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Physiological Stress in Native Brook Trout (*Salvelinus Fontinalis*) During Episodic Acidification of Streams in the Great Smoky Mountains National Park

Keil Jason Neff
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Keil Jason Neff entitled "Physiological Stress in Native Brook Trout (*Salvelinus Fontinalis*) During Episodic Acidification of Streams in the Great Smoky Mountains National Park." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Environmental Engineering.

John S. Schwartz, Major Professor

We have read this thesis and recommend its acceptance:

R. Bruce Robinson, Theodore B. Henry, Randall W. Gentry

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Thesis
Presented for the
Master of Science
Degree
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Keil Jason Neff
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Abstract

Episodes of stream acidification are suspected to be the primary cause of the extirpation of native southern brook trout (Salvelinus fontinalis) from six headwater streams in the Great Smoky Mountains National Park (GRSM). During periods of increased flow from storm events, stream pH can drop below 5.0 (minimum of 4.0) for 2-days or longer. To provide evidence that native brook trout are impacted by stream acidification, in situ bioassay experiments were conducted. Changes in stream water chemistry and brook trout physiology were determined during a 36-hour acidic episode at three remote headwater stream sites in the Middle Prong of the Little Pigeon River watershed.

Conductivity, pH, turbidity, stage height and temperature were monitored continuously; and water samples were collected for laboratory analyses (metals, cations, anions, ANC). Native brook trout were put in cages at the three sites and fish were sampled before and after the acid storm event. Physiological stress in brook trout was assessed by measuring whole-body sodium in individual fish sampled before and after the stormflow, and evaluating whole-body sodium loss as a response to acid conditions.

The pH decreased at all three sites during the acidic episode. Stream pH dropped to approximately 5.0 at two sites and 4.66 at the third site. Prior to the storm, there was no difference in the whole-body sodium concentrations in trout between the three sites. Following the storm event, in trout from the site that experienced the lowest pH, whole-body sodium levels were reduced significantly relative to a) the pre-storm condition and b) trout from the other sites.

Results demonstrate that stream acidification can negatively affect native southern brook trout physiology in the GRSM under actual field conditions. Trout lose the ability to regulate critical blood ions, as exemplified by a loss of whole-body sodium, when stream pH was less than 5.0 for 20 hours. Loss of sodium is an important indication of physiological stress in fish exposed to acid waters. This observation supports the hypothesis that episodic acidification of streams could be limiting native brook trout from occupying headwater streams in the GRSM.

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Chapter I: Introduction

The Great Smoky Mountains National Park (GRSM) receives some of the highest rates of atmospheric acid deposition in the U.S. in the form of SO42- and NO32- (Johnson 1992; Shubzda et al. 1995), which is linked to emissions from regional coal fired power plants (Chestnut and Mills 2005). A major concern with acid deposition is storm events can cause stream pH in the GRSM to drop to as low as 4.0 pH (Robinson 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 (Robinson et al. 2004). Acidic stormflow episodes are periods of low pH, low acid neutralizing capacity (ANC) and elevated aluminum (Wigington et al. 1996) in streams experiencing increased flows from precipitation. Although effects have not been observed directly, acidic episodes are suspected to be the primary cause of native brook trout (Salvelinus fontinalis) extirpation in six headwater streams in the GRSM (Moore and Kulp 2006).

Fish can die or experience sub-lethal physiological stress when exposed to acid conditions. The mechanism of acid stress in fish is generally recognized as a disturbance of ion regulation (Packer and Dunson 1970; 1972) that can lead to circulatory collapse and ultimately death (Booth et al. 1988). Hydrogen ions (low pH) interfere with gill ion transport systems and diminish influx and greatly increase efflux of sodium (Booth et al. 1988; Grippo and Dunson 1996). In addition to low pH, the concentrations of monomeric inorganic aluminum (Al_{IM}) and calcium impact loss of body sodium (Baker and Schofield 1982; Hunn 1985). Calcium bound to sites on the gills enhances membrane integrity and provides higher resistance to loss of ions in fish exposed to acid waters (Wood et al. 1990). Monomeric aluminum can displace calcium at gill binding sites causing greater loss of internal ions (Wood et al. 1990), and waters with low calcium and elevated Al_{IM} and H+ have greater potential for acid stress in fish (Cleveland et al. 1991; Ingersoll et al 1990). Whole-body sodium loss can be assessed to test sub-lethal stress of fish exposed to low pH and other chemical factors (Dennis and Bulger 1991; Grippo and Dunson 1996).

The effects of acidic episodes on trout have been investigated in the laboratory and in the field. Gagen and Sharpe (1987b) found brook trout to lose 40% of whole-body sodium in 24-hours from exposure to 5.0 pH and total dissolved aluminum (Al_{TD}) greater than 0.3 mg/L under laboratory conditions. In in situ experiments, 10-86% mortality was observed in juvenile brook trout exposed to acid episodes with 4.7 pH and 0.2 mg/L of Al_{IM} (Baldigo and Murdoch 1997). Sub-lethal physiological stress, exhibited by 30% whole-body sodium loss in brook trout in situ, was documented for a 24-hour exposure to 4.8 median pH and 0.6 mg/L median Al_{TD} (Gagen and Sharpe 1987a).

Previous research on the effects of acidification on brook trout has considered hatchery-raised northeastern strain in streams of the northeastern U.S. (Gagen and Sharpe 1987a). The southern strain of native brook trout in the GRSM is genetically distinct from northeastern brook trout (McCracken et al. 1993) and determining the physiological response of wild southern strain of brook trout to acid stress is of considerable importance to management of this species in the GRSM. Also, unique in the present study, in situ, sub-lethal responses of the southern strain of brook trout were tested at three remote sites with minimal anthropogenic effects. The objective of the present study was to evaluate changes in whole-body sodium in native brook trout during an acidic stormflow episode in the GRSM and relate these changes to differences in stream chemistry.

Chapter II. Materials and Methods

Study Area

Water quality monitoring and native brook trout bioassays were conducted at three remote sites on streams in the GRSM in east Tennessee: Middle Prong of the Little Pigeon River, Ramsey Prong, and Eagle Rocks Prong (Figure 1). Ramsey Prong and Eagle Rocks Prong are tributaries of the Middle Prong. The Middle Prong is a fifth-order mountain stream; Ramsey Prong and Eagle Rocks Prong are fourth-order mountain streams. The streambeds are dominated by boulder and cobble and the gradients of the stream channels increase with elevation (5-12%; Larson et al. 1995).

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. The Middle Prong and Ramsey Prong have baseflow ANC values in the range of 0-50 μeq/L, which the US EPA classifies as extremely sensitive to acidification; Eagle Rocks Prong has baseflow ANC < 0, which the USEPA classifies as acidic. The watersheds are primarily underlain by metamorphosed sandstone, siltstone and shale, and are covered by upland coniferous and deciduous forest undisturbed in the past century (King 1968). The climate of GRSM is perhumid mesothermal with seasonal temperature variation and precipitation distributed throughout the year (Busing 2005). The mean annual temperature during 1978-1992 was 13.2º C at the Gatlinburg SW station; the mean annual precipitation at this location during the same period was 141-cm (Busing

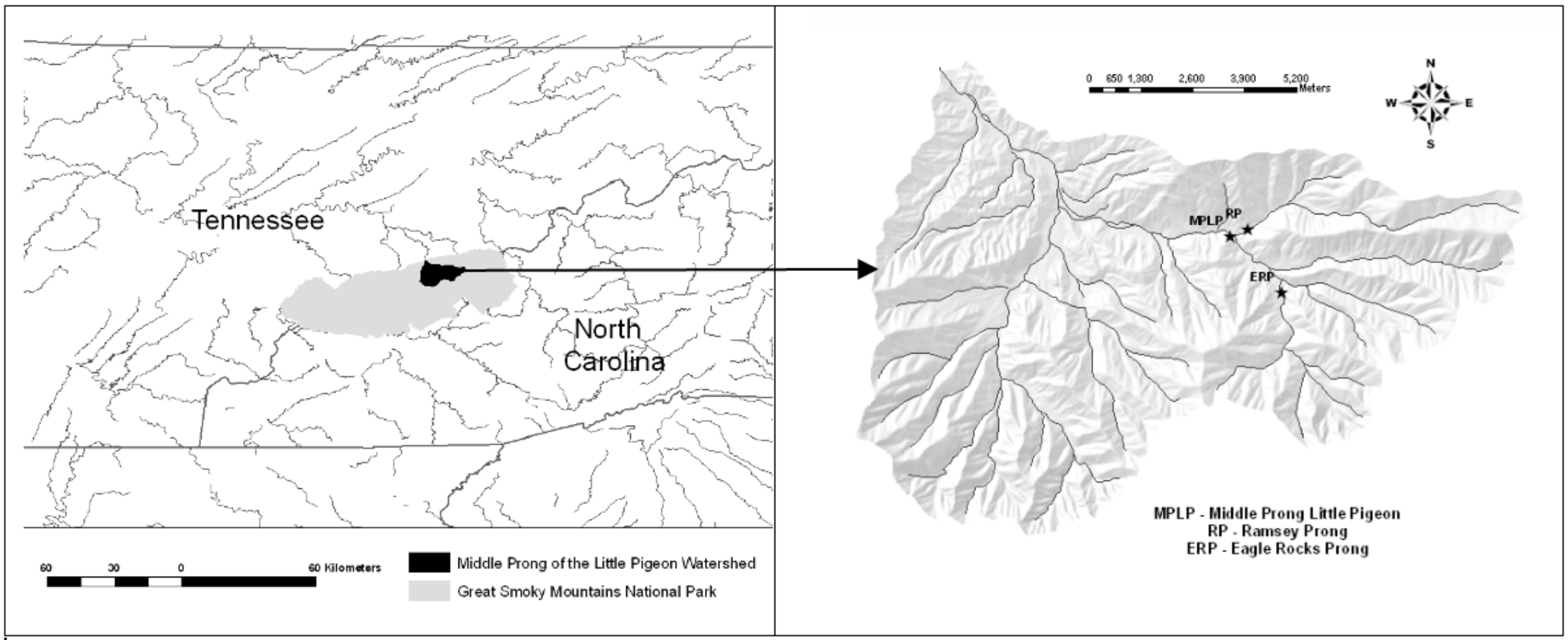


Figure 1. Location of study area and study sites in the GRSM

2005). The Alum Cave Bluffs Parking Area station (1173-m asl) is more representative of the study sites, and recorded a mean annual temperature of 9.9º C and a mean annual precipitation of 200 cm during 1947-1950 (Shanks 1954).

Study Design

The three sites were selected for study on the basis of (i) whether native brook trout had experienced extirpation at that stream location or not, (ii) remoteness (minimum of 2-km from roads) with no current anthropogenic impacts except acid deposition, and (iii) proximity to the lab at the University of Tennessee in Knoxville. The Middle Prong has continuously been inhabited by native brook trout (Bivens et al. 1985; King 1938; Moore and Kulp 2006), and drains waters from Ramsey Prong, Eagle Rocks Prong, and two other 4th-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 1378-m asl (Bivens et al. 1985). Currently, Eagle Rocks Prong only supports 0.1 km of brook trout to an elevation of 920-m asl (Moore and Kulp 2006). In 1985, Ramsey Prong supported 1.0-km of brook trout to an elevation of 914-m asl (Bivens et al. 1985). Now, only 0.2-km of Ramsey Prong supports brook trout to an elevation of 821-m asl (Moore and Kulp 2006).

Water sampling and analyses

Water quality and stage height at the three stream sites were monitored from April 2006 to present. Grab samples were obtained monthly and more frequently during this study. The sampling protocol included field blanks and replicate samples for quality assurance. The means and standard deviations were calculated for selected chemical constituents from 2006 base-flow grab samples at the three sites (Table 1A). Conductivity, pH, turbidity, temperature were measured continuously at 15 minute intervals with a YSI 6920 data sonde. An ISCO 6712 automated water sampler was used to collect samples during stormflows. Precipitation was collected at one open site and three throughfall sites to quantify inputs to the system. Mean values for selected chemical constituents from precipitation and throughfall collection were determined (Table 1B**)**. Chemical analyses were performed at the Civil and Environmental Engineering water quality lab at the University of Tennessee. Water samples were analyzed for the following parameters: conductivity (US EPA method 150.1), pH (US EPA method 120.1), ANC (Mantech PC-Titration Plus); sulfate (SO_4^{2-}), nitrate (NO_3^{2-}), ammonium (NH_4^+) (Dionex IC, Standard Methods 4110); Al, Ca, Cu, Fe, K, Mg, Mn, Na, Si, and Zn (Thermo Electron Intrepid II ICP-AES, vacuum-filtered (0.45-µm) and acidified, US EPA SW-846 Method 6010B). Quality control/ quality assurance samples, in the form of spikes, splits, and replicates, were implemented in each analytical procedure. Ion balances were performed on samples for additional quality assurance.

In situ bioassays

In situ bioassays of adult native southern brook trout were conducted at the three sites in June 2006. Trout used in the bioassays were collected using standard electroshocking techniques (Reynolds 1996) from a reach on the Middle Prong. Test fish (n=120, 20.52 g +/- 8.86 g SD) were randomly distributed and transported in aerated backpack tanks to the three sites (40 trout per site). Fish were given a 24-hour

Table 1: Concentrations of selected chemical constituents in 2006: (A) Stream sites at base flow, (B) Open site precipitation and throughfall

(A)	Middle Prong	Ramsey Prong	Eagle Rocks Prong
pH	5.77	5.67	5.51
ANC (µeq/L)	3.2 ± 6.8	3.5 ± 4.5	-6.0 ± 7.9
NO_3 (µeq/L)	38.9 ± 6.7	31.7 ± 7.3	48.2 ± 6.7
SO_4 (µeq/L)	45.3 ± 5.2	39.6 ± 5.4	52.4 ± 7.4
NH_4 (µeq/L)	0.13 ± 0.10	0.15 ± 0.09	0.17 ± 0.06
Na (µeq/L)	26.7 ± 3.2	24.7 ± 5.8	25.3 ± 5.6
K (µeq/L)	10.4 ± 2.8	10.54 ± 2.0	6.9 ± 1.6
Mg (µeq/L)	24.6 ± 2.6	17.5 ± 1.8	30.4 ± 3.3
Ca (µeq/L)	51.9 ± 5.2	42.1 ± 5.9	52.3 ± 3.3
Al (ppm)	0.07 ± 0.04	0.10 ± 0.05	0.10 ± 0.07

(B)	Open Site	Throughfall
pH	5.68	5.47
Cl (µeq/L)	9.96	9.30
NO_3 (µeq/L)	9.5	10.4
SO_4 (µeq/L)	41.0	39.9
NH_4 (µeq/L)	1.77	6.24
Na (µeq/L)	8.57	9.30
K (µeq/L)	16.9	34.7
Mg (µeq/L)	10.7	16.4
Ca (µeq/L)	42.7	35.7

adjustment period in the cages before the initial fish samples were collected to allow recovery from electroshocking and transport stress.

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. The cages were constructed using 254-mm stainless steel tubing for the frame; 254-mm stainless steel screen was welded to all sides of the frame. Test cages were placed behind large boulders to reduce hydraulic stress to test fish at high flows. 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.

The test period was limited to a maximum of 20 days in which to catch a storm event. Trout were randomly sampled from each of the four test containers at each site at the beginning of the test period (day 1). Trout were sampled every 5th day, and appropriately before and after an acidic episode. All fish were anesthetized and killed, then placed in individual plastic bottles (pre-weighed and acid-rinsed) and transported to the laboratory.

Determination of whole-body sodium concentrations

In the laboratory, all trout samples were immediately put in a cold room (4° C) and within one week were oven-dried at 70° C for 5-7 days. Dry mass was determined for all trout sampled (n=48, 4.99 g +/- 2.20 g SD). Following the procedure of Grippo et al, (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 for analysis of whole-body sodium concentrations using an ICP-AES. To account for differences in trout mass, whole-body sodium was normalized by dividing by wet mass of trout samples (Grippo et al, 1996).

Statistical analyses

For comparisons of pH between sites, single-factor analysis of variance (ANOVA) was utilized. Significant pH differences for ANOVA's were reported at the $p \leq 0.01$ level. Each site was analysed independently to determine if there were differences in whole-body sodium concentrations by date. For each sample date, differences in whole-body sodium were analyzed between sites. Tukey-Kramer honestly significant difference (HSD) tests and ANOVA's were used to determine differences in whole-body sodium concentrations between sites and dates. Significant differences in whole-body sodium concentrations for ANOVA's and Tukey-Kramer HSD tests were reported at the $p \leq 0.05$ level. The JMP platform was used for statistical analyses and plots (SAS Institute Inc. 2005).

Chapter III. Results

Acidic Stormflow Episode

An acidic stormflow episode occurred on the seventh day of the bioassay study period and lasted approximately 36 hours at all 3 sites. No trout died during the study period and trout at all sites were able to maintain balance and normal swimming behaviors. Trout were sampled on days 1, 5, 8, and 10 during the study period; and automated water samples were obtained at the Middle Prong and Ramsey Prong sites during the stormflow (Figure 2). The bioassay period ended on day 10.

