Brook Trout Population Genetic Tools for Natural Barriers in Fragmented Subwatersheds

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ABSTRACT

Barriers to fish movement can cause aquatic habitat fragmentation by reducing the amount of available habitat. The primary goal of my research was to investigate applications of population genetic analysis tools as indicators of barrier effects on brook trout populations in fragmented subwatersheds.

In chapter1, I tested the hypothesis that brook trout population genetic differentiation (F_{ST}) above and below barriers will differ in relation to barrier height and gradient. I also tested the hypothesis that average gene diversity per locus (*H*) and the numbers of alleles (*A*) differed between samples below and above each barrier. There was no significant difference in average number of alleles (*A*) or average gene diversity per locus (*H*) between the above- and below-barrier samples, but linear regression identified a statistically significant relationship between barrier height and F_{ST} values. Unrooted neighbor-joining consensus trees of Cavalli-Sforza and Edwards (1967) chord distances provided evidence of genetic differentiation between samples of resident brook trout above and below natural barriers. Additionally, average total allelic diversity (*A*), average gene diversity per locus (*H*), average number of private alleles per locus per sample, and total alleles per sample differed between Level III Ecoregions.

In chapter 2 I tested the hypothesis that the presence of a barrier, total habitat potentially isolated above a barrier (km), road density, and percent forest cover within a subwatershed (USGS 6th-level Hydrologic Units) were significant habitat

fragmentation factors affecting the effective population size (N_e) of brook trout in the Blue Ridge Level III Ecoregion. Multivariable linear regression indicated that total habitat above the barrier (km) and road density were significant variables retained in the model to predict N_e.

In chapter 3, the objective of the study was to infer relationships between barriers and family structure in brook trout populations. Maximum likelihood analysis of pairwise kinship relationships between above- and below-barrier individuals indicated the presence of parent-offspring relationships between above- and below-barrier individuals at six sites in the Blue Ridge Level III Ecoregion and five sites in the Northern Lakes and Forests Level III Ecoregion, which indicated movement of individuals between the above- and below-barrier locations.

Dedication

I dedicate my dissertation to my former students at Saint Mary's College, Samoa, South Pacific, to my cousin Matthew Hahm, and to those that lost their lives at Virginia Tech on April 16, 2007. One must never take for granted, for a single second, being given the opportunity to pursue one's fullest potential and life dreams.

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Introduction

Habitat fragmentation and barriers to fish movement. - The presence of barriers to fish movement within streams is a significant source of habitat fragmentation that has the potential to affect distributions of fish populations on the landscape (Gibson et al. 2005; Gosset et al. 2006; Sheer and Steel 2006; Poplar-Jeffers et al. 2009). Barriers limit upstream movement of fish and other aquatic life and fragmentation results when stream habitat become inaccessible or unusable for feeding, reproduction, and predator avoidance (Angermeier and Schlosser 1989; Warren and Pardew 1998; Albanese et al. 2004; Knaepkens et al. 2004). Anthropogenic barriers such as dams and stream-crossing structures fragment stream habitats, not only by reducing connectivity between habitat areas, but also by impairing channel functions, such as flow, processing of sediment, and movement of large wood. Natural barriers, such as waterfalls, cascades, and vertical drops, also occur on the landscape and fragment stream habitats. Some waterfalls may be barriers during all flow conditions, whereas others may be barriers only during low flow or high flow, when velocities are too high for fish movement (Reiser et al. 2006). Their presence has historically driven salmonid population structure by limiting upstream dispersal and recolonization in relation to glacial activity and presence of glacial refugia (Poissant et al. 2005; Gomez-Uchida et al. 2009).

Stream restoration effectiveness and biological monitoring. - Monitoring tools for assessing barriers to fish movement currently are based on physical channel characteristics in relation to fish swimming and jumping abilities. Biological monitoring tools are needed to document "biological improvement" of stream systems when habitat fragmentation is reduced by removing barriers to fish movement. Genetic baseline information for fish populations, which needs to be collected only once, can incorporate multi-generational effects of barriers.

Molecular genetic markers have been applied along with tagging studies to explore gene flow and dispersal and distributions of salmonids (Rieman and Dunham 2000; Rogers and Curry 2004; Wilson et al. 2004). Further information is needed on how to apply population genetic tools to identify barriers to upstream dispersal. This study investigates applications of

microsatellite multilocus genotype genetic diversity and differentiation analysis, individual assignment, and kinship relationships which can all be used to infer the amount of dispersal occurring at a barrier site.

Brook trout as a biological indicator species of barriers to fish movement. - Brook trout *Salvelinus fontinalis* are indicators of high water quality and are valued as the only native trout species of the eastern United States. Genetic markers for brook trout are well established, and long-term, historical, genetic information exists for the species (McCracken et al. 1993; Kriegler et al. 1995; Galbreath et al. 2001; Rogers and Curry 2004). Brook trout can be used as indicators of barriers to fish movement, as this species can move long distances, negotiate steep slopes, and jump high obstacles compared to other fish species within native coldwater fish communities of the eastern United States.

In this study I focused on looking for indicators of reduced genetic diversity or differentiation in relation to physical parameters that were comparable across all sites. Selected physical parameters characterized barrier complexity, including height (vertical drop in meters), and gradient (degrees) and were criteria already established for an upstream dispersal barrier to brook trout, aka height was at least 73.5 cm (Kondratieff and Myrick 2006). I also considered gradient in this study, as slopes of 18-22% were considered a maximum slope for upstream movement of brook trout (Moore et al. 1985; Adams et al. 2000).

Primary goal and objective of research. - Recent work on salmonid population genetics at the landscape scale has documented differences in the distribution of genetic variation due to ecoregion, contemporary and glacial barriers to fish movement, and a combination of effects of these factors (Castric et al. 2001; Costello et al. 2003; Poissant et al. 2005; Whiteley et al. 2006; Guy et al. 2008). Effective large-scale, long-term management of salmonids species requires a knowledge of where genetic variation and differentiation occurs on the landscape by ecoregion and in relation to barriers, so that we can be more aware of fine-scale genetic structuring within populations throughout a species range' (Whiteley et al. 2006). Fish conservation managers need to identify potential source populations of genetic variation, as well as which populations

may be at risk due to reduced genetic variation and inbreeding (Petit et al. 1998; Rieman and Allendorf 2001).

Hence, the primary goal of my research was to investigate applications of population genetic analysis tools as biological indicators of barrier effects on brook trout populations in fragmented subwatersheds of two different Level III Ecoregions of the eastern United States. Natural resource agency personnel can use these tools to appropriately develop long-term monitoring programs and to achieve realistic brook trout population management goals. The primary overall objective of my research was to identify indicators of reduced genetic diversity, population differentiation, and kinship among individuals within brook trout samples above and below natural barriers.

CHAPTER 1. Population genetics of resident brook trout upstream and downstream of natural barriers.

Introduction

Recent work on salmonid population genetics at the landscape scale has documented differences in the distribution of genetic variation due to ecoregion, contemporary and glacial barriers to fish movement, and a combination of effects of these factors (Castric et al. 2001; Costello et al. 2003; Poissant et al. 2005; Whiteley et al. 2006). Effective large-scale, long-term management of salmonids species requires a knowledge of where genetic variation and differentiation occurs on the landscape by ecoregion and in relation to barriers, so that we can be more aware of fine-scale genetic structuring within populations throughout a species range' (Whiteley et al. 2006). It is also important to be aware of where potential source populations of genetic variation occur and which populations may be at risk due to reduced genetic variation and inbreeding (Petit et al. 1998; Rieman and Allendorf 2001). This chapter's introduction describes what is known about how barriers and ecoregions influence salmonid population structuring and describes what is known specifically about the brook trout *Salvelinus fontinalis* the native trout of the eastern United States. The brook trout is an appropriate indicator species for a study of genetic effects of barriers because reliable microsatellite markers are well established, and long-term, historical, genetic information is available (McCracken et al. 1993; Kriegler et al. 1995; Galbreath et al. 2001; Rogers and Curry 2004).

Barriers to fish movement. - The presence of barriers to fish movement within streams is a significant source of habitat fragmentation that has the potential to affect distributions of fish populations on the landscape (Gibson et al. 2005; Gosset et al. 2006; Sheer and Steel 2006; Poplar-Jeffers et al. 2009). Barriers limit upstream movement of fish and other aquatic life and fragmentation results when stream habitat becomes inaccessible or unusable for feeding, reproduction, and predator avoidance (Angermeier and Schlosser 1989; Warren and Pardew 1998; Albanese et al. 2004; Knaepkens et al. 2004). Anthropogenic barriers such as dams and stream-crossing structures fragment stream habitats, not only by reducing connectivity between

habitat areas, but also by impairing channel functions, such as flow, processing of sediment, and movement of large wood. Natural barriers, such as waterfalls, cascades, and vertical drops, also occur on the landscape and fragment stream habitats. Some waterfalls may be barriers during all flow conditions, whereas others may be barriers only during low flow or high flow, when velocities are too high for fish movement (Reiser et al. 2006). Their presence has historically driven salmonid population structure by limiting upstream dispersal and recolonization in relation to glacial activity and presence of glacial refugia (Poissant et al. 2005; Gomez-Uchida et al. 2009).

All "barriers" in this study are natural barriers, such as waterfalls, vertical drops, and cascades, and I focus on them because of their documented historical significance in population structuring of salmonids, in relation to glaciations. In particular, previous research has documented the role of waterfalls in population structuring of the native trout of the eastern United States, the brook trout (Poissant et al. 2005). This document will refer to a "barrier" meaning a barrier to upstream fish movement. In addition, a barrier to upstream movement can potentially result in an upstream barrier to gene flow, which will result in genetic isolation. The possibility of upstream dispersal of breeding adults will affect genetic diversity, effective population size, and kinship relationships when access to potential breeding habitat is isolated above that barrier (Shrimpton and Heath 2003; Gibson et al. 2005; Hudy et al. 2010).

Quantifying a barrier. - Information on specific conditions that constitute a barrier to movement for particular fish species is limited. Considerable information is available for the Salmonidae, largely because the effects of dams on commercially significant salmon populations of the western United States have been well documented (Gibson et al. 2005). Depending on the species' swimming and jumping ability, conditions that create barriers to fish movement include: excess drop, high or low velocity, inadequate depth, turbulent flow, and excessive channel slope. Barriers also occur where fish dispersal behavior is affected due to changes in sound, light, or flow. These changes affect fish adaptive behaviors that are instinctual responses to abnormal flow conditions (Nestler et al. 2007), dissolved oxygen levels (Lutz 1995), or presence of

predators (Schilt 2007).

Flow variability such as inadequate water depth or high stream flow events associated with storms or runoff can function as barriers to movement at different times of the year (Belford and Gould 1989; Adams et al. 2000; Bates 2003). Reiser et al. (2006) measured the vertical distances from downstream pool water surfaces to water surfaces at crests of natural barriers, pool crest depths, and flow velocities, and compared these measurements to known (jumping and swimming abilities) of salmonids. Barrier conditions resulted when plunge pool depths were too low or velocities were higher than burst swimming speeds for a species. Although Reiser et al. (2006) acknowledged that temperature was an important factor that can affect jumping ability of salmonids, the authors did not quantify temperature and its relationship to salmonid movement in their study.

Timing of such flow-based barriers may especially have an effect on successful dispersal when fish species are spawning at specific times of the year (Stuart 1964; Bjornn and Reiser 1991; Heard 1991). Reiser et al. (2006) investigated effects of varying flow velocity at natural barriers at different times of the year and determined "flow windows" for successful passage by salmonids. They estimated the number of potential passage days based on when ideal flow conditions coincided with the spawning migration of various salmonid species. For example, in Ward Creek, Alaska, sockeye salmon *Oncorhynchus nerka* had 15 and steelhead *Oncorhynchus mykiss* had 81 potential passage days.

In addition, physical complexity and flow variability, together, may influence the likelihood that particular cascades and waterfalls will act as barriers to fish movement (Reiser et al. 2006). For example, a vertical drop that is 1.2 meters at low water levels or flows may be a barrier, but this same vertical drop may not be a barrier when water levels and flows are high, thus reducing the total height a fish has to jump (Adams et al. 2000). Although vertical falls are more likely to limit upstream movement than steep slopes, channel gradient has also been investigated as a factor potentially limiting fish movement (Moore et al. 1985; Adams et al. 2000). Channel gradients that are too high for successful fish passage are the result of flow

velocities that are higher than fish can negotiate (Reiser et al. 2006).

Barriers and brook trout. - Information is limited on what specifically constitutes a barrier to brook trout. Kondratieff and Myrick (2006) and Brandt et al. (2005) conducted trials of brook trout jumping ability in the lab and Adams et al. (2000) and Moore et al. (1985) conducted work on brook trout ability to negotiate natural barriers in the field. Kondratieff and Myrick (2006) investigated how waterfall height, plunge pool depth, and fish length affected the ability of brook trout to jump over a flashboard flume artificial waterfall. This artificial waterfall was kept at a constant velocity of 1.89 m/s, based on maximum sustained swimming velocity for brook trout (Peake et al. 1997). For brook trout, 8.6 – 34.0 cm total length (TL), Kondratieff and Myrick (2006) documented a 73.5 cm maximum jump height with a 40.0 cm minimum pool depth below the waterfall at baseflow in the lab. As plunge pool depth decreased, the maximum jump height decreased, as jump height from a 10.0 cm deep pool was only 33.5 cm. Total length also affected jumping ability, as brook trout greater than 15.0 cm TL could jump higher than brook trout less than 15.0 cm TL (Kondratieff and Myrick 2006). However, smaller brook trout were able to jump a greater number of times their body length. For example, brook trout 10.0-15.0 cm TL could jump 4.7 times their body length and brook trout 20.0+ cm TL could jump 2.9 times their body length (Kondratieff and Myrick 2006). Adams et al. (2000) documented a 21.0 cm TL brook trout ascending a 1.5 m high falls and a 9.0 cm TL brook trout ascending a 0.7 m high falls in high-gradient streams in the western United States, which suggests that brook trout jumping performance in the field may be different from performance in the lab (Kondratieff and Myrick 2006).

Brandt et al. (2005) investigated jumping ability of age-0 brook trout over an experimental waterfall that was fed by a flume, kept at a constant velocity of 0.47 m/s, which was about the known maximum sustained swimming velocity for 10.0 cm brook trout (Peake et al. 1997). The water temperature was kept at 11.0ºC ± 1ºC as well. This experiment investigated effects of fish length, waterfall height, plunge pool depth, and waterfall width on age-0 brook trout jumping ability. Brook trout 4.4-10.4 cm TL could not jump waterfalls higher

than 16.0 cm with an 8.0 cm deep plunge pool or 22.0 cm with a 10.0 cm plunge pool. Age-0 brook trout were significantly less successful at jumping a waterfall that was just 10.0 cm wide, as six times as many attempted jumps were needed for successfully negotiating this narrow width (P <0.05; F=0.0106) (Brandt et al. 2005).

Field investigation of brook trout has documented channel gradient as a factor that affects dispersal. Moore et al. (1985) documented maximum upstream slopes of 8-18% in the Great Smoky Mountains National Park and Adams et al. (2000) documented a maximum upstream slope of 22% for brook trout movement in headwater streams of the western United States.

Stream temperature is well documented as a significant factor in brook trout distribution, as brook trout are not found in streams that exceed 24ºC (MacCrimmon and Campbell 1969; Meisner 1990; Raleigh 1982; Piccard et al. 2003). However, previous field study has not documented a strong correlation between stream temperature and brook trout movement (Curry et al. 2002).

Previous research does support that brook trout movement is highly correlated to changes in flow (Bjornn and Reiser. 1971; Adams et al. 2000; Curry et al. 2002). Although Reiser et al. (2006) did not include brook trout in their investigation, passage windows during the brook trout spawning season would occur during the fall, primarily September and October (Bjornn and Reiser 1971; Gowan and Fausch 1996; Curry et al. 2002). It's possible that brook trout individuals may not be able to negotiate barriers every year, depending on physical complexity and flow velocity variability, and there may only be limited windows of time when passage is possible from year to year (Adams et al. 2000; Reiser et al. 2006).

Therefore, a complete conceptual model of a barrier to brook trout movement for a given barrier considers the jumping and swimming capability, temperature and flow conditions, physical complexity of the barrier (including vertical drops and slopes), and the timing of when the species is moving. Research to date considers 73.5 cm the maximum jump height for the brook trout where a 40.0 cm minimum pool depth occurs below the barrier (Kondratieff and Myrick 2006). The maximum flow velocity for the species is 1.89 m/s, based on maximum

sustained swimming velocity (Peake et al. 1997). Although movement has not been significantly correlated with temperature, brook trout are not found in streams that exceed 24ºC (MacCrimmon and Campbell 1969; Meisner 1990; Raleigh 1982; Piccard et al. 2003). Maximum upstream slopes for the brook trout are 18-22% (Moore et al. 1985; Adams et al. 2000) and breeding season dispersal typically occurs during September and October (Bjornn and Reiser 1971; Gowan and Fausch 1996; Curry et al. 2002).

However, this study applied knowledge already known about barriers to brook trout movement at known sites to investigate the usefulness of genetic tools to establish that a barrier exists. Currently, specific information on physical criteria that constitutes a barrier to brook trout movement is limited, as Kondratieff and Myrick 2006, Brandt et al. 2006, and Adams et al. 2000 are the only focused studies in the scientific literature on specific physical criteria for brook trout. Flow, temperature, and long-term variability of brook trout movement in relation to the natural barrier sites were not measured, as these datasets were not available consistently and from a long-term timescale for the sites investigated. Height of a natural barrier and gradient were the only reliable and comparable variables to measure across all sample sites at one point in time.

Population genetics and dispersal. - Availability of genetic markers for species and the ability to assign individuals to populations of origin offers useful tools for defining dispersal among populations (Rannala and Mountain 1997; Cornuet et al. 1999; Pritchard et al. 2000). In addition, reduced genetic diversity upstream of a putative barrier and differentiation between populations above and below barriers could be used as indicators of a lack of gene flow due to limited upstream dispersal.

In this study I focused on looking for indicators of reduced genetic diversity or differentiation in relation to physical parameters that were comparable across all sites. Selected physical parameters characterized barrier complexity, including height (vertical drop in meters), and gradient (degrees) and were criteria already established for an upstream dispersal barrier to brook trout, aka height was at least 73.5 cm at baseflow (Kondratieff and Myrick 2006). I also

considered gradient in this study, as slopes of 18-22% were considered a maximum slope for upstream movement of brook trout during the summer and fall breeding season over a wide range of flows (Moore et al. 1985; Adams et al. 2000).

Salmonid genetics in relation to barriers. - Salmonid populations that are isolated by barriers are likely to lose genetic diversity due to loss of gene flow with other populations, and to increased impact of random genetic drift in small populations (Yamamoto et al. 2004). Loss of genetic diversity may affect persistence of a population through decreased fecundity, growth, survival, competitive ability, and adaptive potential (Quattro and Vrijenhoek 1989; Vrijenhoek 1994; Morita and Yamamoto 2002).

Salmonid researchers have investigated effects of barriers to movement on trout population genetic diversity by applying allozyme and microsatellite markers. Previous work on salmonid genetics in relation to barriers included studies of rainbow trout *Oncorhynchus mykiss*, bull trout *Salvelinus confluentus*, coastal cutthroat trout (*Oncorhynchus clarkii clarkia*) (Currens et al. 1990; Neraas and Spruell 2001; Wofford et al. 2005; Whiteley et al. 2006; Deiner et al. 2007; Small et al. 2007; Guy et al. 2008) and brook trout (Poissant et al. 2005) and the following literature review summarizes those findings.

Rainbow trout genetics and barriers. - Currens et al. (1990) sampled rainbow trout in the Deschutes River, Oregon and showed that a population isolated above a barrier on the White River diverged from other populations based on allozyme allele frequency data. Deiner et al. (2007) sampled rainbow trout from 20 sites above and below barriers in the Russian River, California and applied 22 microsatellite markers. Results showed that the number of alleles was significantly lower above barriers in comparison to below barriers. Constructed unrooted neighbor-joining (abbreviated NJ) consensus trees had shorter branch lengths for below-barrier samples as compared to above-barrier samples, meaning that the above-barrier sites had a greater genetic distance, or had allele frequencies less similar to other sites. Small et al. (2007) sampled rainbow trout from 23 sites in the Spokane river drainage above and below barriers and also documented longer branch lengths on the NJ consensus tree for populations isolated above

waterfalls, indicating a sample that was further differentiated, or less genetically similar to other populations.

Bull trout genetics and barriers. - Neraas and Spruell (2001) sampled bull trout above and below Cabinet Gorge Dam, Clark Fork River, adjacent to the Idaho and Montana border. They applied eight microsatellite loci to estimate connectivity between populations within a basin. Results suggested substantial genetic differentiation between samples collected above and below Cabinet Gorge Dam, as visualized by the Cavalli-Sforza and Edwards (1967) chord distance NJ consensus tree. Individual-based assignment test data also supported differentiation between above- and below-dam samples. The Swamp Creek site, above the dam, also had a high frequency for a rare allele, SFO*156.

Coastal cutthroat trout genetics and barriers. - Wofford et al. (2005) and Guy et al. (2008) sampled coastal cutthroat trout in the Camp Creek, Oregon watershed and tested the hypotheses that habitat fragmentation due to barriers to coastal cutthroat trout movement could result in genetic differentiation, and that genetic diversity would be shaped by ecoregional differences. Results showed that there was higher genetic diversity in the Coast Range as compared to the Cascades due to relative differences of interaction between drift and gene flow for each ecoregion. Tributaries connected with mainstem habitats tended to have high levels of allelic richness and samples above barriers tended to have low levels of allelic richness, as levels of gene flow did not counteract effects of drift.

Brook trout genetics and barriers. - Specific studies on the effects of barriers on brook trout genetics are limited. Poissant et al. (2005) applied nine microsatellite loci to 12 brook trout populations in seven river drainages and investigated contributions of historic and current hydrography and effects of barriers on population genetic structure for populations out of migration-drift equilibrium. Allelic diversity above barriers was significantly lower than below barriers, and the unrooted NJ consensus tree showed a lack of above-barrier movement. Results of this study showed no correlation between population differentiation and current landscape hydrology, but showed correlation to historic patterns of geomorphology, as genetic distance was

strongly correlated to historical drainage patterns. The presence of current barriers to migration maintained historic patterns of population differentiation in brook trout populations out of migration-drift equilibrium in this case.

Brook trout genetic marker research and population structure. - Population genetic marker analysis has been used to investigate population structure on the landscape and throughout species ranges (Petit et al. 1998; Whiteley et al. 2006). For the brook trout species, allozyme and mitochondrial DNA marker research indicated sources of genetic variance that included historical association with major drainages or glacial refugia (Schmidt 1986; Perkins and Krueger 1993; Danzmann et al. 1998) and microsatellite marker research indicated current association with major drainages or regions (Hebert et al. 2000; Castric et al. 2001; Rogers and Curry 2004: Stott et al. 2010). The following literature review summarizes these findings.

The historic range of brook trout extends from the headwaters of the Mississippi River in Minnesota, to coastal drainages from Maine to Virginia, and south to the headwaters of the Chattahoochee River in northern Georgia (MacCrimmon and Cambell 1969). Brook trout exhibit diverse life history strategies, ranging from resident populations that move only within their natal stream to anadromous populations that migrate between fresh and saltwater. Brook trout also exhibit adfluvial behavior, in which individuals migrate as juveniles to freshwater lakes and return as adults to streams for spawning (Huckins et al. 2008; Ridgway 2008). Research on anadromous and adfluvial brook trout populations provided evidence that population and genetic structuring were influenced by life history strategy (Hebert et al. 2000; Castric et al. 2001; Curry et al. 2002; Rogers and Curry, 2004; Poissant et al. 2005; D'Amelio et al. 2008).

Brook trout allozyme marker data from throughout the range of the species identified two separate Northern and Southern Appalachian strains based on the presence of diagnostic allele frequencies at the CK-A2*100 (native Southern Appalachian) and CK-A2*78 (hatchery derived Northern strain) loci. This data documented differentiation between northern and southern strain populations (Stoneking et al. 1981; Galbreath et al. 2001; Davis 2008). Davis (2008) analyzed

compiled brook trout allozyme marker data from 56 populations and found that all populations with a high frequency of CK-A2*78 allele clustered together, using Nei's (1978) genetic distance criterion. This study also provided further evidence to support the New River as the geographic boundary between the northern and southern strain through geographical analysis of the presence of northern and southern-strain dominant brook trout populations on the landscape (Guffey 1998; Palmer and Hallerman 2000; Hall et al. 2002; Davis 2008).

Mitochondrial DNA analysis of brook trout populations throughout the species' range has been used to investigate historical lineages and colonization from glacial refugia. Previous research results suggested that brook trout recolonized their northern range from the Appalachian region or from a Mississippian source (Mandrak and Crossman 1992; Bailey and Smith 1981). Danzmann et al. (1998) identified 61 mitochondrial haplotypes and six clades for the brook trout species. This study showed that fish from the Appalachian zone were unlikely sources for recolonization of the northern glacial zone and the high frequency of the C clade haplotypes supported the Mississippian refugium as the likely source (Bailey and Smith 1981; Danzman et al. 1998). Additionally, the Danzmann et al. (1998) study suggested more than one refugial location as a source for recolonization of the northern extent of the brook trout range and populations outside of northern glaciated regions showed the greatest haplotypic diversity.

Hall et al. (2002) investigated the mid-Atlantic brook trout populations, which may represent a transitional group between more genetically diverse southern populations and less diverse northern populations. This study documented that relationships by major drainage (Chesapeake and Ohio) were most significant, as visualized by a NJ consensus tree. Brook trout populations in the mid-Atlantic extent of their range were more genetically diverse as compared to populations in the northern extent of their range, supporting the concept that non-glaciated regions resulted in greater genetic diversity due to longer time periods for differentiation (Hall et al. 2002).

Hebert et al. (2000) applied six microsatellite loci to 24 sample sites of brook trout in three national parks of eastern Canada to quantify the effect of habitat and hydrography on population structure. Results of this study suggested that each sample from 24 locations

represented a genetically definable population and that populations clustered together according to national park, as visualized by the Cavalli-Sforza and Edwards (1967) NJ consensus tree. In this case, anadromy functioned as a source for gene flow between adjacent rivers.

Castric et al. (2001) applied six microsatellite loci to 30 samples of brook trout in six river drainages to test the hypothesis that patterns of genetic differentiation among drainages reflected colonization events. This hypothesis was designed to investigate how genetic differentiation could be explained through recolonization from glacial refugia. Results of this study revealed a lack of genetic structure by drainage and a lack of migration-drift equilibrium among sampled populations.

Rogers and Curry (2004) applied six microsatellite loci to 12 sample sites to indirectly measure dispersal and spatial patterns of genetic diversity among brook trout populations using individual-based multilocus genotype assignment tests. They tested the hypothesis that genetic structure was influenced by drainage pattern and predicted that a greater distance between sample locations would result in greater genetic divergence. Results of this study showed that increased geographic distance did not result in genetic divergence between populations and that twelve sample sites represented five source populations.

Stott et al. (2010) applied 12 microsatellite loci markers to 11 samples of brook trout from streams connected to Lake Nipigon, Minnesota tributaries of Lake Superior, and Isle Royale and investigated population structure of coaster brook trout. This study documented Isle Royale brook trout populations as genetically and geographically distinct, as visualized by the Cavalli-Sforza and Edwards (1967) chord distance NJ consensus tree. There was some conflicting information when considering the mitochondrial and microsatellite genome that needs further investigation, as revealed by brook trout populations in Tobin Harbor (Quinlan 1999).

Further information needed. - Previous research to assess within and among-population genetic diversity and differentiation of salmonids has included comparisons of samples above and below barriers and in stream sites in different watersheds (Wofford et al. 2005; Deiner et al.

