GROWTH AND MORTALITY UNDER LOW pH OF TWO STRAINS OF BROOK TROUT *(SALVELINUS FONTINALIS)*

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By

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Director: Dr. Thomas Martin
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Abstract

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Two genetically distinct strains of brook trout *Salvelinus fontinalis,* northern hatchery derived (NBKT) and native southern (SBKT), occur in southern Appalachia, an area susceptible to acid deposition. Two experiments were conducted to determine differences in growth under low pH between these strains. In the first experiment, 44 individuals of each strain (88 total fish) were collected from wild populations and maintained in the Aquatic Ecology laboratory at Western Carolina University. Following an acclimation period of at least 30 days in laboratory well water (pH 7.4, 13.4°C), fish were exposed to either pH 5.6 (experimental) or pH 7.4 (control) using a randomized block design with both treatments represented in each of two blocks. pH was maintained in the experimental treatment by the addition of dilute (0.6 mol) sulfuric acid (H_2SO_4). Fish were fed live chopped earthworms at a ration of 3% body weight per day. Fish were measured for wet weight (g) and total length (mm) at the beginning of the experiment and every 14 days thereafter for 8 weeks (56 days). Growth in wet weight and instantaneous growth rates for both total length and wet weight were significantly different between the strains ($p < 0.05$), but not between treatments. Growth in total length, combined for both

strains, was significantly different between both pH treatment and strains, but the interaction of strain and treatment was not significant for any growth measurements, indicating that treatment effect was similar for both strains. Mortality was significantly higher for southern brook trout, but was not affected by pH treatment for either strain. Slow growth and high mortality of SBKT may have been the result of stress due to laboratory conditions.

Experiment 2 used only fish that showed positive growth in experiment 1. There were 18 southern strain brook trout that showed positive growth in experiment 1, and all were used in experiment 2. Two tanks containing 9 fish/strain/treatment were used, and maintained at either approximately pH 5.2 or pH 7.4. All fish were fed the same rations as described above. pH did not have a significant effect on any growth measurements. Mean weight of SBKT decreased throughout the experiment, and only 1 fish of this strain showed positive growth in wet weight. Growth was significantly different between strains. Changes in mean relative condition factor were significantly different between strains, with southern strain fish decreasing and northern strain fish maintaining steady values. Mortality was significantly higher in the low pH treatment, but was not different between strains.

SBKT that showed negative growth in experiment 1 were held separately under neutral pH conditions and monitored for growth recovery. These fish showed negative growth in experiment 1 had zero mortalities and positive mean instantaneous growth rates

that were significantly higher than mean growth rates for the same individuals in experiment 1.

Introduction

The southern Appalachian Mountains are home to two genetically distinct strains of brook trout (*Salvelinus fontinalis*) (Stoneking et al. 1981; McCracken et al. 1993; Hayes et al. 1996; Galbreath et al. 2001). Pure populations of northern hatchery-derived brook trout (which have been introduced through stocking) and native southern Appalachian brook trout are fixed for alternative alleles at the creatine kinase A2 (CK-A2) locus and have shown significant allele frequency differences at 9 of 16 polymorphic loci (McCracken et al. 1993). Guffey et al. (1998) estimated a mean genetic similarity (Nei 1978) of I = 0.840 between the two strains using mitochondrial DNA (mtDNA), which is consistent with similarity estimates between strains and subspecies of other salmonid fishes (Galbreath et al. 2001; Guffey et al. 1998). For example, genetic similarities for morphologically distinct subspecies of cutthroat trout (*Oncorhynchus clarki*) range from 0.743 to 0.928 (Guffey et al. 1998). In addition, southern Appalachian brook trout show higher levels of mtDNA variation among populations than northern hatchery-derived strains, which suggests the possibility that southern populations contain more unique locally adapted gene-complexes (Hayes et al. 1996; Guffey et al. 1998). Hybridization with northern hatchery-derived strains threatens to disrupt these gene-complexes, and several authors have argued the need for management approaches that will minimize future losses of pure southern brook trout populations (Stoneking et al. 1981; McCracken et al. 1993; Hayes et al. 1996; Guffey et al. 1998).

The introduction of non-native alleles into southern brook trout populations via hybridization is the latest in a series of events over the last two centuries that may have greatly reduced the range of pure southern brook trout populations in southern Appalachia. King (1937) reported that habitat destruction from logging in the early 1900s caused the average elevation of reproducing brook trout populations to increase from 2000 feet to 3000 feet in Great Smoky Mountains National Park (GSMNP). The creation of GSMNP in 1926 helped stem habitat loss, but subsequent introductions of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) are thought to have prevented downstream migration by brook trout, and may have caused further migration of brook trout into higher elevations due to upstream encroachment by rainbow trout (Kelly et al. 1980). Kelly et al. (1980) reported the average minimum elevation for brook trout populations in 1972-1977 to be 1500 feet higher than in 1900. However, several brook trout populations were apparently protected from rainbow trout competition by natural waterfall barriers, which prevent upstream migration, and these protected populations had not moved significantly since the 1940s (Kelly et al. 1980).

High elevation streams may have served as refuges from competing species, but they may also provide sub-optimal habitat conditions, further threatening the long-term viability of these populations. The pH of streams in GSMNP is correlated with elevation, with pH decreasing as elevation increases (Silsbee and Larson 1982). A high proportion of surface water (as opposed to groundwater) input and the presence of thin soils due to steeper slopes in higher elevations function to decrease the contact time between acidic rainwater and soils and bedrock. This results in less time for acid neutralization to occur

(Allan 1995; Silsbee and Larson 1982). Acidification of rainwater occurs naturally when CO_2 dissolves to form carbonic acid, typically resulting in rain with a pH of 5.7 (Allan 1995). However, the pH of rain in southern Appalachia is typically much lower, with averages of approximately 4.7 (NADP 2003). Much of the cause for this lower pH has been linked to the influence of anthropogenic inputs of inorganic acids H_2SO_4 and HNO_3 from industrial coal-burning activities and petroleum burning vehicles, respectively (Fromm 1980; Sullivan 1990; Allan 1995).

The deleterious effects of acidic water on salmonids are well known. Menendez (1976) found 100% mortality of hatchery-reared brook trout exposed to pH 4.5 in the laboratory over a five month period. Menendez also found significantly reduced growth in the fish exposed to pH 4.5 and 5.0 compared to the control (pH 7.0). In addition, a model developed by Marschall and Crowder (1996), based on extensive reviews of published literature, predicted growth rates for adult brook trout at a pH of approximately 5.0 to be only 50-80% of growth rates at pH 6.5. However, they found that lowered pH resulted in increased mortality only for age-0 fish and not for adults. Based on these data, they predicted a large reduction in the number and proportion of large fish and a decline in total fish populations size.

Mortality and reduced growth under low pH may result from disruption of the active transport of Na^+ across the gill epithelia, causing lethal reductions of plasma NaCl (Packer and Dunson 1970; Fromm 1980). The inhibition of Na^+ can be caused either by direct competition between $[H^+]$ ions and Na^+ ions for active sites on a carrier molecule, or by a general effect of $[H^+]$ ions on the metabolism of cells involved in the active

transport of Na^+ (Packer and Dunson 1970). In addition, low environmental pH can decrease the pH of blood, thereby decreasing the O_2 carrying capacity, which may cause insufficient transfer of O_2 from red blood cells to tissues (Menendez 1976; Fromm 1980). Recent evidence from GSMNP has shown that the pH in some high elevation streams has continued to decline over recent decades, and may have caused the downstream migration of resident native brook trout populations (Steve Moore, 2003, personal communication, Great Smoky Mountains National Park, Gatlinburg, Tennessee).

Many studies have illustrated the differences in pH tolerance among fish species, but fewer have focused on within-species differences. Robinson et al. (1976) found significant differences in acid tolerance in the laboratory among seven inbred hatchery strains, suggesting that acid tolerance may be heritable. In contrast, Lachance et al. (2000) examined growth and egg mortality in the laboratory between a presumed acid tolerant brook trout strain and two strains from neutral waters in Quebec, but found no evidence for heredity of acid tolerance. However, this study was performed on trout from close geographic proximity, and the strains may not have been genetically divergent enough to reveal differences. A recent study revealed significant differences in acid and thermal tolerance, measured as time to loss of equilibrium, between wild northern derived hatchery strain and native southern Appalachian strain brook trout (Cornelison 2005)

The primary purpose of this study was to determine if there are differences in growth between naturalized, hatchery-derived, northern strain brook trout and native, southern Appalachian strain brook trout under chronically low, sublethal, pH conditions.

Secondarily, the experimental design allowed for a test of differences in growth rate of the two strains under laboratory conditions, independent of pH.

