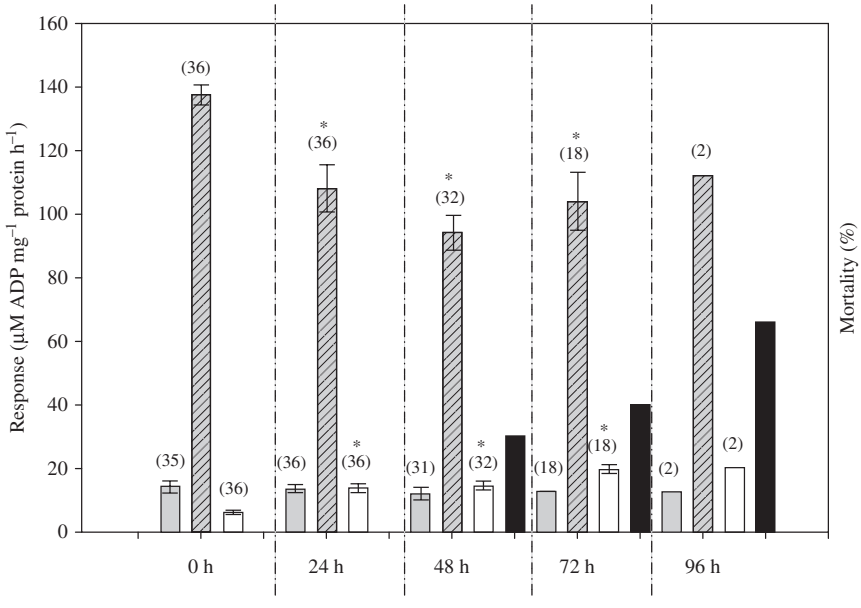

FIG. 4. Physiological response of *Salmo salar* smolts exposed to ambient water chemistry conditions at East Bear Brook, eastern Maine, during spring 2006: Na^+- and K^+-ATPase (μM ADP mg^{-1} protein h^{-1}) (▭), plasma chloride (▨) and plasma glucose (μ mole) (□) and mortality (%) (■). Values are mean ± S.D. Sample size is given in parentheses above each bar. *, a significant statistical difference ($P < 0·05$) when compared with 0 h.

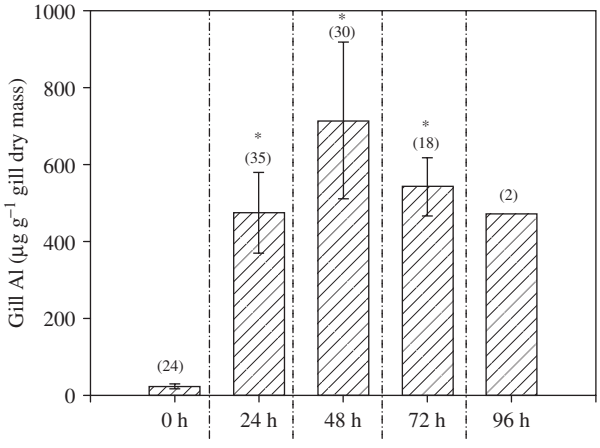

FIG. 5. Gill aluminium concentrations of *Salmo salar* smolts exposed to ambient conditions at East Bear Brook, eastern Maine, during the 2006. Values are mean ± S.D. Sample size is given in parentheses above each bar. *, a significant statistical difference ($P < 0{\cdot}05$) when compared with 0 h.

sites (79·9–162·1 μg l^{-1}) with similar pH. Additionally, East Bear Brook had DOC concentrations <1·9 mg l^{-1} whereas the other study sites were all >4·23 mg l^{-1}. The observed mortality rate of *S. salar* smolts exposed to East Bear Brook was 40% whereas no smolt mortality was observed at other sites with similar pH and aluminium concentrations. *Salmo salar* smolts exposed to East Bear Brook often appeared dark in colouration and at times were either slow to respond, or unresponsive, to human presence near the tanks.

DISCUSSION

The results of this study suggest that the physiological responses of *S. salar* smolts to water chemistry conditions in eastern Maine are variable but can be reliably predicted under certain conditions. Negative physiological effects are strongly correlated to pH and can occur rapidly (within days) under natural conditions that exist in some rivers in the north-eastern U.S.A. Additionally, the results suggest that *S. salar* smolts utilizing rivers and streams in eastern Maine with low DOC concentrations are probably more vulnerable to aluminium toxicity during low pH events than *S. salar* smolts residing in streams with moderate to high concentrations of DOC.