2007; Small et al. 2007; Guy et al. 2008). Observed differences in metrics of genetic diversity due to barriers by applying microsatellite markers have included: genetic differentiation (F_{ST}), heterozygosity (H), and number of alleles (A) (Castric et al. 2001; Taylor et al. 2003; Yamamoto et al. 2004; Poissant et al. 2005; Wofford et al. 2005; Whiteley et al. 2006; Deiner et al. 2007; Small et al. 2007; Guy et al. 2008; Gomez-Uchida et al. 2009). Previous research also indicated that sources of genetic variance among brook trout populations included historical association with major drainages or glacial refugia (Schmidt 1986; Perkins and Krueger 1993; Danzmann et al. 1998) and that presence of barriers influenced regional population structure of brook trout by preserving these historical genetic signatures (Poissant et al. 2005). Higher genetic diversity was documented in unglaciated regions of the southern extent of the brook trout range as compared to the glaciated northern extent of the brook trout range (Danzmann et al. 1998; Hall et al. 2002).

The historic range of brook trout extends from the headwaters of the Mississippi River in Minnesota, to coastal drainages from Maine to Virginia, and south to the headwaters of the Chattahoochee River in northern Georgia (MacCrimmon and Cambell 1969). The Northern Lakes and Forests (abbreviated NLF) and Blue Ridge (abbreviated BR) Level III Ecoregions are ecoregions within the native range of the brook trout that still have high density populations remaining. These Level III Ecoregions represent a comparison between an ecoregion that is glaciated to an ecoregion that is unglaciated within the native range of the species. Brook trout population genetic structure and patterns of genetic diversity are affected by historical and contemporary barriers on the landscape. Barriers to brook trout dispersal have resulted on the landscape due to glaciations, and Poissant et al. (2005) documented that the presence of barriers preserved historical patterns of brook trout genetic diversity due to recolonization from glacial refugia. Research in the BR Level III Ecoregion on historical preservation of brook trout population genetic diversity due to barriers is limited, but there is no recorded history of glaciations in the historic geological literature (Hocutt 1979). Historic presence of glaciations (NLF) or lack of glaciations (BR) affects population genetic structuring on the landscape and understanding how this occurs in different ecoregions is important for baseline understanding of

population genetic diversity within ecoregions and for prioritization of populations within an ecoregion.

Studies have been done on large-scale ecoregional comparisons of genetic diversity and differentiation using microsatellite markers for western native trout species (Currens et al. 1990; Neraas and Spruell 2001; Wofford et al. 2005; Whiteley et al. 2006; Deiner et al. 2007; Small et al. 2007; Guy et al. 2008), anadromous and adfluvial populations of brook trout in Great Lakes region (Hebert et al. 2000; Castric et al. 2001; Curry et al. 2002; Rogers and Curry 2004; Poissant et al. 2005; D'Amelio et al. 2008; Stott et al. 2010) but further work is needed in the unglaciated BR Level III Ecoregion populations overall and resident populations in the glaciated NLF Level III Ecorgegion.

Barriers to fish movement result in reduced upstream dispersal, which can result in reduced gene flow and genetically isolated populations that are at risk due to inbreeding and effects of genetic drift (Slatkin 1985; Wofford et al. 2005). Further information is needed on how to apply population genetic tools to identify barriers to upstream dispersal. This study investigates applications of microsatellite multilocus genotype genetic diversity and differentiation analysis, individual assignment, and kinship/parent-offspring relationships which can all be used to infer the amount of dispersal occurring at a site. Chapter 1 of this dissertation includes discussion of the genetic diversity, differentiation, and individual assignment analysis, Chapter 2 of this dissertation includes discussion of effective population size, and Chapter 3 of this dissertation includes discussion of the kinship relationships.

This is the first study to investigate barriers of different heights and gradients that influence population genetic diversity and differentiation of brook trout populations in two different ecoregions of the eastern United States. Identifying thresholds of height and gradient that influence population structuring of brook trout can be used to identify barriers that pose the greatest risk to brook trout populations on the landscape and this information would be useful for improving long-term conservation and management strategies for the species throughout its range. Understanding population structuring and genetic diversity patterns that exist relative to

historic patterns driven by glaciations and current patterns on the landscape need to be understood at the ecoregional scale for further well-informed management decisions. Populations can be compared within an ecoregion and between ecoregions to identify priority high genetic diversity source populations or populations at risk that can be targeted for restoration. This study, specifically, applies microsatellite markers to brook trout samples above and below natural barriers in the NLF (glaciated) and BR (unglaciated) Level III Ecoregions (Bailey 2005).

Goal and objective. - Hence, the goal of this study was to investigate applications of population genetic analysis tools as indicators of barrier effects on brook trout populations in different Level III Ecoregions of the eastern United States. The objective of my research was to test the hypothesis that brook trout population genetic differentiation measured as (F_{ST}) above and below barriers differed in relation to barrier height and channel gradient. I also tested the hypothesis that average gene diversity per locus (H) values and the numbers of alleles per locus (A) differed between populations above and below barriers to fish movement in relation to height and channel gradient. Finally, I tested the hypotheses that the number of alleles per locus (A), average gene diversity per locus (H), and total alleles will differ between the BR and NLF Level III Ecoregion. The null hypotheses were that brook trout population genetic differentiation (F_{ST}) between above- and below-barrier samples would not differ in relation to barrier height and channel gradient. In addition, the average gene diversity per locus (H) and the numbers of alleles per locus (A) would not differ above and below barriers in relation to height and channel gradient. I predict that heights and channel gradients that result in a barrier to upstream movement would result in a higher F_{ST} value as the above sample is further differentiated from the below sample. I predict that the average gene diversity per locus (H) and number of alleles per locus (A) would be lower above heights and channel gradients that are barriers to upstream movement because those populations would become isolated from gene flow and more likely to be affected by genetic drift. I also predict that genetic diversity (total alleles, A, and H) will be higher in the unglaciated BR Level III Ecoregion as compared to the glaciated NLF Level III

Ecoregion.

Methods

Study areas. - I selected 18 natural barrier sites in subwatersheds (USGS 6th-level Hydrologic Units) of the NLF (six sites in Wisconsin, Michigan, and Minnesota) (Figure 1.1, Table 1.1) and the BR (12 sites in Virginia and North Carolina) (Figure 1.2, Table 1.1) Level III Ecoregions that were at least 68% forested (Bailey 2005; Hudy et al. 2008). I used ArcMap, Version 9.2 (ESRI, Inc.) and USGS National Hydrological Dataset, 1:24,000 high resolution data layers to subdivide 5th level, 10-digit hydrologic unit code watersheds into 6th level, 12-digit hydrologic unit code subwatersheds (Seaber et al. 1987).

Natural barriers to movement of brook trout were identified through field reconnaissance and were at least 73.5 cm maximum jumping height. This height was considered a barrier for 8.6 – 34.0 cm TL brook trout with a 0.40 cm minimum pool depth below the waterfall at baseflow by Kondratieff and Myrick (2006). I also selected three sites in the BR Level III Ecoregion that did not have barriers and started the designated start of the downstream sample reach 50 meters from the nearest road or confluence.

I selected sites according to ecoregion so that selected streams within each specific ecoregion would have similar physiogeographic characteristics for comparisons and analyses based on ecoregional patterns. I selected natural barrier sites found within the same subwatershed (USGS 6th-level Hydrologic Unit) because this is the smallest watershed size with compiled data available on brook trout populations, and this is the spatial level most relevant to management for fish conservation (4,100 to 12,700 hectares for subwatersheds in this study) (Seaber et al. 1987; Fausch et al. 2002).

Sites were also selected based on historic and current survey information that identified locations with sufficient brook trout population densities to collect at least 30 juveniles and adults above and below each natural barrier. A sample of 30 individuals was considered a minimum for reliably estimating genetic diversity of populations (McCracken et al. 1993; Rogers and Curry 2004). Selected sites represented a broad range of barrier heights and channel

gradients.

Study site measurements. - I measured the total stream length sampled above and below each barrier site. I also estimated the total stream length above each barrier to characterize the amount of habitat potentially isolated by an upstream barrier to brook trout movement using ArcMap, Version 9.2 (ESRI, Inc.) and USGS National Hydrological Dataset, 1:24,000 high resolution subwatershed data, digital line graph hydrography files and digital orthophoto quad data layers to achieve 98.5% accuracy (Table 1.2).

Barrier height was measured in the field using a standard rod and level, or was estimated by subtracting the difference in elevation between GPS points recorded directly above the barrier and directly below the barrier based on Google Earth high resolution satellite imagery of 1000 pixels if the barrier height was taller than the standard rod (~10 meters). Channel gradient was estimated as arctan (degrees) from the lowest to highest point of each barrier (Table 1.1). Stream-reach sample distances ranged from 50 meters to 585 meters, as sampling was completed at a given site once a sample of 30 brook trout fin-clips was acquired.

Brook trout sampling. - I used single-pass electrofishing techniques to collect brook trout at each site. I anesthetized fish with clove oil (Taylor and Roberts 1999). I measured total length and weight, and clipped a ~50 mg piece of fin from each trout (Rogers and Curry 2004). Fin-clips were stored and preserved in separate vials with 95% ethanol. I calculated brook trout sample densities above and below each barrier site by calculating the total number of sampled brook trout divided by the total area sampled (m^2) (sampled length*average bankfull width; Table 1.2).

Microsatellite markers and genotyping. - DNA extraction, polymerase chain reaction (PCR), and genotyping protocols were developed at the United States Geological Survey, Conte Anadromous Fish Laboratory (Turners Falls, Massachusetts). I extracted DNA from 897 fin-clips and PCR-amplified microsatellite-bearing fragments at eight loci (Table 1.3) using an MJ DNA Engine Dyad PTC-220 thermocycler. Microsatellite loci were multiplexed (Henegariu et al. 1997, Table 1.3) for cost-effective genotyping using an Applied Biosystems, Inc. ABI 3100-Avant Autoanalyzer and GeneMapper software. I scored genotypes using Peak Scanner v1.0

(Applied Biosystems, Inc).

Between-sample analysis for above- and below-barrier samples

Between sample comparisons of genetic diversity included comparing allelic diversity above and below a barrier, using the parameters, number of alleles per locus (*A*), average gene diversity per locus (*H*), number of private alleles per locus, and total alleles. Between sample comparisons included using *F* statistics to calculate F_{ST} values and R_{ST} values as a measure of population differentiation. The following population genetic diversity and population genetic differentiation sections refer to comparisons between above and below samples at each site.

Population genetic diversity. - I used Create 1.2 (Coombs et al. 2008) to prepare input data files for genetic analysis. I applied Arlequin 3.1.1. (Excoffier et al. 2005) genetic software to quantify genetic variation by calculating number of alleles per locus (*A*) and average gene diversity per locus (*H*) (Neraas and Spruell 2001; Rogers and Curry 2004; Yamamoto et al. 2004). I applied a Wilcoxon sign-rank test for non-normalized data and t-test for normalized data, when the normality test passes, using SAS 9.2 (SAS Institute Inc. 2008) to test the null hypothesis that *H* and *A* did not differ for paired samples above and below each barrier site. I also tested the null hypothesis that the average number of private alleles per locus and the average number of total alleles did not differ for paired samples above and below each barrier site. I also applied GENEPOP (Rousset 2007) to test for deviation from Hardy-Weinburg equilibrium (abbreviated as HWE) at each locus and for linkage disequilibrium for all locus pairs within a population.

Private alleles. - I applied HP-RARE 1.0 (Kalinowski 2005) to quantify the number of unique alleles, or private allele richness in sampled populations to compare their presence above and below barriers. The HP-RARE 1.0 program applied a rarefaction statistical technique that estimated the expected number of private alleles in a sample based on the probability of the allele occurring in a given sample and sample size, as larger samples were expected to contain more alleles (Kalinowski 2004, 2005). I also compared allele presence across all above- and below-barrier samples to identify alleles that were unique to a sample of 30 individual brook trout. The

presence of private alleles below but not above a barrier could indicate lack of gene flow.

Population genetic differentiation. - Various measures of genetic distance have been developed to characterize differences in allele frequencies between and among populations (Weir and Cockerham 1984; Slatkin 1995; Takezaki and Nei 1996). I estimated pairwise genetic distance metrics using Arlequin 3.1.1. (Excoffier et al. 2005) and R_{ST} Calc (Goodman 1997), including distance based on the number of different alleles, F_{ST} (Weir and Cockerham 1984), and Slatkin's distance application based on differences in allele sizes due to stepwise mutation (Slatkin 1995), R_{ST}, to quantify differentiation between above- and below-barrier samples. The F_{ST} metric is considered more conservative when applying <20 microsatellite loci to quantify genetic distance for recent divergence within populations (Whiteley et al. 2006). The F_{ST} value represents difference in allele frequencies when comparing samples due to what alleles are present currently, without considering historic changes in alleles over time (Weir and Cockerham 1984). The R_{ST} value represents differences in allele frequencies when comparing samples due to allele size differences resulting from stepwise mutations over time (Slatkin 1995). Although a low mutation rate is assumed for all sampled brook trout populations in this study, the R_{ST} metric can be used to quantify genetic distance due to divergence over longer time scales that the F_{ST} metric may not identify (Slatkin 1995).

I compared multilocus genotypes for each above-barrier sample to each below-barrier sample for each site (15 above-barrier samples, 15 below-barrier samples, for the BR Level III Ecoregion; and six above-barrier samples, six below-barrier samples, for the NLF Level III Ecoregion) to calculate a site-specific F_{ST} and R_{ST} value. I used linear regression to test the significance of the relationship among barrier height (m), channel gradient (degrees), and F_{ST} and among barrier height (m), channel gradient (degrees), and R_{ST} using SAS 9.2 (SAS Institute Inc. 2008). I tested relationships using logistic regression as well, but resulting models were not a good fit for the data.

Among population analysis

Among population comparisons of all sampled populations included genetic distance

measures and individual assignment analysis. Genetic distance applications describe relationships between populations based on the difference in represented allele frequencies. This measure of differentiation between populations can be used to infer whether populations are intermixing and if breeding is occurring among individuals. Individual assignment applications assign individuals to a source population based on multilocus genotypes at more than one loci for all individuals in all samples. Individual-based assignment tests commonly apply genetic distance analysis or Bayesian cluster analysis techniques to assign a probability that an individual belongs to a source population (Rogers and Curry 2004; Excoffier and Heckel 2006).

Genetic distance. - Genetic distance applications describe relationships between populations based on the difference in represented allele frequencies. This measure of differentiation between populations can be used to infer whether populations are intermixing and if breeding is occurring among individuals. A GENEPOP (Rousset 2007) input file of allele frequency data for all sampled populations was converted into PHYLIP input file format. I then applied a series of programs within PHYLIP (Felsenstein 1995) to construct NJ and consensus trees for all sites within Level III Ecoregions based on genetic distances among sampled populations. Populations were separated into above- and below-barrier samples for each site and were grouped by BR or NLF Level III Ecoregion.

I produced 1,000 bootstrap datasets of the original allele frequency dataset using SEQBOOT in PHYLIP (Felsenstein 1995). The program GENEDIST in PHYLIP used these 1,000 bootstrap datasets to construct a genetic distance matrix of Cavalli-Sforza and Edwards (1967) chord distances (Dcc) for all sampled populations. Cavalli-Sforza and Edwards (1967) chord distance has the highest probability of correct tree topology among distance measures for microsatellite data sets (Takezaki and Nei 1996). The GENEDIST output was the input for the NEIGHBOR application of PHYLIP that produced 1,000 replicates of an unrooted NJ tree for all sampled populations. I produced a consensus tree of the 1,000 replicate NJ trees using the CONSENSE application (Felsenstein 1995). I applied TREEVIEW (Page 1996) to plot and visualize the final consensus tree.

Individual assignment. - I applied GeneClass2 (Piry et al. 2004) to assign multilocus genotypes of individuals to the sampled populations and to identify first-generation migrants (Excoffier and Heckel 2006). I also applied Bayesian model-clustering methods using Structure 2.3.1. (Pritchard et al. 2000) to assign individuals to populations and to investigate structure among populations. Each simulation had three replicates and used a burn-in period of 5,000 replications (Pritchard et al. 2000). I tested the probability that two source populations (one above-barrier and one below-barrier) existed for each sampled barrier site for 60 individuals. I also tested the probability that one to 30 source populations existed for 897 individuals for 15 above-barrier and 15 below-barrier sampled populations in the BR Level III Ecoregion. I tested the probability that one to 12 populations existed for 360 individuals for six above-barrier and six below-barrier sampled populations in the NLF Level III Ecoregion.

Analysis of molecular variance (AMOVA)

AMOVA analysis partitions variation within, among, and between sampled individuals and populations. I applied Arlequin 3.1.1. (Excoffier et al. 2005) to conduct an analysis of molecular variance (AMOVA, Excoffier et al. 1992) by applying the Weir and Cockerham (1984) distance method based on the number of different alleles. Within population genetic analysis assumes Hardy-Weinburg and linkage equilibria and the ability to quantify gene flow and genetic drift are related to their presence (Excoffier and Heckel 2006; Slatkin 2008). I used AMOVA to quantify levels of genetic variation between groups, among samples within groups, among individuals within samples, and within individuals for all BR Level III samples (12 above and 12 below samples, with no non-barrier sites) and all NLF Level III populations (six above and six below samples). I grouped samples at each site according to their above-barrier (group 1) and below-barrier (group 2) location to quantify levels of genetic variation due to a barrier. I also combined above and below-barrier samples for each site and grouped them by Level III Ecoregion to quantify levels of genetic variation due to ecoregion, with NLF as group 1 and BR as group 2.

Results

Barrier height and channel gradient. - Heights for the 18 barrier sample sites ranged from 0.91 meter to 91.4 meters (Table 1.1), with all barrier sample sites meeting the minimum 73.5 cm height determined by Kondratieff and Myrick (2006). The three non-barrier sites were considered 0.00 meter barrier heights. Barrier channel gradients ranged from 1 to 90 degrees for the non-barrier and barrier sites (Table 1.1).

Genetic effect of barriers

I sampled DNA from 1,257 brook trout spread across 21 sites (Table 1.1, Figures 1.1, 1.2) at eight microsatellite loci (Table 1.3) to quantify genetic diversity and differentiation between above- and below-barrier samples.

Genetic diversity for above and below samples. - The following describes the genetic diversity results for comparisons between above- and below-barrier samples. The average number of alleles (*A*) per locus above barriers ranged from 1.63 to 11.0, with a mean of 6.37±0.50 (SE). The average number of alleles (*A*) per locus below barriers ranged from 3.00 to 9.88, with a mean of 6.55± 0.40. There was no significant difference in average number of alleles per locus (*A*) between the above- and below-barrier samples (Wilcoxon sign rank P=0.985). The average gene diversity per locus (*H*) above barriers ranged from 0.16 to 0.79, with a mean of 0.62±0.03. The average gene diversity per locus (*H*) below barriers ranged from 0.39 to 0.78, with a mean of 0.64±0.02 (Table 1.4). There was no significant difference in average gene diversity per locus (*H*) between the above- and below-barrier samples (Wilcoxon sign rank P=1.000). The average number of private alleles per locus above barriers ranged from 0.00 (0 total private alleles for 30 individuals) to 1.75 (14 total private alleles for 30 individuals), with a mean of 0.38±0.08 (Table 1.4). The average number of private alleles per locus below barriers ranged from 0.00 (0 total private alleles for 30 individuals) to 1.25 per locus (10 total private alleles for 30 individuals), with a mean of 0.48±0.08 (Table 1.4). There was no significant difference between the average number of private alleles above and below barriers (Wilcoxon sign rank P=0.16). The total alleles above barriers ranged from 13 to 88, with a mean

of 51±4. The total alleles below barriers ranged from 24 to 79, with a mean of 52±3. There was no significant difference between the average number of total alleles above and below barriers (Wilcoxon sign rank P=0.43).

F_{ST} and R_{ST}. - The following results discuss genetic differentiation values for F_{ST} and R_{ST} values that compare allele frequencies between above- and below-barrier multilocus genotype samples. Genetic differentiation (F_{ST}) values ranged from 0.001 at WAUP, HAYM, NFKS, and SHOE to 0.349 at APOR (Table 1.6). The R_{ST} values ranged from 0.001 at COLE and PRBR to 0.264 at STMY (Table 1.6).

Barrier height and F_{ST} values were positively related, (r^2=0.38, F=8.48, P=0.011) (Figure 1.3) as were F_{ST} and the combined variables of barrier height and channel gradient (r^2=0.41, F=4.43, P=0.034). Correlations among F_{ST}, height, and channel gradient were significant between height and channel gradient (Spearman's rho=0.562, P=0.02) and between F_{ST} and height (Spearman's rho=0.514, P=0.04), but not between F_{ST} and channel gradient (Spearman's rho=-0.012, P=0.961). Therefore, barrier height was identified as the preferred predictive variable (MLR height, P=0.011; channel gradient, P=0.443). I applied this analysis to 16 of 21 sites, as the F_{ST} and R_{ST} values for the three non-barrier sites were removed, plus I removed the Apple Orchard Falls (APOR) and Saint Mary's Falls (STMY) sites. The F_{ST} and R_{ST} values for the APOR site were removed due to a high percentage of relatedness among sampled individuals and for the STMY site due to the sample being out of Hardy-Weinberg and linkage equilibria. Both of these conditions would bias the F_{ST} and R_{ST} estimation methods applied in this study which assume Hardy-Weinburg and linkage equilibria (Weir and Cockerham 1984; Slatkin 1995).

There was no significant relationship between R_{ST} values and barrier height (r^2=0.12, F=1.96, P=0.183), between R_{ST} and channel gradient (r^2=0.00, F=0.000, P=0.983), or between R_{ST} and the combined variables of barrier height and channel gradient (r^2=0.15, F=1.13, P=0.352). Power analysis for the relationship between R_{ST} and height showed that power was lower for the R_{ST} values (0.26, $P > 0.05$), with a greater coefficient of variation (119.63) as

compared to power analysis for the relationship between F_{ST} and height (0.78, $P > 0.05$), with a smaller coefficient of variation (74.47).

There was no significant relationship between R_{ST} values and barrier height as well when all 21 sites were included in the analysis (r^2=0.08, F=1.74, P=0.203). In this case, including all sites reduced the r^2 value and made the relationship even less significant. The relationship between F_{ST} and height was significant when all 21 sites were included in the analysis (r^2=0.25, F=6.33, P=0.021), but including all sites also reduced the r^2 value and made the relationship even less significant.

Genetic diversity comparisons between Level III Ecoregions. - The mean total number of alleles per site (above- and below-barrier samples combined) for the NLF Level III Ecoregion was 80±5 (SE), and the mean total number of alleles per site for the BR Level III Ecoregion was 54±4, with a statistically significant difference between the Level III Ecoregions (t = 3.975, $P <$ 0.001). The mean number of alleles per locus (A) per site (above- and below-barrier samples combined) for the NLF Level III Ecoregion was 10.11 ±0.61, and the mean number of alleles per locus (A) per site for the BR Level III Ecoregion was 6.73±0.47. There was a statistically significant difference between A per site for the Level III Ecoregions (t =-5.662, $P > 0.002$). The average gene diversity per locus (H) per site (above- and below-barrier samples combined) for the NLF Level III Ecoregion was 0.72±0.02, and the average gene diversity per locus (H) per site for the BR Level III Ecoregion was 0.61±0.03. There was a statistically significant difference between H per site for the Level III Ecoregions (t = -2.902, $P > 0.034$). The mean number of private alleles per locus per site for the NLF Level III Ecoregion was 0.78±0.12 and the mean number of private alleles per locus per site for the BR Level III Ecoregion was 0.29±0.05. There was a statistically significant difference between the average number of private alleles per locus per site for the Level III Ecoregions (t= -4.516, $P > 0.001$).

In the BR Level III Ecoregion, private alleles were found in the above sample but not the below sample at the STAT and STAU sites. The following sites had private alleles that were only found at that site among all 21 sampled sites in this study: CBCR, CORN (non-barrier) and

STAT (2 private alleles each) and NFKS (non-barrier), SHCS, and STAU (1 private allele each). In the NLF Level III Ecoregion, private alleles were found in the above sample but not the below sample at two of the six sites: HAYM and SPBK (Table 1.5). The following sites had private alleles that were only found at that site among all 21 sampled sites in this study: HAYM (4 private alleles) and SPBK and WAUP (1 private allele each).

Unrooted neighbor-joining consensus tree. - A clustering algorithm based on similarity in allele frequency per sample was applied to determine whether samples above and below a barrier site resembled one another genetically. Figure 1.4 represents the unrooted NJ consensus tree of Cavalli-Sforza and Edwards (1967) chord distances among 15 above-barrier and 15 below-barrier samples of brook trout in the BR Level III Ecoregion sites. Bootstrap values for 1,000 replicates between above (a) and below-barrier (b) samples ranged from 774 (between CRTR "a" and "b" samples) to 1,000 (between "a" and "b" samples for APOR, CASC, CBCR, SASS, and STAT). This means that 774 out of 1,000 (77%) and 1,000 out of 1,000 (100%) of the bootstrapped NJ trees had the same construction. Bootstrap support is considered good if it is at least 60% (Small et al. 2007; Stott et al. 2010), so bootstrap support in this case is good to excellent.

Within the BR Level III Ecoregion consensus tree only the CRTR and APOR above and below sites did not cluster together, but were on separate branches of the tree, meaning that the above and below samples of these two sites were differentiated from one another. The longer branch length between the above and below samples for the CRTR and APOR sites indicates a greater genetic distance from one another as compared to other sites in the BR Level III Ecoregion. This relationship was supported by excellent bootstrap values of 100% support (1,000 out of 1,000 of the NJ trees had the same construction), for the APOR site and a bootstrap value of 94% support (939 out of 1,000 of the NJ trees had the same construction) for the CRTR site. In addition, the APOR site was the genetically differentiated from all other sites in the BR Level III Ecoregion as seen by the separate and long (more genetically distant) branch length for the above (aAPOR) and below (bAPOR) samples (100% bootstrap support) (Figure 1.4).

Figure 1.5 represents the unrooted NJ consensus tree of Cavalli-Sforza and Edwards (1967) chord distances among six above-barrier and six below-barrier) samples of brook trout in the NLF Level III Ecoregion. Within the consensus tree only the above-barrier (a) and below-barrier (b) components of the MORG site were on completely separate branches, with a bootstrap value of 1,000 (1,000 out of 1,000 (100%) of the NJ trees had the same construction), indicating further genetic distances from each other compared to other above- and below-barrier pairs of sites. Above- and below-barrier samples clustered together at all other sites in the NLF Level III Ecoregion. Bootstrap values for 1,000 replicates between above- (a) and below-barrier (b) samples ranged from 765 (765 out of 1,000 (77%) of the NJ trees had the same construction) (between WAUP "a" and "b" samples) to 1,000 (1,000 out of 1,000 (100%) of the NJ trees had the same construction) (between "a" and "b" samples for MORG and PRBR). The MORG site was the most genetically distant from the PRBR and SPBK sites and it is genetically differentiated from all other sites in the NLF Level III Ecoregion (100% bootstrap support) (Figure 1.5).

Individual assignment. - I applied individual assignment analysis to assign individual brook trout to their source population using multilocus genotype data. Results of this analysis can be used to calculate the likelihood that an individual is assigned to the above-barrier or below-barrier sample at each site. Results of individual assignment using STRUCTURE 2.3.1 (Pritchard et al. 2000) probability distributions showed that K=15 was the mostly likely number of source populations for the 15 above-barrier and 15 below-barrier) samples for the BR Level III Ecoregion. This result indicates that it is less likely that sites are differentiated into above- and below-barrier populations, as each site is most likely a combination of above- and below-barrier individuals, as indicated by K=15 or 15 source populations. For this ecoregion, there was 97% correct assignment of individuals to their source populations when K=15. Similarly, results of individual assignment probability distributions showed that K=6 was the most likely number of source populations for the six above-barrier and six below-barrier samples of the NLF Level III Ecoregion. For this ecoregion, there was 91% correct assignment of individuals to their

source populations when *K*=6. The 97% correct assignment of individuals to their source population for the BR Level III Ecoregion and the 91% correct assignment of individuals to their source population for the NLF Level III Ecoregion represent an excellent rate of assignment, which further is reflected in the Q values for each individual.