Methods – Experiment 1

Fish Collection and Housing

Fifty-eight brook trout of each strain (116 total) were collected by backpack electrofishing from seven streams in western North Carolina and transported to the Aquatic Ecology lab at Western Carolina University. Neutral pH streams (i.e. pH 6.5-7.5) were designated for collection based on current genetic typing records obtained from the North Carolina Wildlife Resources Commission (NCWRC) (see Appendix A for list of streams). Fish with a length range of approximately 120 mm to 180 mm, based on visual estimates in the field, were collected to minimize size variation in the experiment. These lengths approximately represent age 1+ brook trout (Etnier and Starnes 1993). All fish were held in four 280 L living streams: 1.8 x 0.5 x 0.5 m (Frigid Units, Inc., Toledo, Ohio). Upon collection, individual fish were implanted with Passive Integrated Transponder (PIT) tags to allow unique ID of fish. Fish were acclimated for a minimum of 30 days in the laboratory. Water temperature was maintained at approximately 14°C by mixing well-water (20°C) and chilled well-water (10°C) in head tanks. One head tank supplied each of two living streams with a flow-through rate of approximately 700 ml/min. During acclimation, fish were fed approximately 3% body weight per day (calculated by obtaining an average wet weight of a random subsample of fish on the day

of collection) with fresh, chopped earthworms (*Lumbricus terrestris*), following the protocol of Jacobsen (1977).

Thirteen fish (11 northern, 2 southern) died during acclimation. Since it was necessary to use only a number of fish divisible by four in order to get equal representation across all four treatments, and only 47 northern brook trout remained, only 44 fish of each strain were used in the experiment. Extra fish were excluded based on their deviation from the mean weight of the entire population (i.e. the largest and smallest fish were excluded). Supplemental aeration with air stones maintained approximately 95% oxygen saturation. Submersible pumps (Beckett Corp., Irving, Texas) were placed in each tank to create a current and black plastic sheeting provided partial refuge from direct light exposure following the suggestions of Dave Mount (personal communication, US EPA, Duluth, MN).

Experimental Design

Four experimental tanks were aligned parallel to each other and divided into two blocks to minimize error variation. Within each block one tank was randomly assigned as a treatment or control, based on a coin flip, with the other assigned to the opposite. Thirty days after the last collection date, all fish were anaesthetized using 50 mg/L clove oil (Anderson et al. 1997), and measured for wet weight (g) and total length (mm), representing the initial weight and length for the experiment. Fish were not fed at least 24 hours prior to each measurement to minimize weight differences caused by feeding and gut clearance. Eleven fish of each strain (22 per tank) were randomly allocated to

either a treatment or control tank. However, some non-random adjustments were made to ensure similar size distribution in each tank.

Immediately following measurement and allocation, the pH of treatment tanks was lowered and maintained at approximately 5.6 by the addition of dilute (0.6 M) sulfuric acid (H_2SO_4) using peristaltic pumps. pH and temperature were measured at least twice daily, with extra weekly measurements taken during late evening or early morning (i.e. between 12pm – 6am) to ensure that daily measurements reflected consistent levels. Photoperiod was adjusted to follow the local pattern in Cullowhee, NC.

Growth was monitored in the first two weeks by measuring wet weight and length of a random subsample of five fish/strain/tank. However, this was deemed insufficient due to the high variation in fish size, which caused estimates to be biased. Therefore, all subsequent two-week growth measurements were done on all fish in the experiment. Growth data from the first two weeks was not included in the statistical analysis. Dead fish were removed and measured as soon as they were found, and dorsal muscle tissue samples were taken using a 14-gauge Soft Tissue Biopsy Device (Anchor Products Company, Addison, Illinois). Tissue samples were placed in separate labeled microcentrifuge tubes, and frozen at -70°C for later electrophoretic analysis.

Fish were fed fresh chopped earthworms (*Lumbricus terrestris*) at 3% body weight per day in two daily rations (i.e. 1.5% per feeding), following the protocol of Jacobson (1977). The amount of feed was adjusted daily based on fish mortality and biweekly following growth measurements. The experiment concluded after eight weeks (56 days).

Strain Verification

All fish were collected from streams previously identified by the North Carolina Wildlife Resources Commission (NCWRC) as containing pure northern or pure southern populations of brook trout. Further verification of source population ancestry for all experimental fish was based on allele frequencies observed for creatine kinase: 100% CK-A2*100 = native Southern Appalachian strain, 100% CK-A2*78 = northern derived hatchery strain. This follows the protocol of Galbreath et al. (2001). At the end of the experiment all fish were anaesthetized in 50mg/L Clove oil (Anderson et al. 1997), and dorsal muscle tissue samples were taken as described above. These tissue samples, as well as the samples from fish mortalities, were analyzed by cellulose acetate gel protein electrophoresis for creatine kinase. Procedures followed the protocol of Hebert and Beaton (1993) and Galbreath et al. (2001). Staining procedures followed Hebert and Beaton (1993), Guffey (1998), and Galbreath et al. (2001).

Methods – Experiment 2

Fish Selection

At the conclusion of experiment 1 all acid treatments were turned off, and the water was allowed to return to neutral pH (ca. 7.4). Twenty days after experiment 1 concluded, 18 fish of each strain (36 total) from the previous experiment were randomly allocated to either an experimental (pH 5.2) or control (pH 7.4) treatment. Only fish that showed positive growth throughout experiment 1 were used in experiment 2, because it was possible, though not confirmed, that negative growth of individuals from experiment 1

was due to starvation. The use of only positively growing fish minimized the possibility of using fish that had stopped feeding during experiment 1, and also functioned as a selection process of fish that were apparently most tolerant of stressful laboratory conditions. Eighteen southern strain brook trout showed positive growth throughout experiment 1, and all were used in experiment 2. Thirty-one northern strain brook trout showed positive growth throughout experiment 1. Eighteen of these fish were selected for experiment 2 based on their minimal deviation from the average weight of southern brook trout.

Experimental Design

pH of the experimental treatment was maintained at approximately 5.5 for the first two weeks (14 days) of experiment 2. However, this was changed to approximately 5.0 following the first two week measurement in order to increase the treatment effect. Experiment 2 was conducted with the same protocol as described previously for experiment 1, with the exception that temperature for both treatments was raised to approximately 15.8°C, which is within the range of optimal growth temperatures reported for brook trout (14.4 – 16.0°C) (Dwyer et al. 1983). However, it was not possible to maintain this temperature using all four tanks due to the limitations of temperature control described above. Therefore, only two tanks were used. Unfortunately, this lack of replication limited the statistical power of the experiment, and limited the interpretation of results.

Data Analysis

Measurement and analysis protocols were the same for all experiments. Relative condition factor (Kn) was calculated using the following:

$$Kn = W_i/(L_i^m \cdot e^b)$$

where W_i and L_i are weight (g) and total length (mm), respectively, of individual fish at time i, and *m* and *b* are the slope and y-intercept, respectively, of the regression of ln(W) on ln(L) for all fish in the experiment (Table 1.) Instantaneous growth rate was calculated over the entire 56 day period using the following (Ricker 1979):

$$IGR = (\ln X_2 - \ln X_1)/(t_2 - t_1)$$

where X_1 and X_2 are the weights or lengths of individual fish at times t_1 and t_2. Absolute growth rate was calculated using the following (Ricker 1979):

$$G = (X_2 - X_1)/(t_2 - t_1)$$

Table 1. Parameters used for calculation of relative condition factor in both experiments.

	Parameters		
Experiment	*m* (slope)	*b* (y-intercept)	r^2
1	2.968	11.511	0.9159
2	3.033	11.803	0.9087

All statistical analyses were performed using SAS software (SAS Institute, 2000). Tests for the effects of strain and pH treatment on mortality were assessed with analysis of variance (ANOVA) using the GLM procedure. Absolute growth, instantaneous

growth rate, and relative condition factor were analyzed for strain and treatment effects and interactions using the MIXED procedure, with repeated measures included for absolute growth and condition. Statistical significance was determined at an error rate of 0.05 for all tests.

Methods – Recovery Study

Southern strain fish that had shown negative growth in experiment 1 (n=9) were held in a separate tank, maintained at approximately pH 7.4 and temperature 13.1°C, fed similar rations as described above, and monitored for the growth recovery and mortality over the entire time period for experiment 2 (56 days).

Results – Experiment 1

Water Quality

The pH of the experimental treatments was successfully maintained at 5.6, but was more variable than the neutral controls. However, differences between blocks were not significant ($p > 0.05$) (Table 2.) Since each head tank received water diverted from the same source, adjustments for flow rate or temperature on one tank had compensatory effects on the other. These slight fluctuations in the flow rate were the source of much of the variability of pH values. On days 16 and 17, the water inlet of tank 2 was found unattached and not flowing into the tank. This resulted in an acidic episode with measured pH dropping to 4.1. This was the lowest measured pH during the experiment.

Only one mortality was observed during this time. On day 7, the submersible pump in tank 1 stopped working. No replacement was available, so all submersible pumps were removed from each tank. This did not appear to visibly affect feeding activity.

Table 2. Measured values, standard deviation (SD), and number of measurements (*n*) of pH and temperature for experiment 1. Exposure time was 56 days.

