AN ECOLOGICAL COMPARISON BETWEEN TWO STRAINS OF WILD BROOK TROUT, *Salvelinus fontinalis*

By

James Wes Cornelison

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of
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Abstract

AN ECOLOGICAL COMPARISON BETWEEN TWO STRAINS OF WILD BROOK TROUT, *Salvelinus fontinalis*

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Western Carolina University (May 2005)

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The southern Appalachian Mountains are home to an endemic strain of brook trout, *Salvelinus fontinalis*. Protein electrophoretic studies have demonstrated that native Southern Appalachian and northern hatchery-derived wild populations of brook trout are fixed for different alleles for creatine kinase (CK-A2) locus and show significant heterogeneity in allele frequency between strains at an additional 10 of 11 polymorphic loci. The differences observed in these studies are indicative of a substantial genetic divergence within the species and are of a magnitude consistent with sub-specific differentiation recognized among other salmonids. Electrophoretic studies have identified wild brook trout populations in southern Appalachia as being: 1) unaltered native southern Appalachian brook trout versus 2) purely northern-hatchery derived origin or 3) mixed genetic origin, the result of interbreeding between the two strains.

A series of experiments measuring thermal and acidity tolerances and comparing growth/diet characteristics were conducted to determine if any ecological differences exist between these two strains of wild brook trout. Brook trout were collected from various streams and transported to our research facilities where they were individually marked and placed under experimental conditions. Northern strain brook trout demonstrated a significantly higher thermal and acidity tolerance when compared to southern strain brook trout. Diet comparisons revealed northern strain brook trout consumed twice as many organisms as southern strain fish and distribution of prey items across taxa was found to be significantly different between strains. Growth rates were similar between strains but northern brook trout demonstrated a significantly higher survival rate when compared to southern strain brook trout. Results of these experiments indicate northern brook trout outperformed southern strain brook trout under the conditions of this experimental system.

Introduction

The brook trout *Salvelinus fontinalis* is the only salmonid species native to the southern Appalachian Mountains (King 1937; Lennon 1967; MacCrimmon and Campbell 1969). Its original range in streams and rivers extended from about 2,000 feet elevation, upstream to the headwaters (Lennon 1967). Following the turn of the 20th century, the brook trout's original range became significantly reduced resulting in wild populations now being restricted to headwater streams. The reduction in range can be attributed to a number of environmental disturbances associated with logging, road and railroad construction, frequent fires, and harmful fishing practices. In addition, state and federal agencies, as well as private companies, initiated stream stocking programs to supplement the sport fishery with two exotic salmonid species; rainbow trout *Onchorynchus mykiss* and brown trout *Salmo trutta*. These species, as they became established, further reduced the range of brook trout in the region (King 1937; Lennon 1967; Kelley et al. 1980; Bivens et al. 1985; Krueger and May 1991; Flebbe 1994). These stream supplementation programs also included the stocking of hatchery reared brook trout from the northeastern United States, specifically from Bellefonte, Pennsylvania and Berlin, New Hampshire fish hatcheries (McCracken et al. 1993). Attempts to culture native southern brook trout in local hatcheries were unsuccessful (PF Galbreath, personal communication). To an undetermined extent, these hatchery-derived fish have established populations or interbred with wild brook trout in southern Appalachian streams (Lennon 1967; Kreigler et al. 1995).

Recent research, however, indicates that brook trout native to the southern Appalachians represent a distinct strain relative to populations from the northern portion of its range. Fishery managers and fisherman have long suspected phylogenetic differences to exist between northern hatchery-derived brook trout and brook trout native to the southern Appalachians (King 1937; Lennon 1967; Stoneking et al. 1981). Lennon (1967) identified specific differences among the southern Appalachian and northern Appalachian brook trout including size, age, fecundity and morphology and concluded that the two strains were different at either a specific or sub-specific level.

The development of protein electrophoresis in the late 1960s and early 1970s gave scientists and researchers a diagnostic tool for differentiating between strains within a species. Protein electrophoretic studies demonstrated that native southern Appalachian and northern hatchery-derived wild populations of brook trout are fixed for alternative alleles at the creatine kinase A2* (CK-A2*) locus and show significant heterogeneity in allele frequency between strains at an additional 10 of 11 polymorphic loci (Stoneking et al. 1981; McCracken et al. 1993; Kreigler et al. 1995; Guffey 1998). The differences observed in these studies are indicative of a substantial divergence within the species and are of a magnitude consistent with sub-specific differentiation recognized among other salmonids (Stoneking et al. 1981; McCracken et al. 1993; Kreigler et al. 1995; Guffey 1998). Results of this research have identified wild brook trout populations as being: 1) unaltered native southern Appalachian brook trout 2) purely northern-hatchery derived origin or 3) of mixed genetic origin, the result of interbreeding between the two strains (McCracken et al. 1993; Guffey 1998).

In an effort to preserve rare alleles, the North Carolina Wildlife Resources Commission (NCWRC) is currently creating a trout species distribution database for western North Carolina to determine the genetic origin of all wild brook trout populations. A general perception has arisen in recent years that hatchery fish may negatively affect the constitution of wild populations (Allendorf and Ryman 1987; Hindar et al. 1991). This perception is fueled by general observations that the abundance of wild fish often decreases subsequent to the initiation of a hatchery release program, as has been seen in southern Appalachia (Habera and Strange 1993). Krueger and May (1991) noted several ecological effects of salmonid introductions including competition, predation on native fish, environmental modification, and introduction of parasites and disease to native fish. Furthermore, several studies reported significant losses in genetic variation or changes in allozyme frequencies in hatchery populations of salmonid fishes (Allendorf and Phelps 1980). Direct genetic effects from stocked salmonids are caused by interbreeding with native species, while indirect genetic effects may result through selective forces and/or a reduction of effective population size, genetic drift, and inbreeding (Krueger and May 1991). In general, any factor that reduces the abundance or size of a natural population can be interpreted as a negative genetic effect.

Currently, brook trout represent about one fourth of the wild trout resources (stream length) in the southern Appalachian Mountains (Table 1; Habera and Strange 1993).

Table 1. Estimated length of trout streams in the southern Appalachian mountain region.

Agency	Wild Trout (Km)	Brook Trout (Km)
Virginia Department of Game and Inland Fisheries	1,572	1,039
Tennessee Wildlife Resources Agency	938	167
North Carolina Wildlife Resources Commission	2,123	790
National Park Service, Great Smoky Mountains National Park TN/NC	1,185	424
Georgia Department of Natural Resources	3,851	140
South Carolina Wildlife and Marine Resources Department	291	20
Total	9,960	2,580

Recent estimates suggest southern brook trout account for 60% of all wild brook trout populations in western North Carolina (personal communications NCWRC). In addition to the NCWRC, fisheries management agencies in Virginia, Tennessee, Georgia, South Carolina, and Great Smoky Mountain National Park, have initiated efforts to determine genetic origins of wild brook trout populations for future protection. These agencies recognize the importance of genetic diversity in maintaining healthy fish populations and are in the process of designing appropriate management plans. With an ever increasing number of resource users, coupled with mounting threats to habitat quality from land development (Flebbe 1994), acidic deposition (Haines and Baker 1986), and climatic warming (Meisner 1990), sound management plans on a regional scale are now necessary to maintain a healthy fishery.

The main objective of this study was to determine what differences in adaptive characteristics exist between the two strains. Allelic differences indicate a substantial divergence within the species which suggests differences in adaptive characteristics might exist between the two strains. Assessing these differences may ultimately lead to a specific or sub-specific recognition between the two strains.

A series of experiments were designed to determine stress tolerance levels and to describe age, growth, and diet characteristics between strains. Fish were collected from streams determined by electrophoretic studies conducted by the Mountain Aquaculture Research Center, Cullowhee, NC to contain populations of wild northern and southern Appalachian brook trout populations. A non-lethal muscle biopsy was performed on each experimental fish and tissue samples were analyzed for the CK-A2 enzyme loci using cellulose acetate protein electrophoresis following protocols described by McCracken et al. (1993) and Guffey (1998).

Chapter 1: Thermal Tolerance

Introduction

Water temperature plays a large role in limiting the geographic range over which wild populations of fish species are found. Among the myriad of physical, chemical, and biological factors which influence the temperature tolerances of fishes, acclimation temperature is considered to be among the most critical (Elliot 1981; Bettinger and Bennett 2000). Studies have shown a fish population's temperature tolerance is a function of its genetics and its thermal history or acclimations (Vincent 1960; Wahl 1974; Kaya 1978). The relative importance of these two factors varies among species. Wahl (1974) found that domestic strains of brook trout from Pennsylvania hatcheries showed significant differences in upper temperature tolerances. Vincent (1960) demonstrated with convincing evidence that the upper temperature tolerance of wild brook trout was greater than that of a domestic strain. Furthermore, Carline and Machung (2001) concluded that differences in critical thermal maximum (CTMax) levels between wild and domestic strains of brook trout are genetically based.

Experimentation to determine temperature tolerances of fishes are usually measured via either temperature dynamic (i.e. critical thermal methodology, CTM) or static (i.e. incipient lethal temperature, ILT) (Bettinger and Bennett 2000). Measures of upper thermal limits- the conditions at which experimental fish lose equilibrium (LE) or cease opercular movement (death) in conditions of steadily increasing water temperature-has been termed critical thermal maxima (CTMax) (Kilgoure and McCauley 1986; Becker and Genoway 1979). CTMax requires a progressive change of temperature upward or downward from acclimation and exposure temperatures (Becker and Genoway 1979). Several studies have tested the effect of temperature increase rate in CTMax studies, and rates between 0.3° and 1.0° C per hour are generally recommended (Cocking 1959; Becker and Genoway 1979; Kilgoure and McCauley 1986; Galbreath et al. 2004).