The quality index, Q represents the likelihood (probability) that an individual comes from a represented population, in this case, above-barrier (population one) or below-barrier (population two). The Structure 2.3.1 Q plots (Figure 1.6) show the likelihood value, Q of each numbered individual from 1 to 60, with individuals 1-30 coming from the above-barrier sample and individuals 31-60 coming from the below-barrier sample of each site. Sampled populations showed a range of admixture between above- and below-barrier samples with evidence of differentiation more clearly represented at some sites versus others. If the histogram for an individual showed equal area for the green and red color, this meant the individual was equally likely to come from the above- or below-barrier sample and represented a population with high admixture. For example, the Q plots for the North Fork Stony Creek (non-barrier site), Haymeadow Creek (barrier height 0.9 meter), and Waupee Rapids (barrier height 0.9 meter) sites all showed high admixture, with probabilities close to 0.50 equally that individuals came from either the above-barrier (population one) or the below-barrier (population two) sample. Conversely, Q plots for the Saint Mary's Falls (barrier height 4.0 meters), White Rock Falls (barrier height 11.3 meters), and Crabtree Falls (barrier height 91.4 meters) sites were more clearly differentiated, with the majority of individuals 1-30 (above-barrier sample) having a > 0.90 probability that they came from the above-barrier sample. These bar plots and Figure 1.3 suggest a possible threshold of barrier height effects at approximately 4 meters (Figures 1.3, 1.6).

I also used GeneClass2 (Piry et al. 2004) to assign multilocus genotypes of individuals to the sampled populations and to identify first-generation migrants (Excoffier and Heckel 2006) moving between the above- and below-barrier samples at each site. First-generation migrants inferred above a natural barrier site of the BR Level III Ecoregion ranged from zero at the CRTR site to eight at the BWLU site (Table 1.7), suggesting no upstream dispersal at the CRTR and a

higher rate of upstream dispersal at the BWLU site. First-generation migrants inferred above a natural barrier site of the NLF Level III Ecoregion ranged from three at the PRBR site to nine at the HAYM and WAUP sites, suggesting a higher rate of upstream dispersal overall (Table 1.7).

Population structuring

Unrooted neighbor-joining consensus tree. - There were two clusters of groups of sites that were visually displayed within the NJ consensus tree for the BR Level III Ecoregion (Figure 1.4). The first cluster included the CASC, NFKS, BWLL, CBCR, and BWLU sites with a 951 bootstrap value (951 out of 1000 (95%) of the NJ trees had the same construction). Within this cluster were sites from adjacent subwatersheds within Grayson Highlands State Park (BWLL and BWLU, adjacent subwatershed to CBCR). Davis (2008) identified the CBCR, NFKS, and CASC sites as a Southern Appalachian strain brook trout population based on allozyme frequency data (Southern Appalachian strain, diagnostic allele, CK-A2*100). Additional sites within this cluster, identified by this microsatellite data, are likely to be Southern Appalachian strain populations as well based on how these sites are clustering together due to genetic similarity and the high bootstrap support (951 out of 1,000 (95%) of the NJ trees had the same construction) (Figure 1.4). The other cluster of sites that was apparent included the CRTR, WHRK, SHCS, and SHOE sites with a 911 bootstrap value (911 out of 1000 (91%) of the NJ trees had the same construction). These sites were in adjacent subwatersheds, with the CRTR and WHRK sites in one subwatershed (939 out of 1,000 (94%) of the NJ trees had the same construction) and the SHCS and SHOE in an adjacent subwatershed (963 out of 1,000 (96%) of the NJ trees had the same construction) (Figure 1.4).

There were two clusters of groups of sites that were visually displayed within the NJ consensus tree for the NLF Level III Ecoregion (1,000 of 1,000 (100%) of the NJ trees had the same construction) (Figure 1.5). The first cluster included sites from completely different states within the NLF Level III Ecoregion (PRBR in Minnesota and SPBK in Wisconsin) and (HAYM in Michigan. However, bootstrap support was weak for this cluster construction, with only a 426 bootstrap value (426 out of 1,000 (43%) of the NJ trees had the same construction). The other

cluster of sites included the COLE site in Michigan and the WAUP site in Wisconsin. However, bootstrap support was also weak for this construction, with only a 326 bootstrap value (326 out of 1,000 (33%) of the NJ trees had the same construction) (Figure 1.5).

AMOVA. - Analysis of molecular variance quantified the contribution of genetic variation between groups of brook trout samples (above-barrier versus below-barrier), among sites within groups of brook trout, among brook trout individuals within sites, and within brook trout individuals across sites within each ecoregion. Table 1.8 shows this analysis separately for the NLF Level III Ecoregion (a) and for the BR Level III Ecoregion (b). Results of this analysis showed that genetic variation among samples within groups (group 1 = above-barrier and group 2 = below-barrier), among individuals within samples, and within individuals were significant ($P < 0.000$), for all 12 samples of 30 total individuals (six above and six below) in the NLF Level III Ecoregion (Table 1.8.a.) and all 24 samples of 30 total individuals (12 above and 12 below, with three non-barrier sites removed) in the BR Level III Ecoregion (Table 1.8.b.). Genetic variation between groups of above-barrier samples (group 1) and below-barrier samples (group 2) was not significant ($P < 0.97$ for NLF and $P < 1.00$ for BR). This result means that at each sample site separated by Level III Ecoregion for analysis, there was not a significant difference in genetic variation between the above- and below-barrier sample. The largest source of variation was within individuals (89.2% of total variation) for the NLF Level III Ecoregion (Table 1.8.a.). The largest source of variation for the BR Level III Ecoregion was within individuals (62.9% of total variation) followed by among samples within groups (36.2% of total variation) (Table 1.8.b.).

Sources of variation were significant between groups (group 1 = NLF and group 2 = BR; $P < 0.01$), among samples within groups, among individuals within samples, and within individuals ($P < 0.000$), for combined above- and below-barrier samples for each site (60 individuals per site) (Table 1.8.c.). The largest source of variation was within individuals (65.3% of total variation) followed by among samples within groups (24.5% of total variation) (Table 1.8.c.). These results suggest that the largest sources of variance in brook trout multilocus

genotype sample datasets are due to variation within individuals of any given population, followed by variation due to collecting samples from different site locations. Variation due to differences in samples collected above and below barriers was not significant, showing a limited barrier effect; and variation due to differences in samples collected from different Level III Ecoregions (NLF and BR) was less significant than other sources of variation in this analysis ($P < 0.01$ versus 0.00 for all other sources of variation) (Table 1.8.c).

Discussion

My overall goal was to evaluate measures of genetic diversity and differentiation as indicators of barrier effects on brook trout populations. I collected brook trout fin clip samples at 21 sites (Table 1.1; Figures 1.1, 1.2) at eight microsatellite loci (Table 1.3) to test the hypothesis that genetic variation and population structure in brook trout populations were affected by barriers to movement. This is the first study to investigate natural barriers of different heights and gradients that influence population genetics of brook trout populations in two different ecoregions of the eastern United States. Specifically, this study compares population genetics of resident brook trout in the glaciated NLF Level III Ecorgegion and the unglaciated BR Level III Ecoregion.

Specific physical criteria for defining a barrier to fish movement are limited and some barriers exist during all flow conditions, whereas others may be barriers only during low flow or high flow (Reiser et al. 2006). Specific height criteria identified for a barrier to brook trout movement are based on experiments with brook trout in a constructed waterfall in the lab, kept at constant flow. In these experiments, Kondratieff and Myrick (2006) identified a 73.5 cm maximum jumping height, as a barrier for 8.6 – 34.0 cm TL brook trout with a 0.40 cm minimum pool depth below the waterfall. Specific channel gradients identified as a barrier to brook trout are based on work in the field, which identified a channel gradient of 18-22% as the maximum for brook trout movement (Moore et al. 1985; Adams et al. 2000). Applying these height and channel gradient criteria to define barriers to brook trout movement universally may not be appropriate for all putative barriers to brook trout, as barrier complexity and flow conditions can

vary extensively.

This study investigated natural barriers ranging in height from 0.91 to 91.4 meters and from 1 to 90 degrees channel gradient and applied population genetic analysis that infers upstream dispersal to investigate further what constitutes a barrier to brook trout. Specifically, this study applied genetic diversity, differentiation, and individual assignment applications to microsatellite multilocus genotype data to look for indicators of upstream dispersal. This study attempted to identify thresholds of height and gradient that influence genetic diversity, population differentiation, and population structuring of brook trout. Flow, temperature, and long-term variability of fish movement in relation to the natural barrier sites were not measured, as these datasets were not available consistently and from a long-term timescale for the sites investigated. These sources of variability due to these other factors make height and gradient thresholds unclear and weaken statistical strength of potential relationships.

Results of this study showed that F_{ST} and unrooted NJ consensus trees of Cavalli-Sforza and Edwards (1967) chord distances provided evidence of genetic differentiation between samples of resident brook trout above and below natural barriers. Results of this study also showed that average total allelic diversity (*A*), average gene diversity per locus (*H*), average number of private alleles per locus per sample, and total alleles per sample provided measures for comparison of genetic diversity between Level III Ecoregions. Individual assignment analysis of the above- and below-barrier sample for each site showed that Q values identified a threshold height of barrier effects of population genetic effects at approximately 4 meters. These Q values were supported by a 97% correct assignment of individuals to their source population for the BR Level III Ecoregion and a 91% correct assignment of individuals to their source population for the NLF Level III Ecoregion (Figure 1.6).

The unrooted NJ consensus tree for the BR Level III Ecoregion also identified population genetic structuring based on genetic distance between samples. Similarity in allelic frequencies identified a cluster of sites that included the CBCR, NFKS, and CASC sites. These sites were identified as Southern Appalachian strain by Davis (2008). Other sites within this cluster

included the BWLU and BWLL sites which are in the adjacent subwatershed to the CBCR site. The second cluster of sites included the SHCS and SHOE samples in the same subwatershed and the WHRK and CRTR samples from an adjacent subwatershed. These clusters identified similarity in allelic frequencies, which suggests interbreeding. I recommend managing the streams where these samples were collected as one interconnected "population", which means managing at the subwatershed scale at the minimum, as opposed to the stream reach or stream scale.

Barrier effects and genetic diversity. - I predicted that heights and channel gradients that result in a barrier to upstream movement would result in lower average gene diversity per locus (*H*) and number of alleles per locus (*A*) above the barrier because those populations would become isolated from gene flow and more likely to be affected by genetic drift.

The average number of alleles (*A*) and average gene diversity per locus (*H*) were not significantly different above and below each barrier, which contradicted previous studies that documented significant differences in the *A* and *H* values between above- and below-barrier samples (Castric et al. 2001; Taylor et al. 2003; Yamamoto et al. 2004; Poissant et al. 2005; Wofford et al. 2005; Whiteley et al. 2006; Deiner et al. 2007; Small et al. 2007; Guy et al. 2008). These previous studies, were conducted on other salmonid species, or in other areas of the brook trout range as compared to this study. Whiteley et al. (2006) investigated genetic variation throughout the bull trout's native range and determined that genetic population structure varies regionally and by subbasin depending on variability in gene flow, drift, colonization, and barriers (Costello et al. 2003; Yamamoto et al. 2004).

In this study, private alleles were identified only within the above-barrier sample, indicating limited gene flow and a difference in genetic diversity above versus below the barrier for the STAT and STAU sites in the BR Level III Ecoregion and the HAYM and SPBK sites in the NLF Level III Ecoregion (Table 1.5). The presence of private alleles identified in the above-barrier sample and not in the below-barrier sample may indicate a barrier to upstream dispersal. However, this is based on the assumption that all representative alleles were sampled in the

below- and above-barrier population, which may or may not be true based on the percentage of habitat that was sampled. Percentages of total habitat sampled for the above-barrier sample ranged from 0.28 to 10.8%.

Barrier effects and genetic differentiation. - I predicted that heights and channel gradients that result in a barrier to upstream movement would result in higher F_{ST} and R_{ST} values as the above sample is further differentiated from the below sample. I tested relationships between barrier height, gradient, F_{ST}, and R_{ST} values to investigate effects of barriers on genetic differentiation. The only statistically significant relationship I found was between F_{ST} and barrier height (r^2=0.38, F=8.48, P=0.011). This relationship suggests that as the barrier height increases, the F_{ST} value between the above- and below-barrier sample will increase. This relationship was based on one site and would not be the case if the tallest barrier site was removed from the analysis (Figure 1.3) and Sewall Wright's F_{ST} island model assumes equilibrium between migration and drift. However, most populations are not in equilibrium due to processes occurring at a wide variety of spatial and temporal scales (Castric et al. 2001). In this case, the F_{ST} values for the APOR site were removed due to a high percentage of relatedness among sampled individuals and for the STMY site due to the sample being out of Hardy-Weinberg and linkage equilibria. All of these conditions would affect the assumption of migration-drift equilibrium that the F_{ST} calculation requires (Weir and Cockerham 1984; Slatkin 1995).

The unrooted NJ consensus trees for the BR Level III Ecoregion (Figure 1.4) and NLF Level III Ecoregion (Figure 1.5) showed the above- and below-barrier samples on separate branches for the sites in each ecoregion with the greatest barrier height. That is, the CRTR above and below sites in the BR Level III Ecorgion were differentiated from one another, as the below (bCRTR) and above (aCRTR) site were on separate branches in the tree (94% bootstrap support) (Figure 1.4). This site was the tallest barrier in the BR Level III Ecoregion at 91.4 meters. The MORG above and below sites were differentiated from each other as well (100% bootstrap support) and this site was the tallest barrier in the NLF Level III Ecoregion at 23

meters. These results, with excellent bootstrap support, suggested that barriers affected genetic differentiation between above- and below-barrier brook trout samples for the tallest barrier heights in each ecoregion. Longer branch lengths in the unrooted NJ consensus tree with 100% bootstrap support for the APORa sample in the BR Level III Ecoregion (Figure 1.4) and the MORGa sample in the NLF Level III Ecoregion (Figure 1.5) further indicate divergence due to isolated populations above the barrier.

Individual assignment results, which assigned a likelihood (Q) value to each individual showed further evidence of differentiation between above- and below-barrier samples for the Saint Mary's Falls (STMY), White Rock (WHRK), and Crabtree Falls (CRTR) sites, as seen by individuals 1-30 being most likely assigned to the above-barrier sample and individuals 31-60 being most likely assigned to the below-barrier sample (Figure 1.6). Individual assignment analysis for all sites included in this study showed that Q values identified a threshold height of 4 meters that resulted in population differentiation between the above and below sample. These Q values were supported by a 97% correct assignment of individuals to their source population for the BR Level III Ecoregion and a 91% correct assignment of individuals to their source population for the NLF Level III Ecoregion (Figure 1.6).

A lack of differentiation between samples may indicate populations that have remained connected under certain flow conditions or at certain times (Small et al. 2007). In addition, I only sampled a small percentage of habitat that was available (0.28 to 10.8%, and my sample may or may not completely represent all alleles within individuals.

Ecoregional differences. - I predicted that genetic diversity (total alleles, *A*, *H*, and private alleles) would be higher in the unglaciated BR Level III Ecoregion as compared to the glaciated NLF Level III Ecoregion based on the results of previous research (Billington and Hebert 1991; Danzmann et al. 1998; Hall et al. 2002). My results showed the opposite, as all genetic diversity parameters were significantly higher for the NLF Level III Ecoregion (glaciated) as compared to the BR Level III Ecoregion (unglaciated): average total number of alleles per sample ($t = 3.975$, $P < 0.001$); average number of alleles per locus (*A*) per site (t =-

5.662, $P > 0.002$); average gene diversity per locus (H) per site (t = -2.902, P > 0.034); and average number of private alleles per locus per site (t= -4.516, P > 0.001). Analysis of molecular variance also showed that the % variation (89.2%) was higher within individuals for all NLF Level III Ecoregion sites (Table 1.8.a.) than for all BR Level III Ecoregion (62.9%) (Table 1.8.b.). Guy et al. (2008) documented differences in coastal cutthroat allelic diversity in two different Level III Ecoregions of the western United States, but this is the first study to document a difference in allelic diversity for brook trout from two Level III Ecoregions of the eastern United States by applying microsatellite DNA markers. Davis (2008) documented higher allozyme loci allelic diversity for Northern strain brook trout, which originated primarily in the glaciated Northeastern United States (Galbreath et al. 2001) as compared to Southern strain brook trout according to allozyme data.

Brook trout population genetic patterns on the landscape must be interpreted relative to phylogeographic processes, natural history, stocking history, and to contemporary human alteration of connected stream habitats. In the case of phylogeography, the locations of Pleistocene glacial refugia affected the ability of brook trout to colonize new habitats and thus establish new populations and pass on their genetic material. This dataset offers the opportunity to explore such relationships, as glacial activity and locations of glacial refugia on the landscape affected the NLF Level III Ecoregion, while glacial activity had no affect on the BR Level III Ecoregion (Danzmann et al. 1998; Hebert et al. 2000; Hall et al. 2002; Costello et al. 2003).

Analysis of mitochondrial DNA variation throughout the range of the brook trout documented more than one glacial refugia contributing to the NLF Level III Ecoregion as a result of Pleistocene and more recent Wisconsinan glacial events, which offers a more diverse gene pool for colonization (Danzmann et al. 1998; Hall et al. 2002). In contrast, populations in the BR Level III Ecoregion were descendants only of the historic Ohio River and Shavers Run populations (Danzmann et al. 1998; Hall et al. 2002). The brook trout populations of the BR Level III Ecoregion region have had more time for genetic divergence to occur in non-glaciated areas in comparison to the glaciated populations of the NLF Level III Ecoregion, as Great Lakes

populations were completely extirpated during glacial activity. Great Lakes brook trout populations had less time for divergence, as populations could only reestablish and increase population densities as glaciers receded. Brook trout populations in the BR Level III Ecoregion lacked sources of new variation that colonization from glacial refugia would provide for populations in the NLF Level III Ecoregion.

Populations that I sampled did not cover the entire geographic range that encompasses historic glacial refugia, and previous mitochondrial DNA characterization of Southern Appalachian brook trout populations did not include populations that I sampled. However, the greater geographic extent of individuals from different samples in northern Minnesota, Michigan, and Wisconsin into one combined cluster may be evidence of the wide-ranging effect of Pleistocene glaciation on brook trout population structuring. Brook trout migrated from refugia to the NLF Level III Ecoregion landscape as viable habitat became available as glaciers receded, giving rise to a combination of fish from northern Minnesota, Michigan, and Wisconsin within the same cluster. The results of this study are a reminder of the importance to consider ecoregional differences in genetic diversity and population structuring of fish within stream habitats in relation to the historic presence of glaciation and recolonization after glaciers have receded (Pielou 1991; Costello et al. 2003; Whiteley et al. 2006).

Stocking. - However, populations that have been stocked extensively may not follow expected genetic signatures in relation to glaciation. Resident brook trout population diversity, differentiation, and structuring within a landscape also need to be interpreted in relation to the presence and possible introgression of alleles from stocked fish. All sampled brook trout populations in this study were at one time managed as a sport fishery (Virginia Department of Game and Inland Fisheries database; North Carolina Wildlife Resources Commission stocking website; Wisconsin DNR Catchable Trout database; Michigan DNR Fish Stocking database; Fish and Wildlife Information Service, 2005, 2009; Great Lakes Fisheries Commission database). Although documentation is limited, it is highly likely that current wild-breeding brook trout represented in my samples have been influenced by stocked brook trout.

Stocked fish can be identified by diagnostic alleles at specified allozyme loci known for stocked strains (Currens et al. 1990). Introgression with wild fish can be determined by investigating the extent of admixture between diagnostic stocked alleles and native alleles for screened allozyme loci. The presence of admixture between stocked and native strains has varied by study. For example, some studies have documented admixture between stocked and wild rainbow trout individuals (Hansen et al. 2000), while others have not (Small et al. 2007). Additionally, dominant native strains may only persist above barriers to upstream dispersal while fish below barriers are admixed with stocked strains (Small et al. 2007). Although stocking has occurred, these studies and others have shown that natural genetic diversity and population structuring still occurs to a large extent throughout the range of the brook trout species (Danzmann and Ihssen 1995; Danzmann et al. 1998; Burnham-Curtis 2001; Hall et al. 2002; Rogers and Curry 2004; D'Amelio et al. 2008; Wilson et al. 2008). Further investigation of the sampled populations from this study would be needed to identify diagnostic hatchery strain alleles and rates of introgression between hatchery and wild brook trout strains.

Management implications and population structure. - Whiteley et al. (2006) investigated genetic variation throughout the bull trout's native range and determined that genetic population structure varies regionally and by subbasin depending on variability in gene flow, drift, colonization, and barriers (Costello et al. 2003; Yamamoto et al. 2004). Poissant et al. (2005) showed that the presence of contemporary barriers influenced gene flow and regional population structure and that interpreting genetic diversity information from populations that are not at equilibrium requires a combination of contemporary and historical perspectives at a landscape scale. Management strategies should take this variability in genetic population structure and perspective into account.

Population genetics is essential for determining how a species population unit is defined and how populations disperse among each other on the landscape (Waples and Gaggioti 2006). This study provides evidence that supports a management strategy for brook trout populations at the subwatershed scale based on NJ consensus tree clustering of populations that show genetic

similarity and mixing of individuals at the subwatershed scale, which has often been the spatial scale identified most relevant to management for fish conservation (Seaber et al. 1987; Fausch et al. 2002). The results of this study further emphasize the need to develop management strategies according to ecoregion as well, as indicated by the significantly higher difference in genetic diversity for the NLF Level III Ecoregion as compared to the BR Level III Ecoregion.

Historically, fisheries management work has focused on populations and in-stream habitat at the reach scale (Frissell et al. 1986; Roper et al. 1997; Bohn and Kershner 2002). However, further investigation and restoration initiatives relevant to fish populations have acknowledged that management at the watershed scale is more likely to be successful (Reeves et al. 1995; Roper et al. 1997; Bohn and Kershner 2002). Species have various habitat units at different spatial scales and different habitat units are needed for different functions and life history strategies of a species (Lord and Norton 1990; Fahrig and Merriam 1994). If population subunits at the stream reach habitat unit scale, for example, remain interconnected through dispersal, they can form an interconnected larger subwatershed scale population, which includes all interbreeding subunits (Wiens 1989; Harrison et al. 1988).

This study identified a genetically-similar cluster of Southern strain brook trout interbreeding subunits within adjacent subwatersheds of the Grayson Highlands State Park, Virginia. The entire spatial extent of the habitat occupied by these interbreeding subunits within the combined adjacent subwatersheds could be designated as a management area for conservation and restoration (Neville et al. 2006; Narum et al. 2008). Identifying and protecting unique genetic diversity among the subunits and maintaining dispersal among the subunits could be objectives for this management area. Epifanio et al. (2003) described a similar approach for defining conservation units and identifying priority areas for bull trout conservation. Using the NJ consensus tree from the work completed by Neraas and Spruell (2001), they identified a large subwatershed with two genetically similar clusters of populations that were targeted for conservation.

Prioritization and management implications. - Removing barriers to fish movement is a

restoration technique that has potential to restore extensive amounts of instream habitats for native fishes (Roni et al. 2002). Managers and decision makers will always have more stream sites to improve than funds available, so they need tools for prioritization. Understanding how particular natural barriers in particular subwatersheds affect population genetic diversity, differentiation, and structuring on the landscape is essential for making decisions about prioritization of restoration sites. This study attempted to identify thresholds of height and gradient that had an effect on population genetics of brook trout due to reduced upstream dispersal. This study identified a potential threshold of 4 meters based on individual assignment that could be used as a prioritization criterion. This is a greater height than the 73.5 cm previously determined by Kondratieff and Myrick (2006) as the result of experimental trials in the lab.

Petit et al. (1998) emphasized the use of allelic richness of a population as a prioritization criteria for species conservation. Allelic richness represents the raw material within a population that will allow organisms to adapt to stochastic events, and populations with high allelic diversity can function as source populations for conservation and restoration. Allelic richness can also be used in long-term analysis to indicate demographic changes over time. The following samples from this study have the highest representative allelic diversity and represent populations that can function as source populations for conservation in the NLF Level III Ecoregion include: HAYM (98 alleles) and COLE (87). For the BR Level III Ecorgion, the following sites have the highest allelic diversity and can function as source populations for conservation: BWLL (75), non-barrier site, NFKS (73), and non-barrier site, SHOE (70).

Low allelic diversity will indicate genetic isolation, due to increased effects of genetic drift, and a lack of gene flow. Low allelic diversity can indicate a population at risk, as well, as a loss of allelic diversity is often considered a loss of adaptive potential (Quattro and Vrijenhoek 1989; Virijenhoek 1994; Morita and Yamamoto 2002). The following sites have low allelic diversity and represent a site that may be at risk due to genetic isolation: APOR, SASS, STMY and WHRK. The APOR site has the lowest allelic richness of all 21 sites in the study (Table

1.4).

Further consideration of the uniqueness of a population can be considered by considering private alleles. In the BR Level III Ecoregion, the following sites had private alleles that were only found at that site among all 21 sampled sites in this study: CBCR, CORN (non-barrier) and STAT (2 private alleles each) and NFKS (non-barrier), SHCS, and STAU (1 private allele each). In the NLF Level III Ecoregion, the following sites had private alleles that were only found at that site among all 21 sampled sites in this study: HAYM (4 private alleles) and SPBK and WAUP (1 private allele each).

Identifying populations that are genetically divergent from other populations is also useful, as these populations represent unique allelic diversity for the species. Samples from this study that stand out as genetically isolated include the APOR above-barrier sample from the BR Level III Ecoregion, which is genetically divergent according to the NJ consensus tree, 100% bootstrap support (Figure 1.4). The MORG in the NLF Level III Ecoregion above-barrier sample is also genetically divergent according to the NJ consensus tree with 100% bootstrap support (Figure 1.5).

Future research needed. - Long-term research studies need to be designed at the subwatershed scale that combine mark-recapture data and population genetic data in stream channels with barriers to upstream dispersal to further investigate upstream dispersal in relation to barrier height and gradient. Sampling needs to occur not just directly adjacent to the barrier location but should include random sampling of individuals in all age classes throughout all available habitat both to ensure a representative sample of all alleles that are present (Whiteley et al. 2006). This improved study design would eliminate sample bias for private allele, allelic diversity, F_{ST}, and R_{ST} estimation. In addition, this study documented gene flow asymmetry with first-generation migrant analysis and this type of study would improve documentation of dispersal of specific fish in relation to barriers at different times of the year and in different flow conditions.

This study also illustrates the value of combining population genetic markers, as the

combination of the allozyme loci and microsatellite data identified a cluster of Southern strain brook trout samples in the subwatersheds of Grayson Highlands State Park, Virginia. Further investigation of the spatial extent of these Southern strain populations and management strategies is warranted. Additionally, a combination of diagnostic allozyme or mitochondrial DNA loci data for hatchery brook trout with wild trout could be used to identify further influence of stocking on population genetic diversity and structuring of native brook trout populations.

TABLES

Table 1.1. Study site abbreviations and descriptions.

Site Abbreviation	Site name	State	Barrier height (m)	Channel gradient[2]
Blue Ridge:				
APOR	Apple Orchard Falls	Virginia	61.0	80
BWLL	Big Wilson, lower	Virginia	2.1	90
BWLU	Big Wilson, upper	Virginia	1.8	5
CBCR	Cabin Creek	Virginia	7.6	80
CASC	Cascades	Virginia	21.3	90
CORN[1]	Cornelius Creek	Virginia	0.0	1
CRTR	Crabtree Falls	Virginia	91.4	80
NFKS[1]	N. Fork Stony Creek	Virginia	0.0	1
SASS	Sassafras Falls	North Carolina	30.0	70
SHCS	Shoe Creek Cascade	Virginia	3.4	15
SHOE[1]	Shoe Creek	Virginia	0.0	1
STAT	Staton's Creek Falls	Virginia	42.7	80
STAU	Staunton River	Virginia	3.8	6
STMY	Saint Mary's Falls	Virginia	4.0	90
WHRK	White Rock Falls	Virginia	11.3	80
Northern Lakes and Forests:				
COLE	Cole Creek	Michigan	4.3	75
HAYM	Haymeadow Creek	Michigan	0.9	7
MORG	Morgan Falls	Wisconsin	23.0	80
PRBR	Portage Brook	Minnesota	6.4	90
SPBK	Spring Brook	Wisconsin	1.5	65
WAUP	Waupee Creek	Wisconsin	0.9	8

[1]Non-barrier site

[2]Channel gradient was estimated as arctan (degrees) based on change in height between the lowest and highest point on each barrier.

