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Physiological Stress in Native Southern Brook Trout During Episodic Stream Acidification in the Great Smoky Mountains National Park

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Abstract Episodic stream acidification from atmospheric deposition is suspected to detrimentally impact native southern brook trout (*Salvelinus fontinalis*) in Great Smoky Mountains National Park (GRSM) headwater streams. To test the hypothesis that episodes of stream acidification cause physiological distress to native trout, caged fish at three sites were exposed to acid episodes during in situ bioassays conducted in June 2006 and March 2007. Stream pH decreased (>0.7 pH units) and total dissolved aluminum (Al_{TD}) increased (>175 μg/L) at all three sites during acid episodes in both bioassays. Whole-body sodium concentrations were significantly reduced (10–20%) following the acid episodes, when preceding 24-h mean pH values of 4.88, 5.09, and 4.87 and corresponding 24-h time-weighted average Al_{TD} concentrations of 210, 202, and 202 μg/L were observed. Lower whole-body sodium concentrations were correlated with elevated H^+ and Al_{TD} concentrations. Loss of sodium ions in native southern brook trout was consistent with physiological distress resulting from acid exposure reported in salmonids in other investigations. Further research is necessary to conclude whether acid episodes are responsible for extirpation of brook trout from headwater streams in the GRSM.

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The Great Smoky Mountains National Park (GRSM) in North America receives elevated rates of atmospheric acid deposition in the form of sulfur and nitrogen compounds (Weathers et al. 2006; Baumgardner et al. 2003; NADP 2006), which contribute to acidification of surface waters and soils (Driscoll et al., 2001). Although there have been reductions in acid deposition over the past two decades (NADP 2006), there has been little recovery of acidified stream waters and soils in the GRSM (Robinson et al. 2008) or other acid-sensitive areas in the eastern USA (Stoddard et al. 2003; Simonin et al. 2005; Webb et al. 2004). Acids enter poorly buffered streams of the GRSM through wet deposition, and from naturally occurring organic acids and accumulated dry deposition flushed from watersheds; and these inputs have been shown to cause pH depressions of 0.5–2.0 units (to as low as 4.1 units) during

stormflows (Cook et al. 1994; Deyton et al. 2008). Acid episodes refer to reductions of acid neutralizing capacity (ANC), accompanied by increased concentrations of H^+ and Al; when ANC ≤ 0, these are termed acidic episodes (Wigington et al. 1996). Episodic stream acidification can have damaging effects on the health of aquatic ecosystems and fish populations (Baker et al. 1996; Baldigo and Lawrence 2001) and is suspected to be a primary cause of native brook trout (*Salvelinus fontinalis*) extirpation in GRSM headwater streams.

Fish can die or experience sublethal physiological stress when exposed to acid conditions (Baldigo et al. 2007; MacAvoy and Bulger 2004; Woodward et al. 1991). The mechanism of acid stress in fish is generally recognized as disturbance of ion regulation that can lead to circulatory collapse and ultimately death (Booth et al. 1988). Increased H^+ concentrations (low pH) interfere with gill ion transport systems and diminish influx and greatly increase efflux of sodium (Grippo and Dunson 1996). In addition to low pH, the concentrations of monomeric inorganic aluminum (Al_{IM}) and calcium impact ion regulatory mechanisms (Baker and Schofield 1982; Hunn 1985). Aqueous aluminum is mobilized from the edaphic to aquatic phase during episodes of acidification (Driscoll et al. 1980). Inorganic fractions of aluminum increase with decreases in pH and predominate in waters below pH 5.0 (Driscoll and Postek 1995; Sullivan and Cosby 1998). Like H^+ toxicity, the primary mechanism of Al_{IM} toxicity is disturbance of gill ion transport (Booth et al. 1988) when pH is 4.2–4.8 (most severe at pH 4.5), and asphyxia when pH is 5.5–6.4 (most severe at pH 6.1) (Neville and Campbell 1988). Toxic metal ions, including Al_{IM}, compete with metal cations (particularly Ca^{2+}) on channel proteins in the gill surface (Di Toro et al. 2001) and facilitate greater loss of critical blood ions (Wood et al. 1990). In waters of low hardness, less dissolved Ca^{2+} is available and fish are more vulnerable to loss of ions induced by elevated Al_{IM} and H^+ (Cleveland et al. 1991; Ingersoll et al. 1990). Assessment of whole-body ion losses (e.g., whole-body sodium loss) from fish can be used to indicate levels of sublethal stress in fish exposed to low pH and other stressors (Dennis and Bulger 1995; Grippo and Dunson 1996; Matsuo and Val 2007).

Episodic acidification events generate water chemistry conditions that are different from those observed in persistent low-pH waters, and these conditions have different consequences on fish physiology. During an acidification event, stream pH can change rapidly and cause disequilibrium conditions to exist for many metal ions, including Al. Inorganic fractions of aluminum, mobilized when stream pH decreases, are toxic to fish (Driscoll et al. 1980; Exley et al. 1991; Gensemer and Playle 1999). Under rapidly increasing pH, aluminum polymerization can occur, both in stream water and within the gill microenvironment, which can result in acute toxicity (Poleo 1995). The consequences of episodic acidification events on fish are dependent on the extent of the change in pH, the duration of the event, and the time interval between successive acid episodes (Calta 2002; Gagen et al. 1993). Because the nature of episodic acidification events is complex and relatively site specific, investigations on the impacts of these events on wild fish physiology and population dynamics are limited (Kocovsky and Carline 2005; VanSickle et al. 1996). The southern strain of brook trout is exposed to acidification episodes in high-elevation streams, which may impact their survival in these habitats.

