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Original Articles

Evaluating acid-aluminum stress in streams of the Northeastern U.S. at watershed, fish community and physiological scales

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ABSTRACT

In spite of overall improvements in air and water quality, biological stress from low pH and high concentrations of inorganic aluminum continue to impact fish and fish habitat in northeastern North America, with independent and interactive effects on individuals, populations and communities. Integrative indicators can therefore be useful in monitoring both impact and recovery across multiple scales. Using coupled water chemistry (pH, conductivity, and base cation and inorganic aluminum concentration), geographic (site elevation and watershed area) and biological (fish diversity, fish abundance, gill aluminum concentration and gill physiology) data, we developed an integrated indicator of acid-aluminum stress across the White and Green mountains in central New England, USA. As has been established in a number of previous studies, preliminary analysis clearly indicated that across all sites, inorganic aluminum concentration was consistently greatest during the spring season. Structural Equation modeling (SEM) revealed that toxic conditions (concurrent low pH and high concentrations of inorganic aluminum) were well summarized with an integrated toxicity score, related to both base cation concentrations and elevation, with sites at higher elevations more likely to experience toxic conditions as well as low base cation concentrations. Fish diversity and abundance generally trended negatively with toxicity score, with fewer cyprinids and sculpins at high toxicity score sites. In spite of considerable variation among individuals, gill aluminum was positively related to toxicity score for both Atlantic salmon and brook trout. Observed elevated gill aluminum levels associated with reduced gill metabolic activity in Atlantic salmon smolts from impacted systems likely result in impaired osmoregulatory function and seawater tolerance. Overall, our results suggest that the integrated toxicity score metric is associated with a syndrome of acute physiological stress, reduced abundance, and low species diversity for sensitive stream fishes in New England, and can likely serve as a reliable indicator of continued impairment or recovery of acid-aluminum vulnerable systems in this ecoregion.

1. Introduction

Ecological effects of anthropogenic pollution occur on multiple biological, spatial, and temporal scales. Understanding how toxic conditions act on those different scales is essential to understanding ecological vulnerability and possible recovery from damaging pollution events. As an effect of human industrial emissions and air pollution, acid rain has had widespread ecological effects in the Northeastern USA (Menz and Seip, 2004). Acidic precipitation is especially damaging when it causes acidic conditions concurrent with environmentally available aluminum, creating acid-aluminum conditions that can be toxic to fish and other aquatic organisms (Teien et al., 2006). Characterizing ecological recovery from acid-aluminum toxicity is complicated by both the scales on which acid-aluminum toxicity acts and the many

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environmental controls of both aluminum and pH. In spite of considerable contemporary improvements in the average pH of precipitation compared to low pH's of the mid twentieth century (improvements facilitated through the Clean Air Act), the acidity of precipitation has still not returned to pre-industrial levels (Likens and Borman, 1974; Likens et al., 1996; Likens et al., 2021; Driscoll et al., 2016). Further, the protracted recovery and vulnerability to acidic conditions in the Northeast may be especially relevant for streams of the region, with watersheds predominated by glacial till soils that have low base cation concentrations as a result of prolonged atmospheric weathering (Yanai et al., 1999).

Today, limited chronic and widespread episodic acidification is still present in high-elevation streams of the Northeast (Burns et al., 2019). Acidic episodes are most likely to occur when hydrologic conditions create a short precipitation soil residence time due to short path length and or high flow rate (Bailey et al., 1987; Bailey et al., 2019). These acidic shifts in surface water conditions tend to coincide with spring snowmelt and precipitation events (Fakhraei and Driscoll, 2015).

Both episodic and chronic acidification of streams have established physiological and population effects on aquatic species at different trophic levels (Schindler et al., 1989). Lower trophic level organisms including aquatic insects and freshwater zooplankton appear to exhibit changes in community structure and decreases in overall abundance during acidic shifts, while fish and other higher trophic level organisms appear to be more resilient to acidic conditions but are far more vulnerable to aluminum and other environmentally available metals at low pH's (Webb, 2004; Carbone et al., 1998; Gensemer and Playle, 1999). In southern Norway, the effects of chronic acidification and coupled aluminum availability have been extreme enough to extirpate Atlantic salmon from their native streams and habitat (Leivestad and Muniz, 1976).

During acidic shifts, aluminum solubility and mobilization increase, especially for aluminum in monomeric and inorganically bound forms (Fakhraei and Driscoll, 2015). Inorganic, monomeric aluminum (hereafter referred to as Al_i) is biologically reactive, with positively charged Al_i-species able to complex with and accumulate in biological tissues (Teien et al., 2006). High concentrations of Al_i may be toxic to organisms through numerous mechanisms, but a widespread and deleterious effect is its ability to complex with other compounds and subsequently reduce the activity of critical enzymes (Shugalei et al., 2013). In both aquatic insects and fishes, the highest concentrations of aluminum are generally found in gill tissues, where gas exchange creates altered pH conditions that stimulate Al_i to complex with biological tissues (Gensemer and Playle, 1999).

Previous studies on Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), and brown trout (*Salmo trutta*) indicated that toxicity effects of low pH and high aluminum conditions likely affect both respiratory and ionoregulatory function in gill tissues (Gensemer and Playle, 1999). Gill sodium–potassium ATPase (NKA) is critical for ion uptake by the gill in freshwater, and appears to be a target of aluminum, resulting in osmoregulatory failure (Gensemer and Playle, 1999). As part of the Atlantic salmon life history, smolts preparing to outmigrate from rivers to the ocean have increased NKA activity to support seawater tolerance (McCormick et al., 2009a; McCormick et al., 2009b). Several studies have shown that moderate acid-aluminum conditions reduce gill NKA activity and reverse seawater tolerance of smolts, while more severe conditions are lethal in freshwater (Kroglund and Finstad, 2003; Monette et al., 2008; Nilsen et al., 2010; Kelly et al., 2015). Furthermore, the timing of acidic and aluminum-rich river conditions may be especially impactful; high spring aluminum concentrations may be concurrent with Atlantic salmon smolt osmoregulatory development (Driscoll, 1985). Pre-smolt, parr stage Atlantic salmon as well as freshwater-resident brook trout may also experience gill accumulation of aluminum and mortality from low-pH high-Al_i episodes (Baldigo et al., 2016). Under the most severe and lengthy acid-aluminum exposures gill lamella filaments may fuse or exhibit hyperplasia in Atlantic salmon (Lacroix et al., 1993).

While the effects of acid-aluminum stress on brook trout and Atlantic salmon are well established in lab and *in situ* studies (Baldigo et al., 2019; McCormick et al., 2012), there is only limited surveying of wild fish populations for community and physiological stress arising from acid-aluminum conditions in streams of the Northeastern United States (Heisler, 2020). Central to understanding these effects are the ecological scales on which acid-aluminum stress can be measured. First, it is necessary to establish how prone to episodic toxicity various watersheds and catchments are as a function of their water chemistry. Next, acid-aluminum stress may act on the fish community scale with effects on range and abundance of sensitive teleost fishes. At a site or habitat level, this may manifest as reduced species diversity when sensitive species are unable to tolerate severe acid-aluminum conditions. Finally, it is likely that acid-aluminum stress acts on the physiological scale in salmonids, with established effects on respiration and osmoregulation. By evaluating these three ecological scales, we provide evidence of how acid-aluminum stress influences salmonid populations in upland catchments of the Northeast. We also provide a basis for using Atlantic salmon and brook trout physiologies and distributions as sentinels for episodic acid-aluminum stress that may be difficult to quantify from purely physical and water chemistry measures.