Table 1.2. Population density for brook trout sampled.

Site[1]	Pop. Density[2] Above (bkt/m^2)	Pop. Density[2] Below (bkt/m^2)	Stream length sampled above (m)	Stream length sampled below (m)
Northern Lakes and Forests:				
HAYM	0.01	0.01	585	403
SPBK	0.05	0.05	198	198
COLE	0.04	0.05	157	124
PRBR	0.02	0.02	157	135
MORG	0.07	0.09	153	133
WAUP	0.03	0.03	215	215
Mean±SE	**0.04±0.01**	**0.04±0.01**		
Blue Ridge:				
BWLU	0.01	0.02	250	200
BWLL	0.02	0.01	150	150
SHCS	0.10	0.06	50	50
STAU	0.08	0.20	100	50
STMY	0.04	0.05	100	100
CBCR	0.10	0.06	100	100
WHRK	0.07	0.03	100	50
CASC	0.02	0.03	150	100
SASS	0.09	0.06	100	100
STAT	0.17	0.18	50	50
APOR	0.03	0.07	119	59
CRTR	0.27	0.08	50	50
NFKS	no barrier	no barrier	350 (total)	
SHOE	no barrier	no barrier	200 (total)	
CORN	no barrier	no barrier	228 (total)	
Mean±SE	**0.09±0.02**	**0.07±0.02**		

[1]Site abbreviations are explained in Table 1.1.

[2]Above-barrier and below-barrier population densities were estimated by calculating the total number of sampled brook trout divided by the sampled length* average bankfull width (m^2).

Table 1.3. Details regarding brook trout microsatellites screened (King et al. 2003, 2005).

Locus	Dye	Allele size range (bp)	GenBank Accession number
Master Mix1:			
Sfo-C88	FAM (blue)	170-205	AY168192
Sfo-C113	FAM (blue)	125-170	AY168193
Sfo-D75	NED (black)	165-250	AY168197
Sfo-D100	HEX (green)	200-275	AY168199
Master Mix2:			
Sfo-C24	FAM (blue)	110-190	AY168187
Sfo-C115	FAM (blue)	225-370	AY168194
Sfo-C129	HEX (green)	215-270	AY168195
Ssa-D237	HEX (green)	270-450	AF525207

Table 1.4. Brook trout population genetic diversity metrics for above and below barriers, including: total alleles, average number of alleles per locus (*A*), average gene diversity per locus (*H*), and average private alleles per locus.

Site[1]	Total alleles above	Total alleles below	*A* above	*A* below	*H* above	*H* below	Private alleles above[3]	Private alleles below[3]
Northern Lakes and Forests:								
WAUP	73	68	9.13	8.50	0.75	0.72	0.63 (5)	0.88 (7)
HAYM	88	79	11.0	9.88	0.79	0.78	1.75 (14)	1.00 (8)
SPBK	69	65	8.63	8.13	0.72	0.70	0.63 (5)	0.75 (6)
COLE	71	73	8.88	9.13	0.72	0.69	0.38 (3)	1.25 (10)
PRBR	61	60	7.63	7.50	0.71	0.68	0.38 (3)	0.88 (7)
MORG	57	53	7.13	6.63	0.66	0.65	0.50 (4)	0.38 (3)
Mean±SE	**70±4**	**66±4**	**8.73±0.55**	**8.30±0.47**	**0.73±0.02**	**0.70±0.02**	**0.71±0.21 (6±2)**	**0.86±0.12 (7±1)**
Blue Ridge:								
NFKS[2]	64	63	8.00	7.88	0.72	0.71	0.63 (5)	0.88 (7)
SHOE[2]	67	58	8.38	7.25	0.73	0.73	0.25 (2)	0.00 (0)
CORN[2]	48	45	6.00	5.63	0.70	0.72	0.38 (3)	0.25 (2)
BWLU	36	42	4.50	5.25	0.47	0.51	0.00 (0)	0.25 (2)
BWLL	58	67	7.25	8.38	0.69	0.73	0.25 (2)	0.63 (5)
SHCS	56	56	7.00	7.00	0.74	0.71	0.50 (4)	0.25 (2)
STAU	41	46	5.13	5.75	0.54	0.50	0.38 (3)	0.50 (4)
STMY	34	51	4.25	6.38	0.47	0.69	0.13 (1)	0.25 (2)
CBCR	36	34	4.50	4.25	0.53	0.52	0.25 (2)	0.25 (2)
WHRK	31	30	3.88	3.75	0.49	0.52	0.00 (0)	0.00 (0)
CASC	47	42	5.88	5.25	0.63	0.61	0.25 (2)	0.25 (2)
SASS	26	24	3.25	3.00	0.45	0.40	0.00 (0)	0.00 (0)
STAT	55	54	6.88	6.75	0.70	0.71	0.63 (5)	0.88 (7)
APOR	13	32	1.63	4.00	0.16	0.39	0.13 (1)	0.13 (1)
CRTR	39	58	4.88	7.25	0.57	0.69	0.00 (0)	0.50 (4)
Mean±SE	**43±4**	**47±3**	**5.43±0.48**	**5.85±0.41**	**0.57±0.04**	**0.61±0.03**	**0.25±0.06 (2±0)**	**0.33±0.07 (3±1)**

[1]Site abbreviations are explained in Table 1.1.

[2] The NFKS, SHOE, and CORN sites are the non-barrier sites, but equal sample distances were designated as the above and below sections.

[3] Private alleles above and below represent the average number of private alleles over all loci in a population (total number of private alleles in parenthesis).

Table 1.5. Private alleles by Level III Ecoregion and site, including the locus and location in relation to the barrier (above, below, or above and below). The CORN and NFKS sites are non-barrier sites.

Site[1]	Locus	Alleles
Blue Ridge:		
CBCR (above and below)	C115	260, 262
CORN (non-barrier)	D75	239
	C115	360
NKFS (non-barrier)	D75	256
SHCS (above and below)	D237	420
STAT (above)	C115	298
(above and below)	D237	490
STAU (above)	D237	474
Northern Lakes and Forests:		
HAYM (above)	C113	158
	C115	354
	C24	122
HAYM (above and below)	D237	484
SPBK (above)	D237	368
WAUP (above and below)	D237	398

[1]Site abbreviations are explained in Table 1.1.

Table 1.6. Brook trout population genetic differentiation including F_{ST} and R_{ST} (measure of population differentiation between above- and below-barrier samples for each site) and barrier characteristics, including height (m) and channel gradient (degrees).

Site[1]	F_{ST}	R_{ST}	Barrier height (m)	Channel gradient (degrees)
Northern Lakes and Forests:				
WAUP	0.001	0.004	0.9	8
HAYM	0.001	0.005	0.9	7
SPBK	0.012	0.002	1.5	65
COLE	0.015	0.001	4.3	75
PRBR	0.009	0.001	6.4	90
MORG	0.005	0.003	23.0	80
Blue Ridge:				
NFKS[2]	0.001	0.012	0.0	1
SHOE[2]	0.001	0.003	0.0	1
CORN[2]	0.027	0.040	0.0	1
BWLU	0.031	0.061	1.8	5
BWLL	0.013	0.011	2.1	90
SHCS	0.006	0.003	3.4	15
STAU	0.056	0.058	3.8	6
STMY	0.151	0.264	4.0	90
CBCR	0.026	0.048	7.6	80
WHRK	0.043	0.092	11.3	80
CASC	0.004	0.013	21.3	90
SASS	0.035	0.036	30.0	70
STAT	0.032	0.003	42.7	80
APOR	0.349	0.195	61.0	80
CRTR	0.068	0.077	91.4	80

[1]Site abbreviations are explained in Table 1.1.
[2]The NFKS, SHOE, and CORN sites are the non-barrier sites, but equal sample distances were designated as the above and below sections.

Table 1.7. The number of first generation migrants for each site estimated by the Geneclass2 (Piry et al. 2004), Rannala and Mountain (1997) criterion.

Blue Ridge:

Site[1]	Migrants above	Migrants below
APOR	1	3
BWLL	2	6
BWLU	8	5
CBCR	2	1
CASC	3	3
CORN	5	4
CRTR	0	7
NFKS	4	4
SASS	1	2
SHCS	6	3
SHOE	5	2
STAT	5	2
STAU	2	3
STMY	4	6
WHRK	2	1

Northern Lakes and Forests:

Site[1]	Migrants above	Migrants below
COLE	8	7
HAYM	9	8
MORG	6	4
PRBR	3	6
SPBK	4	4
WAUP	9	5

[1]Site abbreviations are explained in Table 1.1.

Table 1.8.a. Analysis of molecular variance comparing all above-barrier samples (group one) and below-barrier samples (group two) from the Northern Lakes and Forests Level III Ecoregion (12 samples), total 360 brook trout individuals.

Source of variation	df	Sum of squares	Variance components	% variation	P-value
Between groups	1	3.94	0.05	-1.47	0.97
Among samples within groups	10	202.8	0.29	.33	0.00
Among individuals within samples	348	1025	0.09	2.97	0.00
Within individuals	360	994	2.76	89.2	0.00
Total	719	2226	3.10		

Table 1.8.b. Analysis of molecular variance comparing all above-barrier samples (group one) and below-barrier samples (group two) from the Blue Ridge Level III Ecoregion (24 samples of 30 individuals per sample, except SHCS below sample of 27; three non-barrier sites removed), total 717 brook trout individuals.

Source of variation	df	Sum of squares	Variance components	% variation	P-value
Between groups	1	13.44	-0.09	-2.56	1.00
Among samples within groups	22	1652.85	1.22	36.2	0.00
Among individuals within samples	693	1631.57	0.12	3.50	0.00
Within individuals	717	1519.00	2.12	62.9	0.00
Total	1433	4816.86	3.37		

Table 1.8.c. Analysis of molecular variance comparing the combined above- and below-barrier samples for each site (60 individuals per site, except 57 individuals for the SHCS site) for all samples from the six Northern Lakes and Forests Level III Ecoregion sites (group one) and from 12 Blue Ridge Level III Ecoregion sites (group two; three non-barrier sites removed), total 1,077 brook trout individuals.

Source of variation	df	Sum of squares	Variance components	% variation	P-value
Between groups	1	302.26	0.2031	5.69	0.01
Among samples within groups	16	1718.07	0.8752	24.50	0.00
Among individuals within samples	1059	2811.41	0.1607	4.50	0.00
Within individuals	1077	2513.00	2.3333	65.32	0.00
Total	2153	7344.74	3.5724		

FIGURES

Figure 1.1. Study sites sampled in the Northern Lakes and Forests Level III Ecoregion (Bailey 2005) (six sites) (PRBR=Portage Brook, MORG=Morgan Falls, SPBK=Spring Brook, COLE=Cole Creek, HAYM=Haymeadow Creek, WAUP=Waupee Creek).

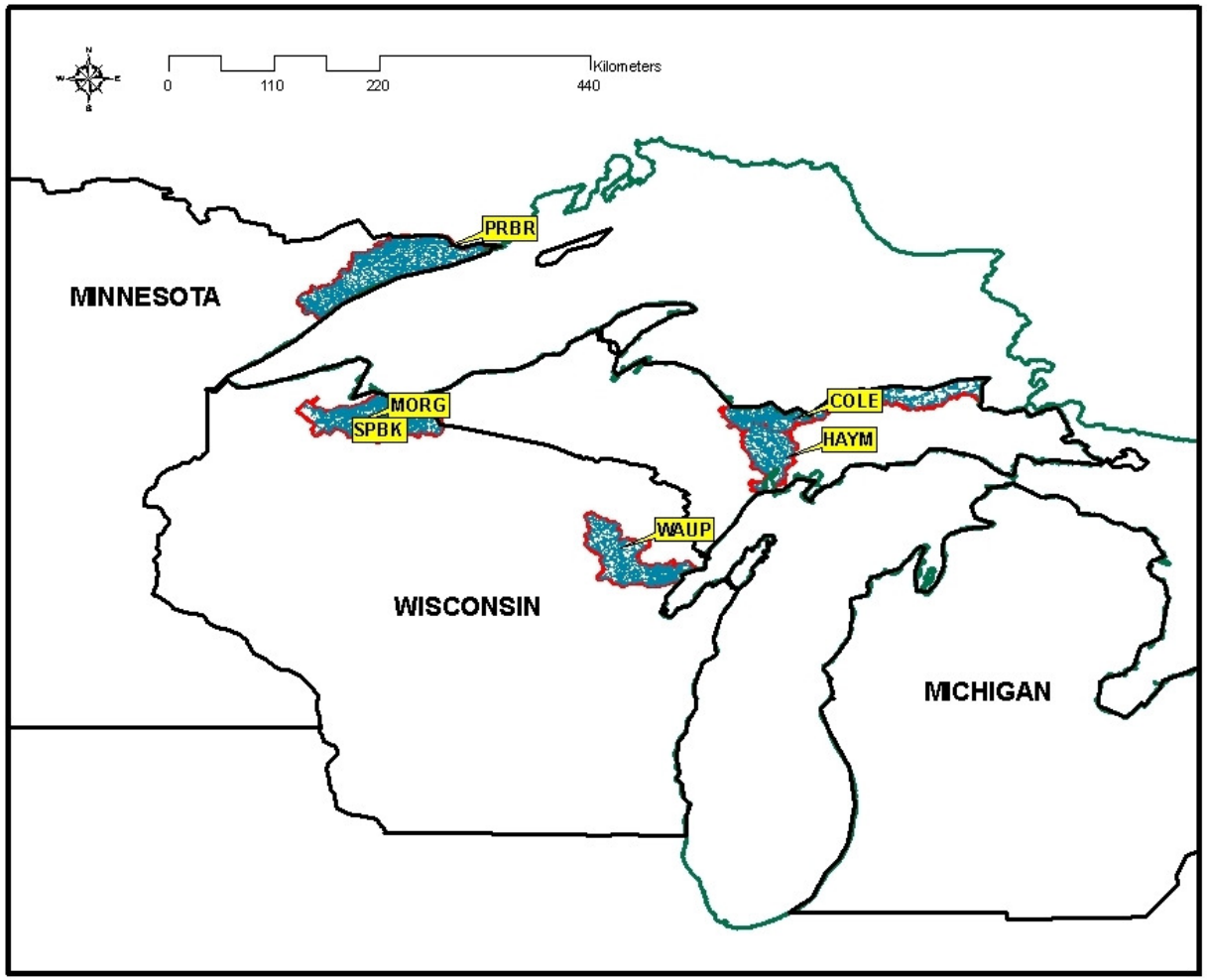


Figure 1.2. Study sites sampled in the Blue Ridge Level III Ecoregion (Bailey 2005) (15 sites) (SASS=Sassafras Falls; CBCR=Cabin Creek; BWLU=Big Wilson Creek, upper; BWLL=Big Wilson Creek, lower; NFKS=North Fork Stony Creek; CASC=Little Stony Creek, Cascades; APOR=Apple Orchard Falls; CORN=Cornelius Creek; SHCS=Shoe Creek Cascade; STAT=Staton's Creek Falls; SHOE=Shoe Creek; STMY=Saint Mary's Falls; WHRK=White Rock Falls; CRTR=Crabtree Falls; STAU=Staunton River).

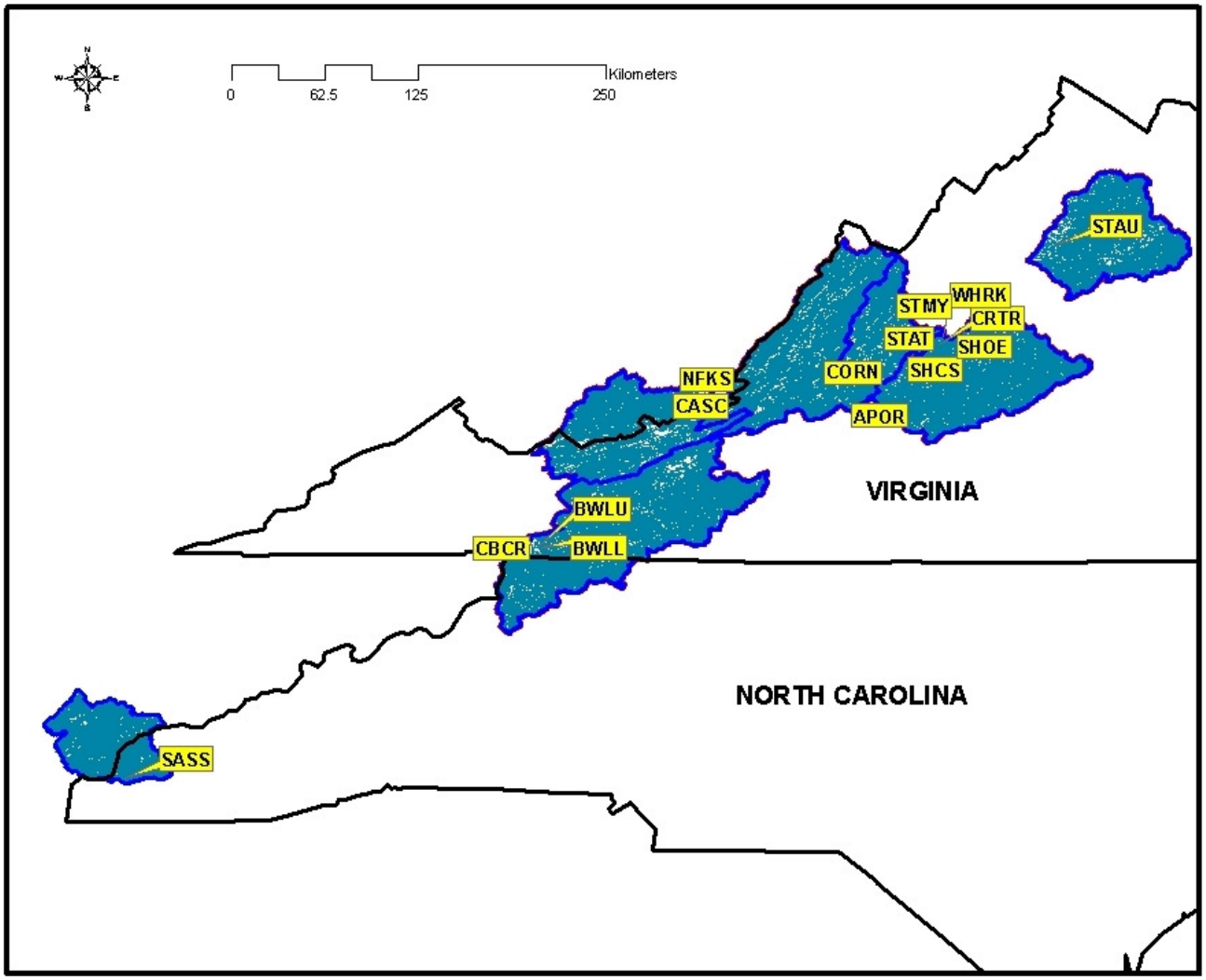
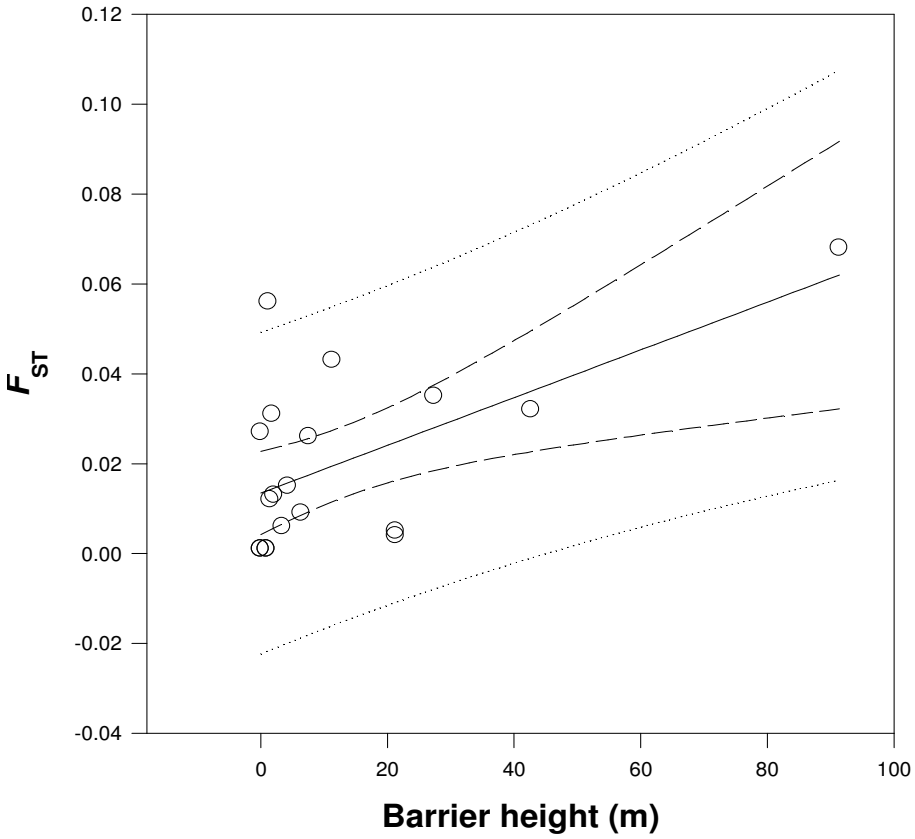


Figure 1.3. F_{ST} values in relation to barrier height with 99% confidence intervals (inner line) and 95% confidence intervals (outer line) for 16 sites (r^2=0.38, F=8.48, P=0.011). F_{ST} for the APOR site was removed due to a high percentage of relatedness among sampled individuals and F_{ST} for the STMY site was removed due to the population being out of linkage equilibrium. The three non-barrier sites were also removed from this analysis.



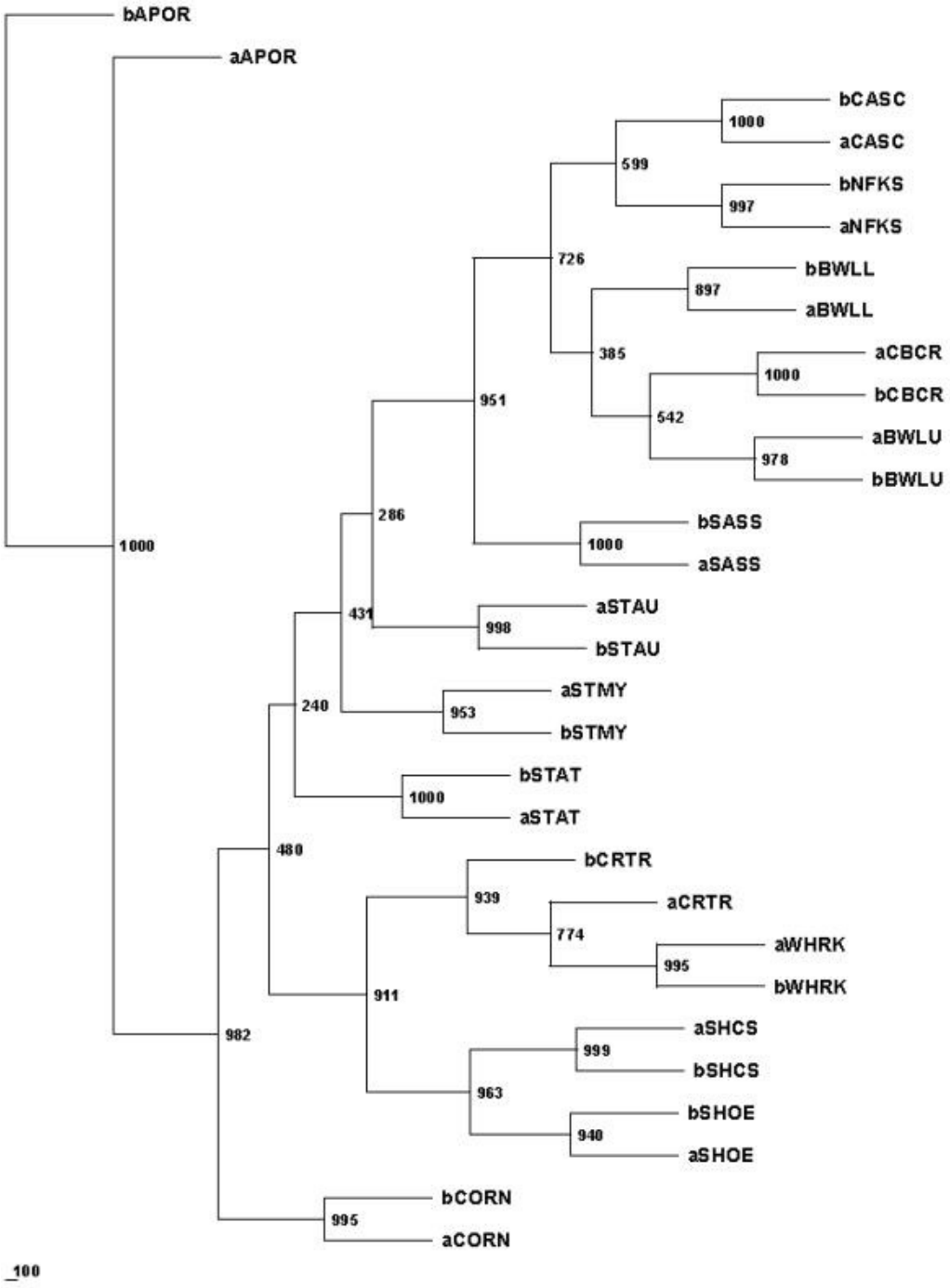


Figure 1.4. Unrooted neighbor-joining (NJ) consensus tree for 30 (15 above-barrier and 15 below-barrier) samples of brook trout in the Blue Ridge Level III Ecoregion. Sample labels with "a" represent above-barrier samples and sample labels with "b" represent below-barrier samples. This consensus tree is based on Cavalli-Sforza and Edwards (1967) chord distance (Dcc) based on allelic variation at eight microsatellite loci. Bootstrap values for 1,000 replicate NJ trees to compile this consensus tree are shown. The unit _100 represents the genetic distance (how genetically different) sites are from each other, with the greater the length of the line, the greater the genetic distance. Site abbreviations are explained in Table 1.1.