Some researchers promote the chronic lethal temperature method test since it employs a much slower rate in temperature increase and more closely represents natural increasing temperatures fish may be exposed to over the summer months (Lutterschmidt and Hutchison 1997). This test allows sufficient time for the fish to acclimate to the chronic thermal stress, providing an environment that more closely simulates the prolonged stress fish might experience in a stream or in controlled culture (Becker and Genoway 1979). In reality, aquatic poikilotherms are rarely exposed to abrupt temperature changes due to the relative thermal inertia of water. Nor are they often exposed to the gradual, but still ecologically rapid, temperature changes required by the CTM method. However, the critical lethal temperature method is logistically complicated, involving numerous experimental tanks whose environmental conditions must be maintained at constant levels over a protracted time period (Kilgoure and McCauley 1986; Bennett and Judd 1992).

In contrast, measurement of CTMax is practical and convenient, and provides a recorded observation for each fish. However, since fish in natural systems rarely encounter such rapid temperature changes as typify CTMax tests, some researchers opt to conduct CTMax tests using much slower heating rates, in the range of 1-2° C/day (Alcorn 1976; Guest 1985; Grande and Andersen 1991). For this study, we chose this alternative CTMax dynamic method for several reasons including (1) the stress sensitivity of wild brook trout, (2) time and labor constraints and, (3) CTMax is the most widely used index to assess relative thermal tolerance (Bettinger and Bennett 2000; Carline and Machung 2001). Our objectives were to compare CTMax between strains of wild northern and southern Appalachian brook trout to determine upper thermal limits for each strain. This study is particularly relevant since the present-day distribution of brook trout is already fragmented (Flebbe 1994). Increased temperatures predicted by various global warming models are likely to further limit suitable brook trout habitat. If trout habitat becomes more fragmented under current warming trends, common local extinctions may become irreversible as avenues for re-colonization are eliminated (Flebbe 1997). Understanding the upper thermal limits of wild brook trout here in southern Appalachia will aid fisheries managers in conservation efforts for this species.

Methods

Fish Collection

Ten streams determined through electrophoretic studies to contain wild northern or southern Appalachian brook trout populations were selected for sampling (Table 2).

Table 2. Stream, watershed and number of brook trout collected according to genetic origin.

Stream	*Watershed*	*Genetic Origin*	*# Collected*
Fisher Creek	Tuckaseegee	Southern	4
Negro Prong	French Broad	Southern	4
Middle Prong	Pigeon	Southern	4
Big Bear Trap	Pigeon	Southern	5
Cove Creek	French Broad	Southern	5
Sugar Creek	Tuckaseegee	Southern	4
Beechflat Creek	Tuckaseegee	Northern	5
Rockhouse Creek	French Broad	Northern	8
Log Hollow Creek	French Broad	Northern	1
Yellowstone Prong	Pigeon	Northern	8

Fish were collected using a battery powered Smith-Root 400B backpack electroshocker between the dates of May 16th – May 18th 2003. Fish were placed in buckets then transferred to 50-L coolers equipped with airstones and transported to the Western Carolina University aquaculture facility in Cullowhee, NC. All streams were within 60 miles of Cullowhee, NC so fish transport did not exceed 75 minutes. Overall, 48 fish were collected. Water temperatures during transport ranged from 6.5° to 9.0° C. At the WCU aquaculture facility, fish were anesthetized with 0.1% clove oil and individually marked with a Panjet™ dye marker for identification to strain and stream of origin. Total length and wet weight for each fish were recorded. A non-lethal muscle biopsy was performed on each experimental fish and tissue samples were analyzed for the CK-A2 enzyme loci using cellulose acetate protein electrophoresis. All fish were confirmed to genetic origin.

Experimental Design

A total of 48 brook trout, 26 southern and 22 northern, were then pooled into an aerated 600L experimental tank and allowed to acclimate for a period of 3 days at 14° C. After 3 days, water temperature was then increased at a constant rate of 2° C/day rate with an 1800-watt immersion heater. An Onset™ datalogger recorded water temperature every 2 minutes. When temperature increased above 25° C, the fish were observed constantly for erratic swimming and gasping behaviors observed in other CTMax experiments (Elliot 1981). The test endpoint for a fish occurred when it lost the ability to remain upright for at least 5 seconds. At this time temperature, time of LE, strain, and stream of origin were recorded for each fish. Fish were then moved to a recovery tank for two days and recovery or death were recorded and percent recovery per stream was calculated.

Condition factors were then recorded for each fish according to the formula: weight x 100/length $(cm)^3$. A possible confounding effect of size on time to LE was investigated following the example of Carline and Manchung (2001), by performing linear regression analyses within strains, testing for a slope of the line significantly different from zero (Data Desk 6.0, Data Description, Ithaca, New York). Variance in time to LE was found to be heteroscedastic among strains and errors were non-normal (Leven's and Sharpiro-Wilk's tests, respectively, SAS/STAT Release 8.02, SAS, Cary, North Carolina). So, data were rank transformed and analyzed via one-way analysis (PROC GLM, SAS/STAT Release 8.02, SAS, Cary, North Carolina) of variance to provide a non-parametric test (Conover and Inman 1981).

Results

The mean CTMax for northern brook trout was 0.3°C higher than that of southern Appalachian brook trout (Table 3). There were significant differences between strains in average rank time to LE ($p<0.001$) with southern strain brook trout being the first to reach LE. Twenty-five percent of southern strain brook trout experienced LE before the first northern strain brook trout.

Table 3. Mean (± standard deviation) fish size and condition factor for northern and southern strain brook trout and results from chronic thermal maximum tests including mean temperature at LE, mean time to LE, and mean rank to LE.

Strain Stream	No.	Rank	Length (mm)	Condition Factor (K)	Time to LE (hr)	Temp at LE (C)
Southern	26	16.5 ± 12.6a	130 ± 27	0.86 ± 0.10	146 ± 3.2	27.0 ± 0.30
Northern	22	33.9 ± 8.8b	135 ± 24	0.84 ± 0.10	149 ± 1.1	27.3 ± 0.10
Southern						
Big Beartrap Cr.	5	17.8 ± 19.0	150 ± 33.0	0.78 ± 0.12	144 ± 6.0	26.9 ± 0.51
Cove Creek	5	7.8 ± 1.9	104 ± 8.0	0.96 ± 0.06	145 ± 0.8	28.9 ± 0.09
Fisher Creek	4	31.6 ± 12.9	147 ± 24	0.88 ± 0.10	149 ± 1.7	27.3 ± 0.08
Middle Prong	4	18.3 ± 7.8	150 ± 24.0	0.83 ± 0.07	147 ± 1.0	27.1 ± 0.15
Negro Prong	4	6.8 ± 5.0	109 ± 10.0	0.87 ± 0.07	144 ± 2.3	26.8 ± 0.21
Sugar Creek	4	18.8 ± 3.3	119 ± 2.0	0.84 ± 0.08	147 ± 0.3	27.2 ± 0.00
Northern						
Beechflat Creek	5	25.4 ± 8.8	121 ± 9.0	0.85 ± 0.13	148 ± 1.1	27.2 ± 0.13
Log Hollow Cr.	1	41	191	0.94	150	27.4
Rockhouse Cr.	8	37.5 ± 8.7	145 ± 16.0	0.88 ± 0.05	149 ±0.74	27.4 ± 0.05
Yellowstone Cr.	8	34.8 ± 8.7	125 ± 25.0	0.78 ± 0.10	149 ± 1.21	27.3 ± 0.05

In the analyses of size, the slope of the regression lines were positive and significantly different from zero (Figure 1). It was evident during the trials that larger fish were outlasting smaller individuals. I concluded size played an equally significant role for both strains in time to LE based on a non-significant interaction.

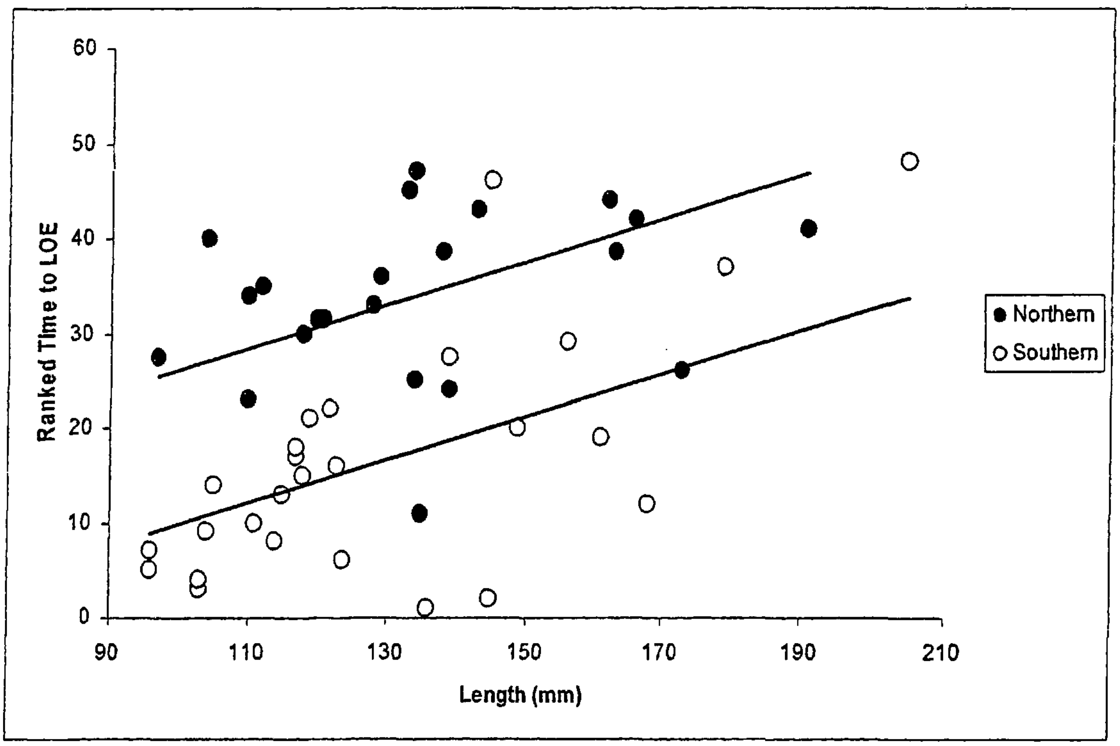


Figure 1. Linear regression analysis of length and ranks according to time to loss of equilibrium (LE).