Measured values						
	pH			Temperature		
Tank	Mean	SD	*n*	Mean (°C)	SD	*n*
1	7.3	0.09	117	13.3	0.54	114
2	5.6	0.41	170	13.4	0.66	114
3	5.6	0.44	172	13.5	0.62	114
4	7.3	0.1	118	13.6	0.71	113

Test Fish

Gel electrophoresis for all tissue samples analyzed confirmed that fish were of either pure southern or pure northern origin. These data were consistent with previous surveys of source populations for all streams used in this experiment. Southern strain brook trout (SBKT) were smaller than northern strain (NBKT) in both wet weight and length at the beginning of the experiment, but differences among treatments were negligible (Table 3). Throughout the experiment, fish were qualitatively observed at the time of feeding. No discernable differences in feeding activity were seen among the treatments.

Mortality

Mortality was significantly different ($F = 8.11$; $df = 1,86$; $P < 0.05$) between strains (Table 4), but not between pH treatments (Table 5). Southern strain fish suffered greater mortality over the course of the experiment (29.5%) relative to northern strain fish (6.8%). Predation of smaller fish was observed on two occasions in neutral (pH 7.4) treatments. Prey consisted of one northern and one southern strain fish. In addition, 4 other fish (3 SBKT, 1 NBKT) were observed missing on a measurement day. It is likely that they were also victims of predation, though this was not observed directly. Since the actual time of death for these missing fish could not be determined, survival time would have been biased and was not analyzed. Mean relative condition factor (Kn) for all observed mortalities (excluding prey), based on post-mortem measurements taken immediately after they were found, was 0.76. This was significantly ($F = 37.6$; $df = 1,86$ $P < 0.0001$) lower than the mean for all surviving fish ($Kn = 1.02$). While this may suggest starvation, the direct cause of this lowered condition is unclear.

Table 3. Mortality, absolute growth, instantaneous growth rate (IGR) and relative condition factor (Kn) of northern (NBKT) and southern (SBKT) brook trout *Salvelinus fontinalis* after 56 d exposure to two pH levels.

	Mean pH											
	5.6						7.4					
	NBKT	S.E	*n*	SBKT	S.E.	*n*	NBKT	S.E.	*n*	SBKT	S.E.	*n*
Mortality (%)	5			36			9			27		
Mean initial wt. g	29.9	2.583	22	23.3	1.947	22	32.5	3.578	22	24.2	2.627	22
Mean Δ wt. g	2.9	0.859	21	0.4	0.746	14	4.5	1.218	20	-0.6	0.968	17
Mean initial ln. mm	148.3	4.277	22	141.0	3.623	22	148.0	5.923	22	139.9	3.946	22
Mean Δ ln. mm	5.0	0.936	21	1.9	0.636	14	6.2	0.760	20	2.1	0.511	17
Mean IGR wt ($g \cdot d^{-1}$)	0.0015	0.0008	21	0.0002	0.0008	14	0.0026	0.0009	22	-0.0001	0.0008	17
Mean IGR ln. ($mm \cdot d^{-1}$)	0.0006	0.0001	21	0.0002	0.0001	14	0.0008	0.0001	22	0.0003	0.0007	17
Mean initial Kn	1.024	0.018	22	0.937	0.041	22	1.072	0.028	22	0.987	0.032	22
Mean terminal Kn	0.995	0.024	21	0.970	0.037	14	1.084	0.020	20	0.973	0.035	17

*Data for changes in weight, length and condition factor, as well as overall instantaneous growth rate are calculated using only surviving fish (n =72)

Growth and Condition Factor

Interactions between treatment, strain, and time had no significant effect on any measurements, indicating that the treatment effect was similar for both strains. Although mean weight of all southern strain fish increased over the 56 day period, this was largely due to the mortality of smaller fish, and is therefore not contradictory to the slightly negative mean growth of individuals shown in Table 4. Mean (SE, n) wet weight and total length of all southern strain mortalities was 18.6 g (1.34, 13) and 137 mm (3.74, 13), respectively, which is lower than the means for all southerns at the beginning of the experiment (Table 4). Interactions of strain and time were significant for wet weight ($F = 6.78$; df = 2,140; $P = 0.0016$) and total length ($F = 5.48$; df = 2,144; $P = 0.0051$), with northern brook trout growing faster in both low and neutral pH (Figures 1 and 2). Growth of all fish in the experiment was significantly ($F = 3.65$; df = 2,144; $P = 0.0284$) affected by low pH treatment when measured as a change in total length over time (Figure 3), but not in wet weight (Figure 4). However, Figure 4 shows slight divergence of treatments with respect to weight over time. While this difference was not significant ($F = 2.04$; df = 2,141; $P = 0.1344$), it is possible that the experimental period was too short to detect it.

Instantaneous growth rate over the entire experiment was significantly different ($F = 12.22$; df = 1,70; $P = 0.0008$) between strains (Table 4), but not between treatments (Table 5). Mean relative condition factor was significantly different ($P < 0.05$) between strains at all measurement periods, but did not change significantly over time. This

indicates that, although southern brook trout grew slower than northerns, relative mean "plumpness" of experimental fish populations remained unchanged.

Table 4. Mean values averaged over two pH levels (pH 5.6 and 7.4) of mortality, absolute growth, instantaneous growth rate (IGR), and relative condition factor (Kn) of two strains of brook trout *Salvelinus fontinalis* after 56 d exposure.*

	Strain					
	NBKT			SBKT		
Mortality (%)	7			30		
	Mean	S.E	*n*	Mean	S.E.	*n*
Mean initial wt. g	31.2	2.190	44	23.8	1.617	44
Mean Δ wt. g	3.7	0.740	41	-0.1	0.625	31
Mean initial ln. mm	148.2	3.610	44	140.5	2.644	44
Mean Δ ln. mm	5.6	0.606	41	2.0	0.395	31
Mean IGR wt ($g \cdot d^{-1}$)	0.0020	0.0003	41	0.0001	0.0004	31
Mean IGR ln. ($mm \cdot d^{-1}$)	0.0007	0.0001	41	0.0003	0.0001	31
Mean initial Kn	1.048	0.017	44	0.962	0.023	44
Mean terminal Kn	1.038	0.017	41	0.971	0.025	31

*Data for changes in weight, length and condition factor, as well as overall instantaneous growth rate are calculated using only surviving fish (n =72)

Table 5. Mean values averaged over two pH levels (pH 5.6 and 7.4) of mortality, absolute growth, instantaneous growth rate (IGR), and relative condition factor (Kn) of two strains of brook trout *Salvelinus fontinalis* after 56 d exposure.*

	Mean pH					
	5.6			7.4		
Mortality (%)	20			16		
	Mean	S.E	*n*	Mean	S.E.	*n*
Mean initial wt. g	26.6	1.674	44	28.4	2.284	44
Mean Δ wt. g	1.9	0.624	35	2.2	0.888	37
Mean initial ln. mm	144.6	2.825	44	144.0	3.572	44
Mean Δ ln. mm	3.7	0.664	35	4.3	0.580	37
Mean IGR wt ($g \cdot d^{-1}$)	0.0010	0.0004	35	0.0014	0.0005	37
Mean IGR ln. ($mm \cdot d^{-1}$)	0.0005	0.0001	35	0.0005	0.0001	37
Mean initial Kn	0.981	0.020	44	1.030	0.022	44
Mean terminal Kn	0.985	0.020	35	1.033	0.021	37

*Data for changes in weight, length and condition factor, as well as overall instantaneous growth rate are calculated using only surviving fish (n =72)

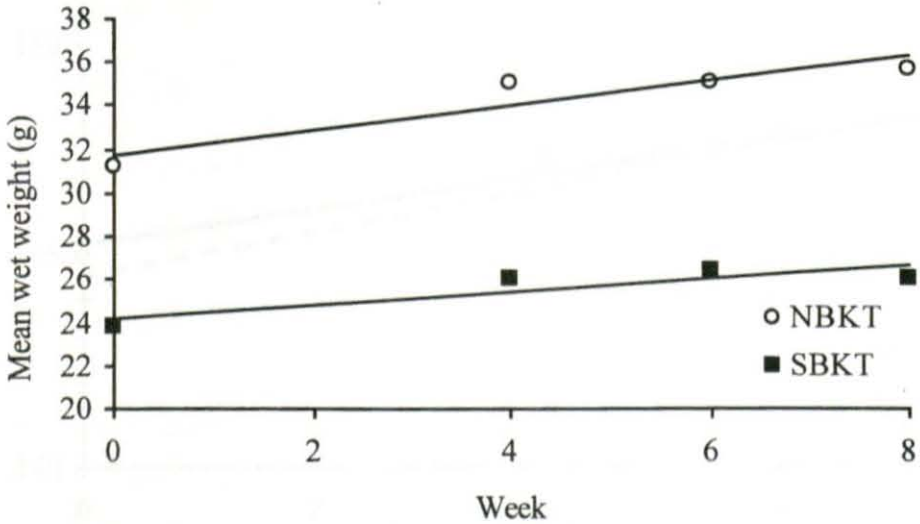

Figure 1. Growth in wet weight over time of northern (NBKT) and southern (SBKT) brook trout. Data points represent means of fish in both pH treatments (pH 5.6 and 7.4).