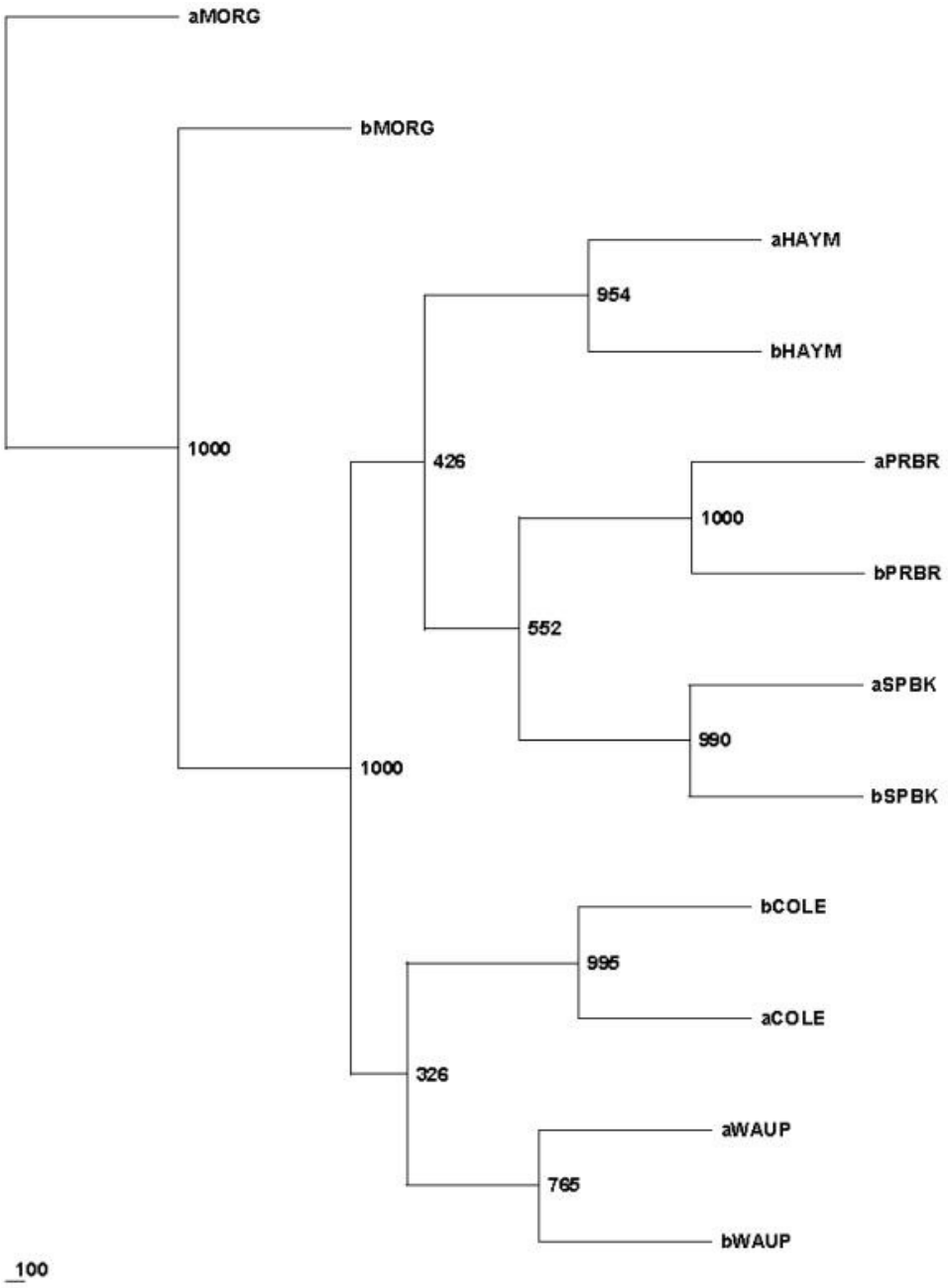
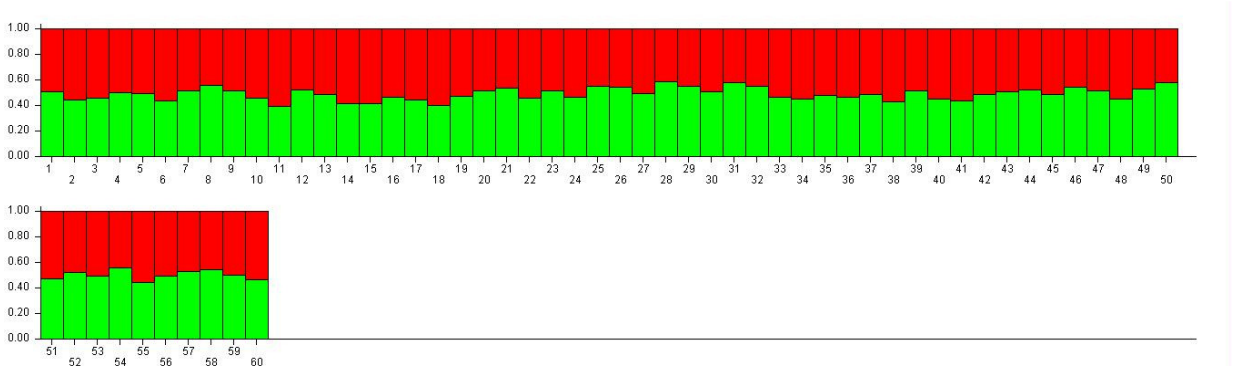


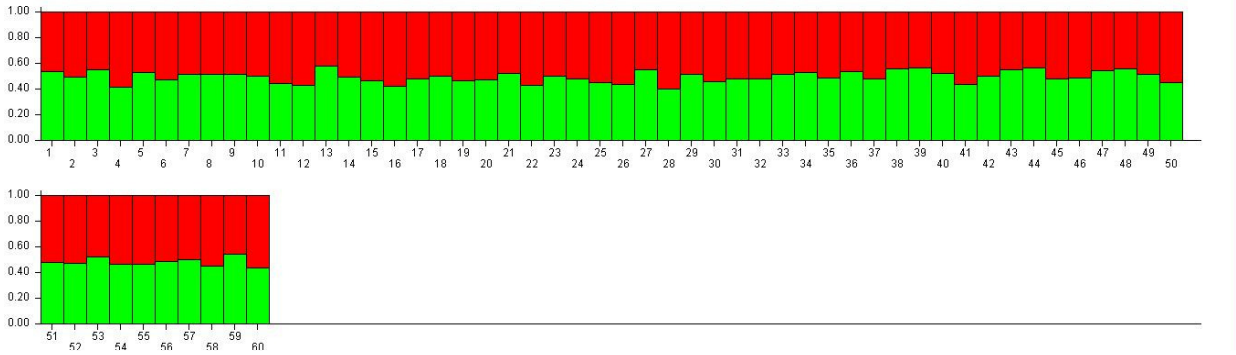
Figure 1.5. Unrooted neighbor-joining (NJ) consensus tree for 12 (six above-barrier and six below-barrier) samples of brook trout in the Northern Lakes and Forests Level III Ecoregion. Sample labels with "a" represent above-barrier samples and sample labels with "b" represent below-barrier samples. This consensus tree is based on Cavalli-Sforza and Edwards (1967) chord distance (Dcc) based on allelic variation at eight microsatellite loci. Bootstrap values for 1,000 replicate NJ trees to compile this consensus tree are shown. The unit _100 represents the genetic distance (how genetically different) sites are from each other, with the greater the length of the line, the greater the genetic distance. Site abbreviations are explained in Table 1.1.

Figure 1.6. Structure 2.3.1. (Pritchard et al. 2000) assignment-test summary plot estimates of Q for individuals at each site. Each numbered individual is represented by a vertical bar, with height proportional to the likelihood (Q) that the individual belongs to each of two inferred clusters (one above and one below). Individuals 1-30 were sampled from the above-barrier sample (30 individuals) of each site (green color) and individuals 31-60 were sampled from the below-barrier sample (30 individuals) of each site (red color). These bar plots suggest a possible threshold of barrier effects at approximately 4 meters.

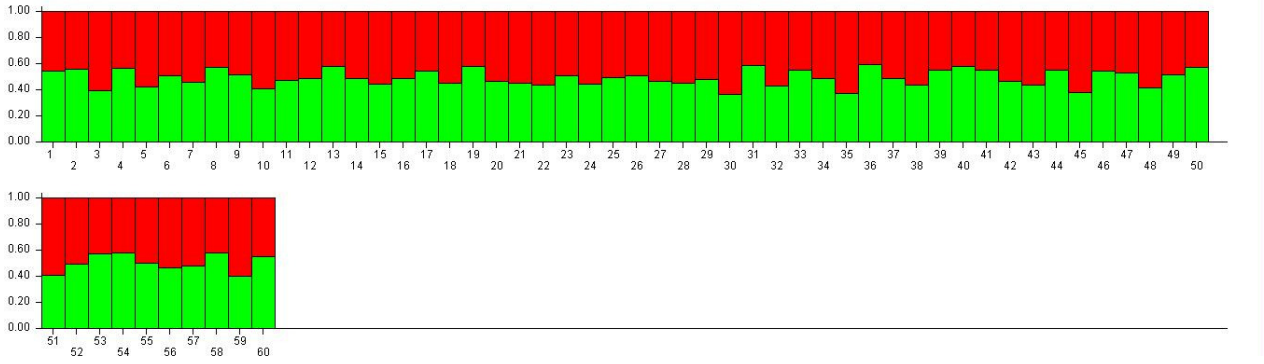
North Fork Stony Creek – barrier height 0 meter



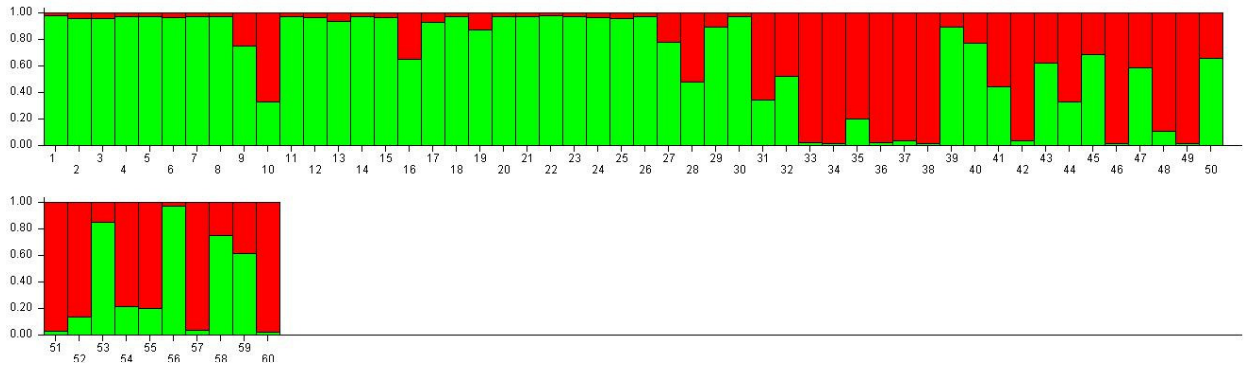
Haymeadow Creek – barrier height 0.9 meter



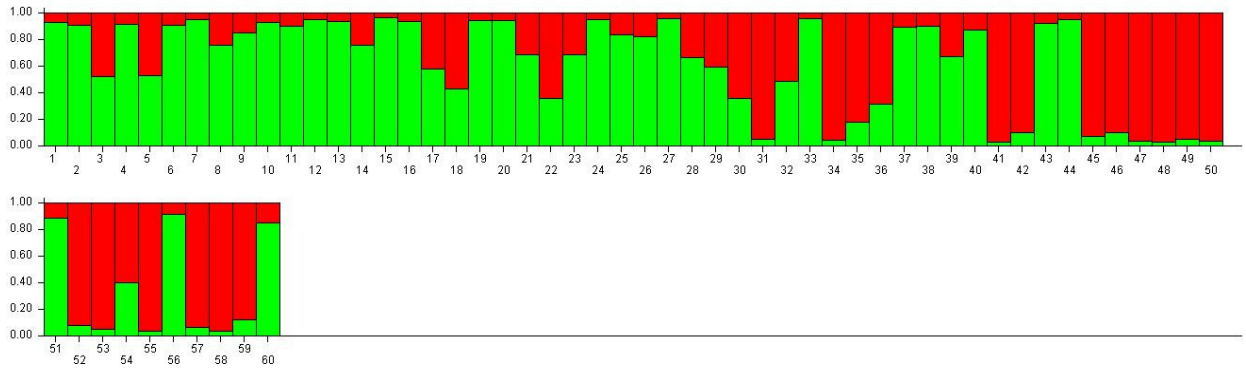
Waupee Rapids – barrier height 0.9 meter



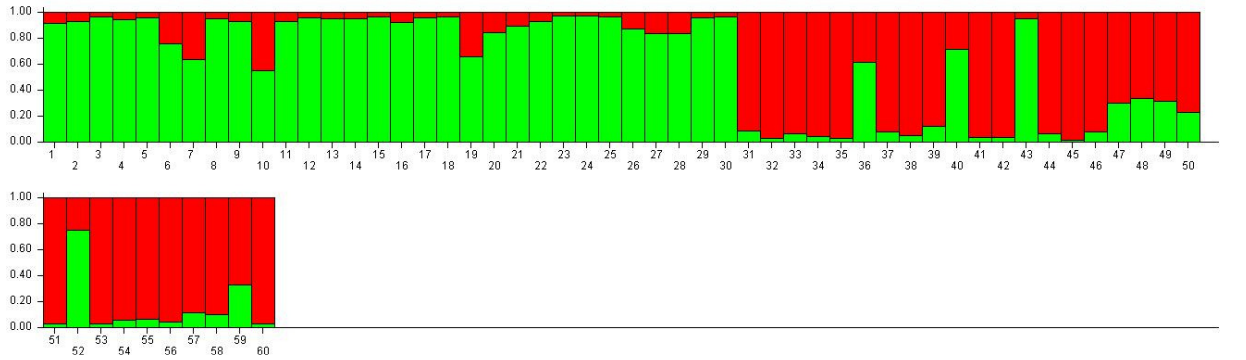
Saint Mary's Falls – barrier height 4.0 meters



White Rock Falls – barrier height 11.3 meters



Crabtree Falls – barrier height 91.4 meters



CHAPTER 2. Effects of habitat fragmentation on effective population size of resident brook trout populations of the southern Appalachian mountains.

Introduction

Stream habitat fragmentation is among the top threats to aquatic populations (Gosset et al. 2006). Factors that may contribute to stream habitat fragmentation by reducing habitat quality and connectivity (Sheer and Steel 2006; Hudy et al. 2008) include barriers to fish movement, sediment from roads, and urbanization (Brasch et al. 1958; Kelly et al. 1980; Warren and Pardew 1998; Johnson and Jones 2000; Curry and MacNeil 2004). Stream habitat fragmentation will vary spatially depending on the species and its functional needs for breeding and foraging. Fish species are known to disperse different distances to access foraging and breeding habitats. For example, previous research documented the blue shiner *Cyprinella caerulea* dispersing 332 meters (Johnston 2000), the leopard darter *Percina panthreina* 200 meters, and the brook trout *Salvelinus fontinalis* 6.6 kilometers (Flick and Webster 1975). In addition, life history strategies of a species may have different habitat needs, with the possibility of fragmentation affecting habitat types differently. For example, the spring-run Chinook salmon *Oncorhynchus tshawytscha* spawn in high-gradient streams, while the fall-run Chinook spawn in low-gradient streams (Salo 1991; Sandercock 1991; Healey 1991).

This study investigated the relationship between habitat fragmentation and effective population size (N_e). The N_e concept was defined by Wright (1931) and represents the "ideal" population size with the same rate of genetic change as the true population being researched. The N_e value of a population has potential be used as an indicator of population genetic change due to breeding success. Breeding success is likely to be reduced in salmonid populations as breeding habitat becomes more fragmented due to reduced habitat quality or inaccessibility. This relationship between breeding habitat fragmentation and a reducing breeding success has been documented in brook trout, brown trout *Salmo trutta*, and Chinook salmon (Raleigh 1982; Curry and MacNeill 2004; Shrimpton and Heath 2003; Gosset et al. 2006).

Chapter 1 of this dissertation identified population structuring of brook trout at the

subwatershed scale based on clusters identified in the unrooted NJ consensus tree of Cavalli-Sforza and Edwards (1967) chord distances, so focusing on fragmentation at the subwatershed scale was appropriate for this study that was investigating effects of fragmentation on brook trout N_e. The subwatershed scale is also considered the spatial level most relevant to management for fish conservation (4,100 to 12,400 hectares for subwatersheds in this study) (Seaber et al. 1987; Fausch et al. 2002) and N_e has been used as an important criteria for genetic conservation in salmonids (Waples 1990; Rieman and Allendorf 2001).

Historically, fisheries work, including issues related to stream fragmentation, has focused on populations and in-stream habitat at the reach scale (Frissell et al. 1986; Roper et al. 1997; Bohn and Kershner 2002). However, further investigation and restoration initiatives relevant to fish populations have acknowledged that management of fragmentation, such as restoring fish passage, at the watershed scale is more likely to be successful (Reeves et al. 1995; Roper et al. 1997; Bohn and Kershner 2002). Species have various habitat units at different spatial scales and different habitat units are needed for different functions and life history strategies of a species (Lord and Norton 1990; Fahrig and Merriam 1994). Habitat fragmentation can impact the ability for dispersal between habitat units by different population subunits. If population subunits at the stream reach habitat unit scale, for example, remain interconnected through dispersal due to restored fish passage, they can form an interconnected subwatershed scale population (Wiens 1989; Harrison et al. 1988; Dunham and Rieman 1999).

Brook trout population genetic data from Chapter 1 also identified effects of fragmentation from natural barriers at the stream reach scale, as high differentiation was identified between the above- and below-barrier samples for the APOR, STMY, CRTR, and MORG sites. This was evident from the high F_{ST} and R_{ST} values for the APOR (F_{ST}: 0.349, R_{ST}: 0.195) and STMY (F_{ST}: 0.151, R_{ST}: 0.264) sites and from the structuring within the NJ consensus trees that showed the above sample on a different branch from the below sample for the APOR, CRTR, and MORG sites (Figure 1.4 and 1.5). These samples were collected directly above and below a natural barrier site, so this was a comparison at the stream reach scale. Previous studies

have investigated habitat fragmentation effects on N_e and have attempted to identify and separate out sources of variation having an effect at different spatial scales (Dunham et al. 1997; Nielsen et al. 2009). This study offered a unique opportunity to focus on natural barrier effects on N_e at the stream reach scale because all samples were collected adjacent to natural barriers from specific stream reaches. Investigated variables at the stream reach scale included barrier height (m) and total stream habitat (km) potentially isolated above each barrier location. Investigated variables at the subwatershed scale included road density (km road/km^2 land) and percent forested land use within each subwatershed. This introduction provides information on stream habitat fragmentation in general and a literature summary of the factors that contribute to salmonid stream habitat fragmentation at the stream reach and subwatershed scales. I then describe the relationship between stream habitat fragmentation and salmonid N_e and other population genetic factors, outside of habitat fragmentation that are known to affect N_e.

This study presents estimates of N_e from brook trout collected from a unique stream reach within a unique subwatershed. This data is based on an estimate of N_e from a sample collected at the stream scale that I have used as a representative sample of N_e from a brook trout population adjacent to a natural barrier for each sampled subwatershed. Keep in mind, this is one estimate from one sample from one point in time and further investigation would be required to compile estimates from more samples throughout an entire subwatershed and from samples from more than one year over time.

Stream habitat fragmentation. - Stream habitat fragmentation can be described as loss of connectivity between different physical habitat units or different habitat functional components, such as breeding and foraging habitat (Poplar-Jeffers et al. 2009). Fragmentation of stream habitats results in reduced habitat complexity which increases risk of population extinction due to increased vulnerability to stochastic events. This is especially the case for small, isolated populations, such as those isolated above barriers to upstream dispersal (Rieman and McIntryre 1995; Lande 1998; Morita and Yamamoto 2002; Letcher et al. 2007). Fragmentation may result due to a loss of accessibility to stream habitat units or due to habitat units becoming less useable

for functions such as breeding and foraging. Stream habitat fragmentation results in a loss of connectivity between populations that may contain unique genetic components or life history strategies for a species.

Stream habitat fragmentation will vary spatially depending on the species, its functional needs for breeding and foraging, and dispersal distances between habitat areas that are used for different functions. Some species may require further migration distances than others as habitat continues to become fragmented (Sheer and Steel 2006). For salmonids, Bryant and Woodsmith (2009) discuss how abundance is influenced by a combination of physical habitat components that function at a variety of spatial scales (Nickelson et al. 1992; Rosenfeld et al. 2000).

Frissell et al. (1986) defined a stream classification system that emphasizes the stream's relationship to its watershed. The classification is hierarchical with the smallest habitat component being microhabitat and the largest habitat component being the stream system at the watershed scale. Smaller habitat components are nested within larger habitat components and processes that affect the larger-scale components will also affect smaller-scale components. Therefore, if habitat fragmentation is occurring at the watershed scale, there will also be affects at the smaller, stream reach scale.

Barriers and salmonids. - The presence of barriers to fish movement within streams is a significant source of habitat fragmentation at the stream reach scale that also has the potential to affect distributions of fish populations at the landscape scale (Gibson et al. 2005; Gosset et al. 2006; Sheer and Steel 2006; Poplar-Jeffers et al. 2009). This document will refer to a "barrier" meaning a barrier to upstream fish movement. Barriers to upstream dispersal result in fragmentation when stream habitats above the barrier become inaccessible or unusable for feeding, reproduction, and predator avoidance (Angermeier and Schlosser 1989; Warren and Pardew 1998; Albanese et al. 2004; Knaepkens et al. 2004).

Natural barriers occur on the landscape and fragment stream reaches directly above and below the natural barrier. Additionally, their presence has historically driven salmonid population structure by limiting upstream dispersal and recolonization in relation to glacial

activity and presence of glacial refugia (Poissant et al. 2005; Gomez-Uchida et al. 2009). All "barriers" in this study are natural barriers, such as waterfalls, vertical drops, and cascades. A barrier to upstream movement can result in an upstream barrier to gene flow, which may result in genetic isolation, increased effects of genetic drift, and reduced N_e above the barrier. A barrier to upstream dispersal for breeding adults will affect N_e when access to potential breeding habitat above the barrier is not possible (Rieman and Allendorf 2001; Shrimpton and Heath 2003).

Roads and stream habitat fragmentation. - Roads and transportation corridors cover more surface area than any other land use in the United States, with an estimated 19% of the total area affected ecologically due to effects of roads and traffic on species, soil, and water interactions (Forman 2000). The distribution of roads within subwatersheds can modify riparian habitat dynamics and disturbance severity, which in turn can fragment stream habitats and negatively affect associated species (Jones et al. 2000). During road construction, fine sediment may enter stream channels and channelization of the stream channel may occur. Disturbance of the stream bed and channelization may fragment spawning habitat and reduce overall aquatic habitat quality. Runoff at stream crossings also can result in sediment inputs. Too much fine sediment can decrease densities of fish populations by fragmenting spawning habitat, reducing spawning success, and by limiting food availability due to sediment impacts on macroinvertebrates (Wood and Armitage 1997; Wheeler et al. 2005). Dunham and Rieman (1999) documented a significant negative relationship between road density at the subwatershed scale and the occurrence of bull trout.

Land use and stream habitat fragmentation. - Urbanization and land use changes are drivers at the watershed scale that can fragment stream habitats for fish by changing temperature, flow, and sedimentation dynamics within stream networks. Urban land uses in the United States are projected to increase from 39.5 million hectares in 1997 to 70.5 million hectares by 2025, which represents a 79% increase (Alig et al. 2004). Previous research on urban land use effects showed that urbanization levels between 8-12% of land area within a watershed resulted in changes to base flow, reductions in fish density and species richness, and increases in species

tolerant to pollution (Wang et al. 1997, 2000, and 2001). Wang et al. (2003) investigated urbanization effects on fish assemblages and found that the area of impervious surface within a watershed was negatively correlated with a coldwater fish biotic index.

Previous research showed that increased percent watershed impervious area was correlated with increased runoff and changes to thermal regimes (Paul and Meyer 2001; Wang et al. 2003). Loss of riparian vegetation due to urbanization affected base flows and reduced shading, which in turn increased stream water temperature (Lyons et al. 1996; Wang et al. 2003). Increases of 1% impervious surface within a watershed resulted in an increase in 0.25 ºC in stream temperature in the midwestern United States, and research in urbanizing watersheds of Long Island, New York documented increased summer stream temperature of 5-8 ºC (Pluhowski 1970).

Aquatic species found in streams have optimal temperature ranges and distributions of these species are based on appropriate temperature regimes of stream channels. For example, brook trout are found in streams that do not exceed 24 ºC (MacCrimmon and Campbell 1969; Meisner 1990; Raleigh 1982). Functioning brook trout redds, which are necessary for successful breeding, have been associated with temperatures between 6 and 8 ºC, groundwater inputs (Witzel and MacCrimmon 1983). Hudy et al. (2008) correlated the presence of successfully reproducing populations of brook trout with 68% or more forested land use. In addition, Stranko et al. (2008) rarely found brook trout in watersheds with urbanized land use exceeding 4%. Declines in brook trout populations also have been related to reductions in in-stream habitat quality and increased siltation of breeding habitat (Raleigh 1982; Curry and MacNeill 2004).

Stream habitat fragmentation and effective population size. - Previous study has showed that the probability of persistence of a given population within fragmented stream habitats was related to the habitat area available and proximity to connected adjacent populations, which were factors that affected N_e (Rieman and McIntyre 1995; Rieman and Allendorf 2001). Additionally, Shrimpton and Heath (2003) showed a significant correlation between N_e and availability of spawning habitat. Shrimpton and Heath (2003) documented a significant relationship between

the N_e of five populations of stream Chinook salmon and spawning habitat (r^2=0.88, $P < 0.05$). As spawning habitats are fragmented, N_e will be affected due to reductions in breeding success and potential genetic isolation of populations above barriers.

Previous research investigated the effects of stream habitat fragmentation due to upstream barriers to fish movement on effective population size of salmonid species as well. For example, researchers in Japan documented isolation of white-spotted charr *Salvelinus leucomaenis* populations above dams, resulting in N_e compared to populations below dams (Morita et al. 2000; Maekawa et al. 2001; Morita and Yamamoto 2002; Yamamoto et al. 2004). Lower N_e resulted in increased genetic drift and decrease in allele diversity over time, as the dam prevented gene flow among adjacent populations. As discussed in Chapter 1 of this dissertation, a loss of allelic diversity is often considered a loss of adaptive potential resulting from reduced growth, survival, or fecundity (Quattro and Vrijenhoek 1989; Virijenhoek 1994; Morita and Yamamoto 2002).

Rieman and Allendorf (2001) further investigated the relationship between fragmentation and reduction in N_e for the bull trout *Salvelinus confluentus*. Management recommendations from this study suggested that small, isolated populations with limited numbers of spawners as a result of fragmentation should be considered at risk and targets for management. Improving connectivity and opportunities for gene flow may mitigate risk in this case.

Population genetic factors affecting effective population size

In addition to environmental factors, such as habitat fragmentation, demographic and population genetic factors also affect N_e. The N_e concept can be described as a rate of change due to variance (variance effective size) or inbreeding (inbreeding effective size) (Wright 1931; Waples 2005). In this case mutation and selection are considered minimal, so the population genetic factors to consider that affect N_e in this case are genetic drift, inbreeding, and gene flow.

Genetic drift. - The definition of variance effective size is based on the assumptions that the effects of natural selection and mutation are minimal and that genetic drift is the primary source of allele frequency change (Wright 1969; Crow and Kimura 1970). Genetic drift is the

random change in allele frequencies that occurs in each generation in a finite population. Effective population sizes that are too low will result in an increase in genetic drift and a continued decrease in allelic richness over time (Mills and Allendorf 1996).

Inbreeding. - Inbreeding effective size refers to the increase of homozygosity per generation within a finite population (Wright 1931; Wang 2005). Inbreeding due to mating of related individuals also can result in a loss of genetic diversity, as the frequency of homozygote genotypes increases to more than expected and the frequency of heterozygote genotypes decreases to less than expected according to the HWE model. According to the HWE model, the expected frequency of heterozygotes at one locus in relation to inbreeding is estimated as: $H_e = 2pq(1\text{-}F)$, where p and q are allele frequencies and F is the decrease in heterozygotes due to inbreeding. F decreases each generation by ½ N_e (inbreeding effective size) of a population (Crow and Kimura 1970).

Gene flow. - As the N_e of a population decreases below the census population size (N), the population loses genetic variation due to genetic drift, and a greater level of gene flow is needed to limit divergence among populations (Frankham 1995; Mills and Allendorf 1996). Wright (1931) determined that under the conditions of migration-drift equilibrium, gene flow (m) will overcome the effects of genetic drift when $m = 1 / 4\ N_e$. Wang and Whitlock (2003) identified a log-linear relationship between gene flow and population size in relation to N_e (r=0.56, $P < 0.00$); they found that migration rate must be higher in smaller populations to maintain N_e and that continuous gene flow can counteract the effects of genetic drift. Increases in gene flow can also counteract effects of inbreeding (Mills and Allendorf 1996; Vila et al. 2003).