Discussion

Among sport fishermen, brook trout have a reputation for requiring colder water than rainbow and brown trout and studies have indicated they are the least thermal tolerant trout species found in western North Carolina (Elliot 1981; Bettinger and Bennett 2000). However, there is a high variation in published measures of CTMax within these species and this is in no doubt related to differences in test protocols, such as pretest acclimation temperature and conditions, heating rate, and water chemistry (Hutchison 1976).

Acclimation temperature may have strongly affected the upper temperature tolerance of fish in this study. It is generally accepted that, for a given fish, CTMax increases with increases in acclimation temperature (Lutterschmidt and Hutchison 1997). Becker and Genoway (1979) indicated that the lethal limits are strongly affected by the temperatures that fish experience prior to tests. They demonstrated that the CTMax for coho salmon was positively correlated to acclimation temperature. They suggested that reporting a single temperature tolerance value or even a range of temperature tolerances for a species is meaningless without reporting the pretest temperature acclimation state. As an alternative, they recommended conducting CTMax tests from different acclimation levels (as with incipient lethal temperature method) at identical temperature increase rates. The results might form a detailed response pattern which could be useful for physiological analysis. We would have expected the observed CTMax in this study to be higher for both strains had the acclimation temperatures been higher; but the literature does not suggest that the difference in CTMax between two groups of fish would change with acclimation temperature.

In contrast, similar studies with salmonids have failed to demonstrate an effect of environmental acclimation on CTMax (Kaya 1978; Lohr et al. 1996). Bettinger and Bennett (2000) found that the upper temperature tolerance of brook trout was least affected by acclimation when compared with 21 temperate fishes. If acclimation temperature does not strongly affect the upper temperature tolerance of salmonids, genetic factors may be responsible.

Other studies have shown that the heating rate during a CTMax trial is positively related to CTMax (Becker and Genoway 1979). We used a heating rate of 2° C/day, which was recommended by Grande and Andersen (1991) and Galbreath et al. (2004), though others have recommended rates varying from 0.3° C/min (Becker and Genoway 1979) up to 1.0° C/min to minimize possible acclimation during the test (Lutterschmidt and Hutchison 1997). However, Galbreath et al. (2004) provided convincing evidence that a temperature increase of 2° C/day provided the greatest sensitivity for detecting differences between three trout species. In the trials reported here, the influence of heating rate on CTMax was not examined

Significant differences in average size and condition factor between strains did not exist in the present study. A lower CTMax temperature has been observed for larger older fish relative to smaller younger ones in some studies; however, these differences have been attributed more to age than size per se (Cox 1974). Size may affect upper CTMax values due to slower rate of heat penetration in larger organisms, and to differences within species due to age, sexual maturity, and other causal physiological factors associated with age. Other studies have indicated that size has only a weak correlation to differences in thermal tolerance measures among same age fish (Elliot 1981; Carline and Machung 2001). Nonetheless, it is generally accepted that as fish increase in size they exhibit a decrease in metabolic rate accompanied with a decrease in activity (Jobling 1983). In the present study, larger fish survived longer before reaching LE than smaller fish, and this was true for both strains.

Wahls (1974) study of 31 strains of domestic brook trout indicates a genetic component for differences in upper temperature tolerance. Furthermore, Vincent (1960) reared domestic and wild brook trout under similar conditions and demonstrated that wild fish had a higher upper temperature tolerance than domestic ones. Both studies concluded that differences in upper temperature tolerance of wild and domestic strains resulted from fundamental genetic differences. These genetic differences most likely developed as hatchery strains were selected for traits such as growth, feed conversion, and disease resistance. The relatively constant water temperatures in many trout hatcheries would not provide the selective pressures necessary for favoring individuals with low or high temperature tolerances. In contrast, natural streams annual temperature deviations might approach both upper and lower CTMax levels.

The present study was unique in that we compared two strains of wild brook trout rather than hatchery versus wild fish. Our results indicate a significant difference in upper thermal tolerance between these strains of brook trout. However, this should be tempered with the fact that southern strain brook trout may have reacted differently to the handling stress involved with this experiment. Northern strain brook trout may have outperformed southern strain brook trout in this experiment due to their history of domestication. Since southern strain brook trout have never been hatchery reared, the inherent stresses involved in this experiment could have resulted in southern strain brook trout reaching LE significantly faster than northern strain brook trout. However, we feel this did not play a major role. All fish were pooled together over a 48 hour period prior to initiation of the trials, and they were not fed so there was no competition for feed. It is my belief that all fish were equally stressed and times to LE between strains were not influenced by these handling stresses.

Chapter 2: Acidity Tolerance

Introduction

Surface water acidification is a serious threat to aquatic ecosystems in North America and Europe (Haines and Baker 1986; Rosseland et al. 1986), and fisheries in many of these locations are thought to have been reduced or lost (Baker and Schofield 1985). Galloway et al. (1976) concluded that H_2SO_4 and HNO_3 are the primary sources of H+ ions that cause acidification in eastern streams. Smelters, electric power plants, and steel mills, (i.e., those industries that use large amounts of coal), are thought to be the principle sources of sulfuric oxides (Haines and Baker 1986; Rosseland et al. 1986). Petroleum burning vehicles are the main sources of nitric oxides (Galloway et al. 1976).

Deleterious changes in stream ecology from acid deposition have been noted in numerous studies (Parsons 1968; Warner 1971; Robinson et al. 1976) and there is concern for wild brook trout populations in southern Appalachia. Wild brook trout populations are now limited to low order, high elevation headwater streams that have a pH/low alkalinity chemistry due to the high acidity in abiotic elements, specifically soil and rock (Cain 1931). Fish populations in high elevation streams are most vulnerable to low pH and related chemical factors because acid-neutralizing capacity tends to increase with distance downstream (Pinder and Morgan 1995). High-elevation headwater streams also limit fish presence because they often lack suitable physical habitat; therefore, the absence of fish species could be the result of low pH, inappropriate habitat, or both (Baker 1990). Southern Appalachian streams are naturally softer and more acidic than streams in the northeastern United States where northern brook trout originated (Cain 1931).

Differences in acid sensitivity have been shown among species of fish and also among strains of the same species. Species particularly tolerant of acid water include *Rutilus rutilu*, *Tribolodon hakonensis* (Swartz et al. 1978) and several South American characins (Dunson et al. 1977). Among North American freshwater fish, numerous studies have shown that brook trout are one of the more tolerant fish to low pH environments (Creaser 1930; Dunson and Martin 1973; Robinson et al. 1976; Swartz et al. 1978). In addition, Robinson et al. (1976) and Dunson and Martin (1973) demonstrated that different strains of brook trout vary in their resistance to lethally low pH environments. They concluded that acid tolerance is a quantitative genetic trait among strains of brook trout.

The objectives of the present study were to determine if any differences in acidity tolerance levels exist between wild northern and southern Appalachian strains of brook trout. Brook trout are generally confined to small tributaries and headwaters of large streams in southern Appalachia. Many of these streams are characterized by low acid-neutralizing (alkalinity, <10mg $CaCO_3$/L), consequently they are susceptible to the effects of acid precipitation (Henriksen 1982; Norton 1982). As in the thermal tolerance study, a dynamic (i.e., critical acid methodology) method was applied in this study to determine lethally low pH levels (see reviews by Robinson et al. 1976; Swartz et al. 1978).

Methods

Fish Collection

See methods section in Chapter 1: Thermal Tolerance for specific details on how fish were collected. Ten streams were selected for sampling (Table 4).

Table 4. Stream, watershed and number of brook trout collected according to genetic origin.

Stream	*Watershed*	*Genetic Origin*	*# Collected*
Fisher Creek	Tuckaseegee	Southern	5
Negro Prong	French Broad	Southern	5
Middle Prong	Pigeon	Southern	4
Big Bear Trap	Pigeon	Southern	5
Cove Creek	French Broad	Southern	6
Sugar Creek	Tuckaseegee	Southern	4
Beechflat Creek	Tuckaseegee	Northern	5
Rockhouse Creek	French Broad	Northern	8
Log Hollow Creek	French Broad	Northern	1
Yellowstone Prong	Pigeon	Northern	8

Experimental Design

A total of 51 brook trout (22 northern, 29 southern) were then pooled together in an aerated 600L experimental tank and allowed to acclimate for a period of 3 days at a pH of 6.8 and temperature of 14° C. Following acclimation, a 0.6M solution of sulfuric acid (H_2SO_4) at a rate of 0.5 mL/min was progressively added to the water using a Perkin Elmer™ metering pump. The level of pH was monitored frequently using an AR15™

pH meter. The test endpoint for a fish occurred when it lost the ability to remain upright for at least 5 seconds. At this time pH, time of LOE, strain, and stream of origin were recorded for each fish. Fish were then transferred to a recovery tank for 2 days after which recovery or death was recorded. All data was analyzed in the same manner as described in the Methods section of Chapter 1: Thermal Tolerance.

Results

The present study indicates that northern brook trout brook have a significantly higher pH tolerance than southern Appalachian brook trout. Average pH at LE for northern brook trout was 3.34 ± 0.24 compared to 3.20 ± 0.10 for southern Appalachian brook trout (Table 5). There were significant differences between strains in average rank time to LE ($p<0.001$) with southern strain brook trout being the first to reach LE.

Table 5. Mean (± standard deviation) fish size and condition factor for northern and southern strain brook trout and results from chronic acidity maximum tests including mean pH at LE, mean time to LE, and mean rank to LE.