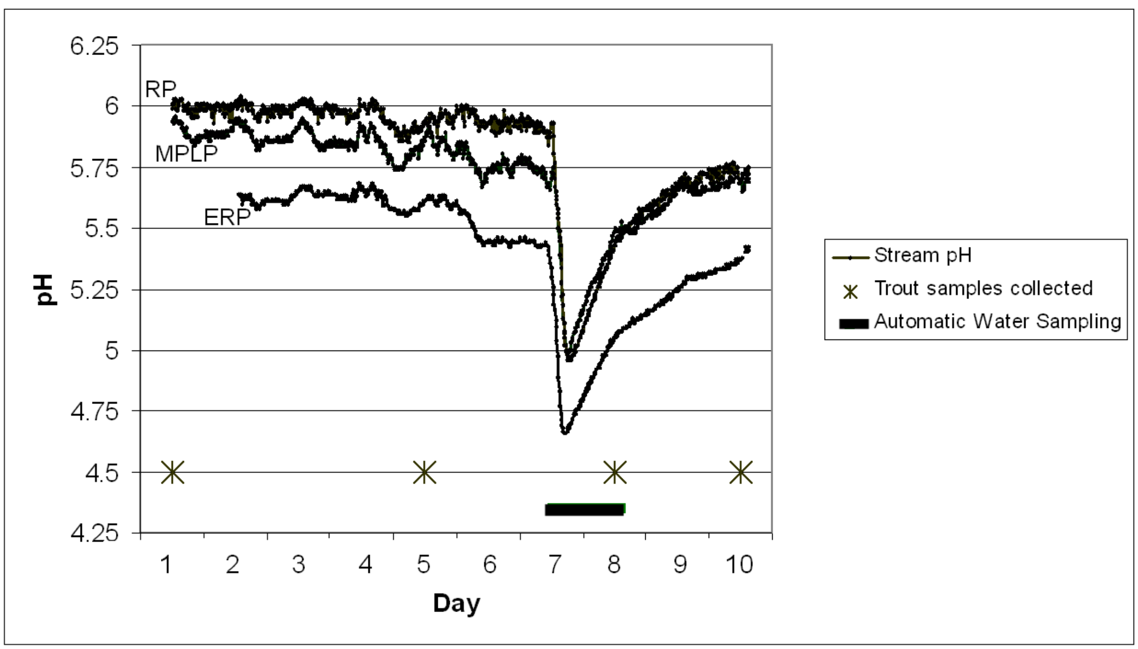


Figure 2: Sampling sequence and pH of Middle Prong (MPLP), Ramsey Prong (RP), and Eagle Rocks Prong (ERP)

Water quality

Durations (in hours) of pH ranges were determined during the bioassay period and during the 36-hour stormflow at the three sites (Figure 3). During the acidic episode, water at Eagle Rocks was significantly more acidic than the other two sites: Middle Prong (mean pH = 5.38) ≈ Ramsey Prong (mean pH = 5.39) > Eagle Rocks Prong (mean pH = 5.00). The means and standard deviations for selected chemical constituents from the stormflow during the in situ bioassay and 2006 episodes were determined (Table 2). The water chemistry data for the study period storm are not presented for Eagle Rocks due to a pump malfunction of the automated water sampler.

Whole-body sodium concentrations

There were no statistical differences by date at the Middle Prong and Ramsey Prong sites (Fig. 4). The trout at Eagle Rocks Prong, the site that experienced the lowest pH, had significantly lower (ANOVA: p=0.012) whole-body sodium concentrations on day 8, the day following the acidic episode, than the other three sample dates (Fig. 5).

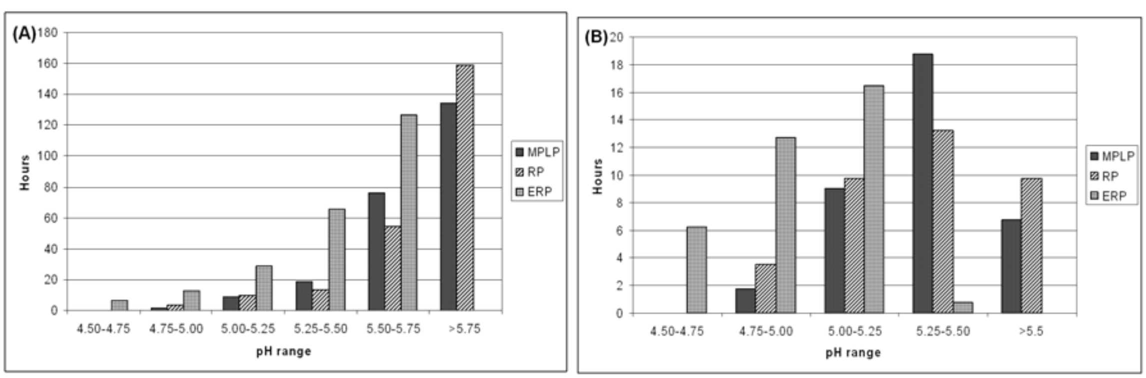


Figure 3: pH durations at Middle Prong (MPLP), Ramsey Prong (RP), and Eagle Rocks Prong (ERP): (A) during bioassay period and (B) during 36-hour stormflow

Table 2: Concentration of selected chemical constituents during acidic stormflow episodes

Parameters	Middle Prong		Ramsey Prong		Eagle Rocks
	Bioassay storm	2006 storms	Bioassay storm	2006 storms	2006 storms
Minimum pH	4.98	4.18	4.97	4.23	4.66/4.42
Al (ppm)	0.17 ± 0.05	0.16 ± 0.07	0.25 ± 0.10	0.21 ± 0.09	0.23 ± 0.10
Maximum Al (ppm)	0.24	0.37	0.41	0.41	0.45
Ca (µeq/L)	55.02 ± 4.60	54.75 ± 5.01	50.38 ± 7.90	51.31 ± 7.15	56.82 ± 6.88
Mg (µeq/L)	25.42 ± 1.64	27.91 ± 4.57	20.76 ± 2.27	20.75 ± 1.79	33.76 ± 4.63
K (µeq/L)	11.00 ± 1.60	11.37 ± 3.36	11.27 ± 2.08	12.36 ± 2.87	9.92 ± 4.07
Na (µeq/L)	22.63 ± 1.17	23.44 ± 2.05	23.21 ± 2.18	24.46 ± 1.23	19.55 ± 2.26
ANC (µeq/L)	5.70 ± 3.41	0.48 ± 6.45	3.13 ± 5.56	3.10 ± 5.72	-9.19 ± 5.91
Conductivity (µS/cm)	15.73 ± 0.63	16.70 ± 1.83	14.38 ± 1.62	14.81 ± 1.87	20.37 ± 2.36

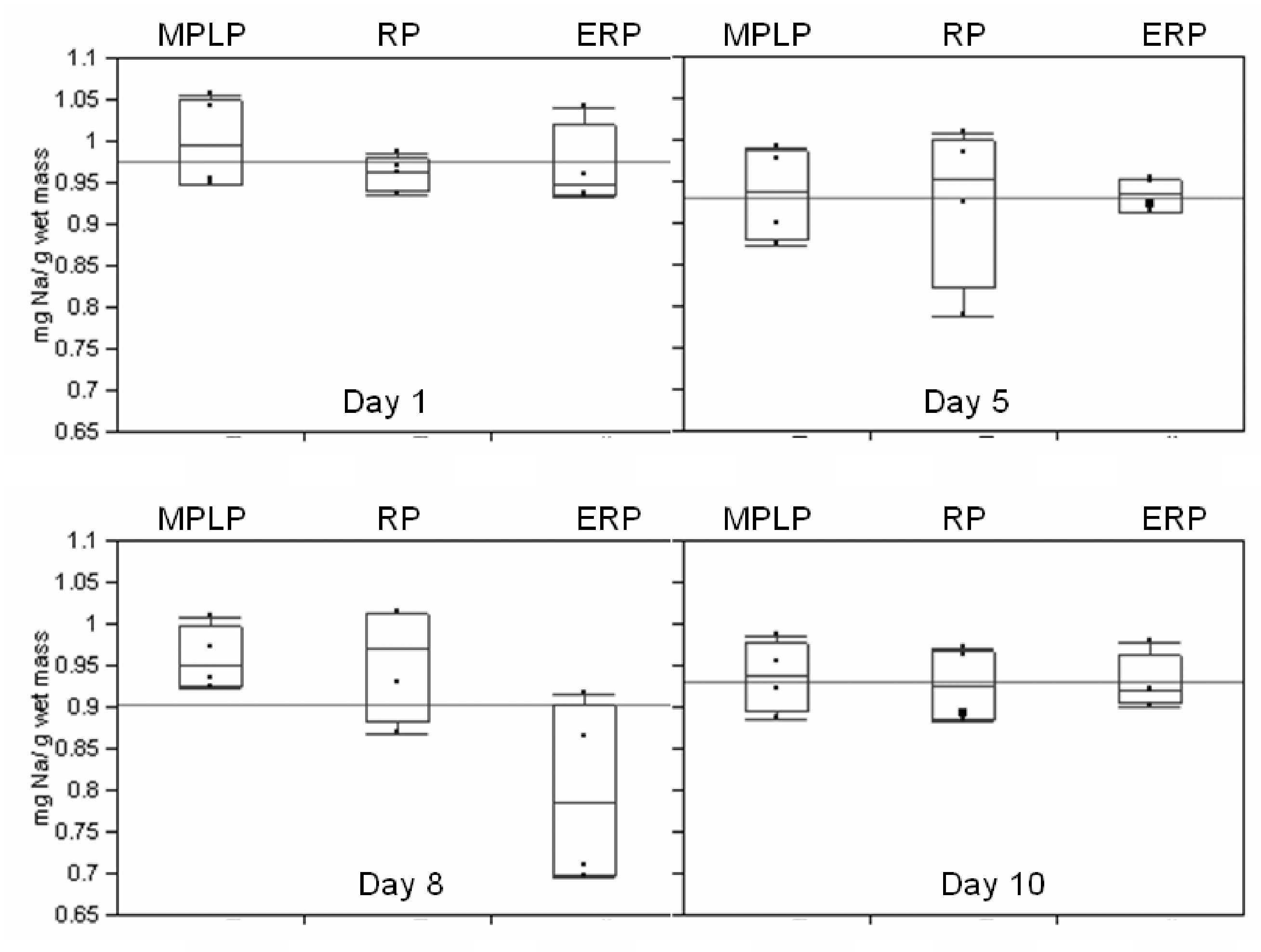


Figure 4: Box plots of whole-body sodium concentrations at Middle Prong (MPLP), Ramsey Prong (RP), and Eagle Rocks Prong (ERP)

With the Tukey-Kramer HSD test, day 8 was significantly different ($p<0.05$) than day 1 and day 5, but not significantly different than day 10. The mean whole-body sodium concentrations (Na (mg) per wet mass (g)) at Eagle Rocks for the four sampling dates in chronological order are as follows: 0.96, 0.93, 0.80, and 0.93 mg/g. Prior to the acidic episode (days 1 and 5) and two days following the storm event (day 10), there were no differences in whole-body sodium concentrations by site. The whole-body sodium concentrations of trout samples were significantly lower (ANOVA: $p=0.027$, Tukey-Kramer HSD: $p<0.05$) at Eagle Rocks than the other sites on day 8 (see Fig. 5). On this date, Eagle Rocks Prong trout had a mean sodium concentration of 0.80 mg/g whereas trout from Middle Prong and Ramsey Prong had sodium concentrations of 0.96 mg/g and 0.95 mg/g respectively.

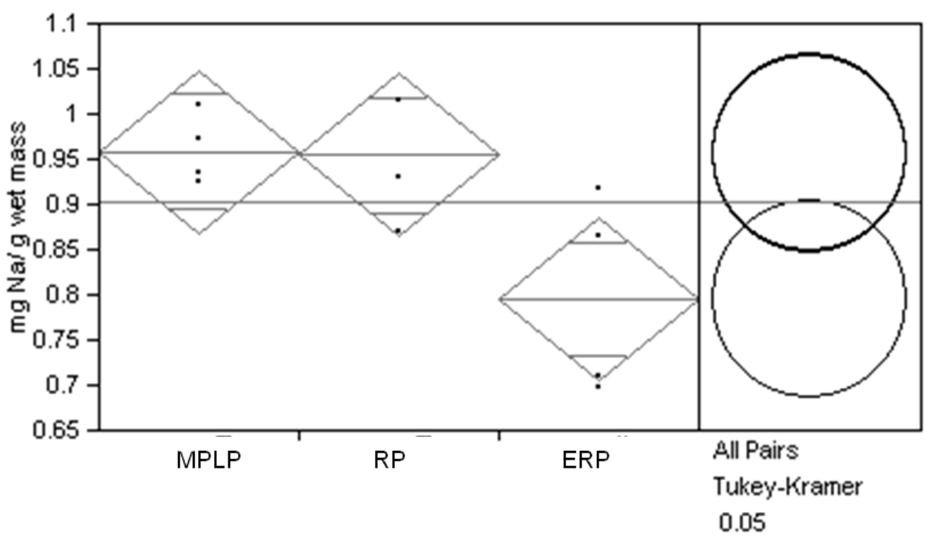


Figure 5: Whole-body sodium concentrations on 6/27/2006 by site at Middle Prong (MPLP), Ramsey Prong (RP), and Eagle Rocks Prong (ERP)

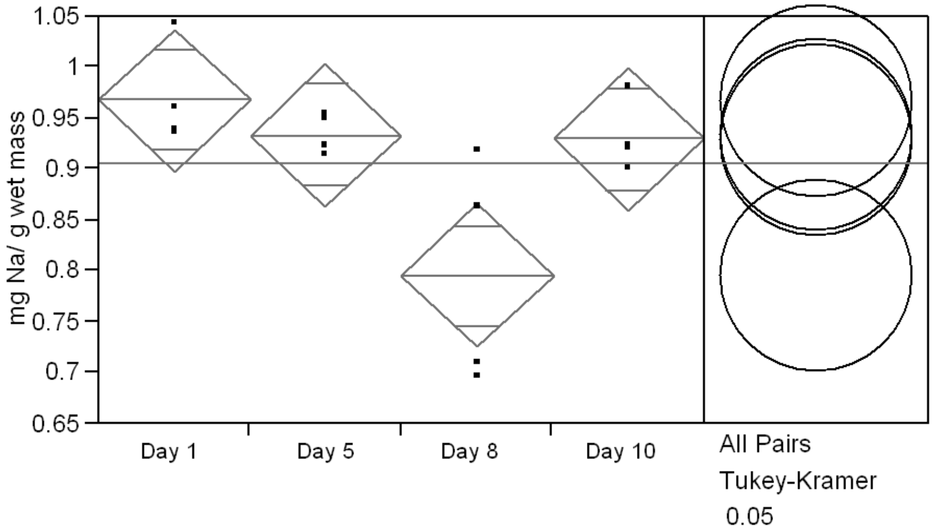


Figure 6 Whole-body sodium concentrations at Eagle Rocks Prong by date

Chapter IV. Discussion

These results demonstrate that acidic stormflow episodes can negatively affect brook trout physiology, as demonstrated by a loss of whole-body sodium, under field conditions in the GRSM. In this particular stormflow, native southern brook trout tolerated pH depression when the minimum pH was 4.97 (13.75-hour durations of pH less than 5.25) and the mean Al_{TD} concentration was 0.25 mg/L. Trout lost the ability to regulate critical sodium ions when the minimum pH was 4.66 and pH was less than 5.0 for 20 hours. Trout at Eagle Rocks Prong lost 15% of their whole-body sodium following the acidic episode. Gagen and Sharpe found 15% sodium loss in northern brook trout exposed to a 24-hour acid episode when the median pH was 5.5 and median Al_{TD} was 0.3 mg/L (1987a). Although no mortality was observed in this study, failure to maintain sodium balance can lead to death in low ionic strength waters (Hesthagen et al. 1999).

The presence of monomeric Al during the acidic stormflow likely contributed to the loss of whole-body sodium. During the acidic episode during the bioassay period, the Middle Prong and Ramsey Prong maximum Al_{TD} concentrations were 0.24 mg/L and 0.41 mg/L respectively (Fig. 6, see Table 2). Eagle Rocks Prong had higher maximum (0.45 mg/L) and mean (0.23 mg/L) Al_{TD} concentrations in 2006 storms than the other two sites (see Table 2). In addition to high concentrations of hydrogen ions (low pH), Al_{IM} is the primary toxic agent responsible for rapid loss of body sodium (Hesthagen et al. 1999). In this experiment, Al_{TD} was used as an estimate of Al_{IM} since monomeric forms of aluminum predominate when pH is less than 6.0 (Driscoll et al. 1984). The solubility

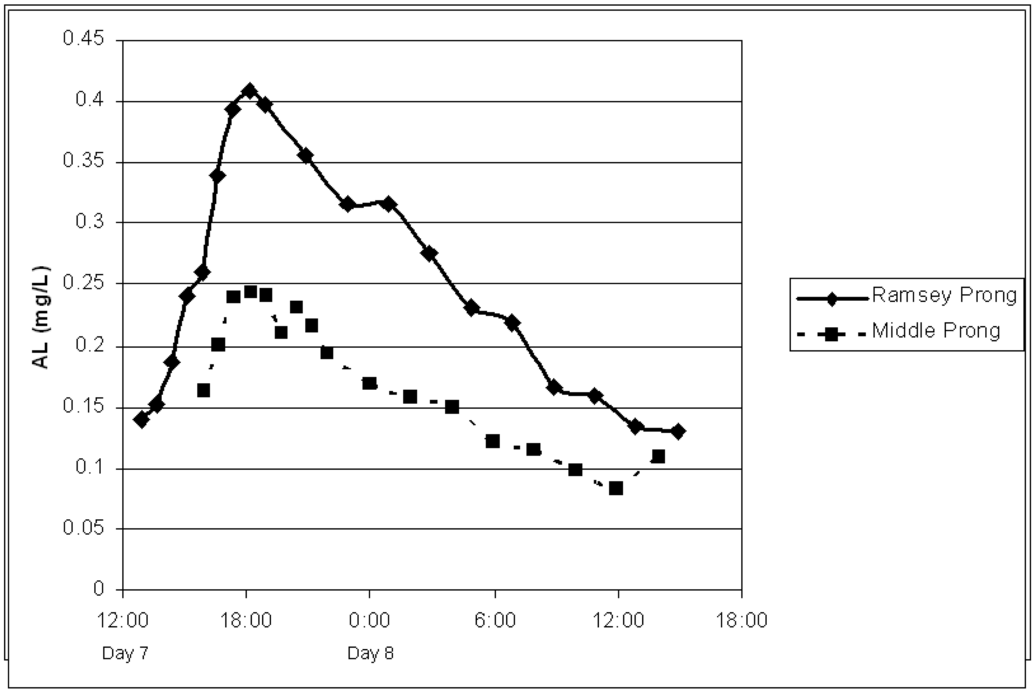


Figure 7: Total dissolved aluminum during acidic stormflow

of aluminum increases exponentially as pH falls below 5.6 with maximum toxicity occurring about pH 5 (Baker and Schofield 1982). Fish toxicity in acidic low ionic strength waters is possible when AlIM exceeds 0.2 mg/L (Baker and Schofield 1982; Hunn et al. 1987).