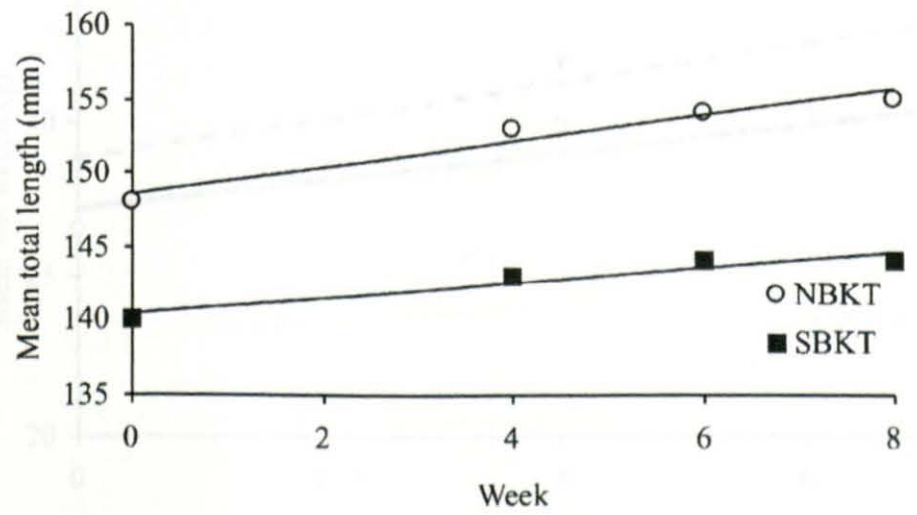

Figure 2. Growth in total length over time of northern (NBKT) and southern (SBKT) brook trout. Data points represent means of fish in both pH treatments (pH 5.6 and 7.4).

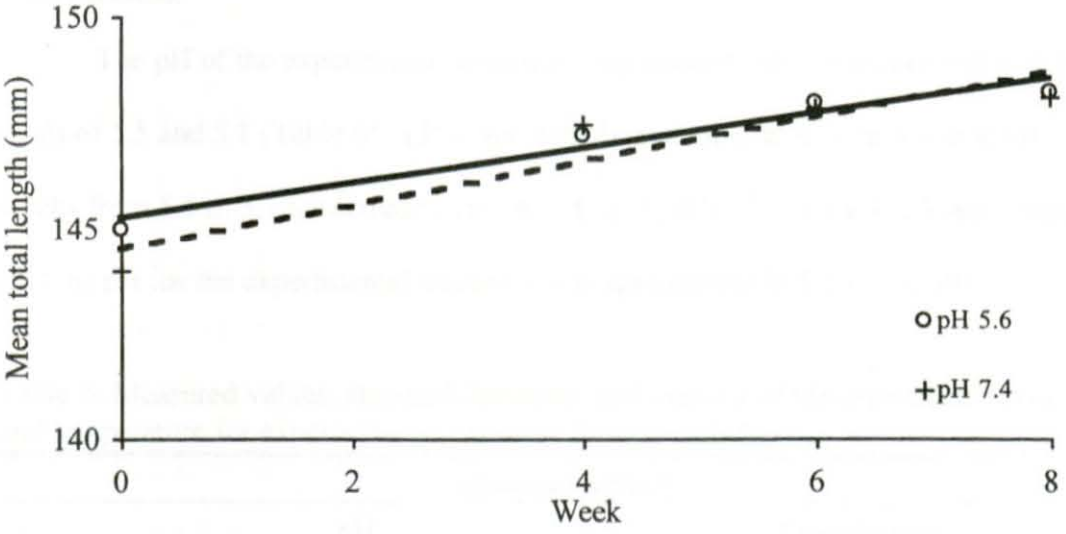

Figure 3. Growth in total length over time for all fish in two treatments (pH 5.6 and 7.4). Data represent means for both strains of brook trout.

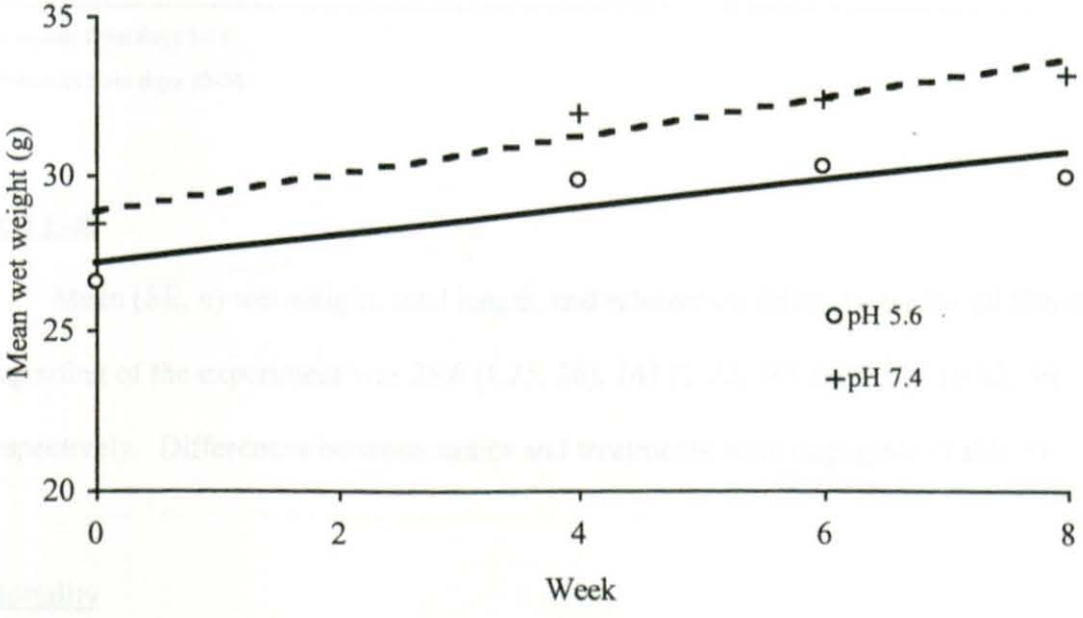

Figure 4. Growth in wet weight over time for all fish in two treatments (pH 5.6 and 7.4). Data represent means for both strains of brook trout.

Results – Experiment 2

Water Quality

The pH of the experimental treatment was successfully maintained at both target goals of 5.5 and 5.1 (Table 6). pH of the experimental treatment was lowered after two weeks from 5.5 to 5.1 to increase treatment effect (Table 6). Overall (56 days) mean (SD, *n*) pH for the experimental treatment was approximately 5.2 (0.38, 99).

Table 6. Measured values, standard deviation, and number of measurements (*n*) of pH and temperature for experiment 2. Exposure time was 56 days.

Measured values						
	pH			Temperature		
Tank	Mean	SD	*n*	Mean (°C)	SD	*n*
1	7.4	0.14	95	15.8	0.77	95
2[a]	5.5	0.36	22	15.9	0.66	95
2[b]	5.1	0.34	77			

a = results from days 1-14.

b = results from days 15-56.

Test Fish

Mean (SE, *n*) wet weight, total length, and relative condition factor for all fish at the beginning of the experiment was 25.6 (1.25, 36), 141 (2.22, 36) and 1.004 (0.02, 36), respectively. Differences between strains and treatments were negligible (Table 7).

Mortality

Mortality was significantly ($F = 4.89$; $df = 1,34$; $P = 0.0341$) different between treatments (Table 8), but not between strains (Table 9). All fish in the control (pH 7.4)

treatment (Table 7) survived. Four fish (11%) in the experimental treatment (pH 5.2) did not survive to the end of the experiment.

Growth and Relative Condition Factor

Interactions of treatment, strain, and time were not significant for any growth measurements. The pH treatment effect was not significant for any growth measurements (Table 7). Southern strain fish had mean negative growth rates in both pH treatments (Table 7), and only one fish in this strain showed positive net gain in weight (1.1g). In contrast, northern strain fish showed positive growth rates that were significantly higher than southern strain fish, regardless of treatment, for weight ($F = 42.66$; $df = 1,28$; $P < 0.0001$), and length weight ($F = 24.76$; $df = 1,28$; $P < 0.0001$) (Table 9). Mean absolute growth over time was significantly different between strains when measured as change in total length over time ($F = 3.39$; $df = 2,57.1$; $P = 0.0408$) (Figure 5).

Condition factor was not correlated to treatment (Table 8), indicating that pH did not have an effect on fish health. Mean relative condition factor decreased for southern strain fish in both treatments (Table 9), but did not change for northern strain fish.