In this study, the driving hypothesis is that acid-aluminum toxicity effects, expressed on both teleost community and physiological scales, are a function of environmental gradients of both aluminum and base cation conditions. We posit and test three linked predictions that stem from this hypothesis. First, across different catchments, sites with the lowest buffer capacity scores will be prone to the highest toxicity scores. Through modeling both a) buffer capacity (as a combination of magnesium concentrations, calcium concentrations, conductivity) and b) toxicity (as a function of pH and inorganic aluminum), we expect sites with higher buffer capacity to experience lower toxicity scores. We also expect site elevation to affect toxicity scores either indirectly through buffer capacity or directly through elevational gradients in soil materials that affect pH and Al_i. Second, on a fish community scale, sites with greater buffer capacities and lower acid-aluminum toxicity scores will exhibit greater alpha-diversity and greater abundance of teleost fishes. Third and finally, on an individual, physiological scale, we predict Atlantic salmon smolts inhabiting sites with higher toxicity scores to exhibit increased gill aluminum concentrations and decreased gill NKA activity due to Al_i inhibition of the enzyme.

These three predictions are evaluated with water chemistry and biological survey data from upland catchments within four watersheds in Southern Vermont and Central New Hampshire. These catchments encompass a range of localized conditions that represent how acid-aluminum stress might function over broader Northeastern landscapes. Furthermore, all sampled catchments have populations of Atlantic salmon and brook trout and are therefore of interest for conservation of these species. Atlantic salmon inhabit a fraction of their native range in the Northeastern United States, and modeling acid-aluminum stress in their native habitat may help locate streams of interest for habitat improvements or conservation interventions (Chaput, 2012).

2. Materials & methods

2.1. Study site selection

In total, 29 sites were selected across the Upper Merrimack (19 sites), Upper Ammonoosuc (2 sites), Saco River (1 site), and West River (7 sites) basins in central New Hampshire and Southern Vermont (Fig. 1 and Table S1). All sites selected were historic Atlantic salmon and brook trout habitat but represent geologically diverse watersheds. The Ammonoosuc and West River basins both drain to the Connecticut River, the largest river basin in New England, and host diverse watershed lithologies with some neighboring streams exhibiting both well buffered

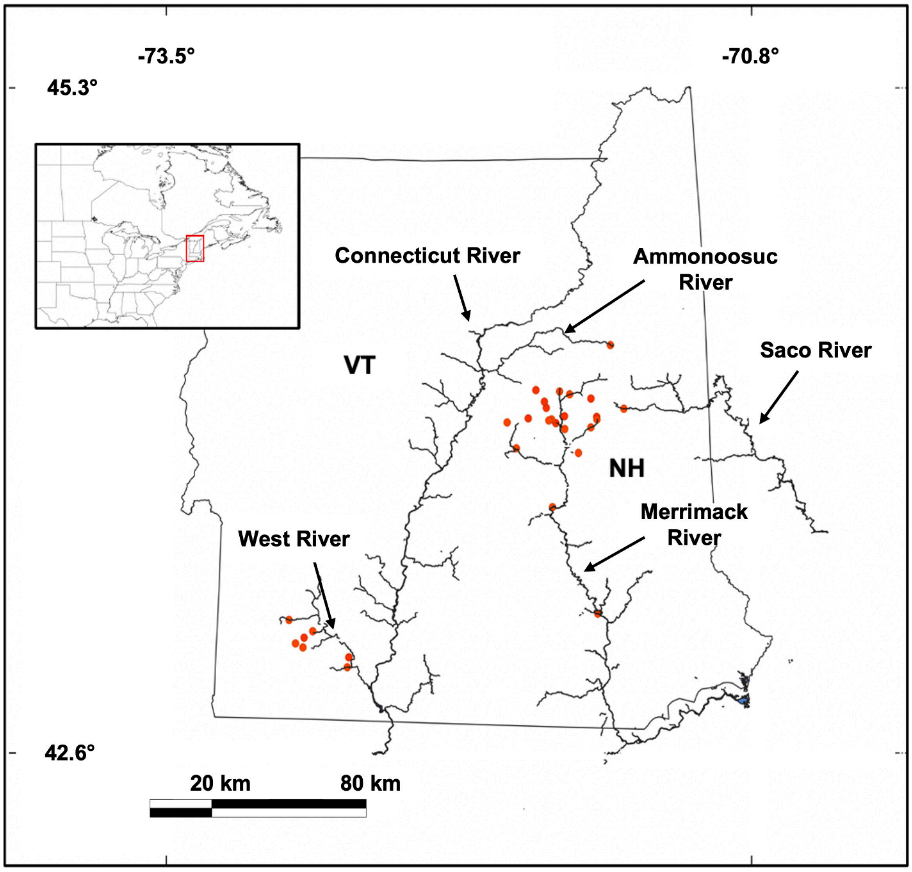

Fig. 1. Site mapping of all 29 water quality and biological sampling sites in this study. Red dots correspond to sampling locations. The sites grouped in the southwest are on the West River Basin, the central grouping is on the Merrimack Basin, with two sites to the north in the Ammonoosuc basin, and one site to the east in the Saco River Basin. The Connecticut river defines the border of New Hampshire to the east and Vermont to the west.

lithologies and lithologies prone to episodic acidification (MassDEP, 2013). In contrast, the Merrimack basin is much smaller than the Connecticut, and the upper basin is characterized by high elevation, poorly buffered bedrock geology and thin surface soils. This makes upper basin streams prone to episodic acidification in the Merrimack (Driscoll et al., 2001). Site drainage area varied from 0.327 km^2 to 6282 km^2 with a median area of 30 km^2 across all sampling sites. The subset of sites where biological sampling took place ranged from 4 km^2 to 59 km^2 in drainage area with a median of 19 km^2 drainage area. Rain gauge data were also compiled from gauges in Hubbard Brook Experimental Forest and Southern Vermont to ensure that sample timing in relation to precipitation events was not confounding measured variables.

Field sampling included *in situ* water chemistry measurements, water collection for laboratory analyses, and biological characterization of stream fish populations. Sites were selected to provide a variety of water chemistry and habitat conditions but were predominantly of special conservation interest for Atlantic salmon recovery efforts (Gephard and McMenemy, 2004).

2.2. Water chemistry sampling

Water chemistry sampling took place four times per year (April, June, September, and late November or early December) at every study site in 2007 and 2008. Sampling times were selected to measure a complete picture of seasonal changes during ice-free times of the year and specifically capture extreme conditions around smolt movements. Atlantic salmon smoltification (physiological and morphological changes associated with the preparation for the transition from freshwater to saltwater environments) and smolt migration occur from mid-April to June in the measured watersheds. Water temperature, pH, and conductivity were all measured *in situ*. Point measurements of pH were made using a portable pH meter (3 Star, Thermo-Orion; https://www.thermoscientific.com) and a low-ion pH electrode (ROSS, Thermo-Orion). The pH meter was calibrated immediately prior to each measurement with two low-ionic strength buffers (Pure Water pH 6.97 and pH 4.10, Thermo-Orion). Measurements were made by suspending the electrode in an area with some gentle water movement but out of direct current and recording the pH at 30 s and after 2 min. Temperature and conductivity were measured with a YSI Model 30 Conductivity probe, temperatures were double checked with a separate calibrated digital thermometer, and all conductivity measurements were temperature corrected.

Total aluminum (Al_t), organic aluminum (Al_o) and Al_i concentration were measured according to Driscoll (1985). Total aluminum samples were passed through a 45 μm nitrocellulose filter and acidified (0.2 % trace-metal-grade HNO_3). Al_o samples were passed through a 45 μm nitrocellulose filter followed by a strong cation exchange column (9.5 mL resin volume, Amberlite 120) at 30 $mL \cdot min^{-1}$ with a calibrated manual syringe pump, then acidified with trace metal grade nitric acid. This processing occurred immediately after collection in the field, after which samples were stored at room temperature until aluminum analysis. Aluminum was measured with graphite furnace atomic absorption spectrophotometry (GFAAS) according to the methods of Kelly et al. (2015). Al_i was calculated as the difference between Al_t and Al_o. Calcium and magnesium concentrations were measured by flame atomic absorption spectrophotometry from separate unacidified water samples taken at the same time and location as Al samples (AAnalyst 100, Perkin Elmer, Wellesley, Massachusetts).