Although effects of H^+ and Al_{IM} toxicity on fish from stream acidification have been investigated extensively during the past four decades (Baker and Schofield 1982; Baldigo and Murdoch 1997; Calta 2002; Simonin et al. 1993), no studies have specifically investigated sublethal toxicity during episodic acidification in situ with the southern strain of brook trout. Much of this research was conducted under simulated conditions in laboratories, while field experiments involving trout have primarily focused on the survival and mortality of hatchery-reared northern strain of brook trout (Baldigo et al. 2007; MacAvoy and Bulger 1995). The southern strain of brook trout is genetically distinct from northeastern brook trout (McCracken et al. 1993) and may have different tolerances for physiological disturbance during acid episodes, as pH tolerances among strains of brook trout have been recognized (Hurley et al. 1989; Robinson et al. 1976). In addition to potential differences in tolerance to acid stress between northern and southern strain brook trout, determination of the effects of acidification on southern brook trout and initiation of remediation strategies are of considerable importance to GRSM fisheries managers. The GRSM, an international biosphere reserve, is the most visited national park in the USA, hosting over 9 million visitors a year (NPS 2007). It is imperative to protect and preserve stream quality and aquatic ecosystems in the GRSM from the impacts of acidic deposition.

The objective of the present study was to evaluate changes in physiological condition in wild southern strain brook trout before, during, and after in situ episodes of stream acidification and relate these changes to differences in stream chemistry. Physiological stress was quantified by whole-body sodium loss, a known indicator of acid stress in trout (Grippo and Dunson 1996). Test sites were selected on three remote headwater streams in the GRSM with no anthropogenic factors except atmospheric deposition of acids; two of these stream sites are known to have lost brook trout populations in the past 20 years. These study sites were concurrently studied to characterize changes in stream water chemistry from baseflow to stormflow, and to

investigate biogeochemical factors resulting in episodic stream acidification (Deyton et al. 2008).

Study Area

Water quality monitoring and native brook trout bioassays were conducted at three remote sites on streams in the GRSM in east Tennessee (Fig. 1): Middle Prong of the Little Pigeon River (N35°42.159', W83°20.067', 820 m), Ramsey Prong (N35°42.257', W83°19.770', 875 m), and Eagle Rocks Prong (N35°41.417', W83°19.183', 965 m). Ramsey Prong (third order) and Eagle Rocks Prong (third order) are tributaries of the Middle Prong (fourth order). Streambeds are dominated by boulder and cobble, and gradients of the stream channels increase with elevation (5–12%) (Larson and Moore 1985). Because of the in situ experimental design, no environmental control was possible; rather, the study design examined toxicological responses over a range of varying water chemistry conditions.

The watersheds selected for study are typical watersheds of the GRSM, and are characterized by steep gradients and thin sandy loams that provide poor buffering capacities. Of 90 stream sites that were sampled bimonthly throughout the GRSM from 1993 to 2002, 2.4% had ANC < 0 μeq/L, 54.2% had 0–50 μeq/L, 41.0% had 50–200 μeq/L, and 3.6% had greater than 200 μeq/L during baseflow (Robinson et al. 2008). The Middle Prong and Ramsey Prong

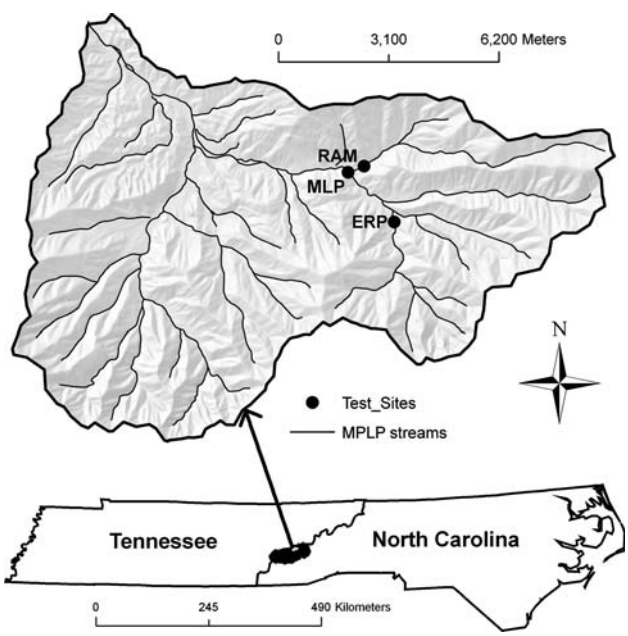

Fig. 1 Location of water quality monitoring and bioassay sites in the Great Smoky Mountains National Park: Middle Prong of the Little Pigeon River (MLP), Ramsey Prong (RAM), and Eagle Rocks Prong (ERP). Base from Seamless Data Distribution System, US Geological Survey, http://seamless.usgs.gov

are extremely sensitive to acidification (baseflow ANC = 0–20 μeq/L); and Eagle Rocks Prong is acidic (baseflow ANC < 0) (Deyton et al. 2008). The watersheds are primarily underlain by metamorphosed sandstone, siltstone, and shale (King et al. 1968), and are covered by upland coniferous and deciduous forest undisturbed in the past century. The climate of GRSM is perhumid mesothermal with seasonal temperature variation and precipitation distributed throughout the year (Busing 2005). From 1928 to 2007, the mean annual temperature was 13.2°C and mean annual precipitation was 141 cm at the Gatlinburg 2 SW weather station, located approximately 30 km west of study sites at an elevation of 445 m (SERCC 2007).