Strain Stream	#	Rank	Length (mm)	Condition Factor (K)	Time to LE	pH at LE
Southern	29	20.0 ± 13.0	136 ± 30	0.88 ± 0.07	69.9 ± 4.6	3.34 ± 0.24
Northern	22	34.3 ± 13.0	144 ± 25	0.92 ± 0.09	73.3 ± 2.8	3.20 ± 0.10
Southern						
Big Beartrap Cr.	5	35.9 ± 6.0	154 ± 11.0	0.87 ± 0.05	73.0 ± 1.0	3.24 ± 0.03
Cove Creek	6	8.9 ± 6.3	115 ± 27.0	0.87 ± 0.07	68.8 ± 1.2	3.33 ± 0.03
Fisher Creek	5	17.3 ± 9.2	116 ± 10.0	0.91 ± 0.04	70.3 ± 1.3	3.30 ± 0.02
Middle Prong	4	31.0 ± 17.3	172 ± 33.0	0.94 ± 0.07	72.3 ± 2.6	3.26 ± 0.06
Negro Prong	5	18.1 ± 5.5	155 ± 15.0	0.82 ± 0.05	70.4 ± 0.7	3.31 ± 0.01
Sugar Creek	4	9.1 ± 6.2	109 ± 9.0	0.85 ± 0.07	64.2 ± 10	3.64 ± 0.63
Northern						
Beechflat Creek	5	25.6 ± 9.3	148 ± 17.0	0.97 ± 0.07	71.3 ± 1.3	3.28 ± 0.03
Log Hollow Cr.	1	37	133	0.91	72.7	3.26
Rockhouse Cr.	8	40.8 ± 8.8	151 ± 28.0	0.92 ± 0.08	74.5 ± 2.4	3.20 ± 0.08
Yellowstone Cr.	8	33.0 ± 16.5	137 ± 29.0	0.89 ± 0.10	73.4 ± 3.6	3.22 ± 0.10

Linear regression analyses revealed that length was significant for both strains in time to LE. The slope of the regression line was significantly different from zero (Figure 2). We concluded size played an equally significant role for both strains in time to LE based on a non-significant interaction.

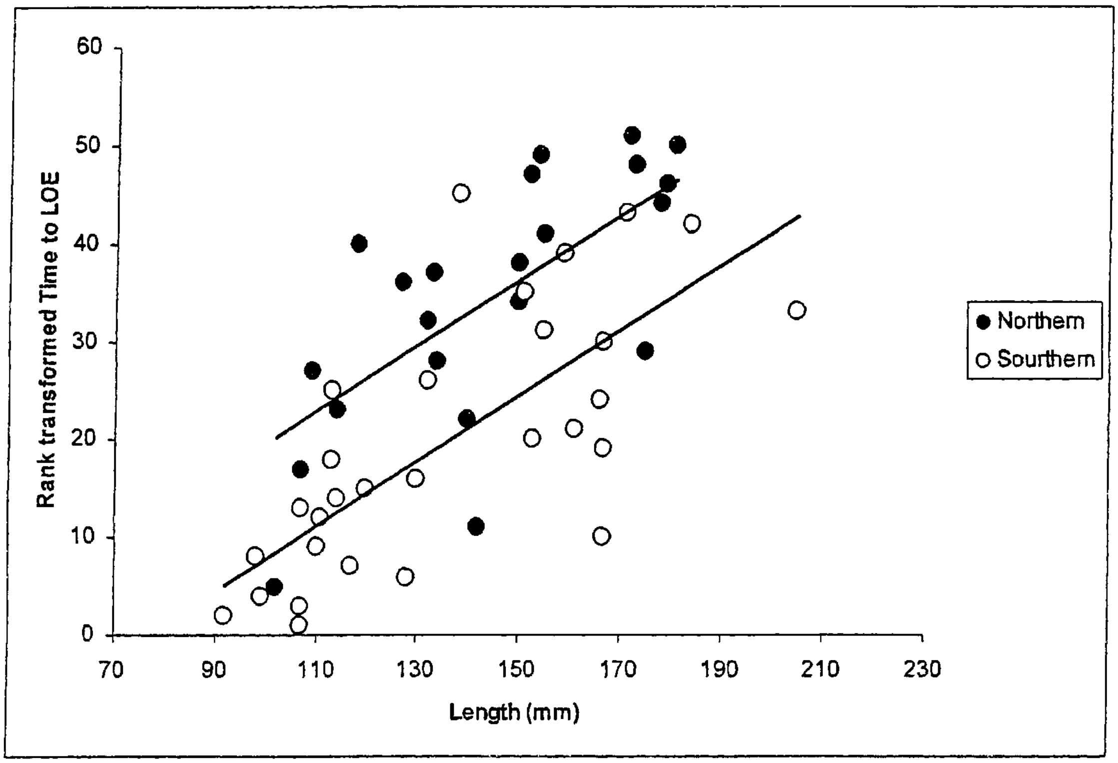


Figure 2. Linear regression analysis of length and ranks according to time to loss of equilibrium (LE).

The behavior of the fish varied with pH. At low and lethal pH values (3.0-3.8) fish initially displayed a short period of extreme activity followed by prolonged periods of inactivity. Prior to loss of equilibrium, activity again increased, as did ventilatory movements. No fish survived after losing equilibrium presumably due to coagulation of gill surfaces resulting in asphyxia (Robinson et al. 1976).

Discussion

Differences in ability to resist lethally low pH environments were demonstrated in this present experiment between northern and southern strain brook trout. This has been demonstrated by other researchers using sulfuric acid solutions (Robinson et al. 1976; Swartz et al. 1978). Their experiments were carried out in both laboratory and partially controlled environments reproducing acidic conditions similar to those caused by mine tailings. They concluded that the brook trout's variable resistance to acidic conditions depended on both genetic and environmental factors. Johnson (1982) has also reported strain differences in resistance times of brook trout in sulfuric acid solutions of pH 3.0-4.0 and concluded that brook trout tolerance to low pH was affected by body size (age), season, temperature, and heredity.

Some researchers have suggested acclimation of fish to acidic conditions may present a confounding factor to resistance times between strains (Vaala 1971; Swartz et al. 1978). Vaala (1971) suggested that low pH acclimation in brook trout is a phenomenon which should be investigated further. Her studies showed that brook trout groups exposed both to near toxic acidity for 5 days, and to chronic acidity for 28 days, demonstrated various hematological changes suggestive of acclimation. These observed changes might provide for increased availability of oxygen thus slowing down anoxia, a major physiological problem of brook trout exposed to low pH. Since fish collections occurred over numerous streams in several watersheds for this experiment, it is possible certain streams exhibit lower annual pH's compared with others. If this is the case, particular brook trout populations might be acclimated to low pH levels creating an "in stream" effect to this study.

Several studies suggest fish size is directly related to survival time at low pH (Johnson 1982). Our data indicate that correlations between size and survival existed but this was equally true for both strains. Robinson et al. (1976) found pronounced size and/or age differences in resistance times; larger brook trout survived longer than smaller brook trout tested. Conversely, Daye and Garside (1974) reported a lack of relationship between size of brook trout and resistance times within samples. Kwain (1975) found the resistance and tolerance of 18-mo-old rainbow trout in low pH environments (sulfuric acid) exceeded that of 4-mo-old rainbow trout. Lloyd and Jordan (1964) did not find any significant correlations between length of rainbow trout and survival times in low pH environments.

Despite the contradictory results, most researchers agree that the tolerance of brook trout to low pH may depend on many factors such as the type of acid involved (i.e., the anion), other cations and anions present, the size of the fish, temperature, previous acclimation history of the fish, etc. Although my results indicate that northern strain brook trout are significantly more tolerant to low pH conditions than southern strain brook trout, it is impossible to isolate which of these factors might be responsible for the observed differences.

The low pH-tests presented in this study have been concerned with the resistance times of brook trout in environments of lethally low pH. While knowledge of pH resistance times of northern and southern strain brook trout in ultimately lethal environments may be useful, information on incipient levels (the lowest pH at which trout can survive indefinitely) of the fish in long-term, low-pH tests may be more valuable. Our lab is currently investigating the effects of long-term, low pH conditions (average 5.0 and 5.6) on the growth and survival of northern and southern strain brook trout. Preliminary results indicate northern strain brook trout exhibit greater growth and higher survivorship compared with southern strain brook trout in conditions of low pH.

Chapter 3: Age and Growth

Introduction

Growth

The age and growth of fishes serves as an important indicator of environmental and physiological conditions (Dianna 1995). Valid age and growth data are required for understanding fish life history and assessing population dynamics. Length, weight, growth, and age data are the cornerstone in the foundation of fishery research and management. Fishes, in general, have indeterminate growth; all ages and sizes have some inherent growth capacity (Dianna 1995). Fishes that grow fast generally have better survival rates since they are less vulnerable to predation. Growth, therefore, can have a direct effect on survival (Dianna 1995).

Many environmental, physiological, and ecological factors can affect the growth of fishes. Several of these factors are especially critical in fragile environments such as headwater streams in western North Carolina. Brook trout population dynamics in western North Carolina are typically regulated by elevation and temperature (Bivens et al. 1985), anchor ice formation (King 1937), competition for habitat (Flebbe 1994), and competition with rainbow trout (Kelly et al. 1980). Studies have shown that hatchery reared fish show greater growth potential than wild fish (Carlander 1987; Kulp 1994).

Age

There are several methods used for estimating the age of fishes such as the comparison of length-frequency distribution, recovery of marked fish (observation), and interpretation of calcified layers laid down in the solid parts of fishes (Hining et al. 2000). The most commonly used technique for estimating the age of fish in temperate climates involves examination of calcified structures (i.e. scales and otoliths) for the presence of annuli. Counting scale annuli has been the most common method by which age has been estimated in North America since 1930 (Carlander 1987). However, recent research suggests that scales are less accurate and tend to underestimate ages due to indistinguishable annuli during periods of slow growth (Beamish and McFarlane 1987; Hining et al. 2000). Beamish and McFarlane found that scales underestimate ages of brook trout by up to 40% and that scale and otolith agreement declines to less than 60% for brook trout of ages 3 and 4 (Kulp 1994). However, the use of otoliths for age estimation has become increasingly common due to convincing evidence that counting annuli in otoliths provides precise age data (Marshall and Parker 1982; Campana 1983; Green et al. 1985; Hining et al. 2000).