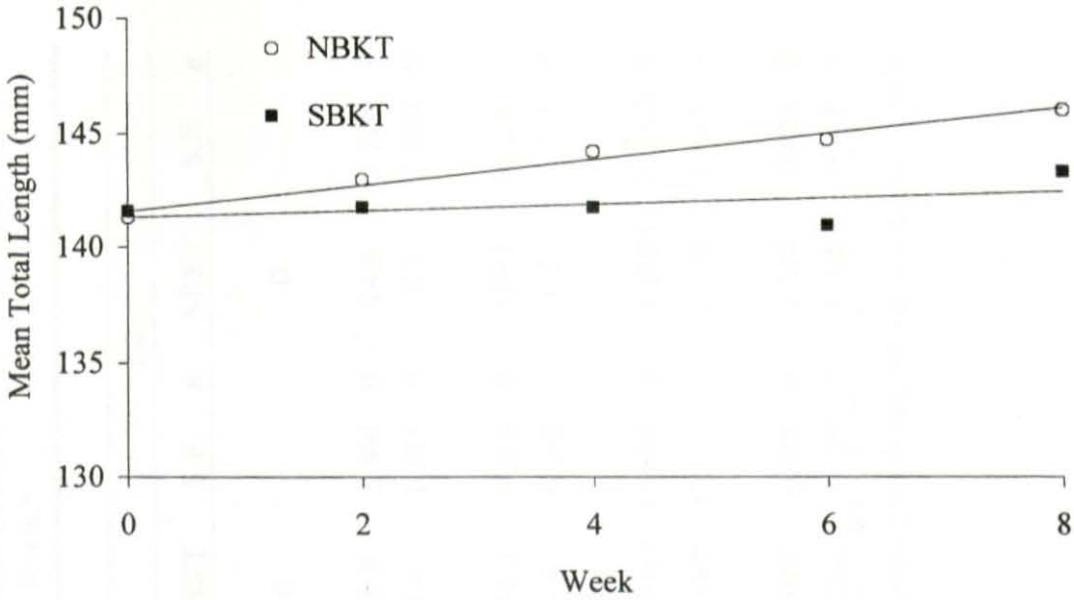

Figure 5. Growth in total length over time for all fish in two treatments (pH 5.6 and 7.4). Data represent means for both strains of brook trout.

Table 7. Mortality, absolute growth, instantaneous growth rate (IGR) and relative condition factor (Kn) of northern (NBKT) and southern (SBKT) brook trout *Salvelinus fontinalis* after 56 d exposure to two pH levels.*

	Mean pH										
	5.6						7.4				
	NBKT	S.E	*n*	SBKT	S.E.	*n*	NBKT	S.E.	*n*	SBKT	S.E.	*n*
Mortality (%)	5			14			0			0		
Mean initial wt. g	25.9	1.885	9	26.2	3.489	9	25.8	2.464	9	24.6	2.308	9
Mean Δ wt. g	2.8	1.118	8	-2.6	0.458	6	3.4	1.133	9	-1.3	1.029	9
Mean initial ln. mm	141.3	3.210	9	144.1	5.980	9	141.2	4.618	9	139.1	4.128	9
Mean Δ ln. mm	4.1	0.990	8	0.0	0.258	6	3.4	0.944	9	0.2	0.278	9
Mean IGR wt ($g \cdot d^{-1}$)	0.0016	0.0006	8	-0.0017	0.0005	6	0.0019	0.0005	9	-0.0009	0.0010	9
Mean IGR ln. ($mm \cdot d^{-1}$)	0.0005	0.0003	8	0.0000	0.0001	6	0.0005	0.0001	9	0.0000	0.0000	9
Mean initial Kn	1.031	0.037	9	0.954	0.036	9	1.017	0.018	9	1.014	0.024	9
Mean terminal Kn	1.050	0.045	8	0.906	0.050	6	1.060	0.029	9	0.960	0.018	9

*Data for changes in weight, length and condition factor, as well as overall instantaneous growth rate are calculated using only surviving fish (n = 32).

Table 8. Mean values averaged over both strains of mortality, absolute growth, instantaneous growth rate (IGR) and relative condition factor (Kn) of all brook trout *Salvelinus fontinalis* in the experiment after 56 d exposure to two pH levels.*

	pH					
	5.6			7.4		
Survival (%)	78			100		
	Mean	S.E	*n*	Mean	S.E.	*n*
Mean initial wt. g	26.1	1.505	18	25.4	2.039	18
Mean Δ wt. g	0.5	0.988	14	1.0	0.826	18
Mean initial ln. mm	142.7	3.306	18	140.2	3.015	18
Mean Δ ln. mm	2.5	0.783	14	2.1	0.654	18
Mean IGR wt ($g \cdot d^{-1}$)	0.016	0.0006	14	0.052	0.0005	18
Mean IGR ln. ($mm \cdot d^{-1}$)	0.031	0.0001	14	0.025	0.0001	18
Mean initial Kn	0.994	0.027	18	1.016	0.015	18
Mean terminal Kn	0.987	0.037	14	1.004	0.020	18

*Data for changes in weight, length and condition factor, as well as overall instantaneous growth rate are calculated using only surviving fish (n = 32)

Table 9. Mean values averaged over two pH levels (pH 5.6 and 7.4) of mortality, absolute growth, instantaneous growth rate (IGR), and relative condition factor (Kn) of two strains of brook trout (*Salvelinus fontinalis*) after 56 d exposure.*

	Strain					
	NBKT	S.E	*n*	SBKT	S.E.	*n*
Mortality (%)	5			14		
Mean initial wt. g	25.9	1.505	18	25.4	2.099	18
Mean Δ wt. g	3.1	0.777	17	-1.8	0.373	15
Mean initial ln. mm	141.3	2.728	18	141.6	3.572	18
Mean Δ ln. mm	4.0	0.686	17	0.3	0.153	15
Mean IGR wt ($g \cdot d^{-1}$)	0.180	0.038	17	-0.127	0.027	15
Mean IGR ln. ($mm \cdot d^{-1}$)	0.049	0.008	17	0.003	0.002	15
Mean initial Kn	1.024	0.020	18	0.985	0.022	18
Mean terminal Kn	1.050	0.024	17	0.936	0.022	15

*Data for changes in weight, length and condition factor, as well as overall instantaneous growth rate are calculated using only surviving fish (n = 32).

Results - Recovery Study

Growth Recovery

Mean (SE, n) pH and temperature were successfully maintained at approximately 7.4 (0.1,85) and 13.3°C (1.01,85), respectively. All southern strain fish that had shown negative growth in experiment 1 survived the second experimental period. Instantaneous growth rate (mean, SE) was positive for wet weight (0.0007 $g \cdot d^{-1}$, 0.0007), and was significantly greater ($F = 8.29$, $df = 1,7$; $P = 0.0109$) than the growth rate of the same individuals in experiment 1 (-0.0015 $g \cdot d^{-1}$, 0.003), indicating a positive recovery of growth in the absence of northern strain brook trout.

Discussion

Based on the results of this study, northern and southern strain brook trout do not appear to differ in their growth under low pH. The low pH treatment (pH ≈ 5.6) in experiment 1 significantly affected growth only when measured as change in total length over time. However, absolute differences were small and differences were not reflected in instantaneous growth rates. It is therefore doubtful that this might be biologically significant. No differences were seen in experiment 2 (pH 5.2). This is in contrast to a study of growth under low pH of hatchery-reared brook trout by Menendez (1976), which found significantly reduced growth at pH 4.5 and 5.0, but not pH 5.5. However, because of the relatively slow growth rates in the present experiments, especially for southern strain brook trout, any further effects of pH on growth may have required a longer experimental period to be revealed.

The significantly greater growth in both experiments of northern strain brook trout, regardless of treatment, suggests that southern strain brook trout may have lower tolerance for laboratory stress. This was not surprising due to the known difficulty of maintaining southern brook trout in artificial settings (Jerry West, personal communication, Western Carolina University, Cullowhee, North Carolina). In addition, because of the hatchery ancestry of northern brook trout populations, it is possible that

the differences in growth observed in this study reflect artifacts of artificial selection for higher growth rates in northern populations. Estimates for the values for the heritability of growth rates for other salmonids (e.g. Atlantic salmon and rainbow trout), on a scale of 0 (no genetic contribution) to 1 (no environmental contribution), range from 0.2-0.4 (Wooton 1990). These values, though not high, offer evidence that intensive artificial selection for growth rates is possible, and may be retained once wild populations are established. While the present study was one of very few, if any, experiments to successfully maintain southern brook trout in the laboratory, it is evident that improved methods will need to be employed for truly meaningful growth data of this strain.

It is also possible that aggressive interactions between the two strains of fish are more detrimental to southern strain fish. Southern strain fish (n = 9) that showed negative growth in experiment 1 recovered growth during the second experimental period in the absence of northern brook trout. Mean instantaneous growth rate ($g \cdot d^{-1}$) was positive for these fish during the second 56-day period, and was significantly greater than that of the first experiment. However, it is also possible that the lower density of these fish during the second period is the cause of improved growth. Several studies have found stocking density to be negatively correlated with growth due to both direct increased stress of aggressive interaction (Marchand and Boisclair 1998; Vijayan and Leatherland 1988) as well as higher consumption rates of more aggressive fish (Boujard et al. 2002; Vijayan and Leatherland 1988). While northern strain fish were not monitored for improvement in the absence of southern strain fish, these data may warrant

further investigation to help determine the primary cause of poor performance of southern brook trout in the laboratory.