Stream chemistry was characterized at the three study watersheds from March 2006 through May 2007 to better understand potential factors that contribute to rapid drops in pH and ANC during acid episodes (Deyton et al. 2008). ANC and pH depressions were observed during all stormflows at the three study sites, ranging from 9.2 to 26.3 μeq/L and 0.65 to 1.27 pH units, respectively. Increased concentrations in sulfate, nitrate, and organic acids were also observed during all stormflows. ANC contribution analysis indicated sulfate was the dominant contributor to ANC depressions among all three watersheds, which provides evidence these streams were impacted by acidic deposition. Nitrate, organic acids, and to a lesser extent base cation dilution were found to be contributors to ANC depressions varying in dominance by watershed and season. Nitrate was a major contributor during spring and early summer months for the Ramsey Prong site compared with the other two study sites. Although average pH declines among the three study sites were similar (0.81–0.92 pH units), average ANC declines were generally larger for Ramsey Prong and Eagle Rocks Prong sites (22 and18 μeq/L, respectively) than for the Middle Prong site (13 μeq/L), which was larger in drainage area. Interestingly, it was observed that significantly larger pH depressions occurred for peak stormflow discharges preceded by long, dry periods. Deyton et al. (2008) provided the necessary background data on water chemistry to support an ecological interpretation of the current study's findings.

The three sites were selected for study on the basis of whether native brook trout had been extirpated at that stream location or not, and remoteness (minimum of 2 km from roads), with no current anthropogenic impacts except acid deposition. The Middle Prong is currently, and has been historically, inhabited by native brook trout (Bivens et al. 1985; King 1939; GRSM fish database), and drains waters from Ramsey Prong, Eagle Rocks Prong, and two other third-order streams that support brook trout. Eagle Rocks Prong supported 4.4 km of allopatric brook trout as

recently as 1985 to an elevation of 1,378 m (Bivens et al. 1985). Currently, Eagle Rocks Prong only supports 0.1 km of brook trout to an elevation of 920 m (GRSM fish database). In 1985, Ramsey Prong supported 1.0 km of brook trout to an elevation of 914 m (Bivens et al. 1985). Now, only 0.2 km of Ramsey Prong supports brook trout to an elevation of 821 m (GRSM fish database).

Methods

Water Chemistry Collection and Analysis

Conductivity, pH, turbidity, temperature, and stage height were monitored at the three stream sites from April 2006 through December 2007 using YSI® 6920 data sondes, in which parameters were measured at 15-min intervals. Automated water samplers (ISCO® 6712 units) were used to collect samples during stormflows. Grab samples were obtained monthly and before and after storm events. In addition, precipitation was collected at one open site and three throughfall sites to quantify inputs to the system (Deyton et al. 2008).

At the University of Tennessee, water samples were analyzed for the following parameters: conductivity [US Environmental Protection Agency (EPA) method 150.1], pH (US EPA method 120.1), ANC (Mantech™ PC-Titration Plus); sulfate (SO_4^{2-}), nitrate (NO_3^{2-}), and ammonium (NH_4^+) (Dionex™ IC, Standard Methods 4110); Al, Ca, Cu, Fe, K, Mg, Mn, Na, Si, and Zn [Thermo-Electron™ Intrepid II inductively coupled plasma atomic emission spectroscopy (ICP-AES), vacuum-filtered (0.45 μm) and acidified, US EPA SW-846 method 6010B]. Total dissolved aluminum (Al_{TD}) reported is acidified and filtered (0.45 μm).

Brook Trout In Situ Bioassays

In situ bioassays of adult native southern brook trout (length > 100 mm) were conducted at the three sites in June 2006 and March 2007. All trout used in the bioassays were collected on the Middle Prong (within 1 km of the test sites) in low-conductivity waters (14–18 μS/cm, 5.4–5.6 pH units, 3–7 μeq/l ANC, 7–15°C) using standard electroshocking techniques (AC, 600–650 V, 1–1.5 A; Reynolds 1996). Test fish ($n = 120$) were randomly distributed and transported (less than 1 km) in aerated backpack tanks to the three sites (40 trout per site) for each bioassay.

Test fish were held at each stream site in 20-L polyethylene cylindrical test containers following the approach of Johnson et al. (1987). To ensure adequate water exchange, 6-mm holes were drilled with a spacing of approximately 40 mm on the bottom and sides of test containers. Openings of test containers were covered with 2-mm-mesh fiberglass screening. Four containers were used at each site and placed in stainless-steel cages ($61 \times 61 \times 61$ cm^3). The cages were constructed using 25.4-mm stainless-steel tubing for the frame; 25.4 mm stainless-steel screen was welded to all sides of the frame. Test cages were placed behind large boulders in recirculation pools to prevent high velocities coming into contact with cages during stormflows. Cage design and in-stream position ensured low velocities in cages at all flow stages. At baseline flows, test containers were not completely submerged to ensure trout had access to the water surface for buoyancy regulation and food. Trout were not fed during the test period because starvation and other caging stresses are minimal during a 20-day testing period (VanSickle et al. 1996).

The in-stream bioassays were conducted for 10 days and included a period prior (2–6 days) to the acid stormflow episode, the stormflow (1–2 days), and a period of recovery (2–6 days) after the stormflow. Fish were sampled one day following the collection of trout to allow recovery from electroshocking and transport. Trout were randomly sampled from each of the four test containers at each site at the beginning of the test period and appropriately before and after acid episodes. All sampled fish were anesthetized and killed, then placed in individual plastic bottles (preweighed and acid-rinsed) and transported to the laboratory. Gill samples were prepared in the field following procedures outlined in chapter 13 of the *NWFHS Laboratory Procedures Manual* (Mumford 2004). Gill sections were excised; and then preserved whole in 10% neutral buffered formalin (10% NBF) for histology.