This study found that plasma chloride and plasma glucose levels in *S. salar* smolts exposed to natural water chemistries were strongly correlated to pH. The use of these indicators of physiological stress allowed pH thresholds at which *S. salar* smolts showed negative physiological response to ambient river conditions in eastern Maine to be estimated. As expected, plasma chloride content decreased and plasma glucose content increased as pH declined. These results are consistent with laboratory studies demonstrating that pH > *c*. 6·0 is not harmful to *S. salar* smolts (Staurnes *et al*., 1995; Monette & McCormick, 2008), as well as previous studies conducted in Norway and Nova Scotia where acid-related physiological effects in *S. salar* have been documented (Lacroix & Townsend, 1987; Staurnes *et al*., 1996). The absence of

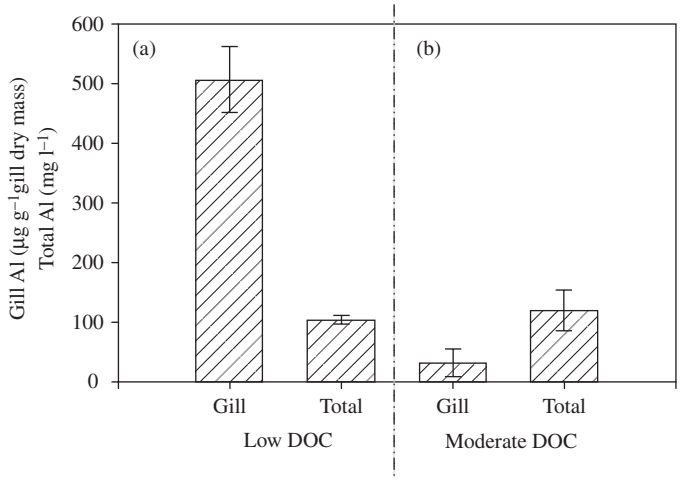

FIG. 6. Comparison of *Salmo salar* smolt gill aluminium after 72 h exposures to (a) East Bear Brook, eastern Maine, with low dissolved organic carbon (DOC) stream water and to (b) other eastern Maine study sites with similar pH and similar levels of total water aluminium but moderate DOC. A 40% mortality rate of *S. salar* smolts was observed under the conditions present at East Bear Brook, whereas no mortality was observed at the other sites. Values are the means ± S.D. of three exposure periods at East Bear Brook and five exposure periods at the other sites consisting of at least 12 fish per exposure.

increased gill aluminium at sites other than East Bear Brook suggests the possibility that these physiological effects are due primarily to the influence of low pH and independent of aluminium. The possibility that aluminium played a role cannot be ruled out, despite the fact gill aluminium in these streams was found not to be a reliable indicator of aluminium toxicity. In East Bear Brook where DOC was low, gill aluminium did appear to be a reliable indicator of more severe physiological responses.

The study also revealed that gill Na^+- and K^+-ATPase activity did not differ in response to stream location, in spite of significant variation in stream pH. This may in part be due to the high variability of up to 50% in gill Na^+- and K^+-ATPase activity among individual *S. salar* smolts. When mean values of gill Na^+- and K^+-ATPase activity were assessed, there was a significant relationship with stream pH and with plasma chloride and glucose. In a laboratory study in which *S. salar* smolts were exposed to acid and aluminium for 6 days, there were significant effects on plasma ions, plasma glucose and salinity tolerance, without significant effects on gill Na^+- and K^+-ATPase activity (Monette *et al.*, 2010). These results contrast with many longer term studies which have found decreased gill Na^+- and K^+-ATPase activity in *S. salar* smolts exposed to low pH. Measurement of gill Na^+- and K^+-ATPase activity employed in the present study provides a measure of the total potential enzyme activity, which is usually reflective of total abundance of the enzyme. It is therefore possible that acid and aluminium could be affecting the *in situ* activity of the enzyme (and thus affecting ion transport) without affecting the gill Na^+- and K^+-ATPase activity. Another possibility is that the freshwater and seawater isoforms of Na^+- and K^+-ATPase that have recently been found in *S. salar* gill tissue (McCormick *et al.*, 2009*a*) may respond differently to acid and aluminium exposure and would not be detected by the measurement of gill Na^+- and K^+-ATPase

activity. In any event, the lack of response of gill Na^+- and K^+-ATPase activity at pH levels that affect plasma chloride suggests that this measurement may not consistently reflect physiological responses in *S. salar* smolts in short-term exposure to acid and aluminium.