Aluminum toxicity during the storm event was probably reduced by the presence of dissolved organic carbon (DOC). Although DOC concentrations were not determined during the study period, concentrations of DOC during baseflows and stormflows have been measured at the three sites. Preliminary results indicate that all three sites have baseflow DOC concentrations from 1.5 to 2.7 mg/L and stormflow DOC concentrations from 2.23 to 4.7 mg/L (Appendix G). Maximum DOC concentrations during stormflows coincide with minimum pH and maximum AlTD concentrations (Appendix G). The toxicity of aluminum is ameliorated by DOC complexing with AlIM (Lawrence et al. 1986); and the DOC concentrations we observed have been shown to decrease concentrations of AlIM in other streams (Baldigo and Murdoch 1997) and likely decrease toxicity in these GRSM streams.

The duration, magnitude, chemical composition, timing and spatial distribution of acidic events are factors influencing the survival and stress response of trout. Consistent with other studies, the duration and magnitude of low pH were found to be the most important factors. The acidic episode during the bioassay period was not the most severe acidic event in 2006. Minimum pH values in 2006 at the Middle Prong, Ramsey Prong, and Eagle Rocks Prong sites were 4.18, 4.23, and 4.42 respectively (see Table 2). We would expect that during these conditions, adult trout at these sites would experience significant physiological stress if not death. Early life stage trout may experience a greater toxic effect to these conditions as it has been documented that early life stages of trout are more sensitive than older ones (Baldigo and Lawrence 2001).

Acid stress and whole-body sodium loss in the brook trout of this study is one possible explanation for extirpation of native brook trout in some streams of the GRSM. However, other explanations are possible. Episodic stream acidification of the magnitudes and durations observed in the GRSM may cause death to brook trout early life stages or affect reproduction in adult fish (Fiss 1993). A negative stress response to acidic stormflows may cause native brook trout to migrate, limiting recolonization, or find chemical refuge from low pH (Carline 1992). Physiological recovery to acidic conditions may dictate the persistence of trout populations.

References

References

Baker JP, Schofield CL (1982): Aluminum toxicity to fish in acidic waters. Water Air and Soil Pollution 18, 343-351

Baldigo, BP, Lawrence, GB (2001): Effects of stream acidification and habitat on fish populations of a North American river. Aquatic Sciences 63, 196-222

Baldigo BP, Murdoch PS (1997): Effect of stream acidification and inorganic aluminum on mortality of brook trout (Salvelinus fontinalis) in the Catskill Mountains, New York. Canadian Journal of Fisheries and Aquatic Sciences 54, 603-615

Bivens RD, Strange RJ, Peterson DC (1985): Current distribution of the native brook trout in the Appalachian region of Tennessee. Journal of the Tennessee Academy of Science 60, 101-105

Booth CE, McDonald DG, Simons BP, Wood CM (1988): Effects of Aluminum and Low pH on Net Ion Fluxes and Ion Balance in the Brook Trout (Salvelinus-Fontinalis). Canadian Journal of Fisheries and Aquatic Sciences 45, 1563-1574

Busing RT (2005): NPP Temperate Forest: Great Smoky Mountains, Tennessee, U.S.A., 1978-1992. Oak Ridge National Laboratory Distributed Active Archive Center

Carline RF, Dewalle DR, Sharpe WE, Dempsey BA, Gagen CJ, Swistock B (1992): Water Chemistry and Fish Community Responses to Episodic Stream Acidification in Pennsylvania, USA. Environmental Pollution 78, 45-48

Chestnut LG, Mills DM (2005): A fresh look at the benefits and costs of the US Acid Rain Program. Journal of Environmental Management 77, 252-266

Cleveland L, Little EE, Ingersoll CG, Wiedmeyer RH, Hunn JB (1991): Sensitivity of Brook Trout to Low Ph, Low Calcium and Elevated Aluminum Concentrations During Laboratory Pulse Exposures. Aquatic Toxicology 19, 303-318

Dennis TE, Bulger AJ (1996): Condition factor and whole-body sodium concentrations in a freshwater fish: Evidence for acidification stress and possible ionoregulatory over-compensation. Water, Air and Soil Pollution 85, 377-382

Driscoll CT, Baker JP, Bisogni JJ, Schofield CL (1984): Aluminum speciation and equilibria in dilute acidic surface waters of the Adirondack region of New York State. In: OP Bricker (ed), Geological Aspects of Acid Deposition, 55-75

Gagen CJ, Sharpe WE (1987a): Influence of Acid Runoff Episodes on Survival and Net Sodium Balance of Brook Trout (Salvelinus-Fontinalis) Confined in a Mountain Stream. Annales De La Societe Royale Zoologique De Belgique 117, 219-230

Gagen CJ, Sharpe WE (1987b): Net Sodium Loss and Mortality of 3 Salmonid Species Exposed to a Stream Acidified by Atmospheric Deposition. Bulletin of Environmental Contamination and Toxicology 39, 7-14

Grippo RS, Dunson WA (1996): The Body Ion Loss Biomarker. 1. Interactions Between Trace Metals and Low pH in Reconstructed Coal Mine-Polluted Water. Environmental Toxicology and Chemistry 15, 1955-1963

Fiss FC, Carline RF (1993): Survival of Brook Trout Embryos in 3 Episodically Acidified Streams. Transactions of the American Fisheries Society 122, 268-278

Hesthagen T, Heggenes J, Larsen BM, Berger HM, Forseth T (1999): Effects of water chemistry and habitat on the density of young brown trout Salmo trutta in acidic streams. Water Air and Soil Pollution 112, 85-106

Hunn JB (1985): Role of Calcium in Gill Function in Fresh-Water Fishes. Comparative Biochemistry and Physiology a-Physiology 82, 543-547.

Hunn JB, Cleveland L, and Little EE (1987): Influence of pH and Aluminum on Developing Brook Trout in a low Calcium Water. Environmental Pollution 43, 63-73

Ingersoll CG, Gulley DD, Mount DR, Mueller ME, Fernandez JD, Hockett JR, Bergman HL (1990): Aluminum and Acid Toxicity to 2 Strains of Brook Trout (Salvelinus-Fontinalis). Canadian Journal of Fisheries and Aquatic Sciences 47, 1641-1648

Johnson DW (1992): Nitrogen-Retention in Forest Soils. Journal of Environmental Quality 21, 1-12

Johnson DW, Simonin HA, Colquhoun JR, Flack FM (1987): In situ toxicity testing of fishes in acid waters. Biochemistry 3, 181-208

King PB, Newman RB, Hadley JB (1968): Geology of the Great Smoky Mountains National Park, Tennessee and North Carolina. Professional Paper 587, United States Geologic Survey

King W (1938): A program for the management of fish resources in the Great Smoky Mountains National Park. Transactions of the American Fisheries Society, 68

Larson GL, Moore SE, Carter B (1995): Ebb and flow of encroachment by nonnative rainbow trout in a small stream in the southern Appalachian Mountains. Transactions of the American Fisheries Society 114, 195-203

Lawrence GB, Fuller RD, Driscoll CT (1986): Spatial relationships of aluminum chemistry in the streams of the Hubbard Brook Experimental Forest, New Hampshire. Biogeochemistry 2, 115-135

McCracken GF, Parker CR, Guffey SZ (1993): Genetic Differentiation and Hybridization between stocked hatchery and native brook trout in Great-Smoky-Mountains-National-Park. Transactions of the American Fisheries Society 122, 533-542

Moore SE, Kulp MA (2006): Personal Communication with Great Smoky Mountains National Park Fisheries Biologists. K. Neff, ed., Knoxville, TN

Packer RK, Dunson WA (1970): Effects of Low Environmental pH on Blood pH and Sodium Balance of Brook Trout. Journal of Experimental Zoology 174, 65-72

Packer RK, Dunson WA (1972): Anoxia and Sodium Loss Associated with Death of Brook Trout at Low pH. Comparative Biochemistry and Physiology 41, 17-26

Reynolds J (1996): Electrofishing. Fisheries Techniques, B. Murphy, Willis DW, ed., Bethesda, MD, 221-253

Robinson RB, Roby JC, Buchanan JR, Barnett TW, Moore SE (2004): Storm Event Monitoring in the Great Smoky Mountains National Park. Proceedings of the ASCE EWRI 2004 World Water and Environmental Resources Congress., American Society of Civil Engineers, Salt Lake City, Utah

Shanks RE (1954): Climates of the Great Smoky Mountains. Ecology 35, 354-361

Shubzda J, Lindberg SE, Garten CT, Nodvin SC (1995): Elevations Trends in the Fluxes of Sulphur and Nitrogen in Throughfall in the Southern Appalachian Mountains: Some Surprising Results. Water Air and Soil Pollution 85, 2265-2270

Wigington PJ, Baker JP, DeWalle DR, Kretser WA, Murdoch PS, Simonin HA, VanSickle J, McDowell MK, Peck DV, Barchet WR (1996): Episodic acidification of small streams in the northeastern United States: Episodic Response Project. Ecological Applications 6, 374-388

Wood CM, McDonald DG, Ingersoll CG, Mount, DR, Johannsson OE, Landsberger S, Bergman HL (1990): Whole-Body Ions of Brook Trout (Salvelinus-Fontinalis) Alevins – Responses of Yolk-Sac and Swim-up Stages to Water Acidity, Calcium, and Aluminum, and Recovery Effects. Canadian Journal of Fisheries and Aquatic Sciences 47, 1604-1615

Appendices

Appendix A: Field Activities

This project required labor-intensive field work in the Great Smoky Mountains National Park. Table 3 is the field log for all field related activities conducted in 2006. In the table, Site 1 refers to the Middle Prong of the Little Pigeon River site; Site 2 refers to the Ramsey Prong site; site 3 refers to the Eagle Rocks Prong site. Following this Table is a list of the full names of field participants.

Table 3: Field Log

Date:	**Activity:**	**Personnel**
01/28/06	Initial survey of Site 3	Neff, Neff2, Smith
02/06/06	Specific Site determination (Sites 1 and 2); transport 6 fencepost, 3 sonde casings, and lengths of chain	Robinson, Schwartz, Neff, Dunnavant, Brawley
02/10/06	Fencepost for sonde installation completed at Sites 1 and 2, initial drilling into boulder for anchor at Site 1; grab samples obtained from all 3 Sites; fencepost at Site 1 cut with hacksaw	Neff
02/14/06	Sonde attached to fencepost at Site 1; 1st anchor attached to boulder and sonde casing at Site 1; initial drilling into boulder for anchor at Site 2	Neff
2/17/06	Site 1 sonde installation completed, sonde initialized	Neff, Jackson, Dunnavant
2/27/06	Site 2 sonde installation completed	Neff, Dunnavant
2/28/06	Site 3 sonde installation completed, sondes initialized at Sites 2 and 3	Neff, Jackson
3/4/2006	Composite Sampler moved to staging area; sonde calibration Sites 1&3, sonde data download Sites 1&3, Eagle Rocks survey	Neff, Neff2, Deyton
3/8/2006	Site 3 location change: stage 2	Neff
3/9/2006	Site 3 location change: stage 1; Site 3 sonde installation completed; Site 2 sonde data download; grab samples (Sites 1-3)	Neff, Jackson
3/13/2006	Flow measurements at Sites 1&2; download sondes at Sites 1&2; battery and composite sampler accessories transported to Site 2	Neff, Dunnavant

Date:	**Activity:**	**Personnel**
3/17/2006	Batteries and composite samplers to Sites 1&3, battery seats to Sites 1,2,3	Neff, Justice, Schuh
3/22/2006	Solar panels to Sites 1,2,3; calibration Sites 1&3; flow meter measurements Sites 1&3	Neff, Zimmerman
3/24/2006	Protective tubing to Sites 1&3	Neff, Neff2
3/27/2006	Site 1 composite sampler set up and initialized; Site 2 composite sampler initial set up	Neff, Jackson, Dunnavant
4/01/06	Site 2 solar panel installed; Site 1 composite sample tested with liquid level actuator; Site 1: intake tubing encased; flow meter readings at Sites 1 & 2; sonde @ Site 2 brought back to lab	Neff, Deyton
4/02/06	Site 3 initial composite sampler setup, solar panel installed	Neff, Neff2, Smith
4/05/06	Site 2 sonde re-initialized; Site 1 sonde calibration and download; hobo installed at ranger station	Dunnavant, Dunnavant2
4/07/06	Sites 2 and 3 composite sampler setup complete; Site 3 solar panel rewired (14G) to 12V battery; Sites 2 and 3 programs created and run to capture storm event.	Neff
4/09/06	Samples recovered from Sites 2 and 3 from 040706 storm event.	Neff, Neff2
4/13/06	Launched the hobo at Greenbrier Ranger Station. Took inventory at Sites 1 and 2. Carried new chain to Sites 1 & 2.	Dunnavant, Rucker
4/15/06	Attached hobo rain tipping bucket at Site 3. Programmed extended manual program for composite sampler at Site 3.	Neff, Neff2
4/18/06	Site 3 sonde downloaded and calibrated. Attempted to rewire and initialize hobo. Programmed extended manual program for composite sampler at Site 2. Carried new chain to Site 3.	Neff
4/24/06	Hobo initialized at Site 3. pH calibration Site 3. Composite samples taken from 4/21 storm @ Site 3; sampler re-initialized. Composite samples taken from 4/21 storm @ Site 2; sampler re-initialized.	Neff
4/28/06	Sonde and sonde casing reinstalled at Site 3, composite samplers reprogrammed with rate of change of depth or pH	Neff, Davis
5/5/06	Installed fish cage @ Site 1; downloaded hobo at Ranger Station; installed throughfall collection buckets at Ranger Station, Site 1, Site 2; re-installed hardware at Site 1 and Site 2	Neff, Jackson, Dunnavant, Deyton

Date:	Activity:	Personnel
5/16/06	Trout trial run with Steve Moore, Matt Kulp and GRSM fisheries field crew.	Neff, Robinson, Schwartz, Henry, Dunnavant, Jackson, Deyton, Carter
5/18/06	Trout mortality check, sonde 1 check, Site 3 attempt	Jackson, Deyton
5/22/06	Carried #2 fish cage to Site 1, carried #3 fish cage above waterfall on way to Site 3, Transported composite sampler to Site 1, set up wiring, adjusted position of cage at Site 1, brought back sonde from Site 3 for repair, installed throughfall collection at Site 3.	Neff, Deyton, Jackson, Armistead
5/25/06	Initialized composite sampler and replaced bottles at Site 2, carried fish cage to Site 2, reinstalled sonde casing at Site 3 in more secure location, initialized "teaching" sonde at Site 3, release trout from cage # 1 at Site 1, took 3 trout samples from Site 1,	Neff, Deyton
5/31/06	Completed setup for water quality monitoring at Site 1, initialized composite sampler at Site 1, downloaded and calibrated sondes at Sites 1 and 2, downloaded hobos at ranger station and Site 3, brought back "teaching" sonde for repair, carried cage to Site 3, reinstalled throughfall collection at Site 3	Neff, Deyton, Carter
6/2/06	Installed fish cages at Sites 2 and 3.	Neff, Deyton, Owen
6/7/06	Drilled and installed to anchor cages at Sites 2 and 3.	Neff, Deyton
6/14/06	Carried buckets to install at Sites 2 and 3. Completed cage anchor installation at Site 3.	Neff, Deyton, Conlan
6/19/06	Collected 120 trout with Matt Kulp and GRSM fisheries field crew. 40 trout transported to each Site. Trout weighed and measured and distributed into cages.	Neff, Deyton, Owen, McKenna, Liyana, Carter
6/20/06	Trout sampled from all three Sites for total body sodium analysis. Grab samples from all 3 Sites.	Neff, Deyton
6/21/06	Sonde installed and initialize at Site 3. Battery at Site 3 assumed dead ➔ returned to UTK to be charged.	Neff
6/23/06	Charged battery to Site 3. Composite sampler still not getting power. Brought composite sampler back down to UTK.	Neff
6/24/06	Brought composite sampler back to Site 3 with new battery connection cable. Fixed and reprogrammed composite sampler at Site 3. Trout sampled from all three Sites for total body sodium analysis.	Neff, Smith