Further information needed

Effective population sizes in brook trout. - Few studies have been conducted to estimate effective population sizes of brook trout. The study offers further estimates of N_e from wild brook trout populations in streams, which is valuable for range-wide conservation planning for the species. Kincaid (1995) is the only published study that documents known N_e for the brook trout species in the laboratory and Letcher et al. (2007) is the only study that documents N_e for

brook trout in wild stream populations. Kincaid (1995) estimated N_e for brook trout hatchery stocks from 1988 to 1991. Resulting N_e values ranged from 191 to 332, with an average of 276 fish. Letcher et al. (2007) estimated generation times and N_e for 16 samples of 2001-2003 wild brook trout samples from a western Massachusetts watershed. The brook trout samples from three different years were 834 (2001), 719 (2002), and 971 (2003) total individuals. This study estimated a mean generation time of 1.91 years and N_e estimates of 91.9 (95% C.I. 69.6-125.5) for an isolated tributary, 29.3 (95% C.I. 25.2-33.3) for a small open system, and 113.1 (95% C.I. 93.1-140.7) for a large open system (Letcher et al. 2007). Effective population size estimates for bull trout ranged from 50 to 150 fish based on simulation models (Rieman and Allendorf 2001).

Barriers to fish movement result in reduced upstream dispersal, which can result in genetically isolated populations that are at risk due to inbreeding (Slatkin 1985; Wofford et al. 2005). As N_e decreases, the rate of inbreeding accumulation per generation increases (Kincaid 1995). Hence, Kincaid (1983) recommended a minimum N_e of 100 per generation in hatchery stocks, which predicts 0.50% inbreeding accumulation per generation. A minimum N_e of 200 per generation predicts 0.25% inbreeding accumulation per generation for wild stocks (Allendorf and Ryman 1987). This study offers an opportunity to estimate N_e in wild brook trout populations associated with natural barriers. These estimates can be compared to the Allendorf and Ryman (1987) minimum 200 N_e recommendation and combined with any evidence of inbreeding to identify potential genetic isolation in barrier-associated populations.

Application of one-sample N_e estimation methods. - Two N_e estimation approaches based on microsatellite multilocus genotype data, are advantageous in that they do not require extensive demographic data for a sampled population. There are temporal methods that require samples in more than one generation and one-sample methods based on quantifying linkage disequilibrium (LD). Temporal methods are based on the difference in allele frequencies between samples collected among two generations (Nei and Tajima 1981; Waples 1989). Temporal estimates of N_e are dependent on samples collected in generation one and generation t at a later time and on allele frequency changes between generation one and generation t.

One-sample estimates are not estimates of N_e in the current generation, but estimate N_e of the sampled individuals of the previous generation (Waples 2005). The only software packages available for one-sample N_e estimation using the linkage disequilibrium method are LDNE (Waples and Do 2008) and OneSamp (Tallmon et al. 2008). The LDNE software package offers more versatility in analysis and produces results that are instantly comparable to the results of OneSamp. This study investigates the usefulness of applying the LD method to one sample from one generation to estimate N_e and look for relationships to habitat fragmentation effects.

Further information is needed for long-term conservation planning on habitat fragmentation factors at different spatial scales that affect N_e in wild populations of brook trout. Further information on applying estimates of N_e for making conservation genetic decisions would also be valuable. Recently developed methods need only one sample of microsatellite multilocus genotype data to estimate N_e by applying the LD method (Tallmon et al. 2008; Waples and Do 2008). This study applies the LD method using one sample per site to investigate effects of land use, sediment from roads, and barriers to fish movement on wild brook trout N_e.

Goal and hypothesis to be tested. - Hence, the goal of this study was to determine significant habitat fragmentation factors affecting N_e of resident Southern Appalachian brook trout populations associated with natural barriers. The intent of this study was to investigate effects of the road density and percent forest cover subwatershed variables on N_e estimates for brook trout populations associated with natural barriers at one location within each subwatershed. The objective was to test the hypothesis that barrier height and total stream habitat potentially isolated above the barrier were significant correlates of brook trout N_e at the stream reach scale. I also tested the hypothesis that road density and percent forest cover within a subwatershed were significant habitat fragmentation correlates of brook trout N_e. Estimated N_e values for sampled populations associated with natural barriers and gene flow between above- and below-barrier samples were associated with habitat fragmentation factors to identify populations at risk of losing genetic diversity.

Methods

Study areas. - I selected one natural barrier in each of ten subwatersheds (USGS 6th-level Hydrologic Units) of the Blue Ridge (Virginia and North Carolina), abbreviated as BR, (Figure 2.1, Table 2.1) Level III Ecoregion that were at least 68% forested (Bailey 2005; Hudy et al. 2008). Hudy et al. (2008) correlated the presence of successfully reproducing populations of brook trout with 68% or more forested land use within the associated subwatershed. Selected streams had similar physiogeographic characteristics found within the BR Level III Ecoregion, including high-gradient cold-water streams, forested slopes, and rugged terrain over metamorphic rock (Bailey 2005). There were differences in population genetic diversity identified according to Level III Ecoregion in Chapter 1 of this dissertation, so I only selected sites within the BR Level III Ecoregion to eliminate a source of variability for the purposes of this investigation.

The historic range of brook trout extends from the headwaters of the Mississippi River in Minnesota, to coastal drainages from Maine to Virginia, and south to the headwaters of the Chattahoochee River in northern Georgia (MacCrimmon and Cambell 1969). The BR Level III Ecoregion is an ecoregion within the native range of the brook trout that still has self-reproducing populations remaining. This ecoregion had available locations with sufficient brook trout population densities to collect at least 30 juveniles and adults above and below each natural barrier. A sample of 30 individuals was considered a minimum for reliably estimating genetic diversity of populations (McCracken et al. 1993; Rogers and Curry 2004).

I used ArcMap, Version 9.2 (ESRI, Inc.) and USGS National Hydrological Dataset, 1:24,000 high resolution data layers to subdivide 5th level, 10-digit hydrologic unit code watersheds into 6th level, 12-digit hydrologic unit code subwatersheds because this is the smallest watershed size with compiled data available on brook trout populations. This is also the spatial level most relevant to management for fish conservation (4,100 to 12,700 hectares for subwatersheds in this study) (Seaber et al. 1987; Fausch et al. 2002).

For selected subwatersheds, percent forested land use ranged from 81% to 100%, and

road density ranged from 0.3 to 2.2 km roads /km^2 of land. Percent forested land per subwatershed was calculated based on a compilation of forest type GIS data from the USGS National Land Cover Dataset and National Hydrography datasets; and road density was calculated based on Topological Integrated Geographic Encoding and Referencing System GIS data (1:24,000 where available for both datasets) (Hudy et al. 2008).

Selected natural barrier sites represented a broad range of barrier heights and channel gradients. Natural barriers to movement of brook trout were at least 73.5 cm maximum jumping height at baseflow, which was considered a barrier for 8.6 – 34.0 cm TL brook trout with a 0.40 cm minimum pool depth below the waterfall at baseflow by Kondratieff and Myrick (2006).

Study site measurements. - I measured the total stream length sampled above and below each barrier site (Table 1.2). I also estimated total stream length above the barrier (km) using the ArcMap, Version 9.2 (ESRI, Inc.) measure tool feature on USGS National Hydrological Dataset, 1:24,000 high resolution digital line graph hydrography files at the 1: 6500 scale (Table 2.3).

Barrier height was measured in the field using a standard rod and level, or was estimated by subtracting the difference in elevation between GPS points recorded directly above the barrier and directly below the barrier based on Google Earth high resolution satellite imagery of 1000 pixels if the barrier height was taller than the standard rod (~10 meters). Channel gradient was estimated as arctan (degrees) from the lowest to highest point of each barrier (Table 2.1). Stream-reach sample distances ranged from 50 meters to 150 meters, as sampling was completed at a given site once a sample of 30 brook trout fin-clips was acquired.

Brook trout sampling. - I used single-pass electrofishing to collect brook trout at each site. I anesthetized fish with clove oil (Taylor and Roberts 1999). I measured total length and weight, and clipped a ~50 mg piece of fin from each trout (Rogers and Curry 2004). Fin-clips were stored and preserved in separate vials with 95% ethanol. I calculated brook trout sample densities above and below each barrier by calculating the total number of sampled brook trout divided by the total area sampled (m^2) (sampled length*average bankfull width; Table 1.2).

Microsatellite markers and genotyping. - DNA extraction, polymerase chain reaction

(PCR), and genotyping protocols were developed at the United States Geological Survey, Conte Anadromous Fish Laboratory (Turner's Falls, Massachusetts). I extracted DNA from 1,257 fin-clips and PCR-amplified microsatellite-bearing fragments at eight loci (Table 2.4) using an MJ DNA Engine Dyad PTC-220 thermocycler. Microsatellite loci were multiplexed (Henegariu et al. 1997, Table 2.4) for cost-effective genotyping using an Applied Biosystems, Inc. ABI 3100-Avant Autoanalyzer and GeneMapper software. I scored genotypes using Peak Scanner v1.0 (Applied Biosystems, Inc).

Estimate of N_e using linkage disequilibrium method. - Linkage disequilibrium is the non-random association of alleles at two or more loci (Slatkin 2008). LDNE is a recently-developed linkage disequilibrium-based program that calculates a correlation coefficient for each pair of alleles at each pair of loci using the Burrow's Δ method and applies Weir's (1979) unbiased estimator of Δ, $\Delta = \Delta S / (S\text{-}1)$, where S adjusts for the sample size. This estimator becomes the numerator of the following equation that estimates the correlation coefficient (r_Δ) for each pair of loci, which is the basis for estimating N_e:

$$\hat{r}_\Delta = \frac{\hat{\Delta}}{\sqrt{[\hat{p}(1-\hat{p}) + (h_i - \hat{p}^2)][\hat{q}(1-\hat{q}) + (h_j - \hat{q}^2)]}}, \qquad \text{(eqn 1)}$$

(Weir 1996; Waples 2006; Waples and Do 2008). This equation compares the frequency of allele A at locus i with that of allele B at locus j, where h_i is the observed frequency of AA homozygotes, p hat is the sample frequency of allele A, h_j is the observed frequency of BB homozygotes, and q hat is the sample frequency of allele B (Waples and Do 2008). For each pair of loci i and j, with k_i and k_j alleles, r^2 hat Δ is calculated using equation 1 for each $k_i k_j$ allele combination. The mean r^2 hat $\Delta_{i,j}$ is calculated for each pair of loci, and this value is considered the weighted mean of pairwise r^2 hat $\Delta_{i,j}$ values. This overall weighted mean and the effective sample size are used to estimate N_e using the Hill (1981) formula for the coefficient of variation of N_e as modified by Waples (2006).

England et al. (2006) identified a bias in the N_e estimates using LD methods developed

by Waples (2006) for sample sizes lower than the true N_e of a population. Waples and Do (2008) subsequently developed an empirical bias correction that adjusts for sample size and incorporated this correction into the program LDNE, as illustrated by the equations below for random mating and monogamy (Waples and Do 2008):

Random mating	S ≥ 30	S < 30
$\hat{N}_e$	$\frac{1/3+\sqrt{1/9-2.76\hat{r}^2}}{2\hat{r}^2}$	$\frac{0.308+\sqrt{0.308^2-2.08\hat{r}^2}}{2\hat{r}^2}$
Monogamy	**S ≥ 30**	**S < 30**
$\hat{N}_e$	$\frac{2/3+\sqrt{4/9-7.2\hat{r}^2}}{2\hat{r}^2}$	$\frac{0.618+\sqrt{0.618^2-5.24\hat{r}^2}}{2\hat{r}^2}$

LDNE applies minimum allele frequency values and eliminates allele frequencies that are less than the critical *P*-value options, 0.05, 0.02, and 0.01. Confidence interval options include 95% parametric and jackknife options. The jackknife option performs as well or better when estimating known-population N_e values. The most precise estimates of N_e using LDNE incorporate the $P_{crit} < 0.02$ value and 95% C.I. jackknife option (Waples and Do 2008; Nielsen et al. 2009). Negative N_e values or infinite upward bounds of confidence intervals indicate that sample sizes were too low or that there was a lack of statistical power to estimate N_e (Waples 1989; Waples and Do 2008).

Therefore, I applied Create 1.2 (Coombs et al. 2008) software to prepare data input files for N_e estimation. I applied the linkage disequilibrium method using LDNE software (Waples and Do 2008) to estimate the N_e of the parental generation of each population. I combined the above-barrier sample of 30 individuals and below-barrier sample of 30 individuals for a total of 60 individuals per site. Samples were combined because recent work by Tallmon et al. (2010) documented with simulation data that a sample size of at least 60 individuals was necessary to accurately estimate N_e of small population sizes using LDNE software.

Estimating gene flow. - Population divergence, as represented by the metric F_{ST}, is the

result of net effects on a population from gene flow and genetic drift, when the effects of selection and mutation are negligible (Whitlock and McCauley 1999; Jensen et al. 2005). Sewall Wright (1965) defined F_{ST} as the divergence between randomly drawn gametes among subpopulations. That is, the F_{ST} value quantifies the departure of observed heterozygosity among subpopulations from Hardy-Weinburg expectations due to divergence among the subpopulations. F_{ST} can be related to genetically effective migration, or gene flow, which can be estimated empirically by applying the equation: $F_{ST} = 1/4Nm + 1$, where m=proportion of migrants, N is population size, and Nm is actual number of migrants entering a subpopulation in each generation. This equation can be manipulated to estimate gene flow as: $Nm = [1/F_{ST}-1]/4$ (Wright 1965; Mills and Allendorf 1996). Wright's island model of population structure (Wright 1931) provides the basis for this equation, but it is not based on ecologically valid assumptions; i.e., it assumes constant population sizes and constant migration (Whitlock and McCauley 1999). However, applications of this equation are useful as a rough estimate of gene flow and provide a benchmark for comparison among populations (Neigel 2002). Therefore, using the equation: $Nm = [1/F_{ST}-1]/4$ (Wright 1965; Mills and Allendorf 1996), I calculated F_{ST} values and then estimated gene flow between the above- and below-barrier sample for each site.

Relationship between N_e and fragmentation variables. - I applied multiple linear regression, adjusted r^2 model using SAS 9.2 software (SAS Institute Inc., Cary, North Carolina, USA), to ten samples of brook trout associated with natural barriers in ten subwatersheds, to identify the variables most significantly related to estimated N_e values. The dependent variable in this case was the N_e value for each site. The independent variables considered in this analysis included barrier height (m), road density per subwatershed (km roads /km^2 of land), % forest cover per subwatershed, and total stream length potentially isolated above each putative barrier (km) (Table 2.2); i.e., variables that measured the degree of fragmentation at each site (Hudy et al. 2008).

For the sake of this analysis, the total stream length potentially isolated above each putative barrier functioned as a surrogate for potentially available breeding habitat. However,

successful breeding at the stream reach scale was not verified in this study. The habitat isolated above the barrier is a factor that needs to be considered for the brook trout samples associated with natural barriers for this study, as natural barriers that limit upstream dispersal will fragment potential breeding habitat units. Successful breeding and breeding habitat is directly related to N_e in salmonid populations (Frankham 1995; Ardren and Kapuscinski 2003; Palstra and Ruzzante 2008; Tallmon et al. 2010).

Results

Barrier height and channel gradient. - Barrier heights for the ten sample sites ranged from 2.1 to 61.0 meters at baseflow (Table 2.1), with all barriers meeting the minimum 73.5 cm height at baseflow criterion determined by Kondratieff and Myrick (2006). Barrier channel gradients ranged from 6 to 90 degrees (Table 2.1). Total stream length above each putative barrier location ranged from 0.93 to 54.3 kilometers (Table 2.2). Chapter one of this dissertation identified a significant correlation between height and channel gradient (Spearman's rho=0.562, P=0.02), and barrier height was identified as the preferred predictive variable (MLR height, P=0.011; channel gradient, P=0.443). Therefore, channel gradient was not considered as a predictive variable in this analysis.

Microsatellite genotype and population genetic data. - Chapter one of this dissertation described the quantitative results for genetic diversity for 15 above-barrier and 15 below-barrier brook trout samples (Table 1.4) at eight microsatellite loci (Table 2.4) in the BR Level III Ecoregion. I genotyped 897 individual brook trout at these 15 sites to quantify total allelic diversity, number of alleles per locus (A), and average gene diversity per locus (H). This study investigates ten of these sites, as the result of removing non-barrier sites and randomly selecting one site per subwatershed if more than one was sampled (Table 2.2).

Estimated effective population sizes (N_e). - Estimated N_e and associated ranges based on 95% confidence intervals for brook trout at the ten natural barrier sites for the ten sampled subwatersheds ranged from 3.8 (1.1-9.1) at APOR to 190 (78-653) at the CASC site (Table 2.5). I had to apply the parametric confidence interval method at critical value P = 0.01 in LDNE to fit

a tighter 95% confidence interval and obtain an N_e estimate that was not negative or ∞ at the CBCR and CASC sites. I applied 95% jackknife confidence intervals and $P = 0.02$ critical values at all other sites.

Estimated gene flow. - Estimated gene flow values between the above- and below-barrier samples ranged from 0.47 at the APOR site with a barrier height of 61.0 meters, to 59.6 at the CASC site with a barrier height of 21.3 meters. I found no statistically significant relationship between barrier height and gene flow (r^2=0.05, F=0.41, P=0.54) (Figure 2.2), although power analysis revealed that there was not sufficient power to effectively test this relationship. Although, power was low, I found a statistically significant relationship between estimated gene flow values and N_e , showing an increasing trend in N_e as gene flow increases (r^2=0.47, F=7.08, $P > 0.03$) (Figure 2.3).

Factors driving fragmentation and N_e. - I tested 15 different models of all possible one, two, and three variable combinations using multiple linear regression, adjusted r^2 model (Table 2.6). The most significant model included a combination of the road density (km roads /km^2 of land) and total stream length above the barrier (km) variables (adjusted $r^2 = 0.50$, F=5.46, $P >$ 0.04). The adjusted r^2 model with only the total stream length above the barrier (km) had an adjusted r^2 of 0.22 and the model with only road density had an adjusted r^2 of 0.32 as compared to the adjusted r^2 of 0.50 for both road density and total stream length above the barrier (km). I tested for correlations among N_e, road density, and total stream length above the barrier (km). There was a significant correlation between N_e and road density (Spearman's rho=0.64, P=0.05), showing that this is the preferred predictive variable. Correlations were not significant between N_e and total stream length above the barrier (Spearman's rho=0.37, P=0.29) or road density and total stream length above the barrier (Spearman's rho= -0.13, P=0.73). Linear regression models with N_e and total stream length above the barrier alone (r^2=0.31, F=3.55, $P > 0.10$); and N_e and road density alone (r^2=0.40, F=5.28, $P > 0.05$) were less significant than the combination of N_e with total stream length above the barrier and road density (r^2=0.61, F=5.46, $P > 0.04$). These relationships show that as the road density increases the N_e decreases and as the total stream

length above increases, the N_e increases.

Discussion

The goal of this study was to determine significant habitat fragmentation factors affecting N_e of resident Southern Appalachian brook trout populations associated with natural barriers. The objective was to test the hypothesis that barrier height and total stream habitat potentially isolated above the barrier were significant correlates of brook trout N_e at the stream reach scale and that road density and percent forest cover within a subwatershed were significant correlates at the subwatershed scale. This study offered a unique opportunity to investigate effects of natural barriers on N_e both at the stream reach and subwatershed scales because all samples were collected adjacent to natural barriers from specific stream reaches in separate subwatersheds.

N_e and subwatershed variables. - Of the habitat fragmentation factors tested as correlates of brook trout N_e, the total stream habitat potentially isolated above the barrier was a significantly correlated stream reach scale variable and road density was a significantly correlated subwatershed scale variable. Multiple linear regression indicated that the combination of the road density (km roads /km^2 of land) and total stream length above the barrier (km) variables was the best predictive model for N_e for the ten samples associated with natural barriers in this study (adjusted $r^2 = 0.50$, F=5.46, $P > 0.04$), with road density explaining greater variability in the model as compared to the total stream length above the barrier.

These results support the importance of the relationship among habitat connectivity, reproductive success, and N_e, as estimated N_e values were most significantly related to total stream length isolated above each putative barrier and road density (adjusted $r^2 = 0.50$, F=5.46, $P = 0.04$). Shrimpton and Heath (2003) showed a significant correlation between N_e and availability of spawning habitat for Chinook salmon, and other studies have documented the significant relationship between variability in reproductive success and N_e for steelhead trout *Oncorhynchus mykiss* (Ardren and Kapuscinski 2003; Araki et al. 2007).

The total potential stream length isolated above the barrier was one of the significant variables related to N_e in the model from this study, and this variable represents the potential

effect of a natural barrier on upstream dispersal of breeding adults. This lack of dispersal will affect N_e when access to potential breeding habitat above the barrier is not possible (Rieman and Allendorf 2001; Shrimpton and Heath 2003). The distribution of roads within subwatersheds can also affect breeding success due to direct disturbance to spawning habitat from road construction and indirect effects due to increased sedimentation (Wood and Armitage 1997; Jones et al. 2000; Wheeler et al. 2005).

Dunham and Rieman (1999) documented a significant negative relationship between road density at the subwatershed scale and the occurrence of bull trout in relation to these disturbance factors. In addition, culverts have especially been identified as a significant source of barriers to fish movement and are barriers where roads and streams cross (Gibson et al. 2005; Poplar-Jeffers et al. 2009). Estimates of culvert densities report one culvert per every 5 stream kilometers in Alberta (Tchir et al. 2004) and one culvert every 7.2 stream kilometers in West Virginia (Poplar-Jeffers et al. 2009). Road-stream crossings that are barriers to upstream dispersal can, therefore, limit access to breeding habitat upstream of the barrier for breeding adults, thus directly affecting N_e.

Despite the significant correlation between N_e, total potential stream length isolated above the barrier, and road density, this model still only explains 50% of the variability (adjusted $r^2 = 0.50$). The investigated habitat fragmentation variables did not represent a wide range of variability: percent forested land use ranged from 81% to 100%; road density ranged from 0.3 to 2.2 km roads /km^2 of land; barrier height (m) ranged from 2.1 to 61.0 meters; and total stream length above each putative barrier location ranged from 0.93 to 54.3 kilometers (Table 2.2). The 81 to 100% forested land use within a subwatershed represent a bias, as the only available subwatersheds within the BR Level III Ecoregion where high density brook trout populations could be found directly above and below natural barriers were in subwatersheds with this high of a percent forested land use. Habitat fragmentation variables related to N_e in brook trout populations in this study or other variables not yet investigated may function at different spatial and temporal scales, so significant relationships to explain the other 50% of the variability may

have not yet been detected (Dunham et al. 1997).

In addition, N_e is known to be one of most difficult parameters to estimate, and this study did not measure the population demographic factors that may contribute more to the other 50% variability predicting N_e of brook trout. These factors, for example, may include: sex ratio, number of breeders, and variance in family size (Arden and Kapuscinski 2003; Shrimpton and Heath 2003; Araki et al. 2007). Samples in this study included adults and YOY brook trout to ensure a sample of at least 30 individuals, which may represent different generations and add variability to N_e estimation. Hatchery supplementation may increase or reduce the N_e of wild populations as a result of increased variance of reproductive success (Ryman and Laikre 1991). All sampled brook trout populations from this study were at one time managed as a sport fishery (Virginia Department of Game and Inland Fisheries database; North Carolina Wildlife Resources Commission stocking website), so it is highly likely that current wild breeding brook trout populations have been influenced by stocked brook trout at one or more times in history. Allozyme, mitochondrial, or DNA fingerprinting diagnostic allele data sets were not generated as a result of this study, which could be used to distinguish the extent of introgression between alleles of hatchery and wild individuals (Bartley et al. 1992; Danzmann et al. 1998).

Estimated N_e. - Estimated N_e values and associated ranges for resident brook trout in the BR Level III Ecoregion ranged from 3.8 (95% C.I. 1.1-9.1) at APOR to 190 (95% C.I. 78-653) at the CASC site (Table 2.5). According to Rieman and Allendorf (2001), target effective population size for short-term conservation of a salmonid population is at least 50, and an average N_e of 500 is more likely to maintain adaptive genetic variation over longer periods of time. Kincaid (1983) suggested a minimum N_e of 100 per generation to minimize inbreeding depression in cultured stocks. Allendorf and Ryman (1987) suggested a minimum N_e of 200 per generation to minimize inbreeding depression.

None of the estimated N_e values for samples of brook trout in this study, without associated ranges, were as high as the 500 recommended to maintain long-term genetic adaptability. Sites that were in the 100-200 or higher range recommended to minimize

inbreeding depression (without considering the 95% confidence intervals) included the following: STAT, 143 (54.4-430); and CASC, 190 (78-653). Among samples at the other eight sites, five had estimated N_e values greater than 50 without considering 95% confidence intervals: BWLL, 96.5 (27.6-243); CBCR, 94.4 (34-264); SASS, 68.4 (42.8-501); SHCS, 83.6 (39-297); and STAU, 78.7 (47.8-593). The remaining three samples had estimated N_e values less than 50, without considering ranges based on 95% confidence intervals: APOR, 3.8 (1.1-9.1); STMY, 9.3 (2.1-20.7), and WHRK, 49.0 (17.4-130) (Table 2.5). Keep in mind, results from this study reflect N_e estimates based on only one sample of 60 individuals from one generation of brook trout sampled directly adjacent to natural barrier sites. The percent sampled of the potential total habitat available above the barrier, for example, was only from 0.23 to 10.8% of the total habitat which may not be a complete representation of the entire population (Table 2.3).

The samples with the lowest estimated N_e values in this study also had the lowest estimated gene flow values relative to other samples and the study identified a statistically significant relationship between N_e and gene flow, although power was low (r^2=0.47, F=7.08, P > 0.03). For example, the APOR sample had an estimated N_e and range based on a 95% C.I. of 3.8 (1.1-9.1) and estimated gene flow of 0.50, which were the lowest such values for all sites. The STMY sample had the second-smallest estimated N_e and range based on a 95% C.I. of 9.3 (2.1-20.7) and second-smallest estimated gene flow (1.40). The CASC sample had the highest estimated N_e and range based on a 95% C.I. of 190 (78-653) and also had the highest estimated gene flow (59.6) (Tables 2.5, Figure 2.3). These results showing an increasing trend in N_e with increasing gene flow and emphasize the importance of maintaining or restoring connectivity among brook trout populations for maintaining N_e and genetic diversity.

One generation of N_e estimation.- Populations with N_e values < 50 are at the highest risk of loss of genetic diversity (Rieman and Allendorf 2001), which for this study would include three of the ten sites represented in this study: APOR (3.8), STMY (9.3), and WHRK (49.0). Populations with N_e values <100 are at risk for loss of genetic diversity and increased inbreeding according to studies by Kincaid (1983), which would include five of the other ten sites

represented by this study. Effective population size, gene flow, and breeding success are naturally variable in populations from one generation to the next. I note that the data presented here are based on one sample at a specific point in time, and one-sample estimates are not estimates of N_e in the current generation, but estimate N_e of the sampled individuals of the previous generation (Waples 2005).

The data presented here can be used to identify populations that may be most at risk only based on one previous generation. In this case the sites with the lowest N_e values (APOR, STMY, and WHRK) were also identified at risk due to reduced genetic diversity in Chapter 1 of this dissertation due to reduced genetic diversity (Table 1.4), so the low N_e values are further indication that these populations are at risk due to genetic isolation. However, the data presented here may also indicate natural fluctuation and further sampling would be needed to investigate the long-term trends in N_e in these populations. Increased time between samples will yield a more reliable indicator of true long-term trends for N_e and gene flow, with recommended sampling of at least 60 individuals ≥ five generations apart for populations of 100-500 individuals (Tallmon et al. 2010).

Management implications. - This study showed a direct relationship between estimated N_e, accessibility to breeding habitat (isolated above natural barriers), and loss of usefulness of breeding habitat due to disturbance (road density). This study also showed a direct relationship between estimated N_e and gene flow. If needed, restoration and improvement of breeding habitat may offer opportunities for increasing the N_e of brook trout populations at risk, where spawning habitat is limited. Increases in gene flow through improved habitat connectivity may increase N_e, which may counteract effects of inbreeding over time (Mills and Allendorf 1996; Vila et al. 2003). In carefully considered contexts, demographic augmentation of resident populations with "migrants" from well-chosen source stocks may be appropriate.