A common indicator of stress in fish is increased plasma cortisol levels (Moyle and Cech 2000), and several researchers have found significant differences in the regulation of this corticosteriod between hatchery and wild fish (Woodward and Strange 1987). These differences may also be heritable (Barton 2002), and it is possible that wild northern derived hatchery brook trout have inherited some resistance to stress from their hatchery-selected ancestors. It is likely that this is the reason that northern strain fish were more successful in both of the present experiments. Also, responses to handling stress have been found to differ widely among (Barton 2002) and within (Davis and Schreck 1997) salmonids. While this study may suggest differences in long-term stress reactions, it is unclear whether these differences may be present in short-term stress situations, such as handling via fishing.

The negative growth rate of southern strain brook trout seen in experiment two is unrealistic and unsustainable in natural populations over long time periods, and is therefore almost certainly due to the effect of prolonged laboratory stress and not reflective of true long-term growth in the wild. Cornelison (2005) examined growth of wild northern and southern strain fish in an outdoor raceway using a similar feeding ration, and found no differences between strains. It is possible that the more natural setting of outdoor raceways provided a more realistic representation of growth than was seen in this experiment. However, survival of southern strain brook trout in Cornelison's experiment was low (41%), and because growth was calculated only for surviving fish,

differences between strains may have been masked if mortality was associated with low or negative growth.

The increased mortality of fish in the low pH (5.2) treatment of experiment 2 is cause for some concern, especially if the patterns of acid precipitation continue to lower pH levels of higher elevation streams, where many pure southern brook trout populations reside (Kelly et al. 1980). In addition, although low pH did not affect growth of northern and southern strains differently in either of the present experiments, other complicating factors associated with acidic deposition may threaten brook trout populations in southern Appalachia. For example, many fish kills and growth reductions of fish in natural acidic environments are enhanced by the presence of toxic aluminum (Al), which is leached from soils and sediments during acidic episodes (Wood et al. 1988; Ingersoll et al. 1990; Wilson et al. 1996). Other anthropogenic factors, such as siltation, harvesting, and exotic species introductions (e.g. rainbow trout), have also been shown to have negative effects on brook trout populations (Marschall and Crowder 1996). However, because the effects of these factors are closely related to different life-history stages of brook trout (e.g. harvesting generally affects only adult fish), brook trout populations may be able to survive one or a few factors, but not combinations (Marschall and Crowder 1996). Future experiments attempting to determine changes that might occur in wild Appalachian brook trout populations should take these complications into account, as pH alone may not be a sufficient predictor. Ingersoll et al. (1990) found that effects of pH on survival and growth of hatchery brook trout were significantly correlated to different life-history stages, with sensitivity decreasing as fish mature from eggs to swim-up fry. All

fish in the present experiment were estimated to be approximately age 1+, based on length at age data given for brook trout by Etnier and Starnes (1993). Although low pH may not directly affect growth of adult brook trout, southern Appalachian populations could still be threatened by poor recruitment, due to lowered survival of eggs and fry.

Conclusions

The results of this study found no differences between northern and southern strains due the effect of low pH in growth and mortality. However, the overall poor performance of southern strain brook trout in the laboratory may have masked potential effects of low pH, especially with respect to growth. In addition, the significant mortality of brook trout exposed to pH 5.2 highlights concern for the fate of remaining native brook trout populations in southern Appalachia, an area susceptible to acid precipitation and acidic episodes. It is encouraging that southern strain fish were successfully maintained in the lab. Because this is one of only a few, if any, instances of success, it may offer hope for the possibility of future laboratory experiments with southern brook trout. However, protocols will have to be improved to achieve more realistic growth rates for southern strain fish.

Literature Cited

Literature Cited

Allan JD. 1995. Stream ecology: the structure and function of running waters. Kluwer Academic Publishers. Dordrecht, The Netherlands.

Anderson WG, McKinley RS, Colavecchia M. 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. North American Journal of Fisheries Management 17: 301-307.

Barton BA. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparitive Biology 42:517-525.

Boujard T, Labbe L, Auperin B. 2002. Feeding behavior, energy expenditure and growth of rainbow trout in relation to stocking density and food accessibility. Aquaculture Research 33:1233-1242.

Cornelison JW. 2005. An ecological comparison of two strains of brook trout (*Salvelinus fontinalis*). M.S. thesis, Biology Department, Western Carolina University, Cullowhee, NC.

Davis LE, Schreck CB. 1997. The energetic response to handling stress in juvenile coho salmon. Transactions of the American Fisheries Society 126:248-258.

Dwyer WP, Piper RG, Smith CE. 1983. Brook trout growth efficiency as affected by temperature. The Progressive Fish-Culturist 45(3):161-164.

Etnier DA, and Starnes WC. 1993. The fishes of Tennessee. University of Tennessee Press. Knoxville, Tennessee.

Fromm PO. 1980. A review of some physiological and toxicological responses of freshwater fish to acid stress. Environmental Biology of Fishes 5(1): 79-93.

Galbreath PF, Adams ND, Guffey SZ, Moore CJ, West JL. 2001. Persistence of native southern Appalachian brook trout populations in the Pigeon river system, North Carolina. North American Journal of Fisheries Management 21: 927-934.

Guffey SZ, McCracken GF, Moore S, and Parker CR. 1998. Southern Appalachian brook trout. In Ecosystem Management for Sustainability: Principles and

Hayes JP, Guffey SZ, Kriegler FJ, McCracken GF, and Parker CR. 1996. The genetic diversity of native, stocked, and hybrid populations of brook trout in the southern Appalachians. Conservation Biology 10(5): 1403-1412.

Hebert PDN, Beaton MJ. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis: a practical handbook. Helena Laboratories, Beaumont, Texas.

Ingersoll CG, Mount DR, Gulley DD, La Point TW, Bergman HL. 1990. Effects of pH, aluminum, and calcium on survival and growth of eggs and fry of brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 47:1580-1592.

Jacobsen OJ. 1977. Brown trout (Salmo trutta L.) growth at reduced pH. Aquaculture 11:81-87

Kelley GA, Griffith JS, Jones RD. 1980. Changes in distribution of trout in Great Smoky Mountains National Park, 1900-1977. United States Fish and Wildlife Service. Technical Paper 102. 10pp.

King W. 1937. Notes on the distribution of native speckled and rainbow trout in the streams at Great Smoky National Park. Journal of the Tennessee Academy of Science 12: 351-361.

Lachance S, Berube P, Lemieux M. 2000. In situ survival and growth of three brook trout (*Salvelinus fontinalis*) strains subjected to acid conditions of anthropogenic origin at the egg and fingerling stages. Canadian Journal of Fisheries and Aquatic Sciences 57: 1562-1573.

Marchand F, Boisclair D. 1998. Influence of fish density on the energy allocation pattern of juvenile brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 55:796-805.

Marschall EA, Crowder LB. 1996. Assessing population responses to multiple anthropogenic effects: a case study with brook trout. Ecological Applications 6(1):152-167.

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.

Menendez R. 1976. Chronic effects of reduced pH on brook trout (*Salvelinus fontinalis*). Journal of the Fisheries Research Board of Canada 33(1): 118-123.

Moyle PB, Cech JJ. 2000. Fishes: An Introduction to Ichthyology. 4th edition. Prentice-Hall, Inc. Upper Saddle River, NJ.

NADP (National Atmospheric Deposition Program/National Trend Network). 2003. Hydrogen ion concentrations as pH from measurements made at the field laboratories, 2003. http://nadp.sws.uiuc.edu/isopleths/maps2003/phfield.gif.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

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.

Ricker WE, 1979. Growth rates and models. In: W.S. Hoar, D.J. Randall and J.R. Brett (Editors). Fish Physiology, Vol. VIII, Bioenergetics and growth, Academic Press, Inc., Orlando, Florida, USA, pp.: 677 - 743.

Robinson GD, Dunson WA, Wright JE, and Mamolito GE. 1976. Differences in low pH tolerance among strains of brook trout (*Salvelinus fontinalis*). Journal of Fish Biology 8: 5-17.

Silsbee DG, Larson GE. 1982. Water quality of streams in the Great Smoky Mountains National Park. Hydrobiologia 89: 97-115.

Stoneking M. Wagner MJ, Hildebrand AC. 1981. Genetic evidence suggesting subspecific differences between northern and southern populations of brook trout (*Salvelinus fontinalis*). Copeia 4: 810-819.

Sullivan TJ. 1990. Historical changes in surface water acid-base chemistry in response to acid deposition. National Acid Precipitation Assessment Program State of Science and Technology. Report Number 11. Washington, DC.

Vijayan MM, Leatherland JF. 1988. Effect of stocking density on the growth and stress-response in brook charr, *Salvelinis fontinalis*. Aquaculture 75:159-170.

Wilson RW, Wood CM, Houlihan, DF. 1996. Growth and protein turnover during acclimation to acid and aluminum in juvenile rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 53:802-811.