Date:	Activity:	Personnel
6/27/06	Collected trout samples and grab samples from all 3 Sites. Brought Isco storm samples back down from Sites 1 and 2. Brought Isco sampler back to lab from Site 3.	Neff, Schwartz
6/29/06	Collected trout samples from all 3 Sites. Collected grab samples from all 3 Sites. Downloaded and calibrated all 3 Sites.	Deyton, Carter
7/04/06	Checked trout mortality at all 3 Sites. Brought tubing back from Site 1. Brought Isco power cable from Site 3.	Neff, Justice2
7/19/06	Transported Earth Science Isco Sampler to Site 1. Transported Site 1 Isco sampler to Site 3.	Neff, Deyton
7/24/06	Set datums for stage to discharge relationship at Sites 1 and 2. New battery to sampler at Site 1. Collected storm samples from Isco sampler at Site 2.	Neff, Deyton, Wells
7/31/06	Downloaded and calibrated Sites 1 and 2 sondes. Initialized and programmed sampler at Sites 1 and 2. Collected grab and through fall samples from Sites 1 and 2. Through-fall volumes: Ranger Station (8L), Site 1 (8L), Site 2 (7.75L).	Neff, Deyton
8/4/06	Downloaded and calibrated Site 3 sonde. Initialized and programmed sampler at Site 3. Collected grab and through fall samples from Site 3. Through-fall (4.8L).	Neff
8/22/06	Composite sampler returned to Site 1. Dr. McKay's sampler returned to lab. Velocity measurements at Site 2.	Neff, Deyton, Wells
9/9/06	Collected all throughfall (Site 1 (7.5L), Site 2 (1.5*L), Site 3 (6L) RS (11L) and grab samples. Downloaded and calibrated sondes at Sites 2 and 3. Brought Site 1 sonde down for repair. Initial programming of Site 1 sampler.	Neff
9/15/06	Installed and launched hobo at Ranger Station. Installed new solar panel at Site 1. Attached new battery for sampler and programmed and calibrated depth/volume @ Site 1.	Neff, Deyton
9/26/06	Flow measurement @ Site 3 (4PM). Sampler at Site 3 triggered on 9/8 (no good). Replaced funnel @ Site 2. Sampler pump failure. Sampler at Site triggered on 9/15 (no good). Funnel and tubing damaged by bear.	Neff, Deyton

Date:	**Activity:**	**Personnel**
9/29/06	Downloaded and calibrated sondes at Sites 1 and 2. Raised sonde casing 4" at Site 1. Replaced funnel and tubing at Site 1. Flow measurement taken at Sites 1 and 2. Sonde at Site 2 not communicating → brought back to lab. Fixed pump on sampler at Site 2 changing tubing position. Wiring harness for charge to battery damage → brought back to lab to be fixed. Ranger station throughfall and hobo download.	Neff, Deyton
10/04/06	Moved sonde casing up at Site 2. Re-installed sonde with adjusted depth. Battery to Site 2: rewired sampler and calibrated volume. Checked sampler at Site 1. Downloaded and calibrated sonde at Site 3. Downloaded hobo at Site 3. Site 3 throughfall. Flow measurements Site 3.	Neff, Deyton
10/18/06	Collected autosampler samples from Sites 1 and 3. Re-set autosamplers at all three Sites.	Neff, Deyton
10/28/06	Collected autosampler samples from Site 1. Re-set autosamplers at all three Sites. Power failure at Sites 2 and 3	Neff, Deyton
11/03/06	Replaced teaching sonde with Site 1 sonde at Site 1. Throughfall samples taken at Sites 1 (*1.5L) and 2 (*<1L). Replaced funnel and tubing for throughfall at Site 1. Attempted to fix sampler at Site 2. Fixed distributor. Tubing chewed by bear → no head → need replacing and adjusting at Site 3.	Neff, Deyton
11/08/06	Fixed sampler at Site 2. Fixed throughfall at Site 2. Downloaded sonde at Site 1.	Neff, Deyton
11/14/06	Downloaded and calibrated sonde at Site 3. Attempted to resolve sampler issue → dismantled and brought sampler back to lab. Throughfall Site 3 (12L) and OS at RS (4.5 L).	Neff
11/17/06	Site 3 sampler not working. Site 1 storm collected. Site 2 power failure.	Neff, Deyton
12/1/06	Replace motor of autosampler at Site 3. Flow measurement Site 3. Sonde power failure Site 3. Download and calibrate all 3 Sites. Throughfall: (2=8.5 L; 3=4 L).	Neff, Deyton
12/18/06	Installed solar panels at Sites 2 &3.	Neff, Deyton

Neff:	Keil Neff
Neff2:	Laura Neff
Smith:	Timothy Smith
Robinson:	R. Bruce Robinson
Schwartz:	John Schwartz
Dunnavant:	Amanda Dunnavant
Brawley:	Angela Smith
Jackson:	Karen Jackson
Deyton:	Edwin Deyton
Justice:	Rebekah Justice
Schuh:	Daniel Schuh
Zimmerman:	G. Tom Zimmerman
Rucker:	Jonathon Rucker
Davis:	Dustin Davis
Henry:	Ted Henry
Kulp:	Matt Kulp
Moore:	Steve Moore
Carter:	Dan Carter
Armistead:	Shaun Armistead
Owen:	Candice Owen
Conlan:	Todd Conlan
McKenna:	Amanda McKenna

Liyana: Tara Liyana

Justice2: Robert Justice

Wells: Joyce Wells

GRSM field crew: including but not limited to Keith, Brad, Russell, Brian, Shane, Adam, and Ryan

Appendix B: Characteristics of Middle Prong of the Little Pigeon

Characteristics of Trout Habitat

Trout distribution in the Middle Prong of the Little Pigeon River (MPLP) watershed is illustrated in Figure 8. The distribution range of brook and rainbow trout may be controlled by other factors than precipitation events and historical logging events. Slope, elevation, median stream pH, geology (surficial and bedrock), contributing soils, and land-cover are characteristics that may affect the spatial distribution of trout. Understanding the prevalence of these characteristics in relationship to the distribution of trout will provide a broader understanding of trout habitat in the MPLP watershed and in the GSMNP.

Arc-GIS was utilized to characterize the prevalence of physical parameters in sub-watersheds in the MPLP that define the range of trout. These parameters include elevation, slope, soils, surficial geology, bedrock, land-cover, and stream pH. Sub-watersheds will be defined by areas that contribute to stream segments with unique occurrences of trout. Trout occurrence is segregated into four distinct categories: a) brook trout only, b) rainbow trout only, c) rainbow and brook trout, and d) no trout.

GIS spatial data layers were obtained from the NRCS (http://datagateway.nrcs.usda.gov/, soildatamart.nrcs.usda.gov); TNGIS (http://www.tngis.org/); the USDA-FSA Aerial Photography Field Office (ftp://ftp.apfo.usda.gov/); the GSMNP (Glenn Harwell and Richard Schulz); and the USGS (seamless.usgs.gov). Table 4 summarizes the layers obtained from each source. The data frame and all data layers were projected into UTM NAD1927 Zone17N. Once

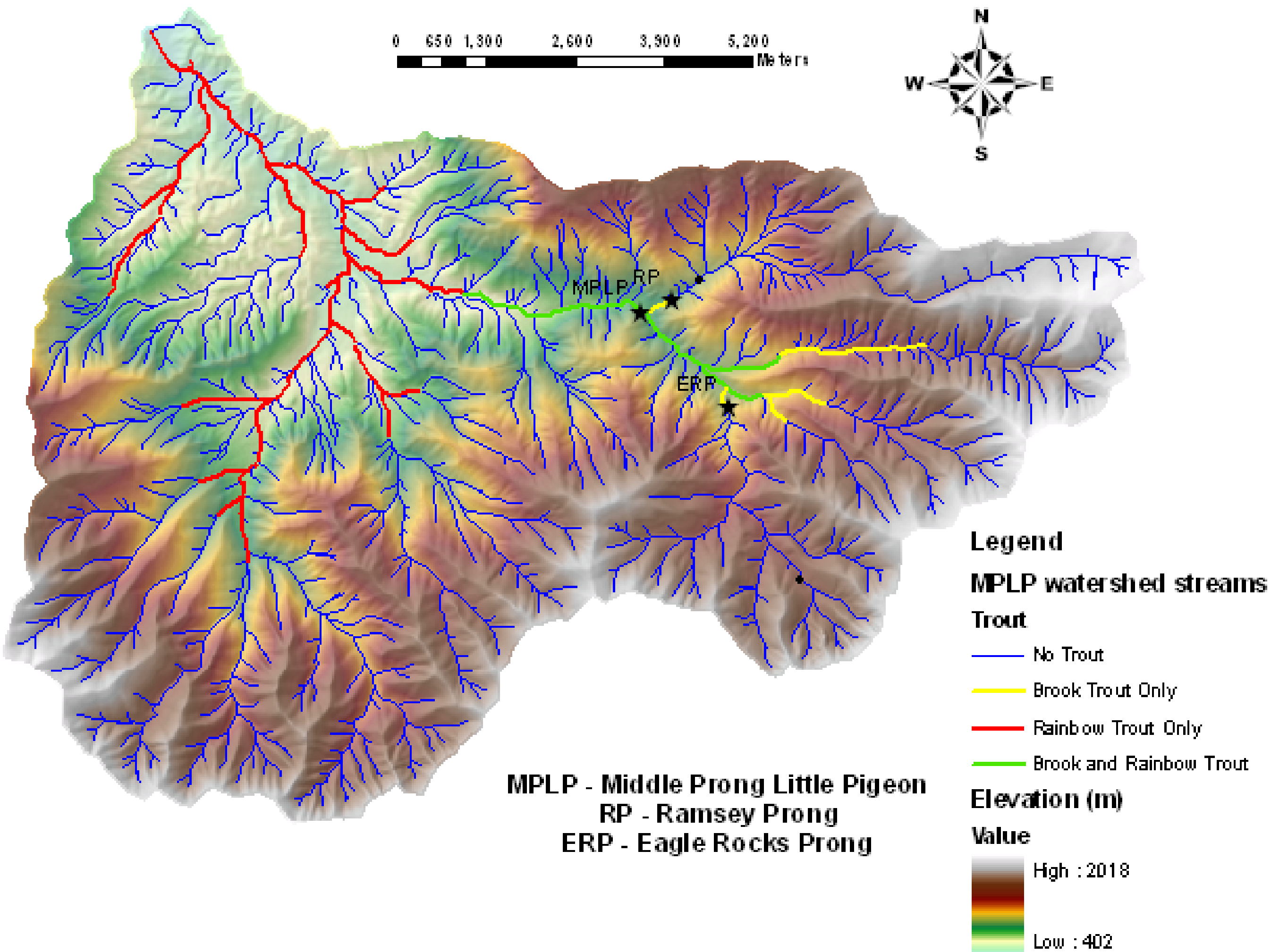


Figure 8: Trout Distribution in the Middle Prong of the Little Pigeon River Watershed

Table 4: GIS Source Layers

Layer	***Source***
TN Land Cover	TNGIS
TN Streams	TNGIS
Common Land Units	USDA-APFO
Soil Survey	USDA-NRCS
Digital Ortho Quads	USDA-NRCS
GSMNP Rivers	Glenn Harwell
GSMNP Site Data	Glenn Harwell
GSMNP Soils	Richard Schulz
GSMNP Surficial Geology	Richard Schulz
GSMNP Bedrock	Richard Schulz
National Elevation Dataset (1/3")	USGS

GSMNP watersheds were delineated; all layers were extracted to the extent of the Middle Prong of the Little Pigeon River watershed.

USGS topographical maps were obtained from the GSMNP fish biologists indicating the range of brook and rainbow trout in the MPLP watershed. Park biologists obtained this data by using standard electroshocking techniques. An additional field was added to the stream layer. Using selection and editor tools, the stream segments with brook, rainbow, or brook and rainbow trout were added to the stream layer in ArcMap.

Stream survey data was brought into ArcMap. Data included UTM coordinates and water quality data (including median pH) from 56 sites across the GSMNP. Stream pH data was interpolated utilizing a number of techniques including i) inverse distance weighted (IDW), ii) kriging and iii) spline. The geostatistical wizard in the geostatistical extension in ArcMap was employed to determine major range, partial sill and nugget for kriging analysis. The spline method was rejected immediately because of the unrealistic interpolated values near perimeter. IDW was preformed several different ways utilizing variable and fixed radius, and different powers and maximum number of points for interpolation.

Three sites independent of the stream survey sites were used to choose the appropriate pH interpolation technique. These three sites are sites currently used for in situ toxicity testing of trout and continuous water quality monitoring. A handheld Trimble DGPS was used to obtain the coordinates of these three sites. The coordinates and mean pH values from these sites were created in a text document and added to ArcMap. An IDW with variable radius, power = 3 and maximum points = 6 produced the interpolated pH surface that most accurately matched the pH values from the three independent sites. The data from the three sites were added to the 56 stream survey sites to produce the final pH interpolation utilizing an IDW with the previously specified inputs (Figure 9).

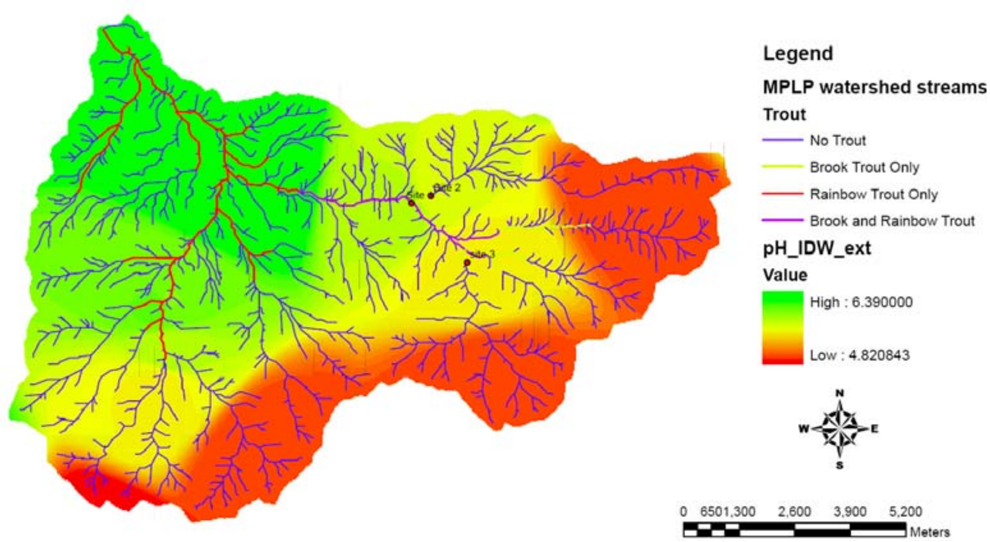


Figure 9: MPLP Interpolated Median Stream pH

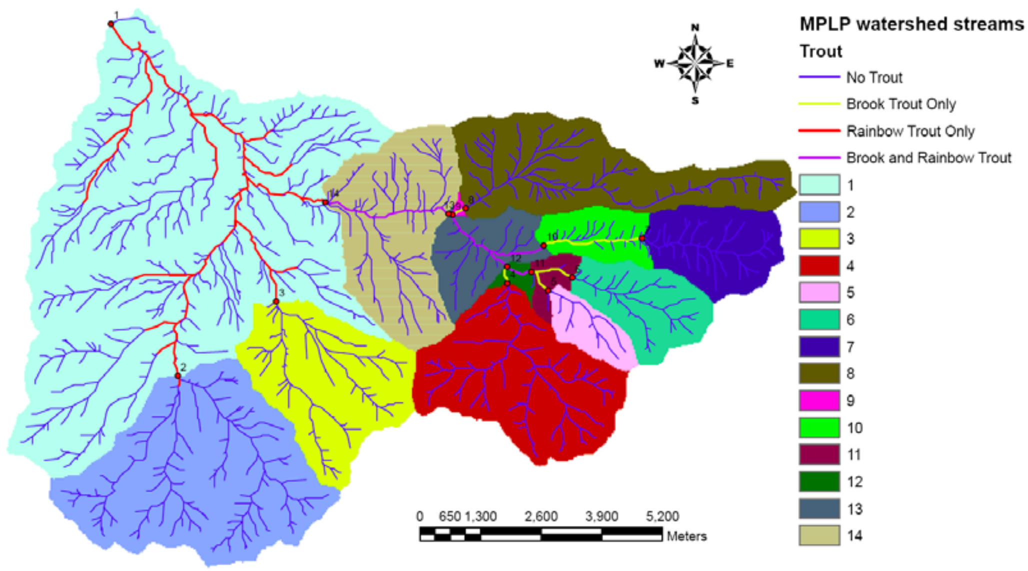


Figure 10: MPLP Sub-Watersheds

Pour points were defined at points in the MPLP watershed where the trout habitat changed. By utilizing fill and flow direction tools in the hydrology toolbox in ArcMap, sub-watersheds were defined at each pour point by using the watershed tool (Figure 10). A new layer was created for each of the 4 trout classifications of the stream network. A 30-meter buffer was applied to each of these layers. This 30-meter buffer was selected to reflect the average distance of runoff on the surface area. Zonal statistics were applied to characterize sub-watersheds and 4 trout buffers. Zonal statistics were applied for pH, elevation, slope, soils, surficial geology, bedrock material, and land cover.

Characterizations of the trout stream buffers provide valuable insight to the habitat of the trout species in the GSMNP. The differences between the streams with rainbow trout only, brook and rainbow trout, and brook trout only provide the most useful comparison. The no-trout category provides additional insight. However, the no-trout

Table 5: 30-m Buffer Zonal Statistics of pH, Slope, Elevation

	Rainbow Trout	Brook and Rainbow Trout	Brook Trout	No Trout
Mean pH	6.23	5.74	5.45	5.65
Mean slope (%)	7.77	11.72	15.93	20.53
Mean elevation (m)	568.5	807.6	1083.78	1065.53

streams do not account for barriers (i.e. waterfalls) to trout migration and other limiting physical barriers (i.e. stream size).

The mean pH of rainbow-only trout streams is 0.49 higher than the mean pH of rainbow and brook trout streams and 0.8 higher than the mean pH of brook-only trout streams. Brook-only trout streams are steeper than brook and rainbow trout streams and rainbow trout streams. The mean elevation of brook-only trout streams is also greater than the other trout stream. Table 5 summarizes these results.