2.3. Biological sampling

Biological sampling took place at 10 sites total, spanning the West River (2 sites), Ammonoosuc (1 site), and Merrimack (7 sites) basins at roughly the same stream access points where water quality data were collected for these sites (Table S1). All biological sampling was conducted between late May and mid-June of 2008. Atlantic salmon parr and smolts encountered during the study were the result of a reintroduction program, where progeny of returning adult Connecticut River or Merrimack River salmon were fry-stoked throughout Connecticut and Merrimack watersheds. Brook trout encountered during the study were predominately wild origin fish. Quantitative sampling of diversity and abundance was conducted via single-pass quantitative electrofishing where species and abundance of all captured teleost fish were recorded over a 150 m electrofishing reach, upstream of the water sampling site. Streams sampled with electrofishing were generally of similar dimensions, so the total fish habitat area sampled was similar between sites. For smolt outmigrant sampling, blocking nets of ½ inch mesh were placed across the entire width of the stream at sample sites leading to fyke traps placed in the thalweg of the stream. Fish were sampled from the net, the fyke trap, and were electrofished just above the net. Nets were set and sampled every 24 h from May 1–6, 2008 and May 29–30, 2008 at sites on Smith Brook and Ball Mountain Brook in the West River Basin, VT.

For both Atlantic salmon and brook trout captured during electrofishing surveys, fish were non-lethally anesthetized (200 mg^{-1} MS-222 neutralized to pH 7.0) and measured for total length, fork length, and weight. Non-lethal gill biopsies were taken by severing six to eight filaments above the septum on the right side (McCormick, 1993) for gill total Al analysis. In smolts, an additional biopsy was taken from the same location on the left side of the fish for measurement of NKA activity. Filaments for gill Al measurements were placed in an acid-washed 1.5 mL microcentrifuge tube, immediately frozen on dry ice, and stored at −80 °C before measurement of gill Al levels. Gill filaments for NKA activity were immediately placed in 100 μl of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA and 50 mM imidazole, pH 7.3) then frozen on dry ice and stored at −80 °C before measurement of gill NKA activity. All work complied with U.S. Geological Survey animal care guidelines under protocol LSC-9066. The number of salmonids captured at each site varied from 13 to 50 salmonids, however at certain sites supplemental electrofishing outside of the sample reach was conducted to bolster the number of salmonids for gill aluminum analysis. This supplementary sampling occurred both at sites with low numbers of salmonids (under 15) in the Merrimack basin, as well as sites of specific conservation interest in the West River basin. These supplemental fish were not included in any community or abundance data.

Gill aluminum was measured according to the method outlined in Monette and McCormick (2008). Each sample had two replicates, and a background correction was made by subtracting the Al present in digestion blanks. Gill Al is represented as a measurement of μg Al g^{-1} gill dry mass. Gill NKA activity was measured by a kinetic assay described by McCormick (1993). Two duplicate 10 μl samples were run in the assay mixture, and two with 0.5 mM ouabain in assay mixture at 25 °C for 10 min. The resulting ouabain-sensitive NKA activity is expressed as μmoles adenosine diphosphate (ADP) mg $protein^{-1}h^{-1}$. Protein concentrations were determined with a bicinchoninic acid (BCA) protein assay. Assays were run and read on a THERMOmax microplate reader with SOFTmax software (www.moleculardevices.com).

2.4. Dataset assembly and analysis

To test the predictions on the watershed, fish community and physiology scales set forth in this study, we created a coupled water chemistry and biological dataset to model toxic conditions and how they relate to fish community structure and physiology. Multiple water chemistry variables covary, including magnesium, calcium, and conductivity measurements that represent soil buffers, as well as Al_i and pH fields that contribute to toxicity. Some of this covariation is likely causal, based on the environmental availability of magnesium and calcium buffers, the measurement of conductivity, and the environmental controls of Al_i (Behera and Shukla, 2015; Fakhraei and Driscoll, 2015). To evaluate the temporal nature of acid-aluminum toxicity, we regressed spring mean Al_i against annual mean Al_i measurements for each site to confirm that sites experience the most severe Al_i conditions in the spring, as suggested by Burns et al. (2019) and Fakhraei and Driscoll (2015).

To represent the multiple causal links between water quality variables in the data, we constructed a structural equation model (SEM) to evaluate the relationship between buffer measurements, toxicity measurements, and site elevation consistent with the methods of Savalei and Bentler (2010) and Malaeb et al. (2000). In the SEM, unmeasured latent variable buffer capacity and toxicity score were calculated. The buffer capacity latent variable (hereafter referred to as buffer capacity) was defined by the covariance structures of measured magnesium (mg/L), calcium (mg/L), and conductivity (μS/cm) and scaled to magnesium concentrations. It has been established that the toxicity of aluminum to fish is driven by both low pH and elevated Al_i (Gensemer and Playle, 1999). Therefore, the toxicity score latent variable was defined by pH and Al_i (μg/L) and scaled to Al_i to represent higher Al_i values and lower pH values with higher toxicity scores. Finally, buffer capacity was regressed on toxicity score to quantify the relationship between the latent variables, and site elevation was regressed on both toxicity score and buffer capacity to test if buffer capacity or toxicity scores changed with elevation. These regressions were tested in a set of model iterations starting from the two latents independent of one another and progressing to the fully defined 4th iteration model.

All models were constructed and calculated in JMP Pro version 15 statistical software with a maximum of 10,000 iterations for model convergence (JMP, 2019). Model fit was assessed with Akaike Information Criterion scores (AICc), Chi-Squared values, and −2 log likelihood scores. Regression and loading scores were evaluated with Wald Z Score tests and evaluated between model iterations (Chou and Bentler, 1990). To evaluate the model performance against measured values of interest, we used a generalized linear mixed model to test the relationship between toxicity score and both pH and Al_i. In the model, season was treated as a nested random effect of year, to ensure that repeat sampling was not influencing the relationships reported. All linear regressions presented in this paper were also assessed with residual by x plots and quantile–quantile plots to determine if data were normally distributed and if model fit could be improved. It is worth noting that there is unequal variance in much of the pH and Al_i data as well as the gill aluminum data, which was considered for whether relationships were compared with regressions or Spearman ρ tests.

To evaluate both the relationships between teleost community and water chemistry as well as teleost physiology and water chemistry, we calculated spring mean toxicity scores and annual mean toxicity scores for each site with biological sampling. A categorical low, moderate, or high toxicity category was assigned based on mean annual toxicity scores. All thresholds were based on the laboratory findings of Monette et al. (2008) and McCormick et al. (2012). The low toxicity score category corresponds to pH above 5.5 and Al_i values below 30 μg/L where no adverse acid-aluminum physiological effects are expected in Atlantic salmon. Moderate toxicity score values roughly correspond to pH values between 5.5 and 5.2 and Al_i at 30–60 μg/L where Atlantic salmon may experience some physiological effects. Finally, sites with high categorical toxicity scores corresponded to pH < 5.2 and $Al_i > 60$ μg/L where mortality is likely for Atlantic salmon.

Teleost community metrics consisted of teleost alpha-diversity and total teleost abundance, and physiological metrics included salmonid gill aluminum measurements and Atlantic salmon smolt NKA ATPase activity. For the fish community scale, both total teleost fish abundance and teleost fish alpha-diversity and toxicity score relationships were tested with linear regressions with each point representing a biological sampling site. Teleost fish alpha-diversity was represented by the number of teleost species present at each sampling site. For the physiological scale, brook trout and Atlantic salmon gill aluminum concentrations were compared against toxicity score but separated by species to account for possible differences in aluminum uptake or sensitivity and assessed with Spearman ρ tests. Atlantic salmon smolt gill aluminum and gill NKA activity were evaluated on a site toxicity categorical basis, and means were compared between low and moderate toxicity score sites with one-tailed, unequal variance, Welch's t-tests to test if smolts from moderate toxicity score sites had reduced NKA function. For freshwater resident Atlantic salmon parr and brook trout, both gill NKA activity and condition factor (Fulton's K) were also compared with toxicity scores and significance was assessed with Spearman ρ tests.