Sites with N_e values lower than recommended for long-term adaptability may indicate the need for restoration of connectivity with other subpopulations within the subwatershed or improvement of breeding habitat. Epifanio et al (2003) describe the value of using population

genetic information to prioritize population subunits on the landscape as part of a larger cluster of interconnected population subunits at the subwatershed scale. Identifying subunits that have low N_e values may indicate a priority population for restoration effort.

Further research. - Long-term monitoring of breeding success and tracking N_e trends of the sites investigated in this study would provide information indicative of trends in N_e, inbreeding, and gene flow for brook trout populations in the BR level III Ecoregion. Effective population size has the greatest utility to capture changes over time, as N_e reflects changes in allele frequency due to successful breeding.

Long-term research designed to sample all available habitat and all age classes above and below natural barriers from this study over ≥ 5 generations would provide a better representation of brook trout population N_e trends over time and risk to sampled populations due to reductions in N_e (Whiteley et al. 2006; Tallmon et al. 2010). Permanent pit-tag readers and mark-recapture data combined with N_e and gene flow estimates could be used to further investigate long-term effects of asymmetric gene flow. Population demographic estimates of the number of breeders sex ratio, number of breeders, and variance in family size would also be useful to identify how these factors of variability affect N_e estimation and relationships to habitat fragmentation factors (Ardren and Kapuscinski 2003; Shrimpton and Heath 2003). Linkage disequilibrium estimation methods could be applied to further investigate one-sample N_e estimates and combined with temporal N_e estimates for brook trout populations to make more effective, long-term management decisions for populations at risk.

TABLES

Table 2.1. Study site abbreviations and descriptions.

Site abbreviation	Site name	Location (county, state)	Barrier height (m)	Channel gradient[1]
APOR	Apple Orchard Falls	Botetourt County, Virginia	61.0	80
BWLL	Big Wilson Creek, lower	Grayson County, Virginia	2.1	90
CBCR	Cabin Creek	Grayson County, Virginia	7.6	80
CASC	Little Stony Creek, Cascades	Giles County, Virginia	21.3	90
SASS	Sassafras Falls	Graham County, North Carolina	30.0	70
SHCS	Shoe Creek Cascade	Nelson County, Virginia	3.4	15
STAT	Staton's Creek Falls	Amherst County, Virginia	42.7	80
STAU	Staunton River	Madison County, Virginia	3.8	6
STMY	Saint Mary's Falls	Augusta County, Virginia	4.0	90
WHRK	White Rock Falls	Augusta County, Virginia	11.3	80

[1]Gradient was estimated as arctan (degrees) based on change in elevation from the lowest to highest point of each barrier.

Table 2.2. Subwatershed fragmentation metrics for ten subwatersheds for the ten sampled natural barrier sites. All non-barrier sites were removed and duplicate sites from the same subwatershed were removed for this analysis to increase variability for fragmentation metrics. Subwatershed numbering was from the USGS 6th level Hydrologic Units compiled for the brook trout range (Hudy et al. 2008).

Subwatershed	Site[1]	Barrier height (m)	Road density (km road/km^2 land)	% Forested	Total stream length above (km)
510479	SHCS	3.4	1.1	90	21.5
510634	APOR	61.0	1.2	99	1.97
5101204	BWLL	2.1	1.8	81	10.1
510167	STAU	3.8	0.5	83	7.66
510428	STMY	4.0	0.3	97	29.5
5101212	CBCR	7.6	1.9	86	0.93
510444	WHRK	11.3	1.6	94	1.54
510760	CASC	21.3	2.2	84	54.3
3701446	SASS	30.0	0.8	100	10.0
510498	STAT	42.7	1.4	98	16.9

[1]Site abbreviations are explained in Table 2.1.

Table 2.3. Total habitat available above the barrier location and percent of total habitat sampled. The total stream length above, or total kilometers of available habitat was estimated using ArcMap, Version 9.2 (ESRI, Inc.) and USGS National Hydrological Dataset, 1:24,000 high resolution, digital line graph hydrography files, zoomed to 1:6,500 scale.

Subwatershed	Site[1]	Total stream length above (km)	Stream length sampled above (km)	Percent total habitat sampled
510479	SHCS	21.5	0.050	0.23 %
510634	APOR	1.97	0.119	6.04 %
5101204	BWLL	10.1	0.150	1.49 %
510167	STAU	7.66	0.100	1.31 %
510428	STMY	29.5	0.100	0.34 %
5101212	CBCR	0.93	0.100	10.8 %
510444	WHRK	1.54	0.100	6.49 %
510760	CASC	54.3	0.150	0.28 %
3701446	SASS	10.0	0.100	1.00 %
510498	STAT	16.9	0.050	0.30 %

[1]Site abbreviations are explained in Table 2.1.

Table 2.4. Details regarding brook trout microsatellites screened (King et al. 2003, 2005).

Locus	Dye	Allele size range (bp)	GenBank Accession number
Master Mix1:			
Sfo-C88	FAM (blue)	170-205	AY168192
Sfo-C113	FAM (blue)	125-170	AY168193
Sfo-D75	NED (black)	165-250	AY168197
Sfo-D100	HEX (green)	200-275	AY168199
Master Mix2:			
Sfo-C24	FAM (blue)	110-190	AY168187
Sfo-C115	FAM (blue)	225-370	AY168194
Sfo-C129	HEX (green)	215-270	AY168195
Ssa-D237	HEX (green)	270-450	AF525207

Table 2.5. Estimated N_e (effective population size) for ten brook trout samples.

Site[1]	N_e ± 95% C.I.	N_e range[2]
APOR	3.8 (2.7-5.3)	(1.1-9.1)
BWLL	96.5 (68.9-146)	(27.6-243)
CBCR, *P*=0.01, parametric CI	94.4 (60.4-170)	(34-264)
CASC, *P*=0.01, parametric CI	190 (112-463)	(78-653)
SASS	68.4 (25.6-433)	(42.8-501)
SHCS (*N*=57)	83.6 (44.6-213)	(39-297)
STAT	143 (88.6-287)	(54.4-430)
STAU	78.7 (30.9-514)	(47.8-593)
STMY	9.3 (7.2-11.4)	(2.1-20.7)
WHRK	49.0 (31.6-80.7)	(17.4-130)

[1]Site abbreviations are explained in Table 2.1.
[2] N_e range was calculated based on 95% jacknife confidence intervals (C.I.) at the *P*=0.02 critical value, except for the CBCR and CASC sites which were calculated using 95% parametric confidence intervals at the *P*=0.01 critical value to achieve a tighter C.I.

Table 2.6. Multiple linear regression analysis r^2 and adjusted r^2 values for habitat fragmentation variables as a predictive model for N_e: barrier height (m), total habitat potentially isolated above the barrier (km), subwatershed road density (km of road/km^2 of land), and subwatershed %forest (total area forested land within subwatershed).

Variables in model	**r^2 value**	**Adjusted r^2 value**
Barrier	0.01	-0.12
% forested	0.24	0.14
Area[1]	0.31	0.22
Road density	0.40	0.32
Barrier, area	0.31	0.11
Barrier, % forested	0.33	0.14
Barrier, road density	0.41	0.24
Road density, % forested	0.46	0.30
% forested, area	0.46	0.31
Road density, area[2]	0.61	0.50
Barrier, road density, % forested	0.46	0.19
Barrier, % forested, area	0.55	0.33
Barrier, road density, area	0.61	0.42
Road density, % forested, area	0.64	0.47
All variables	0.66	0.38

[1] Total km stream length upstream of barrier location.
[2]Road density and area had the highest r^2 and adjusted r^2 value as compared to all single and combined variables (r^2=0.61, F=5.46, P>0.037).

FIGURES

Figure 2.1. Sampled Blue Ridge Level III Ecoregion subwatersheds in Virginia and North Carolina, showing ten investigated subwatersheds.

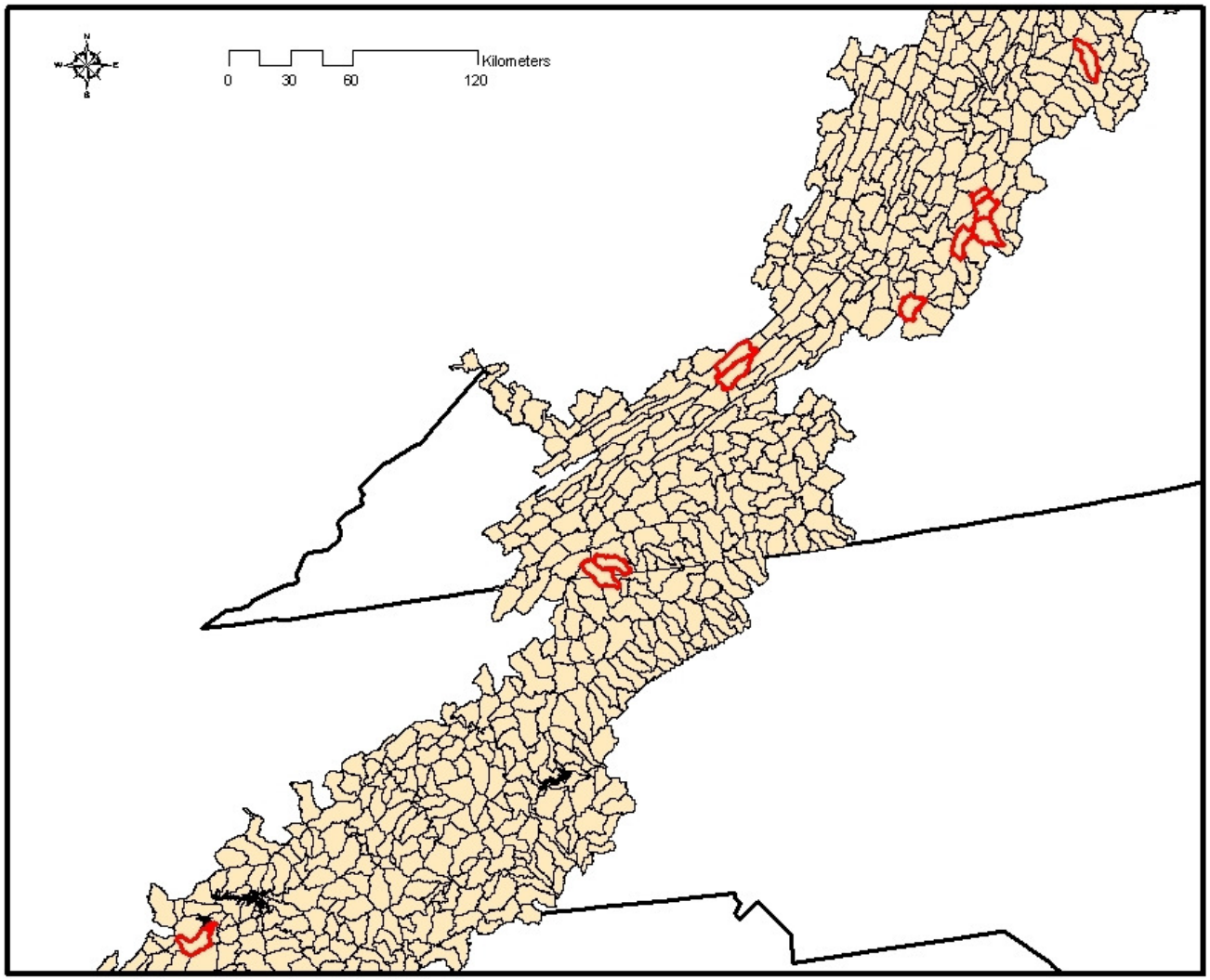


Figure 2.2. Estimated gene flow for ten brook trout samples in relation to barrier height (m). Gene flow was estimated according to: $Nm=[1/F_{ST}-1]/4$ (Wright 1965; Mills and Allendorf 1996) and no statistically significant relationship between barrier height and gene flow was found ($r^2=0.05$, $F=0.41$, $P=0.54$).

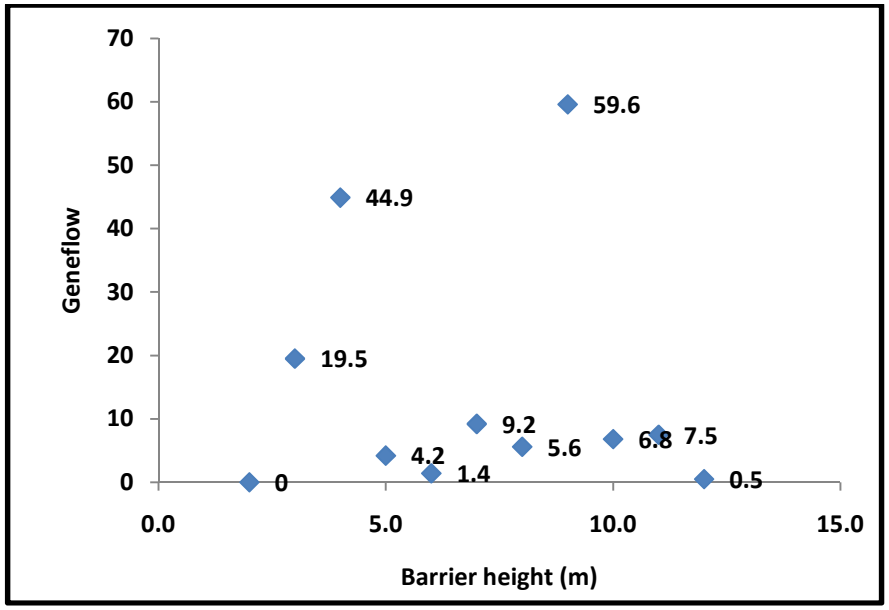
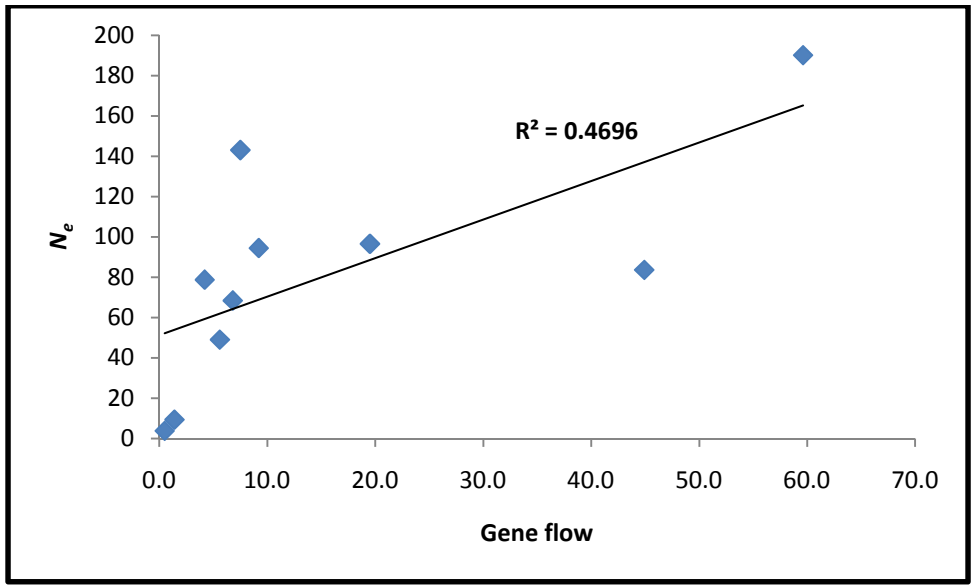


Figure 2.3. Significant linear regression relationship between gene flow and N_e for ten sites, showing an increasing trend in N_e as gene flow increases (r^2=0.47, F=7.08, P > 0.03).



CHAPTER 3. Brook trout kinship upstream and downstream of natural barriers.

Introduction

Barriers to fish movement result in reduced upstream dispersal, which can result in reduced gene flow and genetically isolated populations that are at risk due to inbreeding and effects of genetic drift (Slatkin 1985; Wofford et al. 2005). Further information is needed on how to apply population genetic tools to identify barriers to upstream dispersal. This study investigated applications of microsatellite multilocus genotype kinship, or relatedness, relationships which can be used to infer the amount of dispersal occurring at a site. Documenting how parents and offspring are spatially distributed can be used to infer dispersal and high relatedness can indicate a lack of upstream dispersal due to a limited number of breeders and resulting inbreeding.

A variety of analytical techniques have recently arisen to assess kinship (Belkhir et al. 2002; Kalinowski et al. 2006; Tallmon et al. 2008; Waples and Do 2008; Jones and Wang 2009; Coombs et al. 2010). Genetic monitoring of kinship has measured the degree of relatedness, variance in family group size, and inbreeding coefficients to investigate changes in population genetic diversity over time (Ryman and Laikre 1991; Small et al. 2009). As a result of more recently available analysis techniques, it is now possible to further investigate kinship relationships within brook trout *Salvelinus fontinalis* populations that are potentially affected by barriers to movement (Kalinowski et al. 2006; Hudy et al. 2010). Using kinship as an indicator of dispersal could be used to identify barriers to fish movement and populations at risk due to genetic isolation. The following introduction describes previous research investigating kinship relationships among wild salmonid populations and how similar research techniques can be applied to kinship relationships above and below natural barriers.

Relatedness assessment in wild salmonid populations. - To date, the majority of research on kinship relationships within wild salmonid populations has been applied to breeding strategy with associated redds, hatchery supplementation effects on wild populations, and inbreeding

(Ryman and Laikre 1991; Mousseau et al. 1998; Kuligowski et al. 2005; Herbinger et al. 2006; D'Amelio et al. 2008; Small et al. 2009).

Salmonids breed by establishing redds in specific locations within stream channels where they lay and fertilize their eggs. For example, a brook trout will excavate a redd which may contain one or more nests that contains an egg pocket. Functioning brook trout redds have been associated with temperatures between 6 and 8 ºC, groundwater inputs, high-oxygen water, and flows ≤ 177 liters/second (Witzel and MacCrimmon 1983). Salmonid family groups are associated with redds, and individuals from the same redd are on average more closely related than individuals sampled from different redds (Kuligowski et al. 2005). Kuligowski et al. (2005) found that mean relatedness ranged from 0.246 (half-sibs) to 0.453 (full-sibs) within individual redds.

Formal pedigrees have been constructed for all parents and offspring by generation in hatcheries, but this information is unavailable for most wild populations. Herbinger et al. (2006) documented differences in the number of full-sib families and the number of individuals per full-sib family when comparing hatchery and wild populations of Atlantic salmon. The hatchery populations had a smaller number of full-sib families overall, but each full-sib family had a larger number of individuals, ranging in size from eight to 50 individuals. Wild populations had a greater number of full-sib families, but each full-sib family had a smaller number of individuals, ranging in size from one to seven individuals. The fewer the number of families, the fewer the number of breeders, which may put the population at risk from accumulated inbreeding, increased effects due to genetic drift, and increased variance in breeding success (Ryman and Laikre 1991; Herbinger et al. 2006).

Inbreeding coefficients can be used to monitor relatedness within a population, as a higher inbreeding coefficient may indicate higher relatedness in a population. Inbreeding reduces genetic diversity by decreasing heterozygosity, which can be monitored by calculating inbreeding coefficients in a population over time (Wang et al. 2002). Wright (1965) estimated the inbreeding coefficient as: $F_{IT} = F_{ST} + (1\text{-}F_{ST})\ F_{IS.}$ The F values represent departures of

genotype frequencies from Hardy-Weinburg equilibrium (abbreviated HWE) due to processes occurring within and among populations, notably, mating between closely related individuals (F_{IS}) and differentiation among populations (F_{ST}).

Dispersal and barriers and kinship of brook trout. - Hudy et al. (2010) investigated spatial distribution of brook trout in relation to spawning and early dispersal using kinship and parent-offspring relationships. This study analyzed microsatellite data to infer full-sib family structure and combined this information with mark-recapture data to document mean dispersal distances from the originating redd location of 193 meters for the main channel and 117 meters for tributaries. These limited dispersal distances can be applied to barrier sites to infer dispersal of individuals from above-barrier redds to below the barrier and individuals from below-barrier redds to above the barrier. For example, if offspring from parents identified from above the barrier are found below the barrier, they dispersed downstream over the barrier. If offspring from parents identified from below the barrier are found above the barrier, they dispersed upstream over the barrier.

All "barriers" in this study are natural barriers, such as waterfalls, vertical drops, and cascades that range in height from 0.91 meter to 91.4 meters at baseflow, with all barrier sample sites meeting the minimum 73.5 cm height determined by Kondratieff and Myrick (2006) as an upstream dispersal barrier to brook trout at baseflow in the lab. Some waterfalls may be barriers during all flow conditions, whereas others may be barriers only during low flow or high flow, when velocities are too high for fish movement (Reiser et al. 2006). Kinship relationships can be applied to further investigate how barriers of different heights and variability in flow conditions limit upstream dispersal.

The above studies used mean relatedness values, number of full-sib families, number of individuals per full-sib family, inbreeding coefficients, and parent-offspring relationships to investigate kinship within salmonid populations. Hudy et al. (2010) suggest evidence of brook trout passage due to the occurrence of family members on either side of putative barriers. This study investigates brook trout kinship relationships and dispersal of parents and offspring

adjacent to barriers further at sites with a wide range of heights and gradients. High relatedness and inbreeding coefficient values will also be applied in this study to identify genetically isolated populations above a barrier to upstream dispersal.

Objective of research. - No specific studies have applied microsatellite markers to assess kinship relationships in resident brook trout samples above and below natural barriers to migration that vary in height. Therefore, my objective was to infer relationships between barriers and family structure in brook trout populations and to identify any sites with increased inbreeding to identify populations at risk due to genetic isolation above the barrier. I tested the null hypothesis that mean pairwise relatedness coefficients, r_{xy} (Queller and Goodnight 1989) will not differ between above- and below-barrier samples. I tested a second null hypothesis that barrier height will not affect brook trout mean pairwise relatedness coefficients, r_{xy} (Queller and Goodnight 1989) or numbers of full-sibs per family group (Jones and Wang 2009) for each site. I also tested for the presence of parent-offspring relationships between above- and below-barrier individuals at each site to investigate dispersal across the barrier.

Methods

Study areas. - I selected 18 natural barrier sites in subwatersheds (USGS 6th-level Hydrologic Units) of the Northern Lakes and Forests, abbreviated as NLF (six sites in Wisconsin, Michigan, and Minnesota) (Table 3.1, Figure 3.1) and the Blue Ridge, abbreviated as BR (12 sites in Virginia and North Carolina) (Table 3.1, Figure 3.2) Level III Ecoregions that were at least 68% forested (Bailey 2005; Hudy et al. 2008). Hudy et al. (2008) correlated the presence of successfully reproducing populations of brook trout with 68% or more forested land use within the associated subwatershed.

The historic range of brook trout extends from the headwaters of the Mississippi River in Minnesota, to coastal drainages from Maine to Virginia, and south to the headwaters of the Chattahoochee River in northern Georgia (MacCrimmon and Cambell 1969). The NLF and BR Level III Ecoregion are ecoregions within the native range of the brook trout that still has self-reproducing populations remaining. I selected sites according to ecoregion so that selected

streams within each specific ecoregion would have similar physiogeographic characteristics for comparisons and analyses based on ecoregional patterns. These ecoregions have available locations with sufficient brook trout population densities to collect at least 30 juveniles and adults above and below each natural barrier. A sample of 30 individuals is considered a minimum for reliably estimating genetic diversity of populations (McCracken et al. 1993; Rogers and Curry 2004). For the sake of data organization, results will be presented by ecoregion, but analyses will pool all site data together. This pooling of data is appropriate, as breeding behavior relevant to kinship relationships would not change throughout the native range of the species and no differences in kinship relationships according to ecoregion have been documented by previous research.

I used ArcMap, Version 9.2 (ESRI, Inc.) and USGS National Hydrological Dataset, 1:24,000 high resolution data layers to subdivide 5th level, 10-digit hydrologic unit code watersheds into 6th level, 12-digit hydrologic unit code subwatersheds. I selected natural barrier sites found within the same subwatershed because this is the smallest watershed size with compiled data available on brook trout populations, and this is the spatial level most relevant to management for fish conservation (4,100 to 12,700 hectares for subwatersheds in this study) (Seaber et al. 1987; Fausch et al. 2002). Selected sites represented a broad range of barrier heights and channel gradients. Natural barriers to movement of brook trout were at least 73.5 cm maximum jumping height at baseflow, which was considered a barrier for 8.6 – 34.0 cm TL brook trout with a 0.40 cm minimum pool depth below the waterfall at baseflow by Kondratieff and Myrick (2006). I also selected three sites in the BR Level III Ecoregion that did not have barriers and started the designated start of the downstream sample reach 50 meters from the nearest road or confluence.

All sampled brook trout populations were at one time managed with supplementary stocking (Virginia Department of Game and Inland Fisheries database; North Carolina Wildlife Resources Commission stocking website; Wisconsin DNR Catchable Trout database; Michigan DNR Fish Stocking database; Great Lakes Fisheries Commission database) (Tables 3.2, 3.3).

This study will estimate N_e for wild brook trout and look for evidence of inbreeding. Presence of inbreeding may indicate mixing of stocked and wild fish. In addition, stocked fish, in some cases, have been reported to have lower N_e (Ryman and Laikre 1991). Studies have shown varied results on impacts of introgression of stocked alleles on wild trout populations depending on how many fish were stocked and for how many years (Heggenes et al. 2006; Small et al. 2009). Although allozyme, mitochondrial, or DNA fingerprinting diagnostic allele data sets were not generated as part of this study, these could be used to distinguish the extent of introgression between alleles of hatchery and wild individuals (Bartley et al. 1992; Danzmann et al. 1998).

Study site measurements. - I measured the total stream length sampled above and below each barrier site (Table 1.2). I also estimated the total stream length above each barrier to characterize the amount of habitat potentially isolated by an upstream barrier to brook trout movement using ArcMap, Version 9.2 (ESRI, Inc.) and USGS National Hydrological Dataset, 1:24,000 high resolution subwatershed data, digital line graph hydrography files and digital orthophoto quad data layers to achieve 98.5% accuracy (Table 2.3).

Barrier height was measured in the field using a standard rod and level, or was estimated by subtracting the difference in elevation between GPS points recorded directly above the barrier and directly below the barrier based on Google Earth high resolution satellite imagery of 1000 pixels if the barrier height was taller than the standard rod (~10 meters). Channel gradient was estimated as arctan (degrees) from the lowest to highest point of each barrier (Table 3.1). Stream-reach sample distances ranged from 50 meters to 585 meters, as sampling was completed at a given site once a sample of 30 brook trout fin-clips was acquired.

Brook trout sampling. - I used single-pass electrofishing techniques to collect brook trout at each site. I anesthetized fish using clove oil (Taylor and Roberts 1999). I measured total length and weight, and clipped a ~50 mg piece of fin from each trout (Rogers and Curry 2004). Fin-clips were stored and preserved in separate vials with 95% ethanol. I calculated brook trout sample densities above and below each barrier by calculating the total number of sampled brook

trout divided by the total area sampled (m^2) (sampled length*average bankfull width; Table 1.2).

Microsatellite markers and genotyping. - DNA extraction, polymerase chain reaction (PCR), and genotyping protocols were developed at the United States Geological Survey, Conte Anadromous Fish Laboratory (Turners Falls, Massachusetts). I extracted DNA from 1,257 fin-clips and PCR-amplified microsatellite-bearing fragments at eight loci (Table 3.4) using an MJ DNA Engine Dyad PTC-220 thermocycler. Microsatellite loci were multiplexed (Henegariu et al. 1997; Table 3.4) for cost-effective genotyping using an Applied Biosystems, Inc. ABI 3100-Avant Autoanalyzer and GeneMapper software. I scored genotypes using the Peak Scanner v1.0 (Applied Biosystems, Inc).

I applied COLONY (Jones and Wang 2009) to estimate the genotyping error rate at each locus for each individual in each sample. Genotyping errors can occur due to poor DNA quality in the original sample, substandard PCR plate runs, or genotype scoring errors (Wang 2004). A genotyping error can cause an individual to be incorrectly assigned to a family group, which would in turn affect correct assignment of all of that individual's siblings.