The 30-m buffers for the stream designations were comprised of different soils, bedrock and surficial geology zones. Table 6 shows the relative percents of bedrock material. Table 7 shows the relative percents of surficial geology zones. Bedrock in rainbow-only, brook and rainbow, and brook-only streams are primarily comprised of

Table 6: 30-m Buffer Zonal Statistics of Bedrock

	Rainbow Trout	Brook and Rainbow Trout	Brook Trout	No Trout
Zp: Pigeon Siltstone	3.1%	0.0%	0.0%	2.3%
Zt: Thunderhead Sandstone	12.6%	86.9%	100.0%	57.2%
Ze: Elkmont Sandstone	61.5%	23.1%	0.0%	10.1%
Zrf: Sand/Siltstone	22.7%	0.0%	0.0%	15.9%
Za	0.0%	0.0%	0.0%	7.9%
Other Sandstone/Siltstone	0.0%	0.0%	0.0%	6.6%

Table 7: 30-m Buffer Zonal Statistics of Surficial Geology

	Rainbow Trout	Brook and Rainbow Trout	Brook Trout	No Trout
Qa: alluvium	35100	0	0	2700
Qt: terrace deposit	6300	0	0	17100
Qac: coarse alluvium	653400	182700	4500	692100
Qdb: boulder debris fan	506700	88200	32400	2021400
Qc: colluvium	9900	36900	130500	2945700
Qdf: debris fan	0	0	0	289800

sandstone. Colluvium is the primary surficial geological component of brook-only trout streams. Coarse alluvium and boulder debris fans are the primary surficial geological components of rainbow-only and brook and rainbow trout streams.

Slope, elevation, median stream pH, geology (surficial and bedrock), contributing soils, and land-cover are characteristics that affect the spatial distribution of trout. The prevalence of these characteristics in relationship to the distribution of trout provides a greater understanding of trout habitat in the MPLP watershed and in the GSMNP.

Brook trout occupy a unique habitat in the GSMNP. Brook trout only streams are characterized by steeper slopes, higher elevations, lower stream pH, upland mixed and deciduous forest riparian zones, sandstone bedrock materials and colluvium. Rainbow trout only streams are characterized by more gradual slopes, lower elevations, higher stream pH, upland mixed forest riparian zones, sandstone bedrock materials and coarse alluvium and boulder debris fans. Rainbow and brook trout streams are characterized by an intermediate habitat structure with parameters falling between the brook trout only and rainbow trout only streams.

Unique brook trout streams enable brook trout to establish a niche in which they can tolerate more acidic and steeper waters that rainbow trout cannot survive. The ability of brook trout to tolerate more acidic waters than rainbow trout may ensure that brook trout will not be eliminated from the park if stream pH continues to decline. However, if stream pH increases, rainbow trout range may increase if slope is not a limiting factor. Thus, brook trout populations may be extirpated if stream pH increases (by being out-competed by rainbow trout) and physical barriers prevent brook trout from upstream migration.

Watershed Characteristics

Watershed and sub-watershed characteristics are presented in table 8. Park WS is the Middle Prong of the Little Pigeon River in the GRSM. MPLP is the Middle Prong watershed above the study sites. RP is the Ramey Prong watershed and ERP is the Eagle Rocks watershed.

Table 8: Watershed and Sub-Watershed Geographic Characterizations

	Area (square km)	Maximum Elevation (m)	Mean Elevation (m)	Minimum Elevation (m)	% Anakeesta
Park WS	117.4311	2018	1144.17	402	8.50%
MPLP	38.6928	2018	1403.43	791	3.00%
RP	10.3185	2016	1409.75	838	0%
ERP	10.4976	1799	1441.89	961	11.10%
	Minimum Slope	Mean Slope	Maximum Slope	Stream Length (km)	
Park WS	0%	25.40%	61.03%	353.22	
MPLP	1.01%	25.67%	60.50%	111.68	
RP	1.01%	22.45%	51.76%	30.88	
ERP	1.07%	27.45%	60.50%	28.55	

Appendix C: Standard Operating Procedures for Whole-Body Sodium Analysis of Brook Trout (*Salvelinus fontinalis)*

Principal Investigator: Dr. Theodore B. Henry, Keil J. Neff

Objective: Establish a protocol for determining the whole-body sodium content of brook trout specimens.

Reference: This SOP was developed in the Center for Biotechnology (CEB) from the procedure used by Rich Grippo and was further modified to improve results.

<u>Procedure</u>:

This procedure is designed to measure the sodium content of adult brook trout. Brook trout (southern strain) are captured in the Great Smoky Mountain National Park and are immediately euthanized. Trout specimens are place in sterilized, pre-weighed and acid rinsed 250 ml Nalgene bottles and are refrigerated at 5° C.

1. The wet mass of each trout specimen is measured.
2. Trout specimen is oven-dried at 70 ° C for 5-7 days.
3. Dried trout specimen is allowed to cool for 1 hour.
4. The dry mass of each trout specimen is measured.
5. Approximately 70 ml of trace metal grade nitric acid is added to each sample bottle.
6. Sample bottles are sealed with Parafilm and placed under a fume hood.

7. Each trout specimen is dissolved in acid for 2-3 weeks until foaming has stopped (only a liquefied, amber-colored fish/acid solution remains).
8. Fish/acid solution is poured into acid-rinsed 100-ml graduated cylinder. Using a 5-ml micropipette, amounts of trace metal grade nitric acid are shot into 250-ml sample bottle (rinsing any remaining foam or residue) and then added to the 100-ml graduated cylinder. Using a 1-ml micropipette, nitric acid is added to graduated cylinder until solution volume is exactly 100-ml. Solution is then poured back into sample bottle, covered with Parafilm, and placed under a fume hood.
9. 100-ml fish/acid solution is given a 24-hour period for any biochemical reactions to come to completion.
10. 100-ml of deionized (DI) water is added to an acid-rinsed 100-ml bottle. Using a 1-ml micropipette, 1-ml of DI water is removed bringing total volume to 99-ml. Using a new 1-ml micropipette, fish/acid solution is added to 99-ml DI (solution is drawn from centroid of the 100-ml fish/acid solution; foam on sides of pipette tips are removed with Kim-wipe before adding to new bottle).
11. 1% fish/acid solution is vacuum-filtered using 0.45 µm filter (one rinse cycle).
12. Appropriate sodium standards (acidified 1%) are prepared. Each 1% fish/acid solution is run as a sample in the ICP-AES. Splits, spikes, and USGS check samples are performed for quality assurance.

Appendix D: Trout and Whole-Body Sodium Data

Trout Mass and Whole-Body Sodium Data

Tables 9, 10, 11 and 12 include the mass measurements and sodium concentrations of sampled trout from 6/20/2006, 6/24/2006, 6/27/2006 and 6/29/2006. Bucket ID identifies the sampled trout. The first number in the Bucket ID refers to the site: 1 is Middle Prong of the Little Pigeon River, 2 is Ramsey Prong, and 3 is Eagle Rocks Prong. The second number refers to the bucket location in the cage (numbered 1-4 clockwise starting upper left with top towards hinges) at each site. Wet(g) and Dry(g) refer to wet mass and dry mass of sampled trout respectively. Dry/Wet is the ratio between dry mass to wet mass. Na(mg/L) is the sodium concentration of the dilute acid/trout solution as reported by the ICP-AES. Na(mg) is the whole-body sodium in the trout. Na mg/g dry is the whole-body sodium divided by the dry mass. Na mg/g wet is the whole-body sodium divided by the wet mass.

Table 9: 6/20/2006 Sampled Trout

Bucket ID	Wet (g)	Dry (g)	dry/wet	Na (mg/L)	Na (mg)	Na mg/g dry	Na mg/g wet
1.1	16.932400	4.680700	0.276435	1.612137	16.121370	3.444222	0.9521
1.2	34.784300	8.344900	0.239904	3.293906	32.939060	3.947208	0.9470
1.3	22.585600	5.269800	0.233326	2.380703	23.807030	4.517634	1.0541
1.4	16.562600	4.101300	0.247624	1.720659	17.206590	4.195399	1.0389
2.1	31.086700	7.404200	0.238179	3.043216	30.432160	4.110121	0.9789
2.2	28.491200	6.534500	0.229352	2.806146	28.061460	4.294355	0.9849
2.3	21.064500	5.327800	0.252928	1.967020	19.670200	3.691993	0.9338
2.4	17.317300	4.372800	0.252510	1.661288	16.612876	3.799139	0.9593
3.1	16.423900	4.122300	0.250994	1.708671	17.086710	4.144946	1.0404
3.2	23.835000	6.492400	0.272389	2.224995	22.249950	3.427076	0.9335
3.3	18.369300	4.566300	0.248583	1.760677	17.606770	3.855807	0.9585
3.4	13.314400	3.560100	0.267387	1.245816	12.458160	3.499385	0.9357

Table 10: 6/24/2006 Sampled Trout

Bucket ID	Wet (g)	Dry (g)	dry/wet	Na (mg/L)	Na (mg)	Na mg/g dry	Na mg/g wet
1.1	11.665200	2.847200	0.244076	1.155612	11.556120	4.058767	0.9906
1.2	16.269500	3.788200	0.232841	1.587507	15.875070	4.190663	0.9758
1.3	14.354200	3.165500	0.220528	1.287663	12.876630	4.067803	0.8971
1.4	14.744900	3.542200	0.240232	1.288127	12.881272	3.636517	0.8736
2.1	9.411500	2.121900	0.225458	0.868993	8.689930	4.095353	0.9233
2.2	17.942600	4.821400	0.268712	1.413654	14.136540	2.932040	0.7879
2.3	15.095500	3.479700	0.230512	1.484589	14.845890	4.266428	0.9835
2.4	11.461300	2.584100	0.225463	1.153826	11.538260	4.465098	1.0067
3.1	18.713300	4.796500	0.256315	1.705830	17.058300	3.556406	0.9116
3.2	13.057500	3.186500	0.244036	1.238339	12.383390	3.886204	0.9484
3.3	12.044900	2.785500	0.231260	1.147035	11.470350	4.117878	0.9523
3.4	9.063700	2.225100	0.245496	0.833921	8.339209	3.747791	0.9201

Table 11: 6/27/2006 Sampled Trout

Bucket ID	Wet (g)	Dry (g)	dry/wet	Na (mg/L)	Na (mg)	Na mg/g dry	Na mg/g wet
1.1	28.202100	7.438900	0.263771	2.735036	27.350360	3.676667	0.9698
1.2	14.427400	3.592600	0.249012	1.453248	14.532480	4.045115	1.0073
1.3	18.930700	4.715600	0.249098	1.746696	17.466960	3.704080	0.9227
1.4	18.976500	4.414700	0.232640	1.769130	17.691300	4.007362	0.9323
2.1	39.463900	9.063600	0.229668	3.993567	39.935670	4.406160	1.0120
2.2	21.990200	5.601700	0.254736	1.905423	19.054230	3.401508	0.8665
2.3	11.326060	2.842200	0.250943	1.049771	10.497710	3.693516	0.9269
2.4	8.892700	2.142800	0.240962	0.900492	9.004923	4.202409	1.0126
3.1	40.629700	9.768100	0.240418	3.721300	37.213000	3.809646	0.9159
3.2	14.139300	3.680500	0.260303	1.219076	12.190760	3.312256	0.8622
3.3	28.039900	7.745800	0.276242	1.983366	19.833660	2.560570	0.7073
3.4	16.058000	4.441100	0.276566	1.116685	11.166850	2.514433	0.6954

Table 12: 6/29/2006 Sampled Trout

Bucket ID	Wet (g)	Dry (g)	dry/wet	Na (mg/L)	Na (mg)	Na mg/g dry	Na mg/g wet
1.1	42.896300	10.976100	0.255875	3.798662	37.986620	3.460849	0.8855
1.2	23.109600	5.048400	0.218455	2.204000	22.040000	4.365740	0.9537
1.3	25.138400	5.923300	0.235628	2.314600	23.146000	3.907619	0.9207
1.4	22.123900	4.937300	0.223166	2.179700	21.797000	4.414761	0.9852
2.1	35.004400	7.692200	0.219750	3.362372	33.623720	4.371145	0.9606
2.2	21.402500	4.882700	0.228137	2.075865	20.758650	4.251469	0.9699
2.3	15.140800	3.426500	0.226309	1.346408	13.464080	3.929397	0.8893
2.4	14.545200	3.152300	0.216724	1.28382	12.838200	4.072645	0.8826
3.1	15.837600	3.567100	0.225230	1.424359	14.243590	3.993045	0.8994
3.2	18.679800	4.270000	0.228589	1.718952	17.189520	4.025649	0.9202
3.3	42.191000	10.827900	0.256640	4.126469	41.264690	3.810960	0.9780
3.4	23.021600	5.196700	0.225731	2.114040	21.140400	4.068043	0.9183

Whole-Body Sodium QA/QC

QA/QC samples were prepared and analyzed to ensure results are precise and accurate and comprise at least 20% of all samples. Quality control check samples (QC) were prepared using a standard stock solution. Quality control samples were implemented for each run; QC results are presented in Table 13. A trace metal sample (T-183), with reported most probable value, was obtained from the USGS Standard Reference Sample website. T-183 samples were implemented for each run; T-183 results are presented in Table 14. Laboratory splits and spikes (spiked with standard stock solution) results are presented in Table 15 and Table 16 respectively. Blanks (DI) are prepared from deionized water and acidified 1% with nitric acid. Blank samples were implemented in each run; DI results are presented in Table 17.

Table 13: QC Results

		Na5889
QC-1	13 Sep 2006 12:15:48	0.9857
QC-1	13 Sep 2006 13:15:47	0.9745
QC-1	11 Sep 2006 12:34:08	0.9892
QC-1	11 Sep 2006 13:34:07	0.9878
QC-1	11 Sep 2006 13:38:44	0.9626
QC-1	14 Sep 2006 13:42:29	0.9824
QC-1	14 Sep 2006 14:42:33	0.9634
QC-1	14 Sep 2006 14:47:10	0.9662
QC-1	18 Sep 2006 14:45:52	0.9573
QC-1	19 Sep 2006 09:36:08	0.9938
QC-1	19 Sep 2006 14:32:38	0.9899
QC-1	19 Sep 2006 15:23:26	0.9591
QC-1	6 Oct 2006 11:16:05	0.9811
QC-1	6 Oct 2006 12:11:31	0.9532
QC-1	9 Oct 2006 10:15:18	1.007
QC-1	9 Oct 2006 11:35:39	0.9638
QC-1	9 Oct 2006 12:35:41	0.9125
	Average	0.972324
	Actual	1
	% Difference	2.767647
	Std Dev	0.021634

Table 14: USGS T-183 Sample Check

		Na5889
T183	13 Sep 2006 13:10:57	12.90055
T183	9 Oct 2006 12:30:49	12.54808
T183	6 Oct 2006 12:06:41	12.97629
T183	19 Sep 2006 15:18:37	12.46235
T183	19 Sep 2006 10:31:18	12.57251
T183	18 Sep 2006 15:41:01	12.6204
T183	14 Sep 2006 14:37:43	12.81938
T183	11 Sep 2006 13:29:17	12.64443
	Average	12.693
	Actual	12.7
	% Difference	0.055126
	Std Dev	0.183479
	Rel Std Dev	1.45%

Table 15: % Recover of Spike Samples

7/24_1.1	11 Sep 2006 12:43:22	1.1655504
7/24_1.1spike	11 Sep 2006 12:47:58	1.910804
	Actual Spike	0.7
	% Recovery	106.4648
7/24_1.2	11 Sep 2006 12:52:32	1.6003484
7/24_1.2spike	11 Sep 2006 12:57:07	2.298952
	Actual Spike	0.7
	% Recovery	99.800514
6/24_2.3	13 Sep 2006 12:34:12	1.6031362
6/24_2.3spike	13 Sep 2006 12:38:47	2.268912
	Actual Spike	0.7
	% Recovery	95.110829
6/24_2.2	13 Sep 2006 12:25:02	1.4124686
6/24_2.2splike	13 Sep 2006 12:29:38	2.266116
	Actual Spike	0.7
	% Recovery	121.94963
0629_3.4	9 Oct 2006 12:21:40	1.831462
0629_3.4spike	9 Oct 2006 12:26:15	2.807562
	Actual Spike	1
	% Recovery	97.61
	Total Avg % Recovery	105.83144

Table 16: % Recovery of Split Samples

		Na5889
6/24_1.3	11 Sep 2006 13:01:41	1.3021236
6/24_1.3split	11 Sep 2006 13:06:17	1.2856986
	Difference	0.016425
	% Difference from Avg	1.2694071
6/24_1.4	11 Sep 2006 13:10:52	1.2714864
6/24_1.4split	11 Sep 2006 13:15:28	1.2881272
	Difference	0.0166408
	% Difference from Avg	1.3002588
6/24_2.1	11 Sep 2006 13:20:04	0.9067014
6/24_2.1split	11 Sep 2006 13:24:41	0.9233768
	Difference	0.0166754
	% Difference from Avg	1.8223702
6/24_2.4	13 Sep 2006 12:43:21	1.2300872
6/24_2.4split	13 Sep 2006 12:47:56	1.2221558
	Difference	0.0079314
	% Difference from Avg	0.646869
6/24_3.1	13 Sep 2006 12:52:32	1.6537994
6/24_3.1split	13 Sep 2006 12:57:07	1.64503
	Difference	0.0087694
	% Difference from Avg	0.5316674
6/24_3.2	13 Sep 2006 13:01:44	1.220117
6/24_3.2split	13 Sep 2006 13:06:20	1.2151346
	Difference	0.0049824
	% Difference from Avg	0.4091898
0629_3.3	9 Oct 2006 12:12:29	4.814205
0629_3.3split	9 Oct 2006 12:17:05	4.788644
	Difference	0.025561
	Total % Difference from Avg	0.5323628

Table 17: DI Sample Checks

		Na5889
di	11 Sep 2006 12:38:44	-0.002385
di	13 Sep 2006 12:20:25	-0.002396
di	9 Oct 2006 11:40:15	-0.017005
di	9 Oct 2006 10:19:53	0.00451
di	6 Oct 2006 11:20:41	-0.004415
di	19 Sep 2006 14:37:15	0.074338
di	19 Sep 2006 09:40:44	-0.006924
di	18 Sep 2006 14:50:28	-0.030404
DI	14 Sep 2006 13:47:06	0.001078
di	13 Sep 2006 12:20:25	-0.001532
di	11 Sep 2006 12:38:44	0.017658
	Average	0.002957

Additional Trout Analyses

In the Results (Chapter 2), whole-body sodium analyses were restricted to wet mass. The following figures and summary statistics describe statistical differences when considering dry mass. Box plots and sodium concentrations (mg Na per g dry mass) are illustrated in Figure 11. Figure 12 shows the mean diamond plots and Tukey-Kramer HSD confidence circles of trout whole-body sodium concentration. Figure 13 illustrates the differences of sodium concentration between sample dates at the Eagle Rocks Prong site.