3. Results

3.1. Spatial and temporal variation in water chemistry

There was high variability in pH and aluminum among streams, with Al_i values varying from 0 to over 400 μg/L and pH values ranging from the mid 4′s to low 7′s (Fig. 2). Al_i concentrations were higher in spring relative to other times of the year (Fig. 3). Spring mean Al_i was strongly related with annual mean Al_i on a site basis (Fig. 3; n = 29, slope: 1.18, $R^2 = 0.99$, p-value < 0.001), but was higher in spring for almost all sites.

When run in a Spearman ρ correlation test, acidity (pH) and Al exhibited a significant negative relationship, but the low R^2 value indicates that there are likely other variables that control the availability of Al_i (Fig. 2; n = 229, Spearman ρ: −0.41, p < 0.001). Residuals for the model fit of Al_i on pH also were heteroscedastic; the untransformed data provide poor model fit for linear modeling. While elevation was not incorporated into the model, sites at high elevations appear to exhibit both the lowest pH and highest Al_i conditions of the dataset, as exemplified by the color grading on Fig. 2.

3.2. SEM modelling

Structural equation modeling enhanced predictions of both pH and high Al_i conditions in the dataset. All four models ran successfully with the full n = 229 water quality dataset and, as expected, the toxicity score latent variable had a positive relationship with inorganic aluminum and a negative relationship with pH. It is important to also note that the

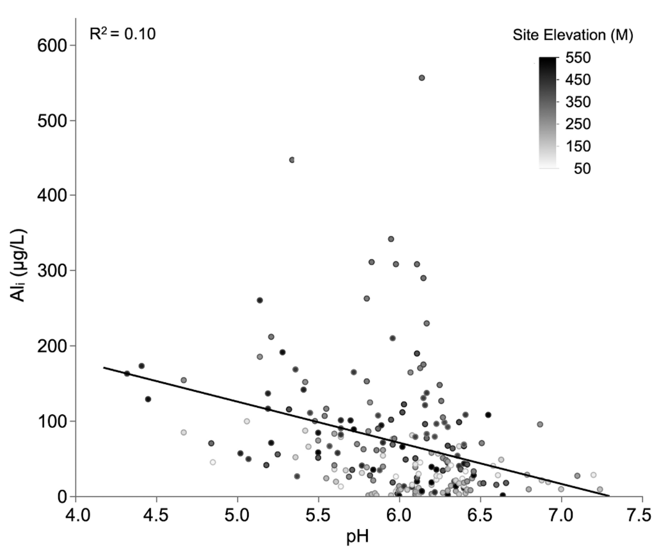

Fig. 2. Al_i plotted against pH for 29 sites, sampled 4 times per year over two years. The negative relationship between pH and Al_i is significant (p < 0.001).

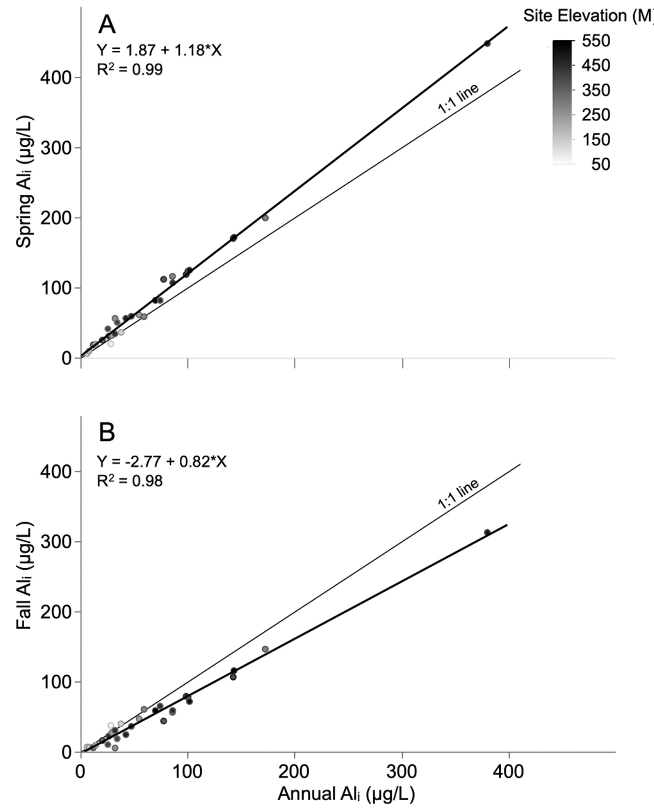

Fig. 3. Spring (A) and fall (B) mean inorganic aluminum regressed against annual mean inorganic aluminum for all 29 sites. The spring and fall mean Ali to annual mean Ali regressions are significant (p < 0.001).

relatively high chi-square statistics for all models signified that the covariance structures for all models were statistically different from those of the dataset. This difference implied an imperfect model fit; however, since these models were not designed to perfectly define the Ca, Mg, conductivity, Al_i and pH control system, and instead model toxic conditions more effectively, the model with the lowest chi-squared was chosen as the best approximation of the system. Buffer capacity had a significant negative relationship with toxicity score in model iterations V2, V3, and V4 (buffer capacity was not regressed on toxicity score in model V1) (V2, coefficient: −42.22, Wald Z: −3.47, p-value < 0.001; V3, coefficient: 41.98, Wald Z: −3.53, p-value < 0.001 V4, coefficient: −35.06, Wald Z: −3.71, p-value < 0.001) (Table 1). Site elevation was negatively related to buffer capacity yet positively related to toxicity score in the V4 model (buffer capacity, coefficient: −0.0008, Wald Z:

Table 1
Model 4 Structural Equation Model parameter estimates.

Loadings	Estimate	Std Error	Wald Z	Prob > |Z|
Buffer Capacity to Conductivity	69.09	4.260	16.22	<0.0001
Buffer Capacity to Calcium (mg/L)	3.850	0.2162	17.81	<0.0001
Buffer Capacity to Magnesium (mg/L)	1	—	—	—
Toxicity Score to pH	−0.00578	0.0012	−4.861	<0.0001
Toxicity Score to Al_i (μg/L)	1	—	—	—
Regressions				
Buffer Capacity to Toxicity Score	−35.06	9.449	−3.710	0.0002
Site Elevation (m) to Buffer Capacity	$-7.89 * 10^{-4}$	$2.34 * 10^{-4}$	−3.370	0.0008
Site Elevation (m) to Toxicity Score	0.1787	0.0371	4.812	<0.0001

−3.37, p-value < 0.001; toxicity score, coefficient: 0.18, Wald Z: 4.81, p-value < 0.001). However, when site elevation was regressed on both buffer capacity and toxicity score, the magnitude of the buffer capacity to toxicity score regression decreased (-41.98 to −35.06). Taken in sum with the significant relationship of site elevation on toxicity score, these results indicate that there are aspects of site elevation that influence toxicity score variability independent of buffer capacity (Fig. 4). The V4 model provided the best representation of the water quality data with the best AICc, chi-squared, and −2 log likelihood scores, as lower AICc, chi-square, and −2 Log Likelihood scores represented a better model fit (Table 2). Variances range considerably among model parameters but denote a greater variability in toxicity score than buffer conditions.

As a function of the model creation and parameterization, both Al_i and pH are related to the toxicity score (Figure S1; Al_i, slope: 1.56, $R^2 = 0.51$; pH, slope: $-9.08*10^{-3}$, $R^2 = 0.50$). Through the scaling relationship, the relationship is positive between Al_i and toxicity score, and negative between pH and toxicity score. The R^2 values for each regression also indicate toxicity can act as a predictor of both low pH and high Al_i conditions. The residual by x plots for the toxicity score vs Al_i and pH fit lines both show more homoscedastic results than the residual by x plot for the raw Al_i vs pH fit, which also indicates a better model fit through the structural equation modeling approach. However, the Al_i versus toxicity score relationships still show some trend in the residuals; there are likely nonlinearity effects between toxicity score and Al_i. There was no significant difference in mean toxicity scores for the years 2007 and 2008 (two-tailed *t*-test, df = 225.7, t = -0.82, p-value: 0.415). Toxicity scores varied considerably among locations with a maximum value of 179, upper quartile of 94.9, lower quartile of 46.6, and median of 72.0 and scores were normally distributed. Mean toxicity scores were lower in the West River Basin in Vermont than the New Hampshire site mean measurements (two-tailed *t*-test, df = 87.7, t = -3.47, p-value < 0.001).