Measurements of wild brook trout populations by the Mountain Aquaculture Research Center have demonstrated that naturalized northern brook trout are on average larger then southern Appalachian brook trout (PF Galbreath, personal communication). King (1937) found the average size of southern Appalachian brook trout to be around 6 inches but could reach lengths of 12 to 18 inches under favorable conditions. Unfortunately, these favorable conditions no longer exist and the majority of wild brook trout populations have been driven to small headwater streams at elevations above 900 m where lower temperatures typically limit production (Kelly et al. 1980).

Over the past 40 years evidence has accumulated that suggests hatchery –reared trout differ considerably from native, wild populations (Carlander 1987). In general, hatchery reared trout have a tendency to exhibit higher growth rates and lower survival rates than wild trout (Carlander 1987). Cooper (1959) cited many field tests where stocked hatchery fish exhibited high natural mortality and pointed out that it is often difficult to ascertain and evaluate the various factors that may be operating in the given situation.

Ample data exists on the growth of lotic and lentic fish populations throughout the United States (Waters 1977; Balon 1980) however, growth data for northern and southern Appalachian brook trout in smaller headwater streams are scarce (Kulp 1994). The objective of this study was to compare age and growth characteristics among pure northern and pure southern brook trout. We hypothesized that northern strain brook trout, due to their history of hatchery selection, would outperform southern Appalachian brook trout over an 8-week period in a common garden experiment.

Methods

Fish Collection

Ten streams determined to contain wild northern or southern Appalachian brook trout populations were selected for fish collections (Table 6).

Table 6. Stream, watershed and number of brook trout collected according to genetic origin.

Stream	*Watershed*	*Genetic Origin*	*No. Collected*
Fisher Creek	Tuckaseegee	Southern	4
Negro Prong	French Broad	Southern	3
Little Creek	Chatooga	Southern	3
Big Bear Trap	Pigeon	Southern	4
Chastain Creek	Tuckaseegee	Southern	4
Beechflat Creek	Tuckaseegee	Northern	5
Flat Laurel Creek	Pigeon	Northern	4
Sams Branch	Pigeon	Northern	2
Log Hollow Creek	French Broad	Northern	6
Bryson Branch	Cullusaja	Northern	5

Fish were collected using a battery powered Smith-Root 400B backpack electroshocker between the dates of June 25th – 28th 2004. Fish were placed in buckets then transferred to 50-L coolers equipped with airstones and transported to the Lonesome Valley Aquaculture Research Station in Cashiers, NC. All streams were within 60 miles of Cashiers, NC so fish transport did not exceed 75 minutes. Overall, 22 northern and 18 southern Appalachian brook trout were collected. Water temperatures during transport ranged from 10° to 13° C. At the WCU aquaculture facility, fish were anesthetized with 0.1% clove oil and a passive integrated transponder (PIT) tag was injected into the dorsal muscle for individual identification. Total length and wet weight for each fish were recorded, along with the ID number from the PIT tag. A non-lethal muscle biopsy was performed on each experimental fish and tissue samples were analyzed for the CK-A2 enzyme loci using cellulose acetate protein electrophoresis. All fish were confirmed to genetic origin.

Experimental Design

A total of 41 fish were placed in an 8' x 40" outdoor concrete raceway prepared nine months in advance with a gravel/boulder substrate bottom, woody debris, and overhead cover to create an artificial stream environment. Boulders and debris were positioned in a manner to promote a laminar flow through the channel. Fish were fed a combination of meal worms, red worms, and grasshoppers at a rate of 5% of biomass every other day. In addition, field observations indicated an abundance of aquatic insect life established in the raceway.

Biomass was calculated according to total weight measurements. At the end of the 8-week period the raceway was drained and fish were collected. Length, weight, and PIT tag numbers were recorded. Instantaneous and absolute growth rates along with mortality rates were calculated for all age classes and compared among strains. All recaptured fish were then sacrificed using an overdose of clove oil. Harvested fish were transported to WCU where the sagittae were removed by the "up through the gills method" (Secor et al. 1992) and stored in micro-centrifuge tubes.

One otolith from each fish was then mounted on a glass microscope slide by heating a small drop of thermosetting plastic resin on a hot plate (Neilson and Green 1981; Hining et al. 2000). The otolith was positioned sulcus side up. After hardening, otoliths were hand-ground using 600-grit wet sandpaper (Campana and Neilson 1985; Secor et al. 1992). Otoliths were viewed using a 10x compound microscope to distinguish and count annuli (Brothers 1987). All otoliths were read independently by three readers, one with considerable past experience.

Statistical Analysis

In the analysis of vital statistics of fish populations, instantaneous growth rates have been recommended because of their utility in related calculations of biomass (Ricker 1958). However, since growth is not positively exponential over a long period in the life of a fish, instantaneous rates of growth ideally should be used for comparatively short segments of the entire growth history. For this reason, instantaneous growth rates were calculated for all fish according to: $\ln(L_2/L_1)/t_2 - t_1$ where L_2 is length at harvest and L_1 is length at time of capture. Absolute growth rates, $L_2 - L_1/t_2 - t_1$, were also calculated and compared with instantaneous growth rates.

Results

The results of this common garden experiment suggest there is little difference in the growth of northern and southern strain brook trout (Table 7). Among surviving fish, there was no difference in the instantaneous growth rate between strains (t-test, df=21, $P>0.05$, calculated separately for length and weight changes).

Table 7. Summary statistics on survival and growth of northern strain and southern strain brook trout held in a common garden experiment over an eight week period. Data reported here are means (and standard errors).

		Absolute Growth Rate		Instantaneous Growth Rate	
Strain	Survival	Length (mm)	Weight (g)	Length (mm/d)	Weight (g/d)
Northern	80%	9	7.9	0.0011	0.0033
SE[2]		(2)	(1.8)	(0.0002)	(0.0010)
Southern	41%[1]	9	7.6	0.0011	0.0045
SE		(2)	(2.8)	(0.0003)	(0.0015)

1 adjusted for 2 mortalities after fish became entangled in bird-exclusion netting that had inadvertently fallen into artificial stream.

2 Standard Error

Among fish for which age could be determined, growth rates were similar among year-classes (Table 8). The only difference may have been for age 4+ fish, however there were only 2 individuals observed to belong to this age class, and they were both northern strain fish.

Table 8. Instantaneous growth rates, broken down by age class, calculated for northern and southern strain brook trout held in a common garden experiment over an eight week period.

	Northern Strain		Southern Strain	
Age Class	(mm/d)	(g/d)	(mm/d)	(g/d)
2+	0.0011	0.0042	0.0013	0.0068
3+	0.0011	0.0039	0.0014	0.0061
4+	0.0006	0.0013		

While there were no significant observed differences in growth over the 8-week experiment, northern strain fish demonstrated significantly higher survival (Fisher's exact test, P=0.008) than southern strain fish. The source of the increased mortality among southern strain fish is unknown. There were no obvious signs of disease or nutritional deficiency among either strain at the end of the experiment and all recovered fish had gained weight and grown in length over the eight week period. But, the lack of significant differences in growth rate could have been affected by the difference in survival. If mortality had been associated with no growth, or loss of weight, estimates of growth rate based on survivors may be biased.

Discussion

Growth rates for both strains were almost identical and consistent with other growth studies performed on strains of brook trout (Cooper 1959; Kulp 1994). Kulp (1994) compared growth rates of northern and southern Appalachian brook trout in streams within the Great Smoky Mountains National Park and found that the northern strain grew larger their first year of life. However, he concluded growth increments were similar in ages two through four. Contrary to what I hypothesized, my results indicate that northern and southern Appalachian brook trout exhibit similar growth patterns at ages 2 or 3. No trout were determined to be less than 2 years of age.

Observed differences in sizes between the two strains could be attributed to a variety of factors other than differences in growth rates. It has long been known that brook trout grow to different sizes in different waters, and there has been much speculation on what factors may determine this. Size differences observed in populations might have more to do with brook trout densities than any other variable. Several eastern Tennessee studies have indicated that southern strain brook trout populations have higher densities than both hybrid and northern populations (Habera and Strange 1993; Kulp 1994). Brown (1945) suggested that the food supply and the degree of crowding played an important role in determining growth and size differences among brown trout.

Kreigler (1995) reported that hatchery brook trout standing stocks had lower net production relative to wild brook trout in Adirondack mountain ponds, and they postulated that hatchery reared brook trout poorly adapted to conditions outside of the hatchery environment. Furthermore, Carlander (1987) reported that stocked brook trout in New York streams grew faster than wild brook trout but experienced higher natural mortality. Additional studies by Kreigler et al. (1995) found that hatchery allele frequencies in heavily stocked hybrid populations were low, suggesting that the reproductive success of hatchery brook trout stocked into wild populations was limited.

Water quality differences between northern and southern Appalachian streams could play a role in population dynamics between strains. Brook trout streams in western North Carolina tend to be headwater reaches characterized by poorly buffered soft waters, low incident light, steep gradients, and seasonal flooding (Harshbarger 1978; Moore et al. 1983). Phillips (1959) attributed the poor survival of hatchery trout in some stocking programs to a function of the mineral content of the stocked waters. He hypothesized that fish transferred from hard-water hatcheries to soft, natural waters may have a survival problem due to the increased energy expanded for osmoregulation. The low densities of northern strain brook trout populations, and the fact that they have failed to expand and proliferate in areas where they have been continuously introduced over the past 70 years, implies the strain is not genetically adapted to southern Appalachian systems. Further studies on population dynamics between the two strains may reveal wild northern brook trout populations are composed of fewer, larger fish.