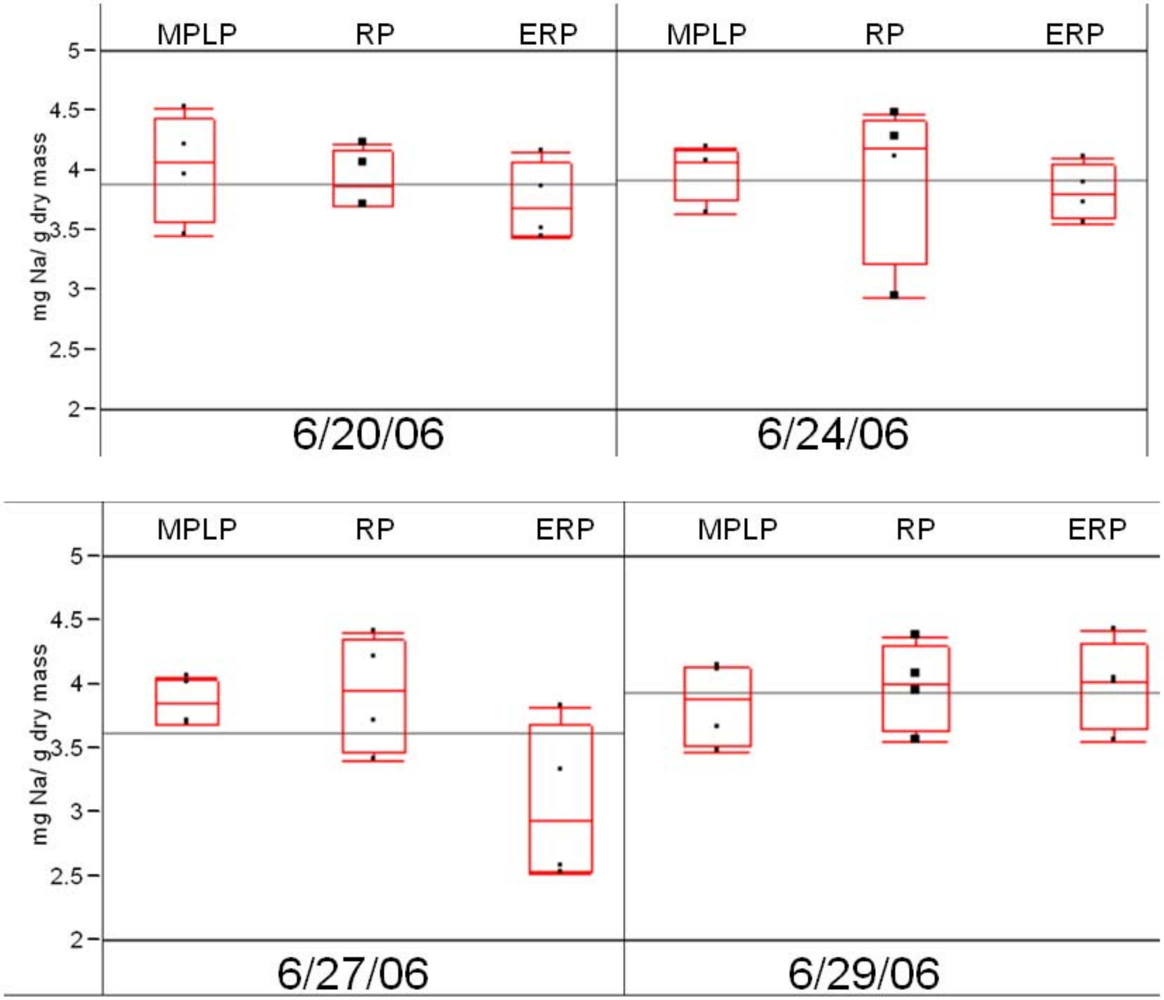


Figure 11: Whole-Body Dry Mass Sodium Concentrations of All Sampled Trout

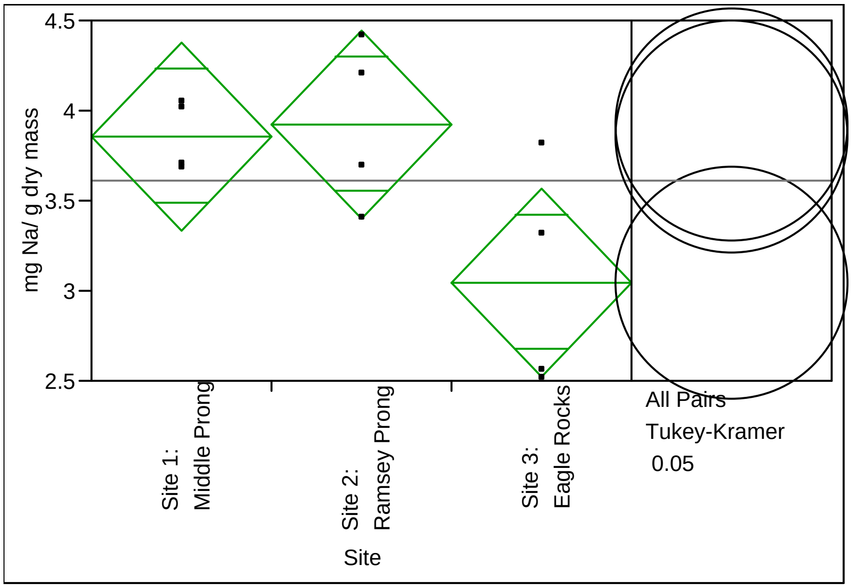


Figure 12: Mean Diamonds of Total Sodium per Dry Mass on 6/27/2006

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Site	2	1.9036910	0.951845	4.4567	0.0452
Error	9	1.9221975	0.213577		
C. Total	11	3.8258885			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Site 1: Middle Prong	4	3.85833	0.23107	3.3356	4.3810
Site 2: Ramsey Prong	4	3.92590	0.23107	3.4032	4.4486
Site 3: Eagle Rocks	4	3.04923	0.23107	2.5265	3.5719

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
2.79201	0.05

Abs(Dif)-LSD	Site 2: Ramsey Prong	Site 1: Middle Prong	Site 3: Eagle Rocks
Site 2: Ramsey Prong	-0.91239	-0.84481	-0.03571
Site 1: Middle Prong	-0.84481	-0.91239	-0.10329
Site 3: Eagle Rocks	-0.03571	-0.10329	-0.91239

Positive values show pairs of means that are significantly different.

Level		Mean
Site 2: Ramsey Prong	A	3.9259000
Site 1: Middle Prong	A	3.8583250
Site 3: Eagle Rocks	A	3.0492250

Levels not connected by same letter are significantly different.

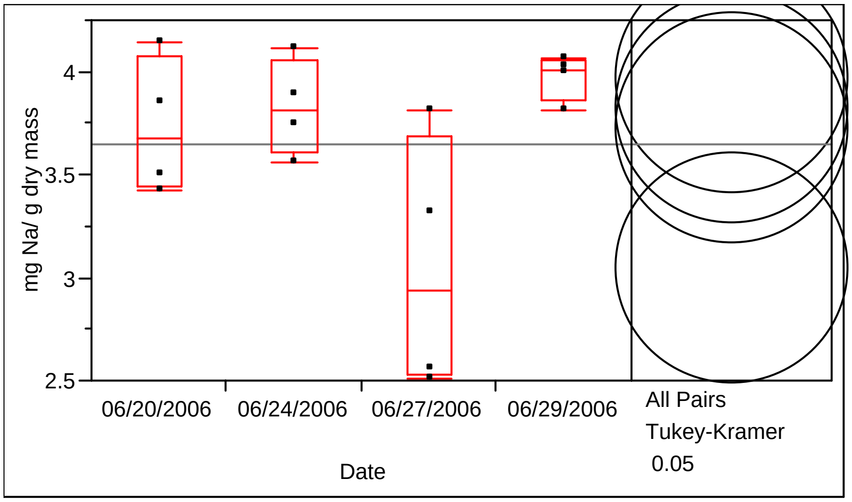


Figure 13: Whole-body Sodium Concentrations at Eagle Rocks Prong at Each Sample Date

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Date	3	2.0165983	0.672199	4.7137	0.0213
Error	12	1.7112512	0.142604		
C. Total	15	3.7278495			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
06/20/2006	4	3.73181	0.18881	3.3204	4.1432
06/24/2006	4	3.82709	0.18881	3.4157	4.2385
06/27/2006	4	3.04923	0.18881	2.6378	3.4606
06/29/2006	4	3.97442	0.18881	3.5630	4.3858

Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
2.96883	0.05

Abs(Dif)-LSD	06/29/2006	06/24/2006	06/20/2006	06/27/2006
06/29/2006	-0.79275	-0.64542	-0.55014	0.13244
06/24/2006	-0.64542	-0.79275	-0.69747	-0.01489
06/20/2006	-0.55014	-0.69747	-0.79275	-0.11017
06/27/2006	0.13244	-0.01489	-0.11017	-0.79275

Positive values show pairs of means that are significantly different.

Level			Mean
06/29/2006	A		3.9744231
06/24/2006	A	B	3.8270920
06/20/2006	A	B	3.7318098
06/27/2006		B	3.0492331

Levels not connected by same letter are significantly different.

Appendix E: Permits

IACUC Permit

An IACUC permit was required for scientific research on animals. The following is the adapted IACUC permit prepared on behalf (and under the direct management) of Dr. Robinson, Dr. Schwartz and Dr. Henry by Keil Neff in the spring of 2006.

Effect of acid deposition on fish and water quality in the Great Smoky Mountains National Park
Title

Dr. R. Bruce Robinson/ Dr. John Schwartz/ Dr. Theodore Henry
Principal Investigator/Instructor

(865)974-2503 rbr@utk.edu Civil and Environmental Engineering 223 Perkins Hall
Wk. Phone *E-Mail* *Department* *Campus Address*

Your signature as P.I., Co-investigator, or Department Head on this application verifies that: (1) the information herein is true and correct and that you are familiar with and will comply with the legal standards of animal care and use established under federal and state laws and policies as well as university policies; (2) the proposal has received approval for scientific and/or educational merit by peer review; and (3) the activities do not unnecessarily duplicate previous experiments.

Signature of P.I./Instructor *Date*

Signature of Co-investigator *Date*

Signature of Department Head *Date*

GENERAL INFORMATION

1. This is a **[X]** New **[ ]** 3-year rewrite

[X] Research **[ ]** Teaching Courses/CE Seminars covered by this protocol:

____

2. **[ ]** Yes **[X]** No This protocol includes the use of farm animals used or intended for use as food or fiber, or livestock or poultry used or for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber.

3. **Veterinary Care**
Who is responsible for clinical care of these animals?
_______N/A____

NOTE: If the veterinarian providing clinical care is anyone other than the Attending Veterinarian (Dr. O'Rourke), an authorization form ***must*** *be filled out and signed by the AV and IO prior to protocol approval. Please contact the OLAC office for further information.*

4. **Funding Source:**

Congressional Earmark funding administered through the US Environmental Protection Agency.

5. **Non-Surgical Procedures** (Pertains to any experimental procedure - including non-surgical, pre-surgical and post-surgical procedures using animals)

[ ] NO [X] YES:

6. **Surgical Procedures** (Pertains to <u>any</u> surgical procedure, including non-survival surgery. If other procedures are done on animals prior to or after surgery, complete applicable sections.)

[X] NO [ ] YES:

7. **Field Studies Involving Wild Animals**

[ ] NO [X] YES: (this section may be obtained from the IACUC office 974-3631)

8. **Hazardous Agents**

[X] NO [ ] YES:

9. **Prescription Drugs/Controlled Substances**

[X] NO [ ] YES:

10. **Euthanasia**

[ ] NO [X] YES:

A. NON-TECHNICAL SUMMARY

Native brook trout have been extirpated from six headwater streams in the Great Smoky National Park (GRSM). Based on long term declines in stream pH, Park resource managers fear that brook trout will continue to be eliminated from streams and may disappear entirely from the Park within about 25-50 years. Although acid deposition and acidic storm events are suspected of being the primary cause, baseline and storm event water quality monitoring coupled with fish sampling, in situ survival tests, and physiological examinations are required to determine whether acid deposition is indeed the cause. This research project will perform monitoring in stream systems that have experienced native brook trout elimination and in those that have not. This work will compare 1) water quality, especially pH and toxic metal concentrations, and 2) survival and stress of trout under baseline and storm event conditions. The results will also be compared between healthy stream reaches in the Park and those that have seen extirpation. The results will be generalized and a predictive model developed.

This research will monitor three stream sites (Little Pigeon River, Ramsey Prong, Eagle Rocks Prong). The Little Pigeon River will serve as a control in that it has not seen trout extirpation. Ramsey Prong and Eagle Rocks Prong have shown historical trout extirpation for unknown reasons. Stream monitoring at each site will consist of 1) continuous water quality measurements with a multi-parameter monitor (sonde), 2) collection of storm events stream samples with an automated sampler, 3) measurement of fish population metrics with standard electrofishing gear (non-lethal method), 4) in situ trout survival tests, and 5) collection of individual fish for further physiology testing.

In situ trout survival tests will be conducted for three storm events and one non-storm flow (base flow). The toxicity of stream water to trout will be investigated by comparing the lethal and sub-lethal responses of trout held in cages at the three sites. Trout will be collected from the Little Pigeon River by electrofishing and transported to the three sites. Four cages constructed of HD-polyethylene pipe will be installed at each site. Forty trout will be randomly assigned to each site (ten fish per cage) for a duration of 20 days. Mortality will be checked at a minimum of once a week and following a rain event. Four trout from each site will be randomly sampled from cages at the beginning of the testing period and following an episodic rain event for the evaluation of the sub-lethal effects of exposure. Trout from each of these samples will be immediately euthanized in 250 mg/L of fish anesthetic (MS-222, Argent Chemical Laboratories, Redman, WA). Three of the four sampled trout will be placed in individual plastic bags and held on ice during transport to the laboratory. These fish will be frozen in the laboratory. The whole-body sodium concentration will be determined following the procedure of Gonzalez and Dunson (1987). The fourth sampled trout will be euthanized and samples of gill tissues will be collected immediately. Gill tissues will be preserved in 10% NBF for histological analysis. Fish gills preserved for histopathology will be excised from the head and processed in paraffin for routine histological examination (Henry and Gizzle, 2004).

1. Gonzalez, R. J. and Dunson, W. A. 1987. ADAPTATIONS OF SODIUM-BALANCE TO LOW PH IN A SUNFISH (ENNEACANTHUS-OBESUS) FROM NATURALLY ACIDIC WATERS.

JOURNAL OF COMPARATIVE PHYSIOLOGY B-BIOCHEMICAL SYSTEMIC AND ENVIRONMENTAL PHYSIOLOGY 157 (5): 555-566.

2. Henry, T. B. and Gizzle, J. M. 2004. Electroshocking-induced injuries in newly transformed juvenile fish. Journal of Aquatic Animal Health 15(2):147-157.

B. ANIMAL HOUSING FACILITIES

In order to provide assurance of humane care and use of laboratory animals, all animal housing facilities on the UTK campus will be operated according to federal laws, AAALAC and NIH Guidelines, and the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching as appropriate. Housing of animals, including those actively on experiment, for periods longer than 12 hours, is restricted to facilities meeting such guidelines and laws.

1. Please check your preferred housing location.

[ ] College of Arts and Sciences Facility in the Walters Life Sciences Building (WLS).
[ ] College of Human Ecology Animal Facility in the Jessie Harris Building.
[ ] College of Veterinary Medicine (CVM) Laboratory Animal Facility VTH/Cherokee
[ ] CVM Large Animal Clinical Sciences in VTH/Cherokee Farm
[ ] CVM Small Animal Clinical Sciences Research Runs
[ ] UT Medical Center Lab Animal Facility
[ ] Joe E. Johnson Animal Research and Teaching Unit
[ ] IACUC Approved Satellite Facility List bldg/room # ____________________
[X] ***Other** List: Great Smoky National Park (Little Pigeon River, Ramsey Prong, Eagle Rocks Prong) in situ

***Any newly established facility must be approved before housing animals.

2. Please list all special housing or husbandry requirements.

Test fish will be contained in HD-polyethylene pipe cages. These cages will have 100+ predrilled holes to ensure adequate water exchange. Wet mass in cages will not exceed 7.5 g/L to avoid stress from crowding (Johnson, 1987). Cages will be submerged and anchored downstream of a large obstruction, such as a large rock, so that fish are not subjected to high current velocities (J. VanSickle, 1996).