3.3. *Fish diversity, abundance, and physiology*

Teleost species encountered during sampling included Atlantic salmon, brook trout, slimy sculpin (*Cottus cognatus*), blacknose dace (*Rhinichthys atratulus*), and white sucker (*Catostomus commersonii*). Teleost richness and total teleost abundance generally varied with toxicity score (Fig. 5). The number of teleost species present declined with toxicity score (Fig. 5; n = 10, slope: −0.028, $R^2 = 0.673$, p-value: 0.0036). Teleost abundance also declined with spring mean toxicity score, but the trend was marginally significant (n = 10, slope: −0.739, $R^2 = 0.374$, p-value: 0.0604). The lowest diversity sites appeared to occupy high elevations, whereas abundance did not appear to trend with any elevational gradients.

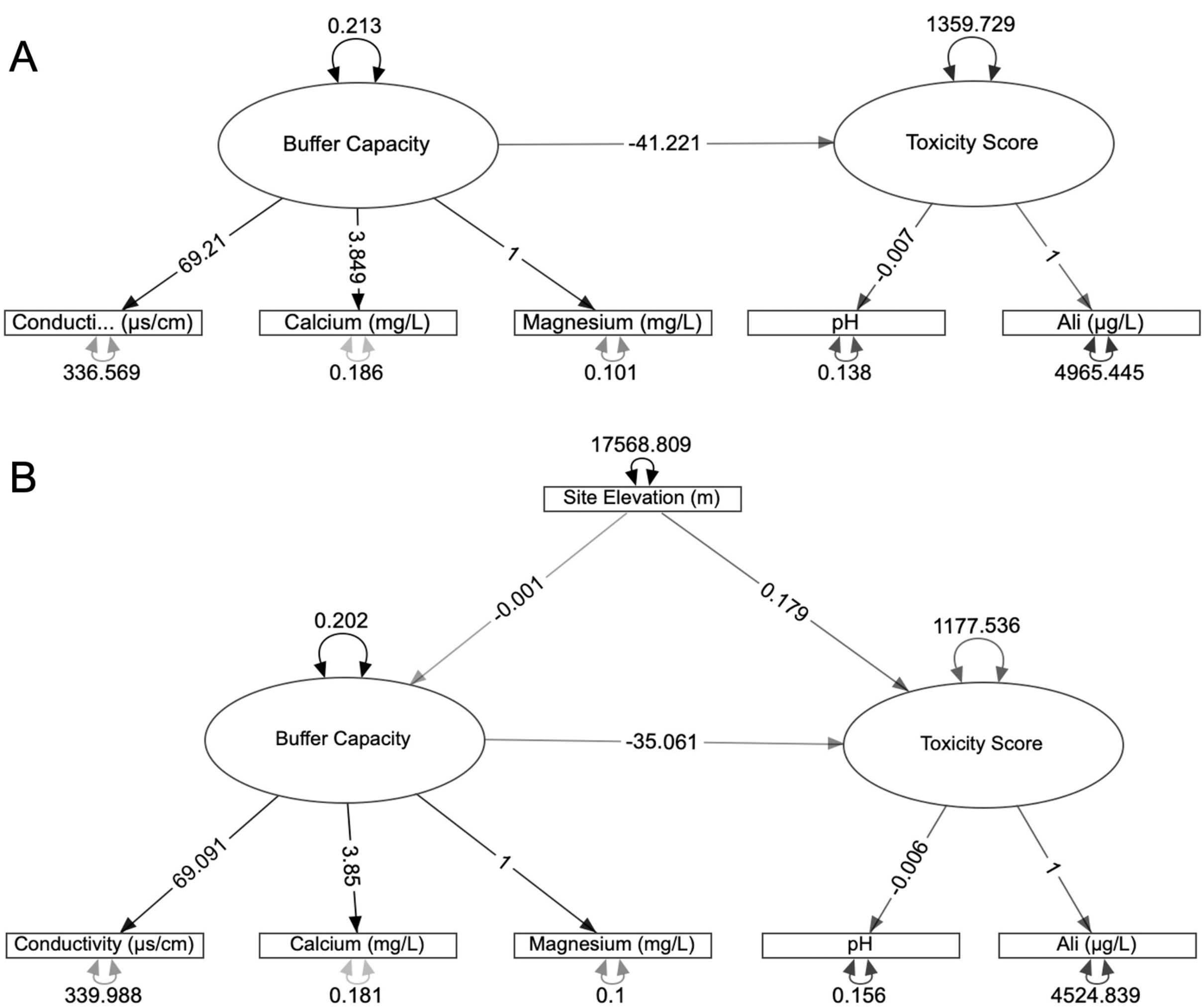

Fig. 4. Structural equation models (SEM) of watershed buffer capacity and toxicity scores both without (A) and with (B) the effect of elevation. Oval fields are synthetic, latent variables and rectangular fields are manifest, measured fields. Regressions or loadings are represented by straight arrows, with solid arrows being statistically significant regressions at α = 0.05. The number on each straight arrow is the regression or loading coefficient. The regressions for buffer capacity to magnesium loading and the toxicity score to Al_i loading are fixed to 1 to act as scalars for the latent variables. Curved arrows represent the variance score for a variable. In this model, buffer capacity and site elevation have significant impacts on toxicity score independent of one another (Table 1).

Table 2
Model Comparison of four possible toxicity score Structural Equation Model models.

Model Name	Number of Parameters	DF	AICc	−2 Log Likelihood	ChiSquare	P-Value
Independent (Null Hypothesis)	12	15	9363.76	9338.31	647.03	<0.0001
Model 1: Buffer Capacity and Toxicity Score Latents	17	10	8841.00	8804.10	112.82	<0.0001
Model 2: Buffer Capacity Regressed on Toxicity Score	18	9	8819.34	8780.08	88.80	<0.0001
Model 3: Site Elevation Regressed on Buffer Capacity	19	8	8809.68	8768.05	76.76	<0.0001
Model 4: Site Elevation Regressed on both Latents	20	7	8780.70	8736.66	45.38	<0.0001

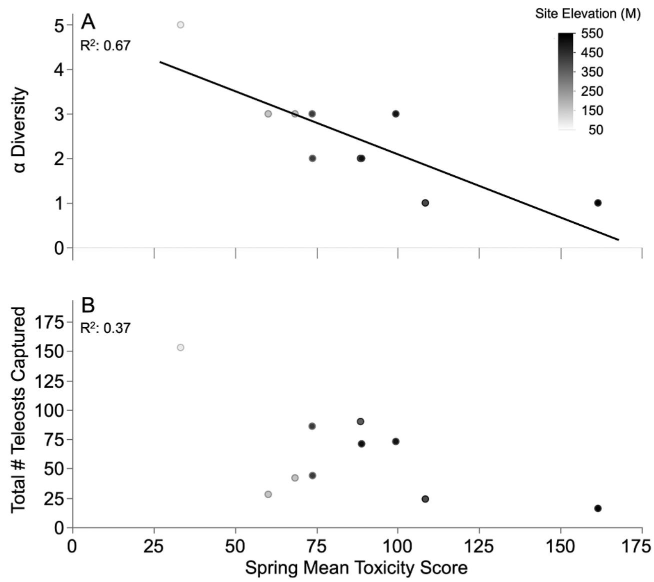

Fig. 5. Teleost species present (A) and teleost abundance (B) plotted against spring mean toxicity score for all sites with biological sampling. Diversity and total numbers were from a standard stream length (150 m) sampled by electrofishing. The negative relationship between species richness and spring toxicity score is statistically significant ($p = 0.004$). The negative relationship between teleost abundance and spring mean toxicity score is a relatively strong trend but not significant ($p = 0.060$).