It is clear that factors other than just pH are affecting physiological responses of *S. salar* smolts. This was shown by the differences in physiological response of *S. salar* smolts to water chemistry at East Bear Brook in comparison to the other eastern Maine study sites. At East Bear Brook and several other sites such as Columbia, Saco and Sprague Falls, pH and total aluminium were similar whereas DOC was much lower at East Bear Brook. The most likely explanation is that the reduced DOC at the East Bear Brook study site led to decreased organic-aluminium complexes, increasing the total amount of toxic inorganic aluminium. In a previous study of eastern Maine rivers, Haines *et al*. (1990) found that streams with DOC averaging 3·5 mg l^{-1} had inorganic aluminium averaging 40 μg l^{-1}, while streams with DOC averaging 10 mg l^{-1} had half the levels of inorganic aluminium. On the basis of the known toxic thresholds of inorganic aluminium on *S. salar* smolts (Kroglund *et al*., 2007), this two-fold increase in inorganic aluminium is likely to have negative consequences whenever pH is <6·0. It should be noted, however, that the control of inorganic aluminium is highly complex and dependent on the interaction of many aspects of stream chemistry. Reduced pH can both increase the amount of aluminium that is dissolved from soil into water and increase its toxicity (Rosseland *et al*., 1990). Inorganic aluminium and especially pH vary strongly with changes in discharge, whereas DOC is less responsive to flow (Haines *et al*., 1990). The concentration of cations and anions, especially fluoride, also play a role in determining the concentration of inorganic aluminium. Although the possibility that other chemical constituents played a role in the results of the study at East Bear Brook cannot be ruled out, it seemed most likely that low DOC and low pH played the primary role in promoting aluminium toxicity based on the elevated gill aluminium measured in *S. salar* smolts exposed to stream water at this site. Thus, the present results support the idea that interactions between aluminium and DOC constitute an important mechanism in reducing the toxic effects of pH and aluminium on fishes in natural waters (Neville, 1985; Roy & Campbell, 1997).

Salmo salar smolts exposed to East Bear Brook, which is more representative of many southern Norwegian rivers (*e.g*. low pH, high aluminium and low DOC) where stream acidification is known to affect *S. salar* smolt survival, experienced a rapid loss of plasma chloride, a rapid increase in plasma glucose, significantly higher gill aluminium content and mortality. East Bear Brook was the only study site where *S. salar* smolts suffered direct mortality. The observed mortality which occurred in *S. salar* smolts exposed to East Bear Brook is thought to be a result of elevated inorganic aluminium concentrations in the brook.

As previously mentioned, aluminium is very dynamic in aquatic environments, its speciation can be strongly influenced by pH and it can affect *S. salar* smolts by disrupting normal gill functions and affecting both osmoregulation and respiration (Gensemer & Playle, 1999). Elevated gill aluminium concentrations (323–1011 μg g^{-1}) measured in *S. salar* smolts at the East Bear Brook study site are believed to be a result of the increased availability of inorganic aluminium under low pH, high aluminium and low DOC conditions occurring in East Bear Brook during this study. These elevated gill aluminium concentrations appear to be responsible

for the physiological impairment and subsequent mortalities of *S. salar* smolts at this study site. In support of this hypothesis is a study conducted by McCormick *et al.* (2009*b*) in which gill aluminium was found to be a strong predictor of both physiological stress and mortality of *S. salar* smolts exposed to similar water chemistries in southern New England.

Although salinity tolerance was not assessed in this study, previous studies have shown that *S. salar* smolts lose salinity tolerance when exposed to acid and aluminium at levels similar to those in the present study (Saunders *et al.*, 1983; Kroglund & Finstad, 2003; Kroglund *et al.*, 2007). Previous studies have also shown when plasma chloride of *S. salar* smolts is decreased to the levels seen in the present study (*e.g.* <110 mM), salinity tolerance is also lost (Kroglund *et al.*, 2007; Monette *et al.*, 2010). In the present study, *S. salar* smolts exposed to ambient conditions of episodically acidified eastern Maine rivers experienced reduced plasma chloride when pH fell to <5·9, and the severity of the physiological stress increased with further declines in pH. Thus, in years when episodic acidification events are sufficient to result in pH reduction <5·9 that leads to loss of plasma chloride, accompanying physiological responses will probably result in the loss of salinity tolerance of *S. salar* smolts. Such a loss in salinity tolerance has been shown to result in reduced ocean survival (Kroglund *et al.*, 2007), threatening the longer term sustainability of *S. salar* populations.

In conclusion, the findings from this study suggest that the negative physiological effects observed in *S. salar* smolts exposed to ambient water chemistries at the study sites can result from either the individual or combined effects of pH and in-stream inorganic aluminium. The findings from this study, and supporting literature, suggest that the presence of moderate to high DOC concentrations may be buffering the potentially lethal effects of inorganic aluminium on *S. salar* smolts at several of the study sites more typical of the natural water chemistries found in Nova Scotia (*i.e.* low pH, high aluminium and moderate to high DOC). Therefore, it appears that the current level of DOC in many eastern Maine rivers is protective to some degree of the negative physiological responses typically associated with low pH and high aluminium concentrations. This is demonstrated by lower gill aluminium concentrations in *S. salar* smolts exposed to high DOC waters relative to *S. salar* smolts exposed to low DOC waters with similar pH and aluminium concentrations. It is important to note, however, that when pH drops to <6·0 coincident with *S. salar* smolt migration, physiological compromise of *S. salar* smolts can occur under certain conditions found in eastern Maine rivers (*e.g.* low pH, high aluminium and low DOC) and could have negative consequences for their marine survival.

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