VanSickle, J.; Baker, J. P.; Simonin, H. A.; Baldigo, B. P.; Kretser, W. A.; Sharper, W. E. 1996. Episodic Acidification of Small Streams in the Northeastern United States: Episodic Response

Project. *Ecological Applications*, Vol. 6, No. 2. (May, 1996), pp. 374-388.

3. Will wire bottom cages be used for rodents? If so, please provide scientific justification for use of wire bottom cages.

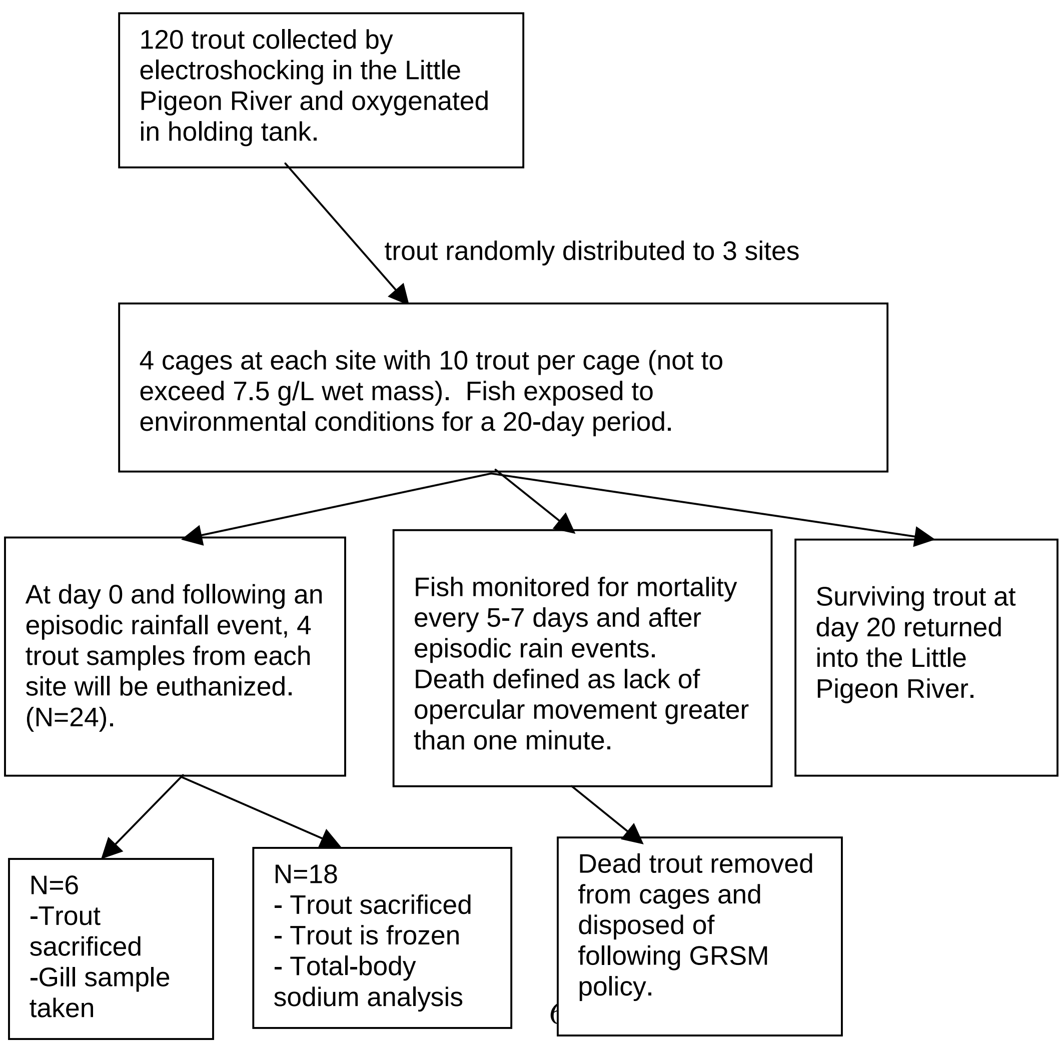
   N/A

4. Environmental enrichment is routinely provided for animals. Please indicate if environmental enrichment is NOT to be provided and justification why it would interfere with research.

   N/A

In accordance with NIH Guidelines and Federal Law, the IACUC formally reviews all animal housing facilities and satellites semi-annually and files a report to the Office for Protection from Research Risks (OLAW) in Washington, DC and the United States Department of Agriculture (USDA).

FLOW CHART (INCLUDING EXACT ANIMAL MANIPULATIONS/PROCEDURES):



C. DESCRIPTION OF ANIMALS

TABLE 1.

Common Name	Strain/Breed	Sex	Weight/Age	Source	# per Day
Brook Trout	***Salvelinus***	***M/F***	***Juvenile/adult***	***Little Pigeon River***	**0-120**

1. Animals will be removed from the animal housing facility:

   [X] No

   [ ] Yes. Please answer the following:
   1. Animals will be taken to (bldg/room number):____________________
   2. Animal manipulations that will be performed in the laboratory include: __________ ____
   3. Estimated total time period live animals will be kept in the laboratory: _______hours
   4. Animals will be returned to the facility YES NO

2. Disposal of animals after completion of activity:

   [ ] Return to production/breeding unit/facility inventory
   [ ] Slaughter, (must conform to the Humane Slaughter of Livestock, 9 CFR, part 313)
   [ ] Sold
   [ ] Transfer to another research project – please list protocol # and Investigator ____________________
   [ ] Adoption
   [X] Euthanatized
   [ ] Returned to owner
   [X] Other (Please describe)___ **Trout not euthanatized will be returned alive into Little Pigeon River**______

D. QUALIFICATIONS OF PERSONNEL

List **all** individuals who will be working with the animals on this project. Include all investigators, student employees, post-doctoral researchers, staff research associates and laboratory assistants who will actually work with the animals. If personnel do not have experience, state how they will be trained.

The **Occupational Health Program** (OHP) is **mandatory** for all personnel who work with laboratory animals.
If an individual having animal contact is not currently enrolled in the Occupational Health Program call The Occupational Health Nurse, 974 5728 for information on enrolling.

TABLE 2.

Name/Degree(s)	OHP*	VA** training	Procedure(s)/Description of **Relevant** Experience
Theodore B. Henry, Ph.D.	**Yes**	**Yes**	**Ph.D. in fish pathology/aquatic toxicology; aquaculture experience**
John Schwartz, Ph.D.	**Yes**	**Yes**	**M.S. Fisheries Science, Ph.D. Environmental Engineering**
Keil J. Neff, B.S.	**Yes**	**Yes**	**Trained by Dr. Henry and Dr. Schwartz**
Amanda Dunnavant, B.S.	**Yes**	**Yes**	**Trained by Dr. Henry and Dr. Schwartz**

****ALL INDIVIDUALS LISTED ON THIS PROTOCOL MUST BE ENROLLED IN OHP AND A "CERTIFICATE OF COMPLETION "MUST BE ON FILE IN THE IACUC OFFICE.**

E. ANIMAL WELFARE

1. **In addition to procedures which obviously cause pain, distress, or discomfort, USDA (Policy 11 & 12) states that any procedure which requires the use of an anesthetic or analgesic to prevent pain or discomfort is by definition a painful procedure (examples of painful procedures include: survival and non-survival surgery, use of Freund's Adjuvant, monoclonal antibody production, food/water deprivation, and application of noxious stimuli).**

According to the above definition does this project involve pain or distress? **[X]** yes **[ ]** no

If this is a teaching protocol, you must search the ucdavis.edu website for alternatives.
If yes, please provide a written narrative that **must** include:

a. at least two databases must be searched or other sources consulted to confirm that less painful alternative methods are not available. **(Web of Science, Chemical Abstracts)**
b. the date of the search **(September 2005)** and the years covered by the search **(1985-2005)**
c. key words and/or search strategy used **(fish bioassays, in situ testing, biomonitoring, episodic acidification, brook trout)**
d. a narrative written in such a way that the IACUC can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough

A general search using Web of Science and Chemical Abstracts (SciFinder Scholar) was conducted with the search terms described in part E1b (see above). Starvation and other caging stresses are minimal during a 20-day testing period and mortalities observed in acidified bioassays during the first 20 days are due to toxic effects (J. VanSickle et al., 1996). Capture of trout using electroshocking equipment is a standard operating procedure for collection of fish (Henry and Gizzle, 2004)

1. Henry, T. B. and Gizzle, J. M. 2004. Electroshocking-induced injuries in newly transformed juvenile fish. Journal of Aquatic Animal Health 15(2):147-157.

2. VanSickle, J.; Baker, J. P.; Simonin, H. A.; Baldigo, B. P.; Kretser, W. A.; Sharper, W. E. 1996. Episodic Acidification of Small Streams in the Northeastern United States: Episodic Response Project. *Ecological Applications*, Vol. 6, No. 2. (May, 1996), pp. 374-388.

This search should consider:

- **Replacement** of existing animal methods with non-animal methods whenever possible.
- **Reduction** of the number of animals needed.
- **Refinement** of research procedures to minimize pain and discomfort.

2. Does the proposed research/course duplicate any previous work? **[ ]** yes **[X]** no If yes, explain why it is scientifically necessary to replicate the experiment. If this is a teaching activity, describe the specific educational goals that will be met through the proposed use of animals.

3. Explain why the proposed species is/are the most appropriate.

Native fish populations have been endangered in regions impacted by acidic precipitation (Johnson, 1987). The GRSM receives the highest acid deposition of any national park (Shubzda et al., 1995). Native brook trout have been extirpated from six headwater streams in the GRSM Park. The protection of brook trout, which is the only native trout in the GRSM, is the highest priority in the Park's fisheries management plan. The GRSM needs to understand and predict the potential for impairment of aquatic resources by acid deposition. Importantly, the GRSM includes five Outstanding National Resource Waters, which the Park is legally responsible for protecting from impairment. If the GRSM is not able to understand and predict potential impairment, then it may be too late to act when the problem becomes unequivocally clear.

1. Johnson, D.W., H.A. Simonin, J.R. Colquhoun and F.M. Flack, 1987. In situ toxicity tests of fishes
in acid waters. Biogeochemistry 3: 181–208.

2. Schubzda, J., Lindberg, S. E., Garten, C. T., and Nodvin, S. C. 1995. Elevational Trends in the Fluxes of Sulfur and Nitrogen in Throughfall in the Southern Appalachian Mountains: Some Surprising Results. Water, Air, and Soil Pollution 85:2265-2270.

4. Describe the steps you have taken to reduce the number of animals in your study. (refining experimental design, replacing animals with *in vitro* procedures, etc.)

Experiments designed according to methods described in the literature, and which satisfy appropriate assumptions for statistical analyses.

5. Provide an explanation of how the numbers of animals to be used were derived. If used in an experiment (test a hypothesis) numbers should be based on scientific and statistical requirements (ex: power tests or previous experience) to achieve objectives.

Since the response variance is unknown, numbers of trout used in this study are based on numbers used in similar studies from the literature search.

. **DURING THE STUDY:**

a. How often will the clinical condition of animals be monitored?

Trout will be monitored at a minimum of once a week and following rain events.

b. Who will monitor the clinical condition of the animals?

Keil J. Neff, Amanda Dunnavant

c. Are animals expected to experience any specific study-induced or related problems (i.e. health problems, pain, distress, complications, etc.) or any health problems as a result of the phenotype of the animal?

No

d. What criteria will be used to assess pain, distress, or discomfort?

Check all that apply:

[ ] Loss of appetite.
[ ] Loss of weight.
[X] Restlessness.
[X] Abnormal resting postures in which the animal appears to be sleeping or is hunched up.
[ ] Licking, biting, scratching, or shaking a particular area.
[ ] Failure to show normal patterns of inquisitiveness.
[ ] Failure to groom, causing and unkempt appearance.
[ ] Guarding (protecting the painful area).
[X] Loss of mobility.
[ ] Red stain around the eyes of rats.
[X] Unresponsiveness.
[ ] Self-mutilation.

[ ] Labored breathing.
[X] Other. (please list): Excessive gill movement

e. What criteria will be used for removing animals from the study prematurely?

Trout will be prematurely removed from study upon death as defined by lack of opercular movement for > 1 minute.

NON-SURGICAL PROCEDURES

1. SITES OF STUDY

Site of experimental work**:** **N/A**
Bldg. Room

2. STUDY PROCEDURES : N/A

Please check the following items that apply and fill out appropriate parts of this section.

[ ] Polyclonal antibody production*****
[ ] Blood***** or other body fluid withdrawal, tissue collection, injections, tail clip, gavaging
[ ] Restraint with mechanical devices (tethers, stanchions, metabolism cages)
[ ] Projects involving food and water deprivation, or dietary manipulation
[] Tumor and disease models***** or toxicity testing
[ ] Anesthesia or analgesia***** (for non-surgical procedures)
[ ] Behavioral studies
[ ] Endoscopy, fluoroscopy, radiology, ultrasound, MRI, CT, PET, or other diagnostic procedures
[ ] Monoclonal antibody production
[ ] Use of tissues, serum, tumor lines, hybridoma, etc.

*** The IACUC has approved guidelines for these procedures. Deviations must be justified.**

POLYCLONAL ANTIBODY PRODUCTION

Describe procedure, antigen and adjuvants used, the ratio of antigen to adjuvant and routes and volumes of injection (Vol. should be < 0.25 ml per injection site; subcutaneous route is recommended):

N/A

BLOOD OR BODY FLUID WITHDRAWAL/TISSUE COLLECTION/ INJECTIONS, TAIL CLIP, GAVAGING

Describe in detail method(s), needle sizes, volume(s) collected or administered, and frequency of collection or injections:

Use cursor to expand the cells as needed. **N/A**

RESTRAINT WITH MECHANICAL DEVICES

Describe device, duration of restraint, frequency of observation, conditioning procedures and steps to assure comfort and well-being:

N/A

PROJECTS INVOLVING FOOD AND WATER DEPRIVATION, OR DIETARY MANIPULATION

Describe methodology. State objective criteria used to assess physical condition and pain, discomfort, stress, and distress during the course of study. Include clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

N/A

TUMOR AND DISEASE MODELS, TOXICITY TESTING

Describe methodology used for tumor/disease induction and/or toxicity testing. State objective criteria used to assess physical condition and pain, discomfort, stress, and distress during the course of study. Include clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

Trout will be exposed to natural waters in situ. The toxic stressor is the natural waters during episodic, low pH events. As the objective of this experiment is to relate characteristics of water quality during these events to toxicity, the important endpoints will include morbidity and death.

ANESTHESIA/ANALGESIA/TRANQUILIZATION (OTHER THAN SURGERY)

N/A

Adequate records describing anesthetic monitoring and recovery must be maintained and available to the attending veterinarian and animal care staff.

Name and qualifications of person administering drugs:_________**N/A**___________

CRITERIA TO ASSESS LEVEL OF ANESTHESIA.
Check all that apply:
[ ] Respiration rate
[ ] Heart rate
[ ] ECG
[ ] Toe pinch
[ ] Tail pinch
[ ] Corneal reflex
[ ] Color of mucous membranes
[ ] Muscular relaxation
[ ] Other (pulse oximeter, respirometer) please list ______________________________

ANESTHESIA RECOVERY MONITORING

a. Will analgesia be provided? [ ] Yes [ ] No
If no, please explain why analgesics are withheld:
N/A

b. What is the anticipated duration for recovery from anesthesia?
N/A

c. How often will animal(s) be monitored during recovery?
N/A

d. What specifically will be monitored?
N/A

e. Who will be monitoring them?
N/A

RECOVERY MONITORING

a. Following anesthesia recovery, what parameters will be monitored?
N/A

b. Who will monitor the animals?
N/A

c. How frequently will animals be monitored?
N/A

BEHAVIORAL STUDIES

Describe in detail types of behavioral manipulations, including placement in testing chambers or apparatus, use of aversive stimuli, duration of test periods, and frequency of test periods:

N/A

ENDOSCOPY, FLUOROSCOPY, X-RAY, ULTRASOUND, MRI, CT, PET, OR OTHER DIAGNOSTIC PROCEDURES

Describe, in detail, methodology and animal manipulations:

N/A

MONOCLONAL ANTIBODY PRODUCTION

Please provide scientific justification explaining why specific *in vitro* monoclonal antibody production methods cannot be used. IACUC monoclonal antibody production guidelines **must** be followed. Any deviation from these guidelines must be specified and scientifically justified below.

N/A

USE OF TISSUES, SERUM, TUMOR LINES, HYBRIDOMA ETC.

All tissues must be MAP or PCR tested to ensure that tissues are free of infectious agents. Please provide evidence of this testing:

N/A

SURGICAL PROCEDURES

The attending veterinarian must be consulted on anesthetic regimens, surgical procedures and post-surgical care.

Adequate records describing surgical procedures, anesthetic monitoring and postoperative care must be maintained and available to the attending veterinarian and animal care staff.

Type of Surgery

__ Nonsurvival surgery: (animals euthanized without regaining consciousness).
__ Major survival surgery: (major surgery penetrates and exposes a body cavity or produces substantial impairment of
physiologic function).
__ Minor survival surgery.
_ Multiple survival surgery?
If yes, provide justification for multiple survival surgical procedures:

1. Surgeon's name and experience with species and procedures to be performed:
N/A

2. Location where surgery will be done:
N/A

3. Describe the pre-op preparation of the animals:
N/A
   a. Food restricted for _____ hours
   b. Water restricted for _____hours

4. Minimal sterile techniques will include (check all that apply):
N/A
   [ ] Sterile instruments
   [ ] Sterile gloves
   [ ] Cap and mask
   [ ] Sterile gown
   [ ] Sterile operating area
   [ ] Clipping or plucking of hair or feathers
   [ ] Skin preparation with a sterilant such as betadine
   [ ] Practices to maintain sterility of instruments during surgery

5. Describe the following surgical procedures:
N/A
   a. Skin incision size and location:

   b. Method of skin closure: (type, suture size, suture pattern, etc.)

   c: Describe surgery in detail:

6. WILL PARALYZING DRUGS BE USED?
N/A [ ] No [ ] Yes ____
Drug Dose

IF YES, PLEASE JUSTIFY THE NEED TO USE PARALYZING DRUGS:

7. Anesthetic Protocol
N/A

TABLE 6.