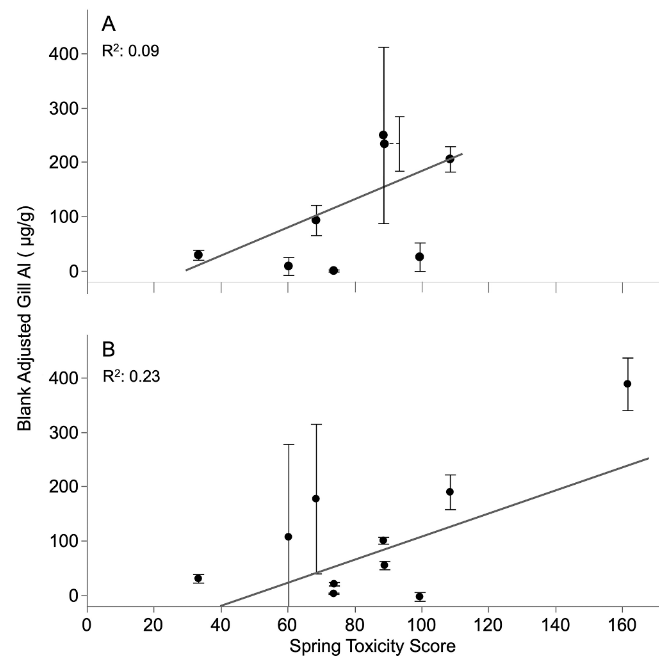

Fig. 6. Mean site gill aluminum plotted against spring mean toxicity score, grouped by species (Atlantic salmon: A, Brook Trout: B). Error bars represent one standard error from the mean. Both Atlantic salmon and brook trout exhibit significant positive relationships between spring toxicity scores and gill Al accumulation (A: $p < 0.001$; B: $p < 0.001$).

There were effects of high toxicity scores on salmonid physiologies for both Atlantic salmon parr and smolts, as well as freshwater resident brook trout. For both salmonid species, there was a positive relationship between gill aluminum levels and spring mean toxicity score (Fig. 6; Atlantic salmon, $R^2 = 0.09$, Spearman ρ: 0.356, p-value < 0.001; brook trout, $R^2 = 0.23$, Spearman ρ: 0.37, p-value < 0.001). Notably there was a great deal of variation in these data, as reflected by the relatively low R^2 values for each relationship. Brook trout gill aluminum concentrations had less variability than Atlantic salmon gill aluminum, as reflected by the higher R^2 for that fit. Gill aluminum was not related to 7-day antecedent precipitation for either Atlantic salmon or brook trout (Atlantic salmon, Spearman ρ: 0.09, p-value: 0.37; brook trout, Spearman ρ: −0.14, p-value: 0.110). Additionally, a slight, negative relationship was found between condition factor and toxicity score for Atlantic salmon parr and brook trout (Figure S2; Atlantic salmon, $R^2 = 0.04$, Spearman ρ: −0.22, p-value: 0.029; brook trout, $R^2 = 0.03$, Spearman ρ: −0.26, p-value: 0.002). Captured brook trout and Atlantic salmon were predominately (age 1 + or 2 +) fish.

Both gill NKA activity and gill aluminum differed significantly for Atlantic salmon smolts inhabiting low and moderate toxicity score sites. For gill aluminum, smolts inhabiting low toxicity score sites had significantly lower gill aluminum concentrations than those inhabiting moderate toxicity sites (Fig. 7B; t = 3.04, df = 24.87, p-value: 0.005). Gill NKA activity was significantly higher in smolts at low toxicity sites compared to those at moderate toxicity sites (Fig. 7A; t = -2.20, df = 15.66, p-value: 0.043). For freshwater residents, Atlantic salmon parr and brook trout, there was not as strong of a relationship between gill NKA activity and toxicity scores and no relationship between Atlantic salmon parr NKA activity and toxicity score (Fig. S3A; R^2: 0.01, Spearman ρ: −0.05, p-value: 0.644) and a stronger yet still insignificant relationship for brook trout (Fig. S3B; R^2: 0.01, Spearman ρ: −0.17, p-value: 0.052).

4. Discussion

Our results suggest that in spite of a substantial reduction in the acidity of precipitation, soil, and surface water (Fuss et al., 2015), a syndrome of acid-aluminum toxicity continues to impact stream ecosystems in New England at multiple levels of biological and spatial organization. On a landscape scale, acid-aluminum stress is highly variable with different reaches of watersheds experiencing different toxicity conditions, but high elevation sites (>450 m) appear to be most vulnerable to toxic conditions. Fish community richness is negatively related to toxicity scores, as non-salmonids do not inhabit sites with moderate to high acid-aluminum stress. Finally, a distinct physiological signal of acid-aluminum stress is observed in salmonids under severe low pH-high Al_i conditions, with high accumulations of gill aluminum in both Atlantic salmon and brook trout and reduced osmoregulatory function in Atlantic salmon smolts preparing to outmigrate. These results highlight the protracted nature of stream biogeochemical and ecological recovery from chronic acidification of Northeastern watersheds, likely due to underlying lithology of the region and slow regeneration of base cations (Likens et al., 2021). Further, they demonstrate

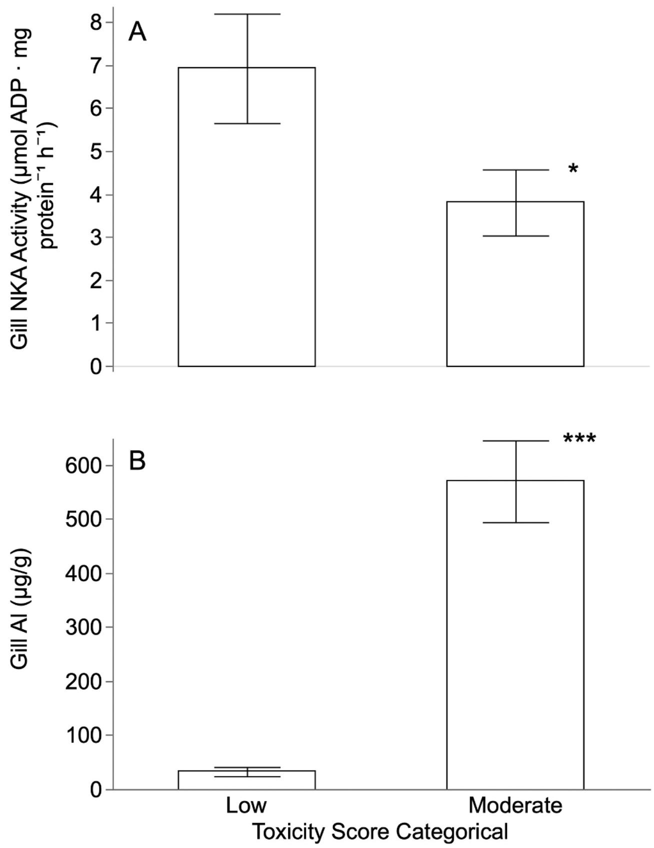

Fig. 7. Gill NKA activity (A) and gill aluminum (B) for Atlantic salmon smolts grouped by toxicity score categories of capture site. Low toxicity score category corresponds to pH above 5.5 and Al_i values below 30 µg/L where we expect to see low adverse acid-aluminum physiological effects. Moderate toxicity score values roughly correspond to pH values between 5.5 and 5.2 and Al_i at 30–60 µg/L. Error bars represent one standard error from the mean. Asterisk indicates significant difference between fish from low and moderate toxicity sites (A: $p = 0.043$; B: $p = 0.005$).

that the combined effects of acid and aluminum can be represented in an integrated biological indicator of exposure to and recovery from biogeochemical stressors.