Wood CM, Playle RC, Simons BP, Goss GG, McDonald DG. 1988. Blood gases, acid-base status, ions, and hematology in adult brook trout (*Salvelinus fontinalis*) under acid/aluminum exposure. Canadian Journal of Fisheries and Aquatic Sciences 45:1575-1585.

Woodward CC, Strange RJ. 1987. Physiological stress responses in wild and hatchery-reared rainbow trout. Transactions of the American Fisheries Society 116:574-579.

Wooton RJ. 1990. Ecology of teleost fishes. Chapman and Hall, London.

Appendices

Appendix A

Sites of Fish Collection

A-1. Stream, watershed, and number of brook trout collected according to genetic origin.

Stream	Watershed	Genetic Origin	# Collected
Beechflat Creek	Tuckaseegee	Northern	14
Log Hollow Creek	French Broad	Northern	24
Yellowstone Prong	Pigeon	Northern	16
Chastain Creek	Tuckaseegee	Southern	17
Fisher Creek	Tuckaseegee	Southern	21
Frady Creek	Tuckaseegee	Southern	13
Sugar Creek	Tuckaseegee	Southern	4

A-2. Raw data for northern brook trout in the low pH treatment from experiment 1. Some values are missing due to mortality.

Stream	pH treatment	Strain	Initial length (mm)	Terminal length	Initial weight (g)	Terminal weight (g)	Initial Kn	Terminal Kn	IGR wt. ($g \cdot d^{-1}$)	IGR ln. ($mm \cdot d^{-1}$)
Yellowstone Prong	5.6	Northern	146	146	26.8	31.4	1.012	1.186	0.0028	0.0000
Log Hollow Creek	5.6	Northern	133	137	23.3	19.0	1.163	0.868	-0.0036	0.0005
Yellowstone Prong	5.6	Northern	124	134	15.6	21.0	0.960	1.025	0.0053	0.0014
Log Hollow Creek	5.6	Northern	157	171	36.0	46.7	1.095	1.100	0.0046	0.0015
Beechflat Creek	5.6	Northern	138	138	20.7	19.4	0.925	0.867	-0.0012	0.0000
Log Hollow Creek	5.6	Northern	159	.	37.1	.	1.086	.	.	.
Log Hollow Creek	5.6	Northern	147	150	25.3	29.2	0.936	1.018	0.0026	0.0004
Yellowstone Prong	5.6	Northern	131	139	21.7	25.8	1.133	1.129	0.0031	0.0011
Log Hollow Creek	5.6	Northern	141	152	24.8	29.1	1.040	0.975	0.0029	0.0013
Yellowstone Prong	5.6	Northern	187	191	59.0	69.0	1.064	1.168	0.0028	0.0004
Beechflat Creek	5.6	Northern	178	185	46.4	46.6	0.970	0.868	0.0001	0.0007
Yellowstone Prong	5.6	Northern	126	129	17.9	18.2	1.050	0.995	0.0003	0.0004
Log Hollow Creek	5.6	Northern	159	165	36.8	42.4	1.078	1.111	0.0025	0.0007
Yellowstone Prong	5.6	Northern	149	161	29.0	37.9	1.031	1.069	0.0048	0.0014
Log Hollow Creek	5.6	Northern	123	126	14.7	13.7	0.927	0.804	-0.0013	0.0004
Log Hollow Creek	5.6	Northern	119	126	18.2	19.2	1.266	1.126	0.0010	0.0010
Yellowstone Prong	5.6	Northern	174	175	43.5	43.5	0.973	0.957	0.0000	0.0001
Beechflat Creek	5.6	Northern	181	181	49.6	53.8	0.986	1.070	0.0015	0.0000
Beechflat Creek	5.6	Northern	129	130	17.9	19.3	0.979	1.031	0.0013	0.0001
Log Hollow Creek	5.6	Northern	155	155	28.6	26.4	0.904	0.834	-0.0014	0.0000
Yellowstone Prong	5.6	Northern	165	168	40.2	42.0	1.054	1.043	0.0008	0.0003
Log Hollow Creek	5.6	Northern	141	149	24.3	27.6	1.019	0.981	0.0023	0.0010

A-3. Raw data for northern brook trout in the control pH treatment from experiment 1. Some values are missing due to mortality.

Stream	pH treatment	Strain	Initial length (mm)	Terminal length	Initial weight (g)	Terminal weight (g)	Initial Kn	Terminal Kn	IGR wt. ($g \cdot d^{-1}$)	IGR ln. ($mm \cdot d^{-1}$)
Yellowstone Prong	7.4	Northern	132	141	22.2	25.6	1.133	1.073	.	.
Log Hollow Creek	7.4	Northern	158	164	33.8	43.3	1.009	1.156	0.0044	0.0007
Beechflat Creek	7.4	Northern	117	123	12.4	16.8	0.908	1.059	0.0054	0.0009
Log Hollow Creek	7.4	Northern	205	216	67.2	86.7	0.921	1.017	0.0045	0.0009
Beechflat Creek	7.4	Northern	135	144	24.0	28.3	1.146	1.114	0.0029	0.0012
Yellowstone Prong	7.4	Northern	146	151	36.8	36.7	1.390	1.254	0.0000	0.0006
Beechflat Creek	7.4	Northern	121	.	16.3	.	1.079	.	.	.
Log Hollow Creek	7.4	Northern	178	179	42.9	40.6	0.897	0.834	-0.0010	0.0001
Log Hollow Creek	7.4	Northern	141	149	26.4	32.6	1.107	1.159	0.0038	0.0010
Log Hollow Creek	7.4	Northern	155	162	31.9	40.6	1.008	1.124	0.0043	0.0008
Beechflat Creek	7.4	Northern	108	115	11.5	13.9	1.069	1.071	0.0034	0.0011
Beechflat Creek	7.4	Northern	167	173	35.6	47.5	0.900	1.081	0.0051	0.0006
Yellowstone Prong	7.4	Northern	184	188	66.4	63.2	1.257	1.122	-0.0009	0.0004
Yellowstone Prong	7.4	Northern	94	.	6.5	.	0.915	.	.	.
Yellowstone Prong	7.4	Northern	172	173	44.4	44.7	1.028	1.017	0.0001	0.0001
Log Hollow Creek	7.4	Northern	171	175	48.1	54.2	1.133	1.192	0.0021	0.0004
Log Hollow Creek	7.4	Northern	175	175	51.5	49.9	1.132	1.097	-0.0006	0.0000
Yellowstone Prong	7.4	Northern	167	170	44.9	45.9	1.135	1.101	0.0004	0.0003
Log Hollow Creek	7.4	Northern	136	143	25.7	31.4	1.200	1.262	0.0036	0.0009
Log Hollow Creek	7.4	Northern	134	144	19.9	27.6	0.971	1.087	0.0058	0.0013
Yellowstone Prong	7.4	Northern	116	129	14.6	20.4	1.096	1.115	0.0060	0.0019

A-4. Raw data for southern brook trout in the low pH treatment from experiment 1. Some values are missing due to mortality.

Stream	pH treatment	Strain	Initial length (mm)	Terminal length	Initial weight (g)	Terminal weight (g)	Initial Kn	Terminal Kn	IGR wt. ($g \cdot d^{-1}$)	IGR ln. ($mm \cdot d^{-1}$)
Fisher Creek	5.6	Southern	129	.	14.9	.	0.815	.	.	.
Chastain Creek	5.6	Southern	136	140	21.1	20.6	0.985	0.882	-0.0004	0.0005
Frady Creek	5.6	Southern	136	139	23.6	28.1	1.102	1.229	0.0031	0.0004
Frady Creek	5.6	Southern	155	.	27.7	.	0.875	.	.	.
Frady Creek	5.6	Southern	165	165	31.1	33.9	0.815	0.889	0.0015	0.0000
Chastain Creek	5.6	Southern	143	146	32.1	33.6	1.290	1.269	0.0008	0.0004
Chastain Creek	5.6	Southern	119	127	15.8	18.5	1.099	1.060	0.0028	0.0012
Chastain Creek	5.6	Southern	170	174	45.6	49.0	1.093	1.096	0.0013	0.0004
Chastain Creek	5.6	Southern	147	146	27.4	28.4	1.014	1.073	0.0006	-0.0001
Fisher Creek	5.6	Southern	125	126	15.0	16.5	0.901	0.968	0.0017	0.0001
Frady Creek	5.6	Southern	117	117	14.3	12.7	1.047	0.930	-0.0021	0.0000
Frady Creek	5.6	Southern	122	.	14.2	.	0.917	.	.	.
Fisher Creek	5.6	Southern	119	121	13.2	14.5	0.918	0.960	0.0017	0.0003
Chastain Creek	5.6	Southern	140	.	24.3	.	1.041	.	.	.
Fisher Creek	5.6	Southern	155	156	27.8	28.3	0.878	0.877	0.0003	0.0001
Chastain Creek	5.6	Southern	162	162	36.0	32.1	0.997	0.889	0.0020	0.0000
Fisher Creek	5.6	Southern	167	168	36.8	34.3	0.931	0.852	-0.0013	0.0001
Fisher Creek	5.6	Southern	160	.	25.8	.	0.741	.	.	.
Fisher Creek	5.6	Southern	129	.	13.8	.	0.755	.	.	.
Chastain Creek	5.6	Southern	131	131	20.7	15.7	1.081	0.820	-0.0049	0.0000
Fisher Creek	5.6	Southern	145	.	20.9	.	0.806	.	.	.
Frady Creek	5.6	Southern	130	.	11.5	.	0.614	.	.	.