	Agent	Dose (mg/kg)	Frequency
Pre-emptive analgesic			
Pre-anesthetic			
Anesthetic			
Analgesic			
Other			

CRITERIA TO ASSESS LEVEL OF ANESTHESIA.
Check all that apply:
__ Respiration rate
__ Heart rate
__ ECG
__ Toe pinch
__ Tail pinch
__ Corneal reflex
__ Color of mucous membranes
__ Muscular relaxation
__ Other (pulse oximeter, respirometer) please list
____

8. Describe any behavioral or husbandry manipulations that will be used to alleviate pain, distress, and/or discomfort.
N/A

9. ANESTHESIA RECOVERY MONITORING
N/A

a. Will analgesia be provided? [ ] Yes [ ] No
If no, please explain why analgesics are withheld:

b. What is the anticipated duration for recovery from anesthesia?

c. How often will animal(s) be monitored during recovery?

d. What specifically will be monitored?

e. Who will be monitoring them?

10. Postsurgical Recovery Monitoring
N/A

a. Following recovery, what parameters will be monitored?

b. Who will monitor the animals?

c. How frequently will animals be monitored?

Hazardous Agents

1. Will animals be subjected to any of the following? No

_______ 1. *Infectious Agents

_______ 2. *Toxic Chemicals or Carcinogens

_______ 3. *Recombinant DNA

_______ 4. *Malignant Cells or Hybridomas

_______ 5. **Radioisotopes

_______ 6. *Human tissue

*Biosafety Committee approval required Approval Number___________________
**Radiation Safety Committee approval required (Please attach approval letter)

2. **If yes**, please complete the <u>attached</u> animal hazard control form.

N/A

3. Complete the following chart. N/A

Legend (prescription) drugs and/or controlled substances

* **Legend Drugs and Controlled Substances** In Tennessee, it is not legal to divert drugs from clinical use to teaching and research use. You, or someone in your group acting as an agent for the group, must have a license for each individual legend drug that you might use in teaching or research. A <u>legend drug</u> is one where the label says “Federal Law restricts this drug to use by or on the order of a licensed veterinarian” or “Federal Law prohibits dispensing without a prescription”. If the drug is a controlled substance, you also need a license from the Drug Enforcement Administration for the specific drugs that you may use. Obtaining and using such

drugs for research needs to be reviewed by the appropriate Committee. **The use of legend drugs and controlled substances must be approved by the appropriate committee.**

Do you have the appropriate licenses and legend and controlled drug Committee approval for the drugs you will use in your project or class?

N/A

[ ] NO* **[ ] YES**

College of Veterinary Medicine personnel contact: Bruce McNeil 974-5670
Medical Center personnel contact Mark Smith 544-9363
All others contact: Brenda Lawson 974-3466

Euthanasia

Methods of euthanasia must comply with the 2000 Report of the AVMA Panel on Euthanasia or other IACUC approved methods. Departures must be justified. A procedure must be in place for a "just in case" situation, even if you don't plan to use it.

1. METHOD OF EUTHANASIA

__X__ Anesthetic overdose.
Drug:___fish anesthetic (MS-222, Argent Chemical Laboratories, Redman, WA)_________
Dose: _____250 mg/L ___________
Route: __________________

_____ Decapitation under anesthesia or tranquilization

_____ Decapitation without anesthesia or tranquilization*

_____ Cervical dislocation under anesthesia or tranquilization

_____ Cervical dislocation without anesthesia or tranquilization*

_____ Exsanguination under anesthesia

_____ Carbon dioxide exposure

_____ Slaughter (covered under the Humane Slaughter of Livestock, 9 CFR, part 313)

_____ Other: Specify:

***PLEASE JUSTIFY ANY DEPARTURE FROM AVMA PANEL REPORT:(EX. CERVICAL DISLOCATION WITHOUT ANESTHESIA)**

2. NAME AND QUALIFICATIONS OF PERSON(S) PERFORMING EUTHANASIA:

Co-PI Theodore B. Henry, Ph.D.; graduate research assistants trained by Dr. Henry.

3. METHOD OF DISPOSAL. EUTHANIZED ANIMAL CARCASSES MUST BE DISPOSED OF APPROPRIATELY.

Please check the appropriate method:

[ ] incinerate
[ ] by Radiation Safety
[ ] carcass will contain hazardous agents and will be autoclaved prior to disposal
[ ] slaughter (covered under the Humane Slaughter of Livestock, 9 CFR, part 313)
[ ] necropsy

[X] other - describe: Trout carcasses in the field will be disposed of according to Great Smoky Mountains National Park Policy. Fish tissues brought back to UT will be 1) completely dissolved in concentrated ultrapure nitric acid for ICP analysis, and 2) preserved in 10% neutral buffered formalin for histological analyses. No other fish tissues will be brought back to UT, therefore will be no tissue to dispose of from our analyses.

National Park Service Scientific Research and Collecting Permit

An application for scientific research and collecting permit was required to conduct research in the Great Smoky Mountains National Park. Keil Neff prepared the application for this report on behalf and under the direct supervision of Dr. Bruce Robinson. The following is the accepted permit in its entirety.

Application For A Scientific Research And Collecting Permit

United States Department of the Interior

National Park Service

Name of the National Park Service area you are applying to: Great Smoky Mountains NP

Type of application:	**Previously assigned NPS study number:**
New Application	n/a
	Previously assigned NPS permit number:
	n/a

Principal investigator:

Dr Bruce Robinson

Mailing address of principal investigator:	**Office phone:**
73E Perkins Hall	8659747730
Dept. of Civil and Envr. Engr.	**Alternative phone:** 8659742503
The University of Tennessee Knoxville, TN 37996 US	**Office fax:** 8659742669
	Office email address: rbr@utk.edu

Institution represented:

University of Tennessee - Knoxville

Co-Investigators:

Name: John Schwartz	**Phone:** (865) 974-7721	**Email:** jschwart@utk.edu
Name: *Keil Neff (*GRA)	**Phone:** (865) 974-8678	**Email:** kneff1@utk.edu
Name: Theodore Henry	**Phone:** (865) 974-1000	**Email:** thenry8@utk.edu
Name: *Amanda Dunnavant (*GRA)	**Phone:** (865) 974-8678	**Email:** adunnava@utk.edu

Project title:

Effect of acid deposition on trout and water quality in the Great Smoky Mountains National Park

Purpose of study:

Native brook trout have been extirpated from six headwater streams in the Great Smoky Mountains National Park. Based on long term declines in stream pH, Park resource managers fear that brook trout will continue to be eliminated from streams and may disappear entirely from the Park within about 25-50 years. Although acid deposition and acidic storm events are suspected of being the primary cause, baseline and storm event water quality monitoring coupled with fish sampling, in situ survival tests, and physiological examinations are required to determine whether acid deposition is indeed the cause. This research project will perform monitoring in stream systems that have experienced native brook trout elimination and in those that have not. This work will compare 1) water quality, especially pH and toxic metal concentrations, and 2) survival and stress of trout under baseline and storm event conditions. The results will also be compared between healthy stream reaches in the Park and those that have seen extirpation. The results will be generalized and a predictive model developed.

Proposed starting date:	**Proposed ending date:**
March 15, 2006	March 31, 2009

Will members of the public be asked to participate in a survey as part of this proposed study?

No

Do you anticipate receiving funding assistance from the U.S. federal government for this study?

Yes

If yes specify the agency(s):

Environmental Protection Agency

Where will data reside upon completion of this project?

Data will reside at the Department of Civil and Environmental Engineering at the University of Tennessee, Knoxville. Data will be provided to the National Park Service if requested.

Location(s) where activities will take place within the National Park Service area(s):

This research will monitor three stream sites (Middle Prong of the Little Pigeon River, Ramsey Prong, Eagle Rocks Prong) within the Middle Prong Little Pigeon watershed.

Site 1: Middle Prong Little Pigeon River: N 35Â°42.159â , W 83Â°20.067â , elevation: ~2700 feet

Site 2: Ramsey Prong: N 35Â°42.257â , W 83Â°19.770â , elevation: 2877 ft

Site 3: Eagle Rocks Prong N 35Â°41.417â , W 83Â°18.183â , elevation: 3168 feet

Methods of access(vehicles, aircraft, boat, snowmobile, foot etc.):

Motor vehicle and foot travel

Appendix F: Study Sites

Middle Prong Little Pigeon River
N 35°42.159'
W 83°20.067'
elevation: ~2700 feet

Figure 14: Middle Prong Water Quality Monitoring Site

Figure 15: Middle Prong Sonde

Ramsey Prong
N 35°42.257'
W 83°19.770'
elevation: 2877 ft

Figure 16: Ramsey Prong Water Quality Monitoring Site

Figure 17: Ramsey Prong Sonde

Eagle Rocks Prong
N 35°41.417'
W 83°19.183'
elevation: 3168 feet

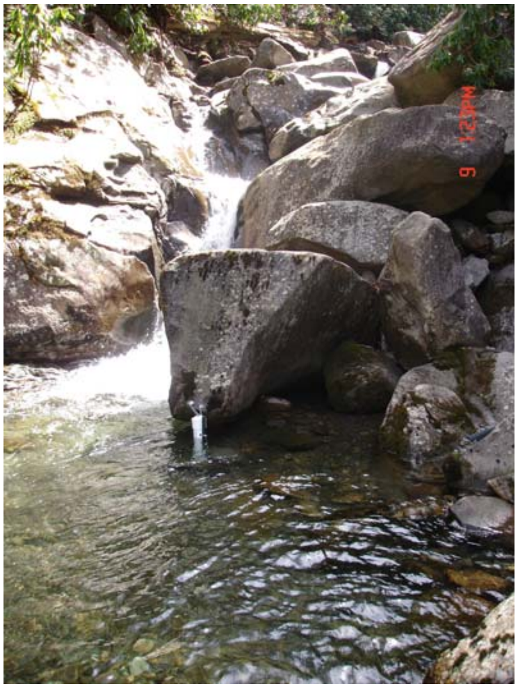


Figure 18: Eagle Rocks Prong Water Quality Monitoring Site

Figure 19: Eagle Rocks Prong Sonde and Trout Cage

Appendix G: Additional Water Quality Results

2007 Dissolved Organic Carbon

Grab samples were obtained for three storms at the study sites in 2007. These grab samples were specifically obtained to determine dissolved organic carbon (DOC) concentrations. Water samples were sent to the Nashville laboratory of Test America, Analytical Testing Corporation for DOC analysis. Results for the January 11th storm are presented in Table 18. Results for the March 1st storm are presented in Table 19. Results for the March 17th storm are present in Table 20.

Table 18: DOC concentrations for 01/11/07 Storm

Middle Prong

Sample	DOC conc (mg/L)
M301 4.5 hr	3.19

Eagle Rocks

Sample	DOC conc (mg/L)
M325 4.5 hr	3.28

Table 19: DOC concentrations for the 3/11/07 storm

Ramsey Prong

Sample	DOC conc (mg/L)	Increase (mg/L)	Percent Increase
M353 Baseflow	2.27		
M360 1.5 hr	3.19	0.92	40.5%
M365 5.25 hr	3.67	1.4	61.7%

Eagle Rocks

Sample	DOC conc (mg/L)	Increase (mg/L)	Percent Increase
M355 Baseflow	2.41		
M384 1.5 hr	2.73	0.32	13.3%
M389 5.25 hr	4.71	2.3	95.4%

Table 20: DOC concentrations for the 3/17/07 storm

Middle Prong

Sample	DOC conc (mg/L)	Increase (mg/L)	Percent Increase
M413 Bflow	1.97		
M422 4.5hr	2.23	0.26	13.2%

Ramsey Prong

Sample	DOC conc (mg/L)	Increase (mg/L)	Percent Increase
M414 Bflow	1.54		
M443 6 hr	4.6	3.06	198.7%

Eagle Rocks

Sample	DOC conc (mg/L)	Increase (mg/L)	Percent Increase
M415 Bflow	2.7		
M456 1.5hr	3.23	0.53	19.6%

Fraction of Time of pH intervals

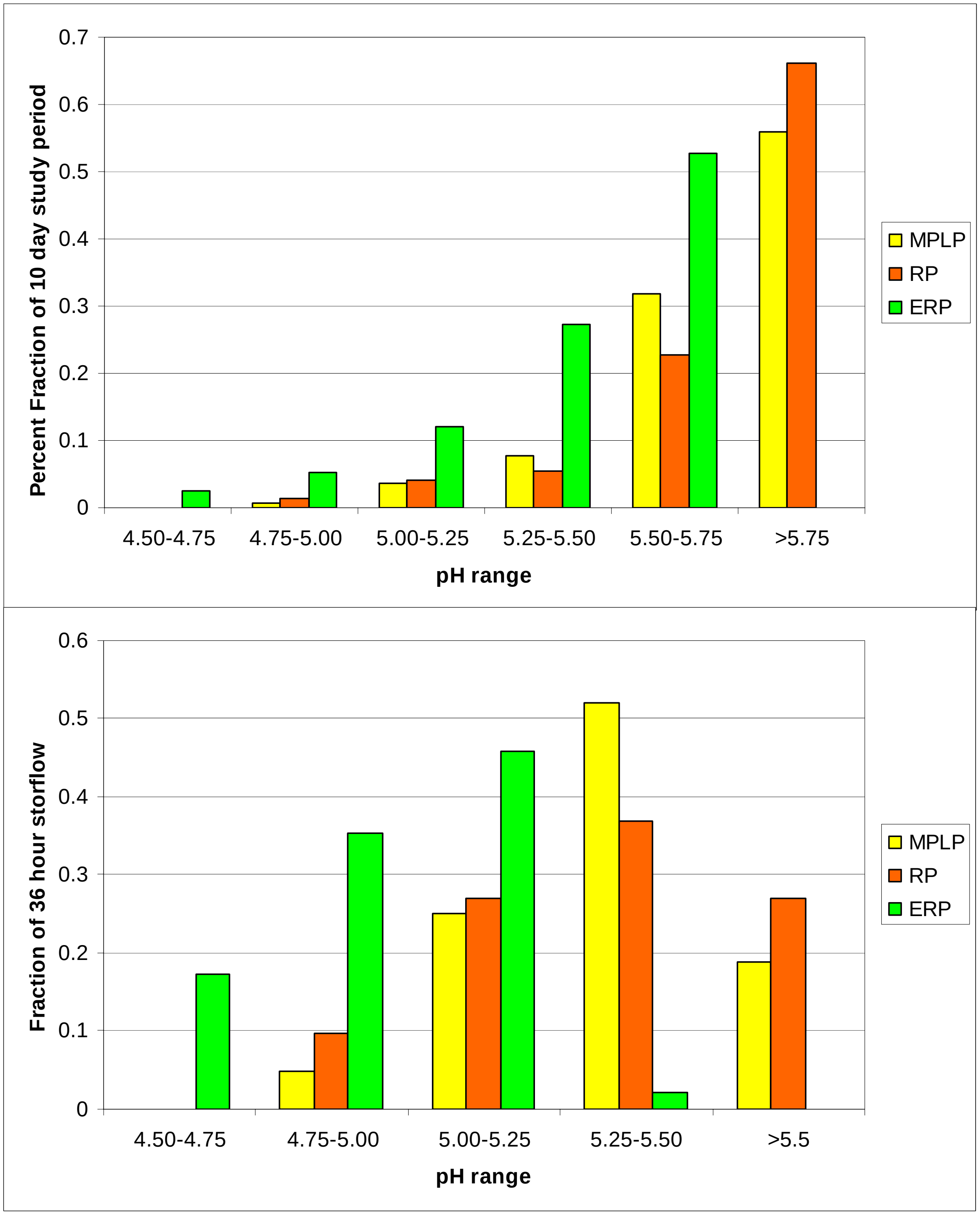


Figure 20:pH durations at Middle Prong (MPLP), Ramsey Prong (RP), and Eagle Rocks Prong (ERP): (A) during bioassay period and (B) during 36-hour stormflow

Vita

Keil Jason Neff was born on January 19th, 1975. He graduated from W. A. Berry High School in Hoover, Alabama in May of 1993. Mr. Neff enrolled in the School of Engineering at Vanderbilt University in August of 1993. He graduated with a Bachelor of Science in Engineering Science and Anthropology in May of 1997. Upon graduation, Mr. Neff took a seasonal position with the National Forest Service in Pisgah National Forest, North Carolina. In September of 1997, he began employment with Duvall & Associates of Franklin, Tennessee where he served as an archeological and osteological technician. From October 1998 through October 1999, Mr. Neff taught English at a South Korean school in Seoul. Upon his return to the United States, he began working for the University of Tennessee and conducted archaeological fieldwork in Townsend, Tennessee. In August of 2002, Mr. Neff began teaching mathematics at South Doyle High School in Knoxville. After four years of teaching, he began graduate studies in the Department of Civil and Environmental Engineering at the University of Tennessee in the fall of 2005, where he focused in Water Resources. Upon completion his Master of Science in Environmental Engineering in August of 2007, Mr. Neff will begin to work towards a Doctorate of Philosophy in Civil Engineering.