Measurements of Al_i and pH in this study fall within the ranges reported by Fakhraei and Driscoll (2015) for surface waters in the Northeast, and by Bailey et al. (2019) for upland catchments in the White Mountains of New Hampshire. Our results also indicate that as sites exhibit greater Al_i concentrations, they tend to experience a greater absolute difference between annual mean Al_i and spring mean Al_i conditions, though the relative difference remains consistent. While both Fakhraei and Driscoll (2015) and Bailey et al. (2019) noted possible biological implications for the more extreme acid-aluminum conditions they report, this study takes those implications a step further by providing quantitative evidence for acid-aluminum impacts on wild aquatic organisms inhabiting low pH-high Al_i conditions. Spatially, the mosaic of different toxicity scores across the Merrimack, West River, Ammonoosuc, and Saco River Basins denotes mixed habitat suitability for stream fishes in upland catchments, with higher acid-aluminum conditions generally in higher elevation basins with smaller catchments (Figure S4; Table 3).

Table 3
Elevational distribution of different toxicity category sites.

Toxicity Score Category	Count	Elevation Range	Upstream Area Range
Low	7	66 m – 349 m	17.9 km^2 – 6,2850 km^2
Moderate	18	228 m – 480 m	10.9 km^2 – 288.4 km^2
High	4	554 m – 494 m	0.3 km^2 – 21.1 km^2

By representing a buffer capacity latent variable as a combination of Ca, Mg, and conductivity and toxicity score latent variable as a combination of Al_i and pH, we were able to confirm the hypothesis that Ca, Mg, and conductivity indirectly affect toxicity through an associated buffering ability. The inclusion of site elevation in SEM model iterations V3 and V4 also suggests that site elevation impacts toxicity score somewhat independently of buffer capacity. A possible cause for this relationship may be elevational gradients in soil organic material with more soil organics complexing with aluminum at lower elevations, thereby making less aluminum available in the Al_i form (Tam, 1987). While soil organic material (carbon, nitrogen, and phosphorous) has been shown to vary on elevational gradients in the Northeast (Bedison and Johnson, 2009), more specific testing would be needed to confirm if soil organic elevational gradients are connected to toxicity score elevational gradients. While none of the SEM model iterations provided a perfect model fit, likely due to the underlying variance in the dataset, the SEM approach allowed for direct comparison of causal structures. For environmental data which may have causal links, the results of this study emphasize how SEM modeling can be a valuable tool for differentiating the effects of different causal structures.

On the fish community level, both abundance and diversity trended negatively with toxicity score as hypothesized (Fig. 5). While sampling did encapsulate a range of toxicity scores (30–160), having 10 sample sites did restrict the resolution of toxicity score conditions, and large variability at moderate and high toxicity score levels somewhat limited what could be said about these relationships. The relationship between richness and toxicity score appeared to be slightly stronger than the relationship between abundance and toxicity score, indicating that richness may be a better community metric to quantify acid-aluminum toxicity than abundance. Studies on macroinvertebrates as indicators of stream health have detailed similar trends, with diversity indices acting as better indicators of pollution or chemically adverse conditions than abundance (Carlisle et al., 2007; Friberg et al., 2010). For the sites with the highest richness of species, up to 3 non-salmonid species were present, and sites with the lowest richness were those only occupied by brook trout. The effects of acid-aluminum toxicity have not been quantified as well in the 3 non-salmonid species in the sample set (*C. cognatus, R. atratulus, and C. commersonii*) yet the trend for them to only inhabit sites with lower toxicity scores reinforces the findings of Baker et al. (1996) that indicated a sensitivity of *C. cognatus* and *R. atratulus* to acidic conditions. However, it is also plausible that site elevation, habitat type, or habitat fragmentation could influence fish diversity on elevational gradients (Askeyev et al., 2017). In support of using biodiversity as a toxicity metric, Ryon (2011) concluded that fish species diversity, abundance, biomass, and community composition can all act as indicators of water quality pollution stress, but that recovery of these metrics varies with environmental gradients as well as pollution gradients. Since this study and Bailey et al. (2019) find significant, positive correlations between Al_i prevalence and altitude in Northeastern catchments, it is reasonable to assume that higher elevation sites may not recover fish diversity and abundance as quickly as lower elevation sites due to more severe and prolonged toxic conditions. But these conclusions must be qualified with understanding that at the highest elevation sites (near the boundaries of the catchments) there are inherent limits to teleost diversity as described by Askeyev et al. (2017).

As hypothesized, gill aluminum was positively related to toxicity score in both resident brook trout and Atlantic salmon populations. While the ability of the gill Al assay has been previously demonstrated for Atlantic salmon, this is the first study to show similar efficacy in wild populations of brook trout in Northeastern watersheds. The significant, positive relationship between toxicity score and gill aluminum for wild brook trout provides a basis for using wild brook trout gill aluminum biopsies as an indicator for acid-aluminum stress; this may be especially valuable given the widespread distribution of native and non-native brook trout across North America and in some cases beyond (MacCrimmon and Campbell, 1969; Hudy et al., 2008). A slight, negative relationship was observed between the condition factors of Atlantic salmon parr and brook trout and their respective site spring toxicity scores (Figure S2). However, gill aluminum content and NKA activity are likely more specific metrics of acid-aluminum physiological stress since condition is highly related to food availability of the local environment for stream dwelling salmonids (Ensign et al., 1990).

For Atlantic salmon smolts, gill aluminum was higher and gill NKA activity was lower in smolts inhabiting sites with moderate or high toxicity scores. The measurements of Atlantic salmon gill Al were slightly higher in this study than those reported in Nilsen et al. (2010), however, toxicity conditions were also more severe at some sites with lower pH values and higher Al_i concentrations than those used in the Nilsen et al. laboratory experiments. Compared to the McCormick et al. (2009a) Atlantic salmon smolt cage experiments conducted in the West River Basin, gill aluminum concentrations reported in this study are slightly lower. This difference may relate to the shorter time frame and more severe acid-aluminum conditions encountered during the cage study. Elevated gill aluminum levels and lower gill NKA activity in outmigrating smolts from sites with moderate toxicity scores indicates an impaired osmoregulatory ability. The decreased NKA activities reported in this study in the range of 1–3 μmol ADP * mg protein^{-1}*h^{-1} for moderate toxicity sites are consistent with levels found in McCormick et al. (2009b) that reduced Atlantic salmon smolt osmoregulatory function and concurrently increased other metrics of physiological stress.

These smolt physiological results have concrete implications for conservation; if recovery strategies for Atlantic salmon are designed to restart ocean-run populations, it is critical to focus habitat recovery, stocking, and migration corridor restoration efforts around watersheds with lower acid-aluminum toxicity scores. Smolts coming from watersheds with increased acid-aluminum toxicity are likely to have increased physiological stresses, which decreases Atlantic salmon migration success (Birnie-Gauvin et al., 2019; Thorstad et al., 2013). The relationships between gill NKA activity and toxicity score were not as strong for brook trout and Atlantic salmon parr, further emphasizing the sensitivity of smolt stage osmoregulation to acid-aluminum conditions (Figure S3). The conceptual model for gill aluminum toxicity effects tested in this study may be a valuable tool for selecting stream reaches for habitat restoration or protection (Fig. 8) and reinforces the conclusion made by Kelly et al. (2015) that gill biopsies can be used for acid-aluminum monitoring.