A-5. Raw data for southern brook trout in the neutral pH treatment from experiment 1. Some values are missing due to mortality.

Stream	pH treatment	Strain	Initial length (mm)	Terminal length	Initial weight (g)	Terminal weight (g)	Initial Kn	Terminal Kn	IGR wt. ($g \cdot d^{-1}$)	IGR ln. ($mm \cdot d^{-1}$)
Chastain Creek	7.4	Southern	126	.	16.6	.	0.974	.	.	.
Fisher Creek	7.4	Southern	140	144	22.4	27.2	0.959	1.071	0.0035	0.0005
Fisher Creek	7.4	Southern	146	.	17.6	.	0.665	.	.	.
Frady Creek	7.4	Southern	201	201	72.5	64.7	1.054	0.941	-0.0020	0.0000
Fisher Creek	7.4	Southern	139	146	23.8	28.1	1.041	1.062	0.0030	0.0009
Fisher Creek	7.4	Southern	132	.	15.5	.	0.791	.	.	.
Fisher Creek	7.4	Southern	139	143	26.1	26.4	1.142	1.061	0.0002	0.0005
Frady Creek	7.4	Southern	126	126	15.7	16.7	0.921	0.980	0.0011	0.0000
Frady Creek	7.4	Southern	122	.	15.4	.	0.995	.	.	.
Frady Creek	7.4	Southern	134	.	17.0	.	0.830	.	.	.
Frady Creek	7.4	Southern	113	116	15.0	15.5	1.218	1.164	0.0006	0.0005
Fisher Creek	7.4	Southern	135	138	21.0	24.0	1.002	1.073	0.0024	0.0004
Chastain Creek	7.4	Southern	155	155	28.5	20.4	0.900	0.645	-0.0060	0.0000
Fisher Creek	7.4	Southern	154	155	28.1	27.0	0.905	0.853	-0.0007	0.0001
Sugar Creek	7.4	Southern	143	146	25.3	26.6	1.017	1.005	0.0009	0.0004
Chastain Creek	7.4	Southern	165	165	34.3	27.4	0.899	0.718	-0.0040	0.0000
Chastain Creek	7.4	Southern	133	137	26.8	22.8	1.338	1.042	-0.0029	0.0005
Fisher Creek	7.4	Southern	133	133	20.9	19.2	1.043	0.958	0.0015	0.0000
Chastain Creek	7.4	Southern	134	134	25.1	24.3	1.225	1.186	-0.0006	0.0000
Fisher Creek	7.4	Southern	125	128	17.1	18.6	1.027	1.041	0.0015	0.0004
Chastain Creek	7.4	Southern	156	156	32.9	34.8	1.020	1.079	0.0010	0.0000
Fisher Creek	7.4	Southern	126	129	14.5	16.9	0.851	0.924	0.0027	0.0004

Appendix B

Measurements – Experiment 2

B-1. Raw data for northern brook trout in the low pH treatment from experiment 2. Some values are missing due to mortality.

Stream	pH treatment	Strain	Initial length (mm)	Terminal length (mm)	Initial weight (g)	Terminal weight (g)	Initial Kn	Terminal Kn	IGR wt. ($g \cdot d^{-1}$)	IGR ln. ($mm \cdot d^{-1}$)
Yellowstone Prong	5.2	Northern	129	.	17.3	.	0.919	.	.	.
Beechflat Creek	5.2	Northern	140	145	25.3	26.4	1.049	0.984	0.0008	0.0006
Beechflat Creek	5.2	Northern	146	155	31.9	41	1.165	1.248	0.0045	0.0011
Beechflat Creek	5.2	Northern	135	142	20.7	21.4	0.958	0.850	0.0006	0.0009
Yellowstone Prong	5.2	Northern	126	130	19.1	20.9	1.090	1.085	0.0016	0.0006
Log Hollow Creek	5.2	Northern	153	156	27.1	32.3	0.858	0.965	0.0031	0.0003
Log Hollow Creek	5.2	Northern	151	152	29.3	31.6	0.966	1.021	0.0013	0.0001
Log Hollow Creek	5.2	Northern	149	150	32.6	31.4	1.119	1.056	-0.0007	0.0001
Yellowstone Prong	5.2	Northern	143	147	29.7	32.7	1.155	1.169	0.0017	0.0005

B-2. Raw data for northern brook trout in the neutral pH treatment from experiment 2.

Stream	pH treatment	Strain	Initial length (mm)	Terminal length (mm)	Initial weight (g)	Terminal weight (g)	Initial Kn	Terminal Kn	IGR wt. ($g \cdot d^{-1}$)	IGR ln. ($mm \cdot d^{-1}$)
Yellowstone Prong	7.4	Northern	162	171	39.5	49.9	1.052	1.128	0.0042	0.0010
Yellowstone Prong	7.4	Northern	116	116	13.4	13.3	0.983	0.975	-0.0001	0.0000
Yellowstone Prong	7.4	Northern	131	137	20.2	23.8	1.024	1.054	0.0029	0.0008
Yellowstone Prong	7.4	Northern	143	147	27.7	31.1	1.077	1.112	0.0021	0.0005
Log Hollow Creek	7.4	Northern	140	145	25.6	30.4	1.061	1.133	0.0031	0.0006
Log Hollow Creek	7.4	Northern	149	151	27.4	30.6	0.940	1.009	0.0020	0.0002
Log Hollow Creek	7.4	Northern	153	153	29.3	27.8	0.928	0.880	-0.0009	0.0000
Log Hollow Creek	7.4	Northern	147	153	29.3	34.4	1.048	1.090	0.0029	0.0007
Log Hollow Creek	7.4	Northern	130	132	20	21.9	1.038	1.085	0.0016	0.0003

B-3. Raw data for southern brook trout in the low pH treatment from experiment 2. Some values are missing due to mortality.

Stream	pH treatment	Strain	Initial length (mm)	Terminal length (mm)	Initial weight (g)	Terminal weight (g)	Initial Kn	Terminal Kn	IGR wt. ($g \cdot d^{-1}$)	IGR ln. ($mm \cdot d^{-1}$)
Fisher Creek	5.6	Southern	146	146	27.1	23.9	0.989	0.872	-0.0022	0.0000
Fisher Creek	5.6	Southern	120	.	13.6	.	0.900	.	.	.
Chastain Creek	5.6	Southern	164	164	33	32	0.847	0.821	-0.0005	0.0000
Chastain Creek	5.6	Southern	156	.	27.3	.	0.815	.	.	.
Chastain Creek	5.6	Southern	144	145	25.9	22.2	0.986	0.827	-0.0028	0.0001
Fisher Creek	5.6	Southern	139	139	27.4	26.6	1.161	1.127	-0.0005	0.0000
Chastain Creek	5.6	Southern	128	128	18	15.1	0.979	0.822	-0.0031	0.0000
Fisher Creek	5.6	Southern	173	173	48.2	44.2	1.052	0.964	-0.0015	0.0000
Frady Creek	5.6	Southern	126	.	15.4	.	0.879	.	.	.

B-4. Raw data for southern brook trout in the neutral pH treatment from experiment 2.

Stream	pH treatment	Strain	Initial length (mm)	Terminal length (mm)	Initial weight (g)	Terminal weight (g)	Initial Kn	Terminal Kn	IGR wt. ($g \cdot d^{-1}$)	IGR ln. ($mm \cdot d^{-1}$)
Sugar Creek	7.4	Southern	144	144	25.3	24.4	0.963	0.929	-0.0006	0.0000
Fisher Creek	7.4	Southern	146	146	27.6	26.2	1.008	0.956	-0.0009	0.0000
Frady Creek	7.4	Southern	147	147	32.5	28.6	1.162	1.023	-0.0023	0.0000
Fisher Creek	7.4	Southern	128	130	18.6	19.7	1.012	1.023	0.0010	0.0003
Fisher Creek	7.4	Southern	156	157	34.6	32.4	1.033	0.949	-0.0012	0.0001
Frady Creek	7.4	Southern	129	129	16.7	15.9	0.887	0.845	-0.0009	0.0000
Fisher Creek	7.4	Southern	146	146	27.3	26	0.997	0.949	-0.0009	0.0000
Chastain Creek	7.4	Southern	140	140	24.6	22.8	1.020	0.945	-0.0014	0.0000
Fisher Creek	7.4	Southern	116	116	14.3	13.4	1.049	0.983	-0.0012	0.0000