Assessment of relatedness. - There are two basic approaches that software developers have used for inferring family relatedness in wild populations using multilocus genotypes. There are pairwise methods that compare each individual within a population to every other individual, considering only one pair of individuals at a time (Queller and Goodnight 1989; Lynch and Ritland 1999; Belkhir et al. 2002; Kalinowski et al. 2006). There are also group-likelihood approaches, that consider all individuals in a population and determine the most likely family groupings of these individuals, such as groups of full siblings (sharing two parents), half siblings (sharing one parent), or unrelated individuals (Wang 2004; Jones and Wang 2009).

I applied Create 1.2 (Coombs et al. 2008) to prepare input data files for tests of deviation from HWE for each locus within a population and for linkage disequilibrium for all locus pairs using GENEPOP (Rousset 2007). I applied the IDENTIX program (Belkhir et al. 2002) to estimate how closely related one individual was to another in each above- or below-barrier sample by applying Queller and Goodnight's (1989) pairwise relatedness estimator. This

software tests the null hypothesis that there is no relatedness among the individuals in a sample. The pairwise relatedness estimator, r_{xy}, is based on the idea that closely related individuals in a sample are more likely to share common alleles. Analysis of r_{xy} metrics accounts for the background level of shared alleles as follows: r_{xy} = number of shared alleles/total number of alleles. The r_{xy} pairwise estimator assumes that $r_{xy} = 0.50$ for a full-sibling (full-sib) relationship, $r_{xy} = 0.25$ for a half-sibling (half-sib) relationship, and $r_{xy}=0.00$ for unrelated individuals (Belkhir et al. 2002). I constructed a histogram of r_{xy} frequencies and calculated a mean r_{xy} value for each above- and below-barrier sample and identified the frequency of brook trout individuals within each sample that were: unrelated (0.00 to 0.24), half-sibs (0.25 to 0.49), or full-sibs ($\geq$ 0.50).

I applied COLONY (Jones and Wang 2009) to estimate the number of half-sibs and full-sibs for each sample of brook trout. Individuals in the same half-sibling family share one parent, and individuals in the same full-sibling family share two parents. This program infers pedigree relationships among parents and offspring in a wild population based on likelihood methods. COLONY assigns probabilities to likely configurations of half-sib and full-sib family constructs in relation to similarities among multilocus genotypes of the entire population (60 individuals in this case). COLONY best estimates half-sib and full-sib family constructions for populations between 50 and 800 individuals.

For this analysis, I combined data for above- and below-barrier samples for each stream site for a total of 60 individuals. This allowed me to investigate mixing of individuals from above and below each natural barrier, as the program assigned individual brook trout to a full-sib family. This procedure was used to infer dispersal between above- and below-barrier samples of brook trout. A complete barrier to upstream gene flow between family groups would show that the individual fish assigned to full-sibling families are all individuals from below the barrier.

I applied ML-Relate (Kalinowski et al. 2006) software to test for the presence of parent-offspring relationships between above- and below-barrier individuals. ML-Relate software calculates maximum-likelihood estimates of relatedness and relationship among individuals using multilocus microsatellite genotype data (Kalinowski et al. 2006). It assigns a maximum

likelihood between pairs of sampled individuals for relationships of unrelated, half-sib, full-sib, or parent-offspring. Pairs of individuals identified with the highest likelihood of parent-offspring that are not that different in likelihood from the full-sib relationship then can be tested for significance. In this case, the putative parent-offspring relationship, with the highest likelihood is tested against the full-sib alternative relationship, with the lowest likelihood. The test is designed to exclude the relationship with the lowest likelihood, so the putative parent-offspring relationship is tested against the full-sib alternative relationship. If the p_{ni}-*value* for the test is low, the alternative hypothesis is rejected and the parent-offspring relationship is the most likely (Kalinowski et al. 2006). This analysis was used to infer upstream movement of parents or offspring above a barrier, or downstream movement of parents or offspring below a barrier.

I used a Wilcoxon sign-rank test for non-normalized data to test the null hypothesis that the relatedness coefficient, r_{xy} did not differ above and below each natural barrier site. I used linear regression techniques to test the significance of the relationship between barrier height and r_{xy} and the relationship between barrier height and mean number of full-sibs per family group.

I applied the fixation-by-loci application in GENEPOP (Rousset 2007) to estimate the inbreeding coefficient for each sample as: $F_{IT} = F_{ST} + (1\text{-}F_{ST})\ F_{IS}$ (Wright 1965). By applying this equation, I calculated the F_{IS} value. A negative or low F_{IS} value in this case means that the observed heterozygosity is greater than the expected heterozygosity for individuals within a sample, and a more positive F_{IS} value represents more inbreeding , or mating between closely-related individuals within a sample (Wang et al. 2002; Herbinger et al. 2006). Although introgression between hatchery and wild brook trout alleles was not verified in this study, the F_{IS} value may differ depending on the extent to which a population is a mix of native and hatchery fish, which is known as the Wahlund effect (Ryman and Laikre 1991; Small et al. 2009).

Results

Barrier height and channel gradient. - Barrier heights for the 18 sample sites ranged from 0.91 meter to 91.4 meters (Table 3.1), with all barriers meeting the minimum 73.5 cm height determined by Kondratieff and Myrick (2006). Barrier channel gradients ranged from 1

to 90 degrees (Table 3.1). In Chapter one of this dissertation I identified a significant correlation between height and channel gradient (Spearman's rho=0.562, *P*=0.02), and barrier height was identified as the preferred predictive variable (MLR height, *P*=0.011; channel gradient, *P*=0.443). Therefore, channel gradient was not considered as a predictive variable in this analysis.

Relatedness above and below barriers. - The mean relatedness values, r_{xy}, ranged from 0.185 to 0.585, with an overall mean of 0.271±0.03 SE, for brook trout samples in the BR Level III Ecoregion and 0.153 to 0.227, with an overall mean of 0.184±0.01 SE, for brook trout samples in the NLF Level III Ecoregion (Table 3.5). There was no significant difference detected between mean relatedness values above and below all combined natural barrier sites (Wilcoxon sign rank *P*=0.87). In addition, there was no statistically significant relationship between mean r_{xy} and barrier height (r^2=0.17, *F*=3.76, *P*=0.07).

Family structure. - The total number of full-sib families ranged from 16 at the APOR site to 41 at the SHOE site in the BR Level III Ecoregion and from 36 at the MORG site to 46 at the COLE site in the NLF Level III Ecoregion (Table 3.6). The mean number of full-sibs per family group ranged from 1.46 to 3.75 with an overall mean of 2.14±0.17 SE for sites in the BR Level III Ecoregion, and from 1.30 to 1.67 with and overall mean of 1.43±0.05 SE for sites in the NLF Level III Ecoregion (Table 3.6). There was no statistically significant relationship detected between mean number of full-sibs per family group and barrier height (r^2=0.01, *F*=0.16, *P*=0.69).

COLONY (Jones and Wang 2009) analysis showed that the majority of sites had mixed full-sib families, which included individuals from both above and below barriers (Table 3.6). For the BR Level III Ecoregion, the number of mixed full-sib families, where above and below-barrier samples were combined, ranged from zero at the APOR site to 10 at the NFKS site. The percentage of full-sib families that were mixed ranged from zero at the APOR site to 36 at the CBCR site (Table 3.6). For the NLF Level III Ecoregion, the number of mixed full-sib families, where above- and below-barrier samples were combined, ranged from three at the COLE site to nine at the MORG and SPBK sites. The percent of full-sib families that were mixed (represented

above and below a barrier) ranged from seven at the COLE site to 25 at the MORG site (Table 3.6). The APOR site (barrier height 61.0 m) had 0 mixed full-sib families and the CRTR site (barrier height 91.4 m) had 1 mixed full-sib families, indicated less dispersal between the above- and below-barrier individuals as compared to other sites (Table 3.6), although the relationship between barrier height and full-sibs was not significant (r^2=0.01, F=0.16, P=0.69).

Parent-offspring relationships. - ML-Relate (Kalinowski et al. 2006) identified pairs of individuals that had the parent-offspring relationship and one individual was from above the barrier and the other was from below the barrier, which indicates dispersal across the natural barrier for the following sites: BWLU, SASS, SHCS, STAT, and STMY sites in the BR Level III Ecoregion and the COLE, HAYM, MORG, PRBR, and SPBK sites in the NLF Level III Ecoregion (Table 3.7). This result indicates dispersal across a natural barrier for ten of the 18 sampled sites in this study. Some sites indicated more than one parent-offspring pair from the same site. For example, the SHCS site had two pairs of parent-offspring relationships involving individual 42, which indicated that two offspring from the same redd location had individual 42 as a parent. ML-Relate (Kalinowski et al. 2006) identified parent-offspring relationships between above- and below-barrier individuals, but, it could not identify whether the parent or offspring moved upstream past a barrier or downstream over a barrier. Further sampling over more than one generation and further applications of parentage software would be needed to identify specific offspring of specific parents (Coombs et al. 2010).

Inbreeding and departures from Hardy-Weinburg equilibrium. - I applied the fixation-by-loci application to estimate the inbreeding coefficient for each sample using the equation, $F_{IT} = F_{ST} + (1-F_{ST})\ F_{IS}$ (Wright 1965) to try to identify sites with high inbreeding. High inbreeding may indicate genetic isolation above a barrier to dispersal. F_{IT} values represent the total departure of the sample from HWE. These values for the BR Level III Ecoregion ranged from 0.018 at the CASC site to 0.369 at the STAU site. Departures from HWE were inferred at the STAU (0.369), APOR (0.258), SASS (0.197), STMY (0.171), and CBCR (0.102) sites. F_{IT} values for the NLF Level III Ecoregion ranged from -0.007 (indicating heterozygote excess) to

0.076, suggesting no evidence of being out of HWE (see Table 3.8). Inbreeding coefficient values (F_{IS}) for the BR Level III Ecoregion ranged from -0.141 (indicating heterozygote excess) at the APOR site to 0.335 (indicating inbreeding or mixing of stocked and wild fish) at the STAU site. F_{IS} values for the NLF Level III Ecoregion ranged from -0.008 at the HAYM site to 0.069 PRBR site (see Table 3.8 for above). F_{ST} values for the BR Level III Ecoregion ranged from -0.002 at the SHOE site to 0.350 at the APOR site, with departures from HWE at the APOR (0.350) and STMY (0.151) sites. F_{ST} values for the NLF Level III Ecoregion ranged from 0.000 to 0.015, indicating no departures from HWE (see Table 3.8).

This analysis indicates that the STAU site may be experiencing inbreeding or mixing between wild and stocked fish. The following sites were identified as out of HWE in the BR Level III Ecoregion: STAU, APOR, SASS, STMY, and CBCR. No sites in the NLF Level III Ecoregion were out of HWE and none were identified as experiencing inbreeding.

Discussion

This is the first study to apply microsatellite markers to assess kinship relationships in samples of resident brook trout from above and below natural barriers. The objective of this study was to infer relationships between barriers and family structure and to identify any sites with increased inbreeding, indicating genetically-isolated populations at risk above the barrier. I did not document a significant difference in mean relatedness values above and below barriers, nor did I document significant relationships between mean relatedness or mean number of full-sibs per family group and barrier height. However, low experimental power and high variability in the data made it difficult to test these relationships robustly. Alternatively, mean relatedness and inbreeding values offer indicators of genetic isolation and parent-offspring relationships indicated dispersal across natural barriers.

Mean relatedness and inbreeding. - Mean relatedness and inbreeding values from this study may be used to identify brook trout populations that are experiencing genetic isolation due to limited dispersal of breeders above a natural barrier. In the BR Level III Ecoregion, a total of seven of 15 sites had mean r_{xy} values at least at the half-sib level (0.25-0.49), and the APOR site

had an above mean relatedness value of 0.811, which is at the full-sib level (≥0.50) (Table 3.5). The APOR site had 54% of individuals that were related, with 24% of the sample full-sibs and 30% of the sample half-sibs (Figure 3.3). Samples from STMY (0.369), SASS (0.335), BWLU (0.328), CBCR (0.270), STAU (0.329), and WHRK (0.270) populations exhibited mean relatedness values within the half-sib range (0.25-0.49) (Table 3.5). All sites in the NLF Level III Ecoregion exhibited relatedness values in the unrelated range (<0.25).

Therefore, the following sites indicated a presence of high relatedness, all sites in the BR Level III Ecoregion: APOR, STMY, SASS, BWLU, CBCR, STAU, and WHRK. COLONY analysis of mixing between individuals of full-sib families above and below natural barriers also indicated no mixing at the APOR site. The lack of mixing between individuals from full-sib families, along with the high relatedness value for the above-barrier sample (0.811) support the hypothesis that the natural barrier (0.61 m) at the APOR site is a complete barrier to upstream dispersal of brook trout. The APOR site is at risk for genetic isolation.

Samples at two of the BR Level III Ecoregion sites that exhibited a mean relatedness value within the half-sib range also exhibited evidence of inbreeding, as indicated by F_{IS} values in the SASS (0.169) and STAU (0.335) sites (Table 3.8). Inbreeding in these cases may be due to the presence of higher relatedness among breeding individuals in each spawning assemblage (Wang et al. 2002). Presence of inbreeding for the SASS and STAU sites may also indicate mixing of stocked and wild fish. Stocked fish, in some cases, have been reported to have lower heterozygosity and have lower N_e, which can increase the impact of genetic drift (Ryman and Laikre 1991). The SASS and STAU sites have been influenced by stocking in the recent past to present (Table 3.2), although studies have shown varied results on impacts of introgression of stocked alleles on wild trout populations depending on how many fish were stocked and for how many years (Heggenes et al. 2006; Small et al. 2009).

The fewer the number of families, the fewer the number of breeders, which may put the population at risk from accumulated inbreeding, increased effects due to genetic drift, and increased variance in breeding success (Ryman and Laikre 1991; Herbinger et al. 2006).

Relatedness value analysis and inbreeding coefficients for the APOR, SASS, and STAU sites suggested a need to increase the availability of diverse spawning pairs of locally-derived brook trout and to increase census size to prevent further inbreeding (Wang et al. 2002; Small et al. 2009). Stocking history should be factored into any supplementation plan in relation to previously stocked census sizes and years. The availability of habitat isolated above the natural barrier may factor into the success of increases in census size and supplementation, especially at the APOR site with only 1.97 km of available habitat above the barrier (Table 2.3).

The presence of inbreeding and higher relatedness values for the SASS and STAU sites may also be due to a small sample size of only 30 individuals from a 100 m stream reach above or below the SASS and STAU sites. These samples may have only included individuals from one redd, which biases our ability to randomly sample the population. Relatedness assessment is affected by our ability to sample the entire population across all available habitats, as opposed to oversampling individuals from a small number of families from a small number of redd locations (Hansen et al. 1997; Herbinger et al. 2006; Hudy et al. 2010).

Indication of dispersal and further study. - Maximum likelihood analysis of pairwise kinship relationships between above- and below-barrier individuals indicated the presence of parent-offspring relationships between above- and below-barrier individuals at six sites in the BR Level III Ecoregion and five sites in the NLF Level III Ecoregion. This finding suggests that individuals moved between the above- and below-barrier location for natural barriers ranging in height from 1.8 to 42.7 meters in the BR Level III Ecorgion and 0.9 to 21.3 meters in the NLF Level III Ecoregion (Table 3.1, 3.7), which indicates dispersal across a wide range of barrier heights. However, this does not indicate whether the dispersal was in an upstream or downstream direction.

In this study, I attempted to document how parents and offspring are spatially distributed in relationship to natural barrier locations to infer dispersal. Further analysis could determine whether parents are moving in an upstream or downstream direction to establish redds, or whether young are dispersing upstream or being swept over the barrier to survive below. Spatial

distribution of breeding habitat and evidence of breeding success for different generations of fish could be monitored through further applications of kinship analysis. Epifanio et al. (2003) described the usefulness of supplementing redd count data with kinship analysis for tracking successful breeding and dispersal of the federally endangered bull trout. Identifying specific offspring and keeping track of breeding activity through kinship analysis could be used to track breeding success of specific individuals.

Bias of relatedness data occurs when a limited number of redds are sampled. I did not identify every individual and assign them to a redd, as was done in Hudy et al. 2010. This is the only way to verify that individuals are from one or more than one redd. COLONY (Jones and Wang 2009) applies a group-likelihood, probability-based method and Queller and Goodnight (1989) estimate a relatedness coefficient based on bootstrapped datasets from combined multi-locus genotypes for the entire sample, in this case, all individuals above or all individuals below each natural barrier. Current kinship analysis methods are based on one generation, which is based on a sample of individuals that resulted from the previous year's breeding event. Collecting more samples of all age classes and identifying individuals from specific redds longitudinally throughout all available habitat above and below all natural barriers within a subwatershed would eliminate any potential bias that these datasets represent (Hansen et al. 1997; Whiteley et al. 2006). Collecting data over ≥ 5 generations and incorporating pedigree analysis (Coombs et al. 2010) with mark-recapture data from permanent pit tag readers could be applied to further document how specific parents and offspring are dispersing from originating redds (Hudy et al. 2010).

TABLES

Table 3.1. Study site abbreviations and descriptions.

Site abbreviation	Site name	Location (county, state)	Barrier height (m)	Channel gradient (degrees)[1]
Blue Ridge:				
APOR	Apple Orchard Falls	Botetourt County, Virginia	61.0	80
BWLL	Big Wilson Creek, lower	Grayson County, Virginia	2.1	90
BWLU	Big Wilson Creek, upper	Grayson County, Virginia	1.8	5
CBCR	Cabin Creek	Grayson County, Virginia	7.6	80
CASC	Little Stony Creek, Cascades	Giles County, Virginia	21.3	90
CORN	Cornelius Creek	Botetourt County, Virginia	0.0	1
CRTR	Crabtree Falls	Nelson County, Virginia	91.4	80
NFKS	North Fork Stony Creek	Giles County, Virginia	0.0	1
SASS	Sassafras Falls	Graham County, North Carolina	30.0	70
SHCS	Shoe Creek Cascade	Nelson County, Virginia	3.4	15
SHOE	Shoe Creek	Nelson County, Virginia	0.0	1
STAT	Staton's Creek Falls	Amherst County, Virginia	42.7	80
STAU	Staunton River	Madison County, Virginia	3.8	6
STMY	Saint Mary's Falls	Augusta County, Virginia	4.0	90
WHRK	White Rock Falls	Augusta County, Virginia	11.3	80
Northern Lakes and Forests:				
COLE	Cole Creek	Alger County, Michigan	4.3	75
HAYM	Haymeadow Creek	Delta County, Michigan	0.9	7
MORG	Morgan Falls	Ashland County, Wisconsin	23.0	80
PRBR	Portage Brook waterfall	Cook County, Minnesota	6.4	90
SPBK	Spring Brook	Ashland County, Wisconsin	1.5	65
WAUP	Waupee Creek rapids	Oconto County, Wisconsin	0.9	8

[1]Gradient was estimated as arctan (degrees) based on change in elevation from the lowest to highest point of each barrier.

Table 3.2. Blue Ridge Level III Ecoregion stocking information for each site (Virginia site information from Virginia Department of Game and Inland Fisheries database; North Carolina information from North Carolina Wildlife Resources Commission stocking website).

Site	Stocking
Apple Orchard Falls, VA	North Creek managed for class II "wild" brook trout and rainbow trout, 1976 to 1994
Big Wilson Lower, VA	Managed for class II "wild" brook trout, rainbow trout, brown trout, 1978 to present
Big Wilson Upper, VA	Managed for class II "wild" brook trout, rainbow trout, brown trout, 1978 to present
Cabin Creek, VA	Managed for class I "wild" brook trout, rainbow trout, 1978 to present
Cascades, VA	Managed for class II "wild" brook trout and rainbow trout, 1977 to present
Cornelius Creek, VA	Managed for class II "wild" brook trout and rainbow trout, 1976 to 1990
Crabtree Falls, VA	Managed for class II "wild" brook trout, 1976 to 1994
Staunton River, VA	Managed for class III "wild" brook trout, 1975 to 2000
North Fork Stony, VA	Managed for class II "wild" brook trout, 1977 to present
Saint Mary's Falls, VA	Managed for class I, II "wild" brook trout, 1975 to present
Sassafras Falls, NC	Tributary to Big Snowbird Creek, "Hatchery supported water" to present
Shoe Creek, VA	Managed for class 1 "wild" brook trout, 1976 to 1993
Staton's Creek, VA	Managed for class II "wild" rainbow trout, 1976 to 1993
White Rock Falls, VA	North Fork Tye River managed for class II "wild" brook trout, 1976-1994

Table 3.3. Northern Lakes and Forests Level III Ecoregion stocking information (Michigan information from MIDNR Fish Stocking Database; Wisconsin information from WIDNR catchable trout database; Minnesota information from Great Lakes Fisheries Commission database).

Site	Stocking (brook trout)
Cole Creek, MI	Cole Creek Pond stocked below research site: 1980-1999 (MI Domestic; Assinica; Assinica/Rome; Owhi; Maine; Temiscame strains)
Haymeadow Creek, MI	1983-2009(Assinica; Assinica/Maine; Assinica/Rome; Maine; Owhi; Temiscame; Iron River strains)
Morgan Creek, WI	1919; 1922-1952; 1954-1956
Portage Brook, MN	1977
Spring Brook, WI	1904-1921; 1922-1952; 1941-1962
Waupee Creek, WI	St. Croix stock, up to and including 2009

Table 3.4. Details regarding brook trout microsatellites screened (King et al. 2003, 2005).

Locus	Dye	Allele size range (bp)	GenBank Accession number
Master Mix1:			
Sfo-C88	FAM (blue)	170-205	AY168192
Sfo-C113	FAM (blue)	125-170	AY168193
Sfo-D75	NED (black)	165-250	AY168197
Sfo-D100	HEX (green)	200-275	AY168199
Master Mix2:			
Sfo-C24	FAM (blue)	110-190	AY168187
Sfo-C115	FAM (blue)	225-370	AY168194
Sfo-C129	HEX (green)	215-270	AY168195
Ssa-D237	HEX (green)	270-450	AF525207

Table 3.5. Mean relatedness, r_{xy}, values (Queller and Goodnight 1989) for brook trout samples above (30 individuals) and below (30 individuals) barriers and for the combined above and below sample per site (all, 60 individuals) in the Blue Ridge and Northern Lakes and Forests Level III Ecoregions.

Site[1]	All	Above	Below
Blue Ridge:			
APOR	0.585	0.811	0.423
BWLL	0.196	0.175	0.192
BWLU	0.294	0.328	0.251
CASC	0.277	0.216	0.217
CBCR	0.277	0.270	0.275
CORN	0.216	0.219	0.219
CRTR	0.221	0.213	0.201
NFKS	0.184	0.168	0.167
SASS	0.334	0.335	0.326
SHCS	0.193	0.181	0.192
SHOE	0.193	0.178	0.167
STAT	0.185	0.188	0.201
STAU	0.313	0.329	0.266
STMY	0.338	0.369	0.255
WHRK	0.261	0.270	0.280
Mean±SE	**0.271±0.03**	**0.283±0.04**	**0.242±0.02**
Northern Lakes and Forests:			
COLE	0.182	0.165	0.178
HAYM	0.153	0.126	0.147
MORG	0.227	0.209	0.220
PRBR	0.188	0.174	0.179
SPBK	0.192	0.163	0.197
WAUP	0.164	0.148	0.156
Mean±SE	**0.184±0.01**	**0.164±0.01**	**0.180±0.01**

[1]Site abbreviations are explained in Table 3.1.

Table 3.6. Family structure inferred from analysis of microsatellite genotypes using COLONY (Jones and Wang 2009) for above- and below-barrier brook trout samples.

Site[1]	Barrier height (m)	FS families[2]	Mean individauls in FS family[2]	Mixed FS families	% FS families mixed per sample
Blue Ridge:					
APOR	61.0	16	3.75	0	0
BWLL	2.1	40	1.50	6	22
BWLU	1.8	27	2.22	7	38
CBCR	7.6	25	2.40	9	55
CASC	21.3	40	1.50	8	35
CORN	0.0	26	2.31	3	27
CRTR	91.4	32	1.88	1	3
NFKS	0.0	40	1.50	10	40
STMY	4.0	21	2.86	2	15
SASS	30.0	20	3.00	7	53
SHOE	0.0	41	1.46	8	32
SHCS	3.4	32	1.78	9	44
STAT	42.7	39	1.54	3	10
STAU	3.8	28	2.14	8	42
WHRK	11.3	26	2.31	3	18
Mean±SE		**30±2**	**2.14±0.17**	**6±1**	**29±4**
Northern Lakes and Forests:					
COLE	4.3	46	1.30	3	10
HAYM	0.9	43	1.40	8	28
MORG	23.0	36	1.67	9	40
PRBR	6.4	43	1.40	5	18
SPBK	1.5	41	1.46	9	37
WAUP	0.9	45	1.33	8	27
Mean±SE		**42±1**	**1.43±0.05**	**7±1**	**27±5**

[1]Site abbreviations are explained in Table 3.1.
[2]FS = Full sibling

Table 3.7. Presence of parent-offspring pairs of individuals among above- and below-barrier locations, identified as significant by hypothesis testing. In this case, the putative parent-offspring relationship is tested against the full-sib alternative relationship as most likely (Kalinowski et al. 2006). The *P* value represents a test of significance for the putative parent-offspring relationship against the full-sib alternative relationship. If the *P* value for the test is low, the alternative hypothesis is rejected and the parent-offspring relationship is the most likely (Kalinowski et al. 2006).

Site[1]	Individual 1 (above-barrier)[2]	Individual 2 (below-barrier)[3]	*P* value
Blue Ridge:			
BWLU	16	37	0.03
SASS	7	39	0.05
	5	31	0.04
SHCS	22	40	0.04
	22	42	0.02
	24	42	0.05
	30	57	0.05
STAT	23	57	0.02
STMY	11	56	0.05
	12	56	0.04
Northern Lakes and Forests:			
COLE	8	39	0.07
	10	39	0.07
HAYM	5	59	0.05
MORG	25	60	0.07
PRBR	22	56	0.06
SPBK	19	53	0.03

[1]Site abbreviations are explained in Table 3.1.
[2]Individuals numbered 1-30 indicate an individual from the above-barrier sample.
[3]Individuals numbered 31-60 indicate an individual from the below-barrier sample.

Table 3.8. Fixation indices across loci per site, as estimated by GENEPOP (Rousset 2007). F_{IS} values represent within-sample departures from Hardy-Weinburg equilibrium (HWE), due to inbreeding and/or other causes. F_{ST} values represent between-sample departures from HWE due to differentiation of samples, and F_{IT} represents total departures of genotypes from HWE. Note: *Denotes a departure of genotypes from HWE. In this case, a negative or low F_{IS} means observed heterozygosity > expected heterozygosity for individuals within sample. A more positive F_{IS} represents possible inbreeding or mixing of native + stocked individuals (Wang et al. 2002; Herbinger et al. 2006).

Site[1]	F_{IS}	F_{ST}	F_{IT}
Blue Ridge:			
APOR	-0.141	0.350	0.258*
BWLL	0.035	0.012	0.047
BWLU	0.017	0.030	0.047
CBCR	0.079	0.025	0.102*
CASC	0.014	0.040	0.018
CORN	0.059	0.026	0.083
CRTR	-0.065	0.069	0.008
NFKS	0.025	0.000	0.025
STMY	0.023	0.151	0.171*
SASS	0.169	0.033	0.197*
SHCS	0.074	0.004	0.078
SHOE	0.096	-0.002	0.094
STAT	0.058	0.031	0.087
STAU	0.335	0.051	0.369*
WHRK	-0.012	0.043	0.032
Northern Lakes and Forests:			
COLE	0.037	0.015	0.051
HAYM	-0.008	0.001	-0.007
MORG	0.066	0.004	0.070
PRBR	0.069	0.007	0.076
SPBK	0.033	0.012	0.044
WAUP	0.012	-0.000	0.011

[1]Site abbreviations are explained in Table 3.1.

FIGURES

Figure 3.1. Study sites sampled in the Northern Lakes and Forests Level III Ecoregion (Bailey 2005) (six sites) (PRBR=Portage Brook, MORG=Morgan Falls, SPBK=Spring Brook, COLE=Cole Creek, HAYM=Haymeadow Creek, WAUP=Waupee Creek).