The significantly higher survival rate of northern brook trout suggests they outperformed southern Appalachian brook trout in the 8-week trial. Northern brook trout have may have outperformed southern Appalachian brook trout in this raceway setting due to their history of genetic selection for desired traits, such as fast growth, feed conversion, and disease resistance. Since southern Appalachian brook trout have no history of genetic selection for desired hatchery traits, the increased mortalities in this hatchery type setting are not surprising. Furthermore, recent feeding experiments conducted here in our labs indicate wild northern strain brook trout readily take trout feed in a controlled setting while southern strain brook trout will not. However, these observations fail to disclose the basic nature of this difference: is it the net effect of the hatchery environment and practice, the impact of a drastic change in environment, the result of hatchery selection, or perhaps a combination of these factors?

Chapter 4: Diet Analysis

Introduction

Understanding the patterns and ways in which animals use food resources is fundamental to the study of any animal population. Fish are often behaviorally sophisticated and exhibit feeding behaviors that point to factors influencing nutrition and growth (Bowen 1986). Salmonids are important predators in cold-water streams, sometimes the only vertebrate predator (excluding salamanders in southern Appalachian), and can potentially determine prey community structure through selective feeding (Connel 1975).

Production in southern Appalachian salmonid populations is low relative to that of salmonid populations in other parts of the country (Habera and Strange 1993). This has been attributed to several factors including reduced invertebrate production, extremes in temperature and streamflow (King 1937), and high stream gradients that limit habitat availability (Lennon 1967). Numerous feeding studies have been performed on salmonids but little is known about the factors governing prey choice under natural conditions (Tebo and Hassler 1963; Connel 1975; Allan 1981). The extensive literature on feeding of salmonids often characterizes feeding as opportunistic (Allen 1941; Elliot 1967; Allan 1981; Cada et al. 1987). Prey includes a diversity of forms; large items are preferred, and new prey appears in the diet as they become available (Allen 1941; Elliot 1967).

The primary objectives of this study were to quantitatively describe food consumption by northern and southern brook trout placed under natural conditions and to investigate factors affecting choice of prey. Previous dietary studies on wild brook trout in southern Appalachia have yielded mixed results (Tebo and Hassler 1963) but to date no studies have been done comparing the diet wild of northern and southern Appalachian brook trout. Several different ways of measuring prey use and analyzing feeding habits have been proposed and used, but little agreement exists that would enable uniformity in analyzing food habit data (Calliet et al. 1996). This study was designed to generate data regarding prey selection between the two strains in a 100m section of stream.

Methods

Fish Collection

See methods section in Chapter 3: Age and Growth for protocol describing fish collections. Ten streams were selected for sampling (Table 9).

Table 9. Stream, watershed and number of brook trout collected according to genetic origin.

Stream	*Watershed*	*Genetic Origin*	*No. Collected*
Fisher Creek	Tuckaseegee	Southern	5
Negro Prong	French Broad	Southern	4
Little Creek	Chatooga	Southern	3
Big Bear Trap	Pigeon	Southern	3
Chastain Creek	Tuckaseegee	Southern	5
Beechflat Creek	Tuckaseegee	Northern	5
Flat Laurel Creek	Pigeon	Northern	4
Sams Branch	Pigeon	Northern	1
Log Hollow Creek	French Broad	Northern	6
Bryson Branch	Cullusaja	Northern	5

Experimental Design

A total of 41 fish (21 northern, 20 southern) were placed in Logan Creek Jackson County, North Carolina. Logan Creek is a second order, high elevation (3,000 ft.) southern Appalachian mountain stream with a bedrock/cobble substrate and strong allochthonous energy input. The riparian zone is composed of mainly hemlock, white pine, rhododendron, and mountain laurel. Brook trout are the only fish species present. Prior to introduction of experimental fish, a 100m section of Logan Creek was measured and barriers were constructed to prevent emigration and immigration. A 1 inch diameter mesh "chicken wire" fence was anchored across the downstream section. This was fitted directly upstream of a weir channel guide (used as an intake for a small hatchery). The upstream section has a natural 10ft waterfall that I determined to be a sufficient barrier to upstream migration. All brook trout already present within the 100m section were removed and placed downstream of the downstream barrier.

A representative sample (approximately 4-5 individual trout) of each strain was sampled via electrofishing one day (over a 24h period) every two weeks over an 8-week period (a total of 4 sampling days). Bowen (1986) recommends a series of diel (24h) collections to determine if the diet varies consistently according to time of day. We sampled fish during morning (7am-10am), afternoon (11am-2pm) and evening (10pm-1am). We waited at least 5 hours between sampling events in order to minimize stress. Individual fish were identified according to PIT tag numbers, time of day recorded and stomach contents were removed and stored in labeled individual vials. Stomach contents were removed using a non-lethal flush pump according to Giles (1980). Samples were preserved in a 10% formalin solution. After 3 days, samples were then transferred to an 80% ethanol solution for long term preservation (Bowen 1986).

Macroinvertebrates were identified to the family level using the head counting method since head capsules often are digested more slowly than the rest of the prey (Allan 1981; Bergman and Greenburg 1994). Partially digested prey not containing an identifiable head were deemed non-identifiable. Prey items were identified using three aquatic macroinvertebrate keys (McCafferty 1981; Merritt and Cummins 1996; Voshell 2002).

To measure prey importance between strains, frequency of occurrence and numerical abundance were calculated and compared. These are common methods used by fisheries biologists to measure prey importance in predatory fish. Frequency of occurrence refers to the proportion or percentage of fish stomachs in which a particular food item was found (Bowen 1986). For example, if 18 out of 22 brook trout sampled contained one or more Perlidae, the frequency of occurrence of Perlidae in the diet would be 0.82 or 82%. Frequency of occurrence information is useful in that it represents what proportion of fish predators sampled consumed at least some of a particular item. However, it can be biased toward relatively scarce food items that occur often but do not contribute significantly in volume or number. Numerical abundance is simply the percent of all stomach contents combined that the prey type comprised (Bowen 1986).

Results

Southern Appalachian and northern brook trout had a dissimilar diet within the 100m section. Numerous samples contained various other body parts indicating fish were feeding and the stomach pump was effective, but identification was impossible since no head capsule was present. Non-aquatic-insects were identified as terrestrials (hymenoptera, lepidoptera, diptera) or other (worms, salamanders, crayfish). The cumulative data for all stomach samples is summarized in Table 10.

A total of 209 prey items were identified from 35 northern brook trout stomachs compared to only 71 from 32 southern Appalachian brook trout stomachs (Table 10). Chironomidae was the most common Diptera present in stomach samples for both strains. Over half, (51%), of southern Appalachian brook trout had empty stomachs compared to 39% in northern brook trout.

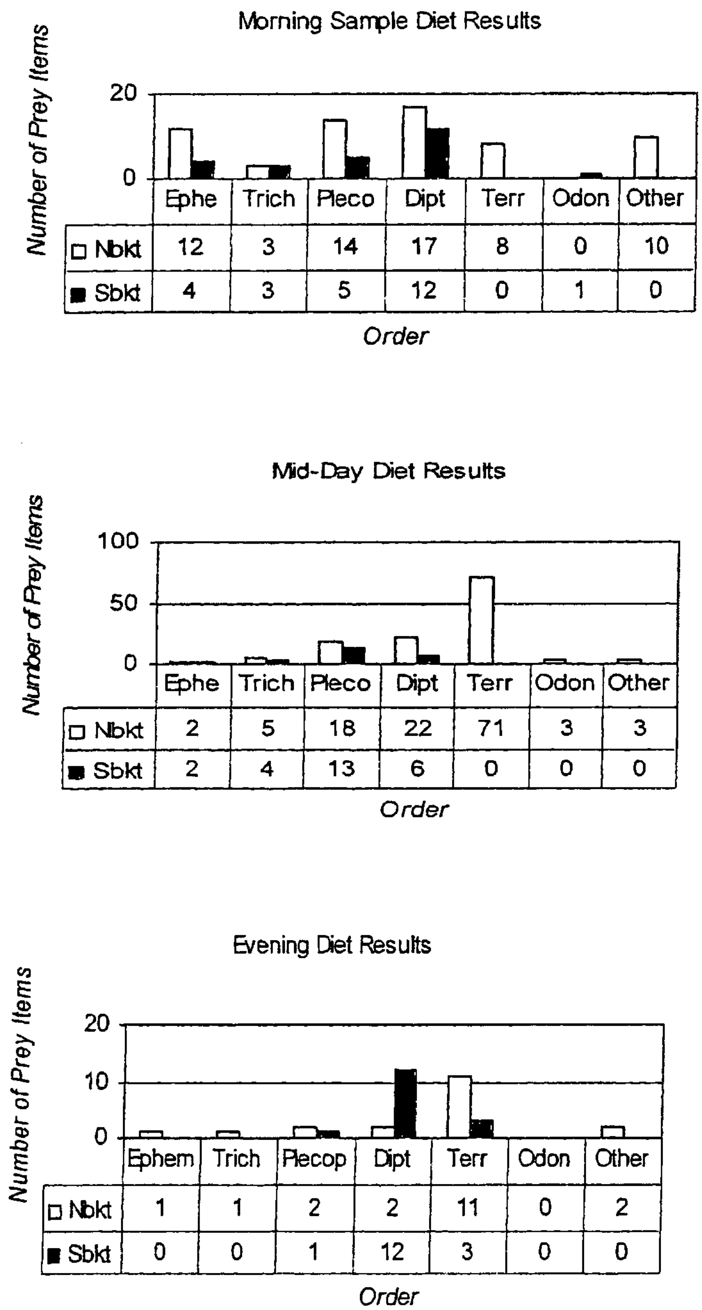
The distribution of prey items across taxa was found to be significantly different between brook trout strains (Chi-Square test of homogeneity $x=40.7$, $p<0.001$). Further, diet overlap measured as percent similarity (Ivlev 1961) was only 58%. Similarities greater than 60% are considered as high overlap (Langton and Bowman 1980). The diet differed primarily in the importance of terrestrial insects (Table 10). Terrestrials were most abundant (43%) and had a high frequency of occurrence (0.22) in northern strain fish, but accounted for only 5% of the diet of southern strain fish. Adult flies (Diptera) were most abundant (43%) and had the highest frequency of occurrence (0.28) in southern Appalachian brook trout samples.