In the laboratory, all trout samples were immediately put in a cold room (4°C) and within 1 week were oven-dried at 70°C for 5–7 days. Wet mass was determined for all trout sampled ($n = 96$, 6.29–63.77 g, median 22.06 g). Following the procedure of Grippo and Dunson (1996), dried trout were put into amounts of trace-metal-grade nitric acid, appropriately diluted with deionized water, and vacuum-filtered through 0.45-μm filter prior to ICP-AES analysis of whole-body sodium. To account for differences in trout mass, whole-body sodium was normalized by dividing by wet mass of trout samples (Grippo and Dunson 1996). Whole-body sodium concentrations are reported as Na (mg) per trout wet mass (g).

Data Analysis

Analysis of variance (ANOVA) was used to model differences in whole-body sodium concentrations among sites and dates, and the Tukey–Kramer honestly significant difference (HSD) test was used to determine differences

among treatment means. Significant differences were reported when $\alpha < 0.05$ for both ANOVA and Tukey–Kramer HSD tests unless otherwise specified. Least squares regressions were performed on whole-body sodium concentrations with the independent variables mean H^+ concentration ($n = 96$, determined for the 24-h preceding fish sampling) and Al_{TD} concentration. Aluminum used in the regression model consisted of: (1) measured Al_{TD} in grab samples at time of trout collection during baseflow conditions ($n = 15$), (2) 24-h time-weighted average of measured Al_{TD} from stormflow samples preceding trout collection after storm events ($n = 5$), (3) estimates from regressions of Al_{TD} by conductivity when baseflow samples were not collected ($n = 3$), and (4) 24-h time-weighted average of estimated Al_{TD} preceding trout collection following stormflow at Eagle Rocks in June 2006 ($n = 1$). Regression models to estimate Al_{TD} were site specific and were significant ($p < 0.0001$): Middle Prong, $Al = -0.418039 + 0.0312671 \times$ conductivity ($R^2 = 0.75$); Ramsey Prong, $Al = -0.341771 + 0.0280474 \times$ conductivity ($R^2 = 0.94$); Eagle Rocks Prong, $Al = -0.942562 + 0.0628421 \times$ conductivity ($R^2 = 0.75$). Conductivity was used because of agreement between laboratory and field measurements. The 24-h mean conductivities from sonde measurements before trout were collected were put into equations to produce final estimates of Al_{TD}. The JMP® platform (SAS® Institute Inc.) was used for all statistical analyses and plots.

Results

Single episodes of stream acidification were captured during both the June 2006 and March 2007 bioassays. Stream pH decreased and Al_{TD} increased at all sites during these episodes (Figs. 2–4). Means and standard deviations were calculated for selected chemical constituents from 2006–2007 baseflow grab samples at the three sites (Table 1). Selected chemical constituents from stormflow water samples are presented in Table 2. All trout survived and were able to maintain equilibrium and normal swimming behaviors at the three sites during the test periods. There was no evidence of increased flow rates through cages during stormflows, which were sheltered from increased water discharge by the structure of cages in recirculation pools. Changes in gill morphology were not observed, and fish recovered normal sodium levels within 3 days after cessation of the acid episodes. Recovery of whole-body sodium provided evidence that cage stress was not responsible for sodium loss. After exposure to acidic episodes in the March 2007 bioassay, coloration of trout appeared faded at all sites.

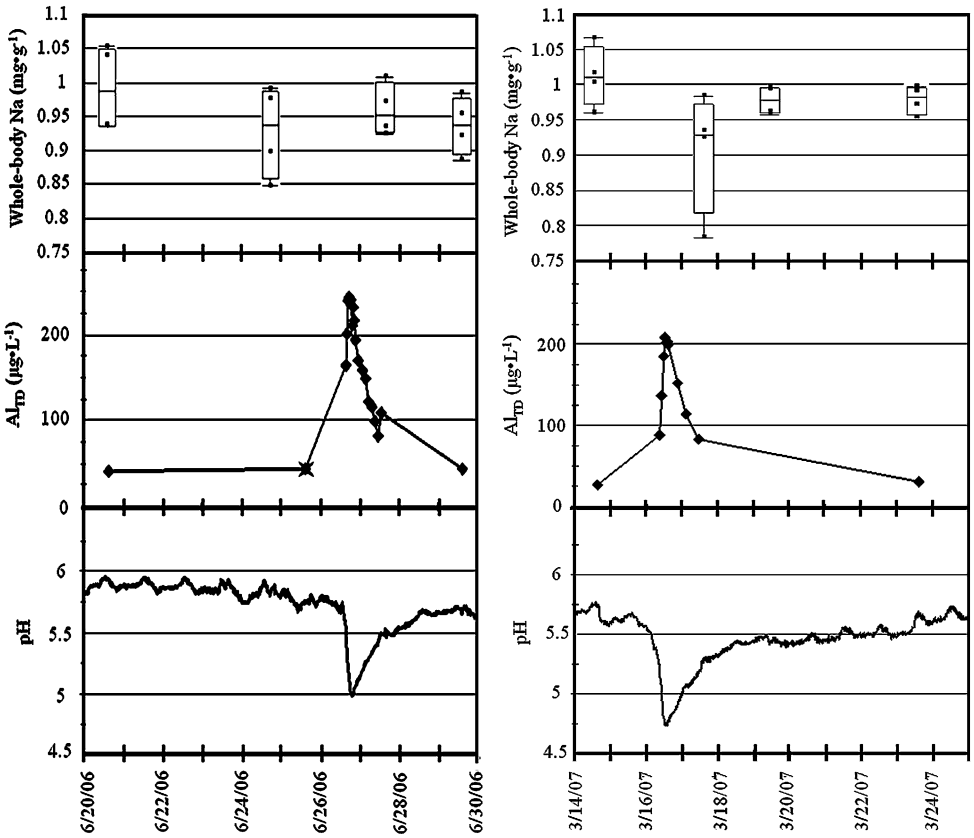

Fig. 2 Whole-body sodium, Al_{TD}, and pH during June 2006 and March 2007 bioassays at the Middle Prong of the Little Pigeon River site, Great Smoky Mountains National Park