Based off the findings of prior research, it is possible that flow, DOC, and timing between low pH-high aluminum episodes and biological sampling may have affected characterization of toxic conditions and physiological stress measurements (Gensemer and Playle, 1999). To account for possible interannual differences in hydrologic conditions, year-to-year toxicity score comparisons showed no difference in mean toxicity score. As for measurement timing, no relationship was found between 7-day antecedent precipitation and gill aluminum. This implies that higher flows associated with high antecedent precipitation were not causing higher physiological measurements of toxicity and that time between biological sampling and high flow events was not confounding physiological metrics. DOC could affect both the biological and water quality fields quantified in this study; studies by Driscoll and Fakhraei

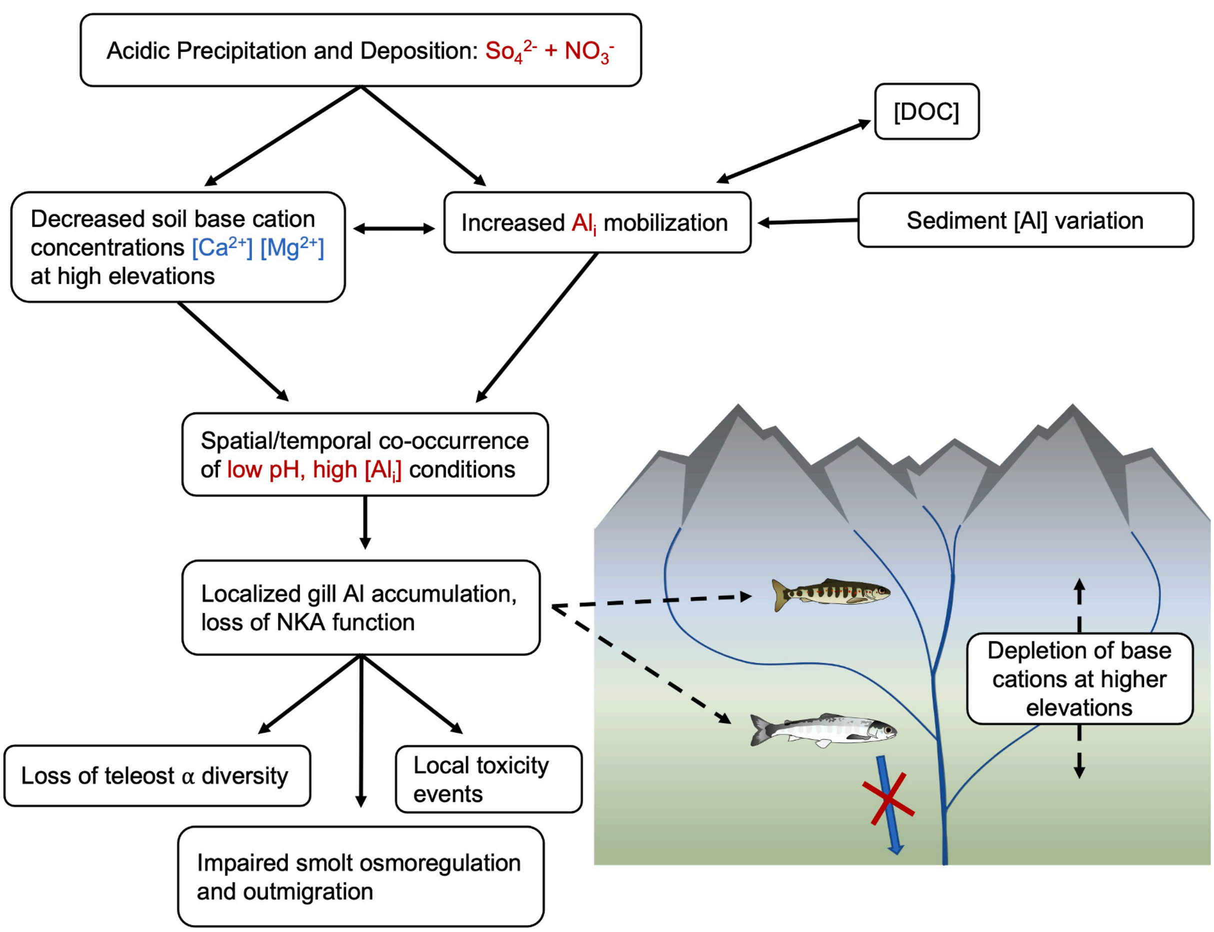

Fig. 8. Conceptual model of the acid-aluminum co-occurrence mechanism and resultant toxic effects. Toxic conditions are likely to occur at higher elevations in watersheds of the Northeast, where brook trout and Atlantic salmon parr occur more frequently than Atlantic salmon smolts. However, the osmoregulatory changes smolts undergo in spring make physiological effects of acid-aluminum conditions particularly potent.

(2015) and Driscoll and Schecher (1990) have both found distinct relationships between DOC, the availability of Al_i, and the occurrence of low pH conditions. If DOC was controlling the availability of Al_i at sites in the sample set, then there could be implications for which sites experienced high or low toxicity scores. However, all sites for this study were selected specifically to exclude high DOC environments, i.e., no low marshland areas and no tannin staining associated with higher DOC environments (Wetzel, 1992; Cory et al., 2006).

Further testing of the acid-aluminum toxicity stress in the Northeast could aim to quantify the biological relationships between acid-aluminum stress, DOC, and forest nutrient cycling. Testing biological toxicity metrics against DOC may elucidate to what extent DOC can alleviate acid-aluminum biological stress. In tropical freshwater environments, high DOC concentrations have been found to significantly reduce the effects of low pH-high Al_i conditions (Trenfield et al., 2012). Contemporary trends show DOC increasing in aquatic environments across the Northeast, so understanding DOC's interaction with acid-aluminum biological stress might help further illustrate biological effects into the future (Monteith et al., 2007; Brown et al., 2017). Finally, further investigation of differential timing of biological recovery can further our understanding of how forest health may affect nutrient cycling in upland catchments. One of the apparent signs of recovery from the widespread acid deposition of the 20th century is the reverse in forest decline associated with increased soil calcium reserves (Battles et al., 2014; Fuss et al., 2015). If calcium availability increases in upland catchments and tree species start utilizing calcium as a growth nutrient, it is possible that less calcium will be available in streams as more calcium is sequestered by forests. This could affect the timing of biological recovery such that forests appear to recover before aquatic species, but ultimately more investigation is necessary to quantify these dynamics.

Upland forest catchments of the Northeast are generally believed to be a climate change resiliency resource—for example, the Nature Conservancy has designated the upland catchments of New Hampshire and Vermont as priority habitat for biodiversity resources in the context of a rapidly changing global climate (Anderson et al., 2014). The results of this study indicate that acid-aluminum toxicity levels may still be high enough in many upland catchments to either limit the abundance of some aquatic organisms or impart negative physiological effects on more sensitive species. Given their additional importance as climate refugia, catchments with lower acid-aluminum toxicity scores may represent priorities for conservation of riverine biological resources in the Northeast. Since the data used in this study are over a decade old, more recent sampling across these watersheds may indicate if acid-aluminum stress has been alleviated at all over the last 13 years; the results of Bailey et al. (2019) indicate that some of the watersheds with categorically high toxicity scores in this study may now experience higher pH conditions.

For salmonids specifically, Northeastern populations face several challenges with climate change; warming waters, increased habitat fragmentation, and changing hydrologic regimes associated with climate change may all negatively affect salmonid populations (Chadwick et al., 2015). While acid-aluminum toxicity may not be as severe as it once was in the mid-20th century, its effects are still consequential for Northeastern salmonids and other acid-sensitive species. Site specific acid-aluminum toxicities are key considerations for conservation and recovery strategies for native species. As recently as 2015, Al_i concentrations in streams of Eastern Canada were found to exceed toxic thresholds (Sterling et al., 2020). Beyond the Northeast ecoregion, environmental damage from acid rain pollution is a contemporary issue. Industrialized countries outside of Europe and North America are still widely dependent on coal-fired powerplants, some of which are directly linked to acid rain in neighboring landscapes (Hao et al., 2001; Larssen et al., 2006). For regions with poorly buffered soils that are susceptible to concurrent low pH and high inorganic aluminum conditions, the integrated toxicity metric presented in this study may serve as a valuable tool for assessing the effects of acidic precipitation on vulnerable aquatic populations.

CRediT authorship contribution statement

Benjamin J. Zdasiuk: Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Celia Y. Chen:** Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization, Project administration. **Stephen D. McCormick:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Keith H. Nislow:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Joel G. Singley:** Validation, Investigation, Writing – review & editing, Visualization. **John T. Kelly:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.109480.

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