Table 10. Total number, numerical abundance, and frequency of occurrence of organisms in southern Appalachian and northern strain brook trout.

	Northern brook trout			Southern brook trout		
Organism	*No. in stomach*	*% of Total*	*Frequency of Occurrence*	*No. in stomach*	*% of Total*	*Frequency of Occurrence*
Diptera						
Chironomidae	17	8.1	0.20	10	13	0.13
Simulidae	4	1.9	0.06	7	9.1	0.09
Dixidae	2	1	0.03	4	5.2	0.03
Tipulidae	15	7.1	0.17	9	11.7	0.09
Culicidae	3	1.4	0.03	3	3.9	0.06
Total	41	19.6	0.23	33	42.9	0.28
Plecoptera						
Peltoperlidae	7	3.3	0.14	6	7.8	0.09
Perlidae	9	4.3	0.11	6	7.8	0.09
Capniidae	19	9.1	0.20	7	9.1	0.13
Total	35	16.7	0.23	19	24.7	0.16
Ephemeroptera						
Siphlonuridae	3	1.4	0.06	0		
Heptageniidae	7	3.3	0.11	1	1.3	0.03
Baetidae	2	1	0.06	1	1.3	0.03
Ephemeridae	4	1.9	0.09	5	6.5	0.13
Total	16	7.7	0.22	7	9.1	0.19
Trichoptera						
Hydrophyschidae	4	1.9	0.09	2	2.6	0.06
Polycentropodidae	1	0.5	0.03	1	1.3	0.03
Philopotamidae	2	1	0.06	2	2.6	0.06
Phryganeidae	1	0.5	0.03	1	1.3	0.03
Hydroptilidae	1	0.5	0.03	1	1.3	0.03
Total	9	4.3	0.14	7	9.1	0.16
Odonata	3	1.4	0.06	1	1.3	0.02
Terrestrials	90	43	0.22	4	5.2	0.08
Other	15	7	0.25	0	0	0

Three crayfish, one salamander, and eleven earth worms were observed in northern brook trout stomachs only and had a frequency of occurrence of 0.25. These were considered large prey items compared to other taxa represented in stomach samples.

There was substantial variation in the observed number of prey items per stomach among sampling intervals and dates (Figure 3). The strongest temporal pattern was apparent in the mid-day collections between the hours of 11am and 2pm. A total of 154 prey items were identified in mid-day collections compared to 125 prey items identified in both morning and evening samples. Both northern and southern Appalachian brook trout demonstrated peak feeding during this period. Feeding rates were lowest for both strains during evening collections between the hours of 10pm and 1am.



Morning Sample Diet Results

	Ephe	Trich	Pleco	Dipt	Terr	Odon	Other
□ Nbkt	12	3	14	17	8	0	10
■ Sbkt	4	3	5	12	0	1	0

Mid-Day Diet Results

	Ephe	Trich	Pleco	Dipt	Terr	Odon	Other
□ Nbkt	2	5	18	22	71	3	3
■ Sbkt	2	4	13	6	0	0	0

Evening Diet Results

	Ephem	Trich	Plecop	Dipt	Terr	Odon	Other
□ Nbkt	1	1	2	2	11	0	2
■ Sbkt	0	0	1	12	3	0	0

Figure 3. Number of prey items in northern and southern strain brook trout stomachs according to sampling periods.

Discussion

Several studies have addressed feeding and stomach content analysis of salmonids in streams outside the southern Appalachian area. The number of prey items in northern and southern Appalachian brook trout in this study were well below the levels reported for brook trout in South Duck River, Manitoba (McNicol et al. 1985) and brook trout in Cement Creek, Colorado (Allen 1941). Within the southern Appalachian region, Cada et al. (1987) and Tebo and Hassler (1963) reported mean numbers of prey items in the stomachs of trout similar to those in this study. No dietary studies have been performed specifically on southern Appalachian brook trout until now.

Both northern and southern Appalachian brook trout fed more during mid-day than at morning or night. The interpretation of feeding activity from diel changes in stomach contents is difficult because of the length of time required for gastric evacuation (Eggers 1977). Other authors have reported feeding activity to be greatest at midday (Elliot 1967, 1970; Metz 1974) and to be continuous over 24h (Allen 1941; Bisson 1978).

Although it is conceivable that fish were overly stressed due to electroshocking and stomach lavage, we believe the influence of such stress was minimal. We noted one mortality during the sampling, and with this exception, all fish returned to the stream had regained equilibrium and seemed unaffected by their experience when released. In other studies where gastric lavage has been used, survival rates have been uniformly high (Light et al. 1983). Although there has been some question to the severity of high-voltage electroshocking in soft water streams of southern Appalachia (Gatz et al. 1986), Hudy (1985) found total mortality to be less than 2% for three-pass depletions with a 700-V output electroshocker.

There are several shortcomings to the manner in which we estimated intake. Four sampling events over an 8-week period may not be adequate to typify feeding patterns of northern and southern Appalachian brook trout. Any type of periodicity other than the four sampling days would have been missed by our sampling regime. Also, it is possible the stomach lavage may not have flushed the entire stomach, specifically the distal portion in larger fish. Studies have shown that as fish length increases the effectiveness of stomach lavage decreases (Meehan and Miller 1978). In contrast, Light et al. (1983) found that only 4 of 198 brook trout (size range 57-355mm) had stomach contents remaining after being subjected to a lavage similar to the one we used. At experiments end, we dissected three stomachs to confirm stomach flushing effectiveness. No prey items were found.

Our results suggest northern strain brook trout and southern Appalachian brook trout planted in a 100m section of stream partitioned the available food in different manners. Northern brook trout had twice as many prey items compared to southern Appalachian brook trout and most frequently consumed terrestrials compared to southern strain fish which relied more heavily on Diptera. Tebo and Hassler (1963) also reported that wild northern brook trout in Ball Creek, North Carolina most frequently consumed terrestrials (47.3%) compared to other aquatic insects during summer months. This may indicate a prey preference and/or a more sophisticated surface prey search engine for these hatchery derived fish. Perhaps years of supplemental hatchery surface feeding manifested itself into genotypic/phenotypic preference for surface water prey items. Also, fish hatcheries are selective environments and selection in the hatchery might affect the level of aggressiveness (Fenderson et al. 1968). Furthermore, Lahti et al. (2001) reported hatchery reared brown trout demonstrated a positive correlation between aggression and growth. Northern brook trout had a lower percentage of empty stomachs and higher average gut fullness than southern Appalachian brook trout at all sampling time periods. In addition, northern strain fish consumed large prey items compared to southern strain fish where no large prey items were observed. This suggests northern brook trout are more aggressive feeders than southern Appalachian brook trout and might have enjoyed a competitive edge over southern strain brook trout.

Conclusions

The results of this ecological comparison suggest these two wild strains of brook trout exhibit differences in adaptive characteristics, particularly diet habits and survivorship. Northern strain brook trout outperformed southern strain brook trout in all experiments. Although statistically significant differences were observed in the thermal and acidity tolerance trials, these results must be tempered with the fact that differences observed were relatively small (Tables 3 & 5). The biological relevance of these differences is unknown. If increased temperatures predicted by various global warming models prove to be true, brook trout habitat could become increasingly fragmented leading to local extinctions. Furthermore, if acclimation played a significant role in these trials, CTMax levels reported here might be misleading. Studies suggest (Becker and Genoway 1979; Lutterschmidt and Hutchison 1997) CTMax levels are strongly affected by temperatures fish experience prior to tests. In the present study, trout were collected from various streams across numerous watersheds. I did not collect any pretest temperature or pH data for the selected streams making it impossible to determine if certain populations might have been acclimated to different pH and temperature regimes.

My data from the diet and growth studies indicates northern strain brook trout enjoyed a competitive edge over southern strain brook trout. Although growth rates were similar, if mortality was associated with no growth, or loss of weight, estimates of growth rates based on survivors might be biased. The drastic change in environment from a natural stream to a raceway setting seemed to have a greater negative impact on southern strain fish resulting in increased mortalities. Northern strain brook trout exhibited a significantly higher rate of survivorship than southern strain fish. Due to their history of genetic selection for desired hatchery traits, northern strain fish may have adapted differently to this raceway setting giving them a competitive advantage over southern strain fish.

Northern strain fish relied more heavily on terrestrials and consumed almost twice as many organisms than southern strain fish. Numerous studies have indicated that competitive behavior is associated with the amount of food consumed by a fish, with the more aggressive fish consuming the most (Jenkins 1969; McCarthy, ID et al. 1992 O' Keefe and Benfey 1996). Northern strain brook trout may be more aggressive feeders and rely more heavily on surface feeding due to years of intense competition for feed and supplemental hatchery surface feeding. Studies indicate hatcheries are selective environments and this selection might affect the level of aggressiveness (Fenderson et al. 1968).

Protecting native southern Appalachian brook trout populations is imperative. Southern brook trout now account for 60% of all wild brook trout populations here in western North Carolina (personal communications, Doug Besler, NCWRC) with the majority being isolated headwater populations. If trout habitat becomes more fragmented, brook trout could ultimately be reduced to a few, small inbreeding populations in headwater refugia, and extirpated from many streams, if no management measures are taken. With an ever increasing number of resource users, coupled with mounting threats to habitat quality from land development (Flebbe 1994), acidic deposition (Haines and Baker 1986), and climactic warming (Meisner 1990), sound management plans on a regional scale are now necessary to maintain healthy populations of southern Appalachian brook trout. Management activities, including changes in stocking practices, habitat management, and angling regulations can be implemented to improve distribution patterns of southern Appalachian brook trout. In addition, Flebbe (1994) noted that restoration of high elevation brook trout streams in western North Carolina could be appropriate in certain areas. Native fish restoration efforts in the Great Smoky Mountains National Park have been successful in replacing sympatric trout populations (brook, brown, and rainbow) with allopatric southern Appalachian brook trout populations (Personal communications, Mallory Martin NCWRC and Matt Kulp GSMNP).