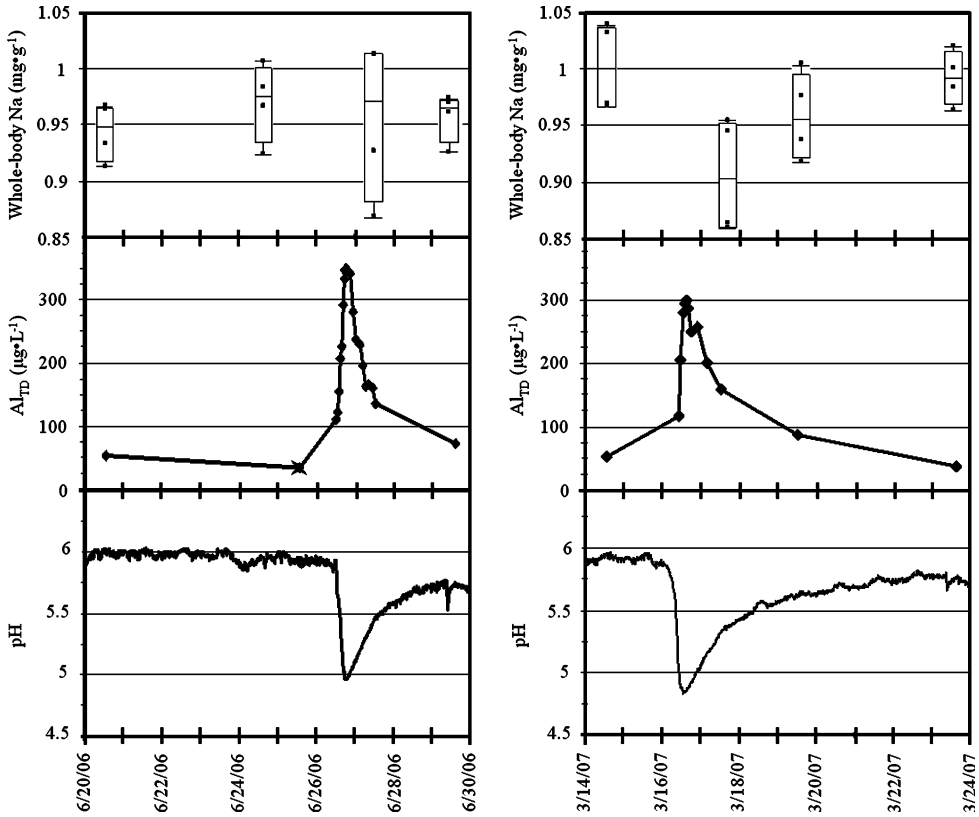

Fig. 3 Whole-body sodium, Al_{TD}, and pH during June 2006 and March 2007 bioassays at the Ramsey Prong site, Great Smoky Mountains National Park

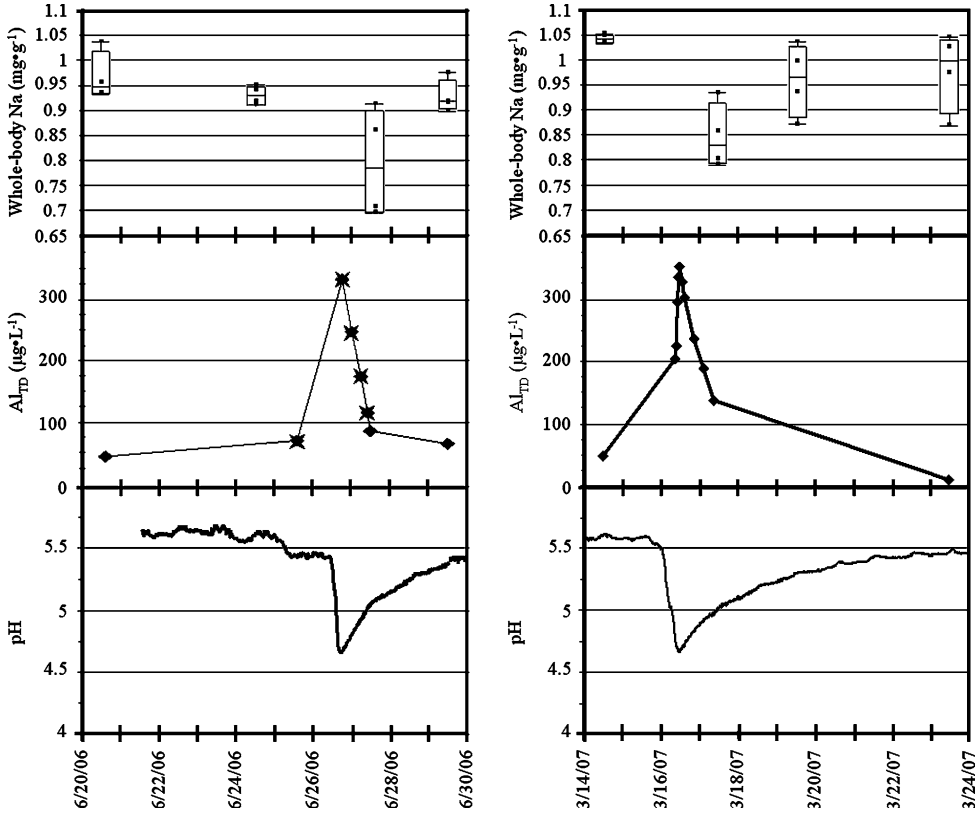

Fig. 4 Whole-body sodium, Al_{TD}, and pH during June 2006 and March 2007 bioassays at the Eagle Rocks Prong site, Great Smoky Mountains National Park

Table 1 Mean values (SD) of selected chemical constituents in baseflow grab samples from test sites in the Middle Prong of the Little Pigeon Watershed, Great Smoky Mountains National Park for the period of April 2006 to July 2007

Chemical parameter	MLP[a] ($n = 22$)	RAM[b] ($n = 22$)	ERP[c] ($n = 22$)
Laboratory pH	5.52 (0.43)	5.44 (0.41)	5.19 (0.40)
ANC (μeq/L)	6.2 (9.3)	3.8 (7.1)	−2.1 (5.1)
NO_3^- (μeq/L)	43.1 (8.4)	35.2 (9.3)	50.1 (6.8)
SO_4^{2-} (μeq/L)	44.7 (4.3)	39.7 (4.5)	51.5 (5.2)
Cl^- (μeq/L)	11.6 (1.8)	11.6 (1.9)	12.0 (2.6)
NH_4^+ (μeq/L)	0.4 (0.2)	0.3 (0.2)	0.8 (1.4)
Na^+ (μeq/L)	27.9 (3.7)	26.9 (5.0)	26.3 (4.2)
K^+ (μeq/L)	10.3 (3.1)	11.4 (2.9)	8.1 (2.5)
Mg^{2+} (μeq/L)	25.2 (2.3)	18.3 (2.1)	30.4 (5.5)
Ca^{2+} (μeq/L)	53.1 (5.5)	43.5 (6.2)	53.8 (4.4)
Al_{TD} (μg/L)	71 (58)	102 (75)	76 (52)

[a] MLP = Middle Prong of the Little Pigeon River

[b] RAM = Ramsey Prong

[c] ERP = Eagle Rocks Prong

The mean whole-body sodium concentration was 0.99 mg/g [standard deviation (SD) 0.05 mg/g, $n = 24$] for trout collected on the first sampling date in both bioassays (Figs. 2–4). Considering all brook trout data from both bioassays, whole-body sodium concentrations were significantly lower (ANOVA $p < 0.001$, Tukey–Kramer HSD $\alpha = 0.01$) immediately following acid episodes (mean 0.89 mg/g, SD 0.09 mg/g, $n = 24$) than in trout collected both before the episode and 2–6 days after the episode.

Sodium losses were greater in trout that were exposed to more acidic conditions. Whole-body sodium concentrations were significantly reduced from the prestorm condition following the acidic episodes (ANC < 0) at Eagle Rocks Prong in the 2006 and 2007 bioassays (Fig. 4, ANOVA $p < 0.001$, Tukey–Kramer HSD $\alpha = 0.01$) and at Ramsey Prong in the 2007 bioassay (Fig. 3, ANOVA $p = 0.0127$; Tukey–Kramer HSD $\alpha = 0.05$) when pH was less than 5.1 units and Al_{TD} exceeded 200 μg/L for 24 h (Table 2). Body sodium concentrations were not significantly reduced following the acid episodes at Ramsey Prong in 2006 or at Middle Prong in 2006 and 2007

Table 2 Selected chemical constituents from samples captured during stormflowsin bioassays and 2006–2007 stormflows at test sites in the Middle Prong of the Little Pigeon Watershed, Great Smoky Mountains National Park

	Chemical parameter	June 2006 bioassay	March 2007 bioassay	2006–2007 storms
Middle Prong of the Little Pigeon River	Minimum sonde pH	4.98	4.74	4.13
	Increase in stage (m)	0.40	0.44	0.85 (max.)
	Number of samples	17	10	119
	Al_{TD} (μg/L)	173(54)[a]	157 (49)[a]	167 (68)[a]
	Max. Al_{TD} (μg/L)	243	208	365
	Ca^{2+} (μeq/L)	55.1(4.7)[a]	65.4 (4.4)[a]	58.2 (7.0)[a]
	Mg^{2+} (μeq/L)	25.4(1.7)[a]	31.4 (1.5)[a]	28.0 (3.9)[a]
	Na^+ (μeq/L)	22.5(1.2)[a]	23.2 (1.3)[a]	22.9 (3.4)[a]
	ANC (μeq/L)	5.5(3.4)[a]	−8.9 (3.8)[a]	−3.8 (7.3)[a]
Ramsey Prong	Minimum sonde pH	4.97	4.83	4.23
	Increase in stage (m)	0.26	0.21	0.92 (max.)
	Number of samples	18	10	76
	Al_{TD} (μg/L)	225(81)[a]	234 (63)[a]	188 (95)[a]
	Max. Al_{TD} (μg/L)	350	300	433
	Ca^{2+} (μeq/L)	50.7(8.1)[a]	61.8 (4.9)[a]	54.0 (8.9)[a]
	Mg^{2+} (μeq/L)	20.9(2.3)[a]	25.8 (2.0)[a]	22.7 (3.5)[a]
	Na^+ (μeq/L)	24.3(1.4)[a]	23.5 (1.3)[a]	24.7 (3.9)[a]
	ANC (μeq/L)	3.1(5.7)[a]	−13.3 (5.4)[a]	−4.5 (13.9)[a]
Eagle Rocks Prong	Minimum sonde pH	4.66	4.67	4.33
	Increase in stage (m)	0.44	0.43	0.99 (max.)
	Number of samples	[b]	10	93
	Al_{TD} (μg/L)	NC[b]	260 (72)[a]	242 (102)[a]
	Max. Al_{TD} (μg/L)	NC [b]	353	447
	Ca^{2+} (μeq/L)	NC [b]	57.3 (3.9)[a]	55.9 (5.8)[a]
	Mg^{2+} (μeq/L)	NC [b]	37.2 (1.3)[a]	33.7 (3.9)[a]
	Na^+ (μeq/L)	NC [b]	18.6 (0.77)[a]	19.2 (2.3)[a]
	ANC (μeq/L)	NC [b]	−18.6 (3.7)[a]	−12.9 (7.3)[a]

[a] Mean (SD)

[b] NC, data not collected due to equipment failure

(Figs. 2 and 3). Additionally, immediately following the stormflow in June 2006, body sodium in trout samples from Eagle Rocks Prong was significantly lower (ANOVA $p = 0.0273$; Tukey–Kramer HSD $\alpha = 0.05$) than in trout from the other two sites, which did not experience as severe acidic conditions.

Regression models, of whole-body sodium concentrations (Figs. 5 and 6) by preceding 24-h [H^+] (body Na mg/g = 1.0006 – 0.0118 × 24-h H + μeq/L, $n = 96$, $p < 0.0001$, $R^2 = 0.36$) and by corresponding 24-h time-weighted average Al_{TD} concentrations (body Na mg/g = 0.9952 – 0.0049 × Al_{TD} μg/L, $n = 96$, $p < 0.0001$, $R^2 = 0.24$), demonstrate that reduced whole-body sodium concentrations were highly correlated with elevated H^+ and Al_{TD} concentrations. The lowest whole-body sodium concentrations occur when H^+ and Al_{TD} were both elevated.

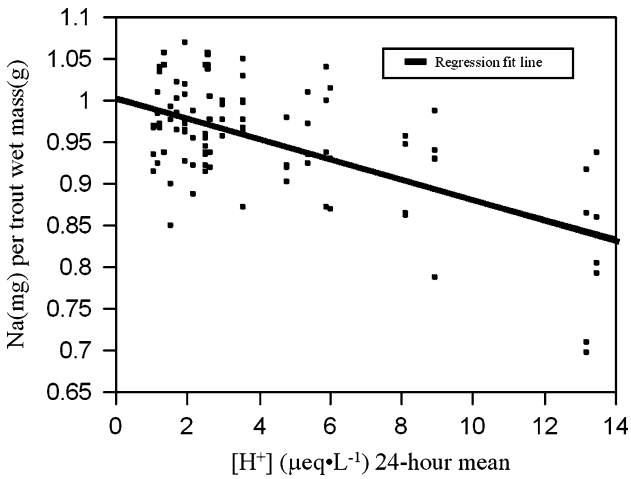

Fig. 5 Least-squares regression of whole-body sodium by hydrogen ion concentration

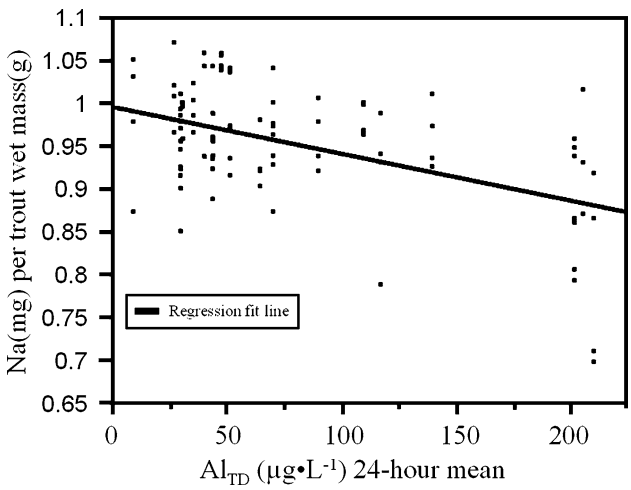

Fig. 6 Least-squares regression of whole-body sodium by Al_{TD} concentration

Discussion

These results demonstrate that, during periods of acute stream acidification from acidic deposition in the GRSM (Deyton et al. 2008), native brook trout lose sodium in a manner that is consistent with physiological distress associated with low pH and elevated Al concentrations (Neff 2007). Whole-body sodium levels were significantly reduced from the prestorm condition only when the 24-h time-weighted average Al_{TD} concentrations exceeded 200 μg/L and pH was less than 5.1. Under these conditions, trout experienced 10–19% loss of whole-body sodium, when monomeric forms of Al are expected to predominate (Johnson et al. 1981). This result is consistent with findings by Gagen and Sharpe (1987) who documented 30% whole-body sodium loss in hatchery-reared northern strain brook trout for a 24-h in situ exposure to 4.8 median pH and 0.6 mg/L median Al_{TD}.

Loss of sodium can lead to fish mortality (Hesthagen et al. 1999) or chronic sublethal stress (Allin and Wilson 1999; Dussault et al. 2005). In the present investigation, fish mortality did not occur; however, the acid episodes captured during the exposure were not the most severe that have been observed at these study sites; minimum pH values at the Middle Prong, Ramsey Prong, and Eagle Rocks Prong sites were 4.13, 4.23, and 4.33, respectively, and had associated Al_{TD} in excess of 300 μg/L (Table 2). Wood and McDonald (1982) indicated that 60–70% loss of sodium and chloride ions is associated with mortality of rainbow trout exposed to acid exposure; this occurred after an acid exposure of 4.0–4.5 pH units. Based on the pH data from June 2006 through March 2007 (Fig. 7), pH conditions at Ramsey Prong reached levels acutely toxic to brook trout (pH < 4.5) five times during the last 6 months of 2006.

The consequences of sublethal sodium loss in trout from acid episodes include downstream immigration (Gagen

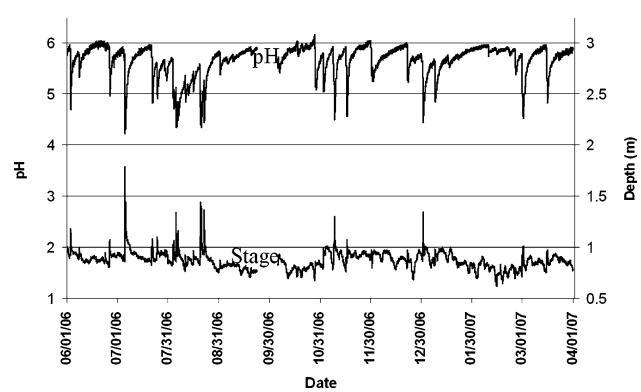

Fig. 7 Stage and pH at the Ramsey Prong water quality site in the Great Smoky Mountains National Park from June 2006 through March 2007

et al. 1994), less successful reproduction (Kaesar and Sharpe 2001), impaired swimming performance (Wilson and Wood 1992), and decreased growth (Cleveland et al. 1991; Mount et al. 1988). The duration, magnitude, chemical composition, timing, and spatial distribution of acid events influences the survival and stress response of trout (Baldigo and Murdoch 1997). Episodes of stream acidification may cause native brook trout to migrate downstream, and physical barriers such as waterfalls and cascades limit recolonization in high-elevation headwater streams. Carline et al. (1992) speculates that adult survival from acid episodes is dependent on watershed and channel morphological conditions that form chemical refugia from low pH during stormflows. However, if chemical refugia do not exist, adult brook trout would spend more energy moving downstream to higher-pH waters, followed by upstream return to occupy headwater reaches without food competition from rainbow trout in lower watershed reaches (Larson and Moore 1985). Poor energetics would reduce fish fecundity over time depending on watershed condition. Adult reproduction may be affected at pH and aluminum concentrations observed in this study (Fiss and Carline 1993). Early-life-stage trout may experience greater toxic effect to acid stress as it has been documented that early life stages of trout are more sensitive than older ones (Baldigo and Lawrence 2001) and exhibit higher sodium turnover rates (Grosell et al. 2002). Although this study evaluated adult trout, it is probable that fry and yearlings impacted by acid episodes can lead to low recruitment and eventual removal from acid-sensitive headwater streams.

Trout populations in higher elevation streams may be at greater risk to acid episodes than trout in streams at lower elevation. A gradient exists in the GRSM such that, with increasing elevation, streams have less buffering capacity and resultant lower baseflow and stormflow pH (Robinson et al. 2008). Native brook trout inhabit 55 km of GRSM streams below 914 m (GRSM fish database), including the Middle Prong (site elevation 820 m); trout in Ramsey Prong (site elevation 875 m) have extirpated. Above 914 m, there are 255 km of brook trout streams to elevations exceeding 1,500 m (GRSM fish database); trout in Eagle Rocks (site elevation 965 m) have extirpated. With a majority of brook trout populating higher elevation streams more susceptible to acidification, it is critical to understand the sensitivity of native brook trout to stream acidification, in relation to baseflow and stormflow water quality and watershed characteristics, in streams throughout the GRSM.

Understanding the ecological processes that lead to brook trout extirpation in some watersheds but not in others is complex, which requires knowledge of: (1) spatial and temporal patterns of episodic stream acidification related to watershed basin area and elevation, geology, and vegetation; (2) life history patterns specifically related to spawning, fry emergence, summer rearing, winter refugia, and home range; and (3) toxicological sensitivity and dose–frequency response of trout related to life stage. Deyton et al. (2008) found that acid episodes varied greatly in peak proton concentration and frequency among the three study sites, particularly in the headwater watersheds of Eagle Rocks and Ramsey Prong. Concentration, duration, and frequency (CDF) of stream pH over time per watershed must be taken into account in relation to life stage (Robinson and Roby 2006). Although this study was only designed to examine the effects of acid episodes on adult brook trout, it importantly found physiological stress occurred in the southern strain. This finding provides the necessary information to develop new hypotheses related to ecological responses from water quality impacts, such as CDF endpoints, that lead to brook trout population declines, and interrelationships with other environmental stressors, i.e., extreme high- and low-flow hydrological events and competition with rainbow trout.

To protect and preserve the quality of brook trout streams in the GRSM and southern Appalachian region, it is imperative to develop critical loads for acid deposition by identifying watershed-scale processes associated with stream acidification, determining system responses to atmospheric deposition, and evaluating impacts to biota (Porter et al. 2005; Sullivan et al. 2007). This study provides a critical ecological response component to ongoing studies in the GRSM to determine critical loads of atmospheric acid pollutants that enter the park and the southern Appalachian region.

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