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109 Student Schedule GM GS MS BIOL Spath, Michael C.
PROD
Screen: SID: 449778157 Course: Term: 053 Printer Code:

S T	Course	Cred	GT	Title	Days	Times	Bldg	Room
				Fall 2005				
	Main Campus			Normal Academic Term	08-24-05 to 12-16-05			
E	BIOL-513-01	3.00		Prin of Gen Microbiolog	MW	0400-0515PM	ST	344
E	BIOL-567-01	3.00		Biostatistics	MW	0800-0950AM	MK	129
E	BIOL-593-02	3.00		AdvStd:Plant Comm Ecolo	MW	1200-1250PM	NS	123
E	BIOL-593-03	1.00		AdvStd:Plant Comm Ecolo	M	0100-0250PM	ST	156
E	BIOL-593-70	3.00		AdvStd:Conservation Bio	MW	0530-0645PM		
		13.00		Total Registered Hours				

Appendices

Appendix A

Thermal Data

A-1. Raw data for acidity tolerance measures for northern strain brook trout.

Stream	Rank	Hours to LE	L(mm)	Wt(g)	K	Temp.
Beechflat	11	145.9	135	21.4	0.87	27.0
Beechflat	23	147.7	110	11.2	0.84	27.3
Yellowstone	24	147.75	139	25.1	0.93	27.3
Rockhouse	25	147.97	134	22.1	0.92	27.3
Yellowstone	26	147.98	173	40.9	0.79	27.3
Yellowstone	27.5	148	97	7.4	0.81	27.3
Beechflat	30	148.27	118	10.3	0.63	27.3
Beechflat	31.5	148.45	120	16.7	0.97	27.3
Beechflat	31.5	148.45	121	16.5	0.93	27.3
Rockhouse	33	148.58	128	20.2	0.96	27.3
Yellowstone	34	148.63	110	8.2	0.62	27.3
Yellowstone	35	148.8	112	9.3	0.66	27.3
Rockhouse	36	148.82	129	18.3	0.85	27.3
Rockhouse	38.5	149.58	138	22.7	0.86	27.4
Rockhouse	38.5	149.58	163	38.3	0.88	27.4
Yellowstone	40	149.65	104	9.2	0.82	27.4
Log Hollow	41	149.75	191	65.6	0.94	27.4
Rockhouse	42	149.82	166	40.6	0.89	27.4
Rockhouse	43	149.83	143	24.8	0.85	27.4
Rockhouse	44	150.1	162	34.6	0.81	27.4
Yellowstone	45	150.55	133	18.6	0.79	27.4
Yellowstone	47	150.9	134	19.6	0.81	27.4

A-2. Raw data for acidity tolerance measures for southern strain brook trout.

Stream	Rank	Hours to LE	L(mm)	Wt(g)	K	Temp.
Big Bear Trap	1	136.18	136	20	0.8	26.3
Big Bear Trap	2	140.33	145	23.5	0.77	26.5
Negro Prong	3	141.4	103	9	0.82	26.6
Negro Prong	4	142.1	103	10.3	0.94	26.8
Cove Creek	5	143.38	96	8.6	0.97	26.8
Negro Prong	6	143.88	124	17.6	0.92	26.8
Cove Creek	7	144.52	96	8.9	1.01	26.8
Cove Creek	8	144.73	114	14.7	0.99	26.8
Cove Creek	9	145.02	104	10.8	0.96	26.9
Cove Creek	10	145.58	111	11.7	0.86	27
Middle Prong	12	146.03	168	37.4	0.79	27
Middle Prong	13	146.22	115	14.2	0.93	27
Negro Prong	14	146.5	105	9.3	0.8	27.1
Sugar Creek	15	146.63	118	14.6	0.89	27.2
Fisher Creek	16	146.72	123	14.8	0.8	27.2
Sugar Creek	17	146.8	117	11.5	0.72	27.2
Big Bear Trap	18	146.95	117	9.3	0.58	27.2
Middle Prong	19	147.12	161	33.1	0.79	27.2
Big Bear Trap	20	147.18	149	28.8	0.87	27.2
Sugar Creek	21	147.22	119	14.5	0.86	27.2
Sugar Creek	22	147.27	122	16.4	0.9	27.2
Fisher Creek	27.5	148	139	24.9	0.93	27.3
Middle Prong	29	148.12	156	30.9	0.81	27.3
Fisher Creek	37	149.42	179	57.2	1	27.3
Fisher Creek	46	150.62	145	24.3	0.81	27.4
Big Bear Trap	48	151.02	205	77.2	0.9	27.5

Appendix B

Acidity Data

B-1. Raw data for acidity tolerance measures for northern strain brook trout.

Stream	pH	Rank	L (mm)	Wt (g)	K	Hours to LE
Yellowstone	3.34	5	102	10.3	0.97	68.55
Beechflat	3.31	11	142	27.3	0.95	69.3
Yellowstone	3.31	17	107	11.0	0.9	70.28
Beechflat	3.31	22	140	29.9	1.1	70.88
Rockhouse	3.31	23	114	13.6	0.92	71.02
Yellowstone	3.28	27	109	12.9	1	71.45
Yellowstone	3.28	28	134	20.0	0.83	71.48
Beechflat	3.27	29	175	50.1	0.93	71.72
Beechflat	3.26	32	132	21.0	0.91	72.22
Beechflat	3.26	34	150	32.9	0.97	72.45
Rockhouse	3.26	36	127	16.5	0.81	72.55
Log Hollow	3.26	37	133	21.3	0.91	72.65
Rockhouse	3.24	38	150	28.6	0.85	73.32
Rockhouse	3.22	40	118	13.7	0.83	73.73
Yellowstone	3.22	41	155	33.0	0.89	73.92
Rockhouse	3.20	44.5	178	58.0	1	74.5
Rockhouse	3.18	46	179	56.1	0.98	75.1
Yellowstone	3.17	47	152	29.9	0.85	75.47
Rockhouse	3.10	48	173	50.9	0.98	77.05
Yellowstone	3.08	49	154	25.7	0.7	78.12
Yellowstone	3.08	50	181	59.0	0.99	78.25
Rockhouse	3.06	51	172	47.2	0.93	78.37

B-2. Raw data for acidity tolerance measures for southern strain brook trout.

Stream	pH	Rank	L (mm)	Wt (g)	K	Hours to LE
Sugar Creek	4.58	1	107	9.6	0.78	48.13
Cove Creek	3.38	2	92	7.8	1	67.08
Fisher Cr.	3.34	3.5	107	11.6	0.95	68.33
Cove Creek	3.34	3.5	99	8.2	0.85	68.33
MiddleProng	3.34	6	128	19.4	0.93	68.67
Cove Creek	3.34	7	117	14.3	0.9	68.72
Sugar Creek	3.34	8	98	7.5	0.8	68.75
Cove Creek	3.33	9	110	11.2	0.84	68.83
NegroProng	3.31	10	167	37.9	0.81	69.27
Sugar Creek	3.31	12	111	12.4	0.91	69.55
Cove Creek	3.30	13	107	10.1	0.82	69.58
Fisher Cr.	3.31	14	114	12.8	0.86	70.03
Sugar Creek	3.32	15.5	120	15.8	0.91	70.22
NegroProng	3.32	15.5	130	17.9	0.81	70.22
Fisher Cr.	3.30	18	113	12.8	0.89	70.42
Cove Creek	3.31	19	167	39.1	0.84	70.52
NegroProng	3.31	20	153	28.4	0.79	70.67
NegroProng	3.31	21	161	32.7	0.78	70.77
NegroProng	3.29	24	166	41.8	0.91	71.07
Fisher Cr.	3.28	25	113	12.6	0.87	71.35
Fisher Cr.	3.28	26	132	22.1	0.96	71.4
BearTrap	3.26	30	167	42.4	0.91	72.12
Bear Trap	3.26	31	155	30.6	0.82	72.15
MiddleProng	3.26	33	205	75.7	0.88	72.38
Bear Trap	3.26	35	151	28.7	0.83	72.48
Bear Trap	3.22	39	159	37.6	0.94	73.55
MiddleProng	3.21	42	184	57.3	0.92	73.95
MiddleProng	3.21	43	171	52.4	1	74.32
Bear Trap	3.20	44.5	138	22.1	0.84	74.5

Appendix C

Age and Growth Data

C-1. Raw age and growth data for northern strain brook trout.

Stream	Initial Length	Ending Length	Initial Weight	Ending Weight	Growth(g) NBKT	Age
Log Hollow	93	107	7.3	15.7	8.4	?
Log Hollow	205	215	93.1	100.6	7.5	3
Log Hollow	150	156	33.1	38.1	5	2
Log Hollow	130	135	43.1	48.1	5	3
Bryson Br.	145	150	24.3	29.5	5.2	2
Bryson Br.	128	133	22.4	23.1	0.7	3
Bryson Br.	169	175	46.5	51.1	4.6	4
Bryson Br.	189	196	68.6	72.1	3.5	4
Flat Laurel	160	165	38.2	43.2	5	2
Flat Laurel	155	159	38.4	43.1	4.7	3
Flat Laurel	189	194	76.3	81.4	5.1	3
Sams Br.	130	135	19.2	24.2	5	3
Sams Br.	117	136	17.7	28.6	10.9	2
Beech	142	157	24.6	35	10.4	3
Beech	133	141	21.8	28.5	6.7	?
Beech	157	187	34.8	73.7	38.9	3

C-2. Raw age and growth data for southern strain brook trout.

Stream	Initial Length	Ending Length	Initial Weight	Ending Weight	Growth(g) SBKT	Age
Fisher Cr.	149	155	30.3	34.2	3.9	3
Chastine Cr.	119	130	15.4	24.3	8.9	2
Chastine Cr.	128	135	19.7	26.3	6.6	2
Negro Prong	111	120	13.1	19.5	6.4	2
Negro Prong	111	127	12.2	23.1	10.9	3
Big Bear	145	154	26.7	34.6	7.9	3
Big Bear	191	196	66.1	74.5	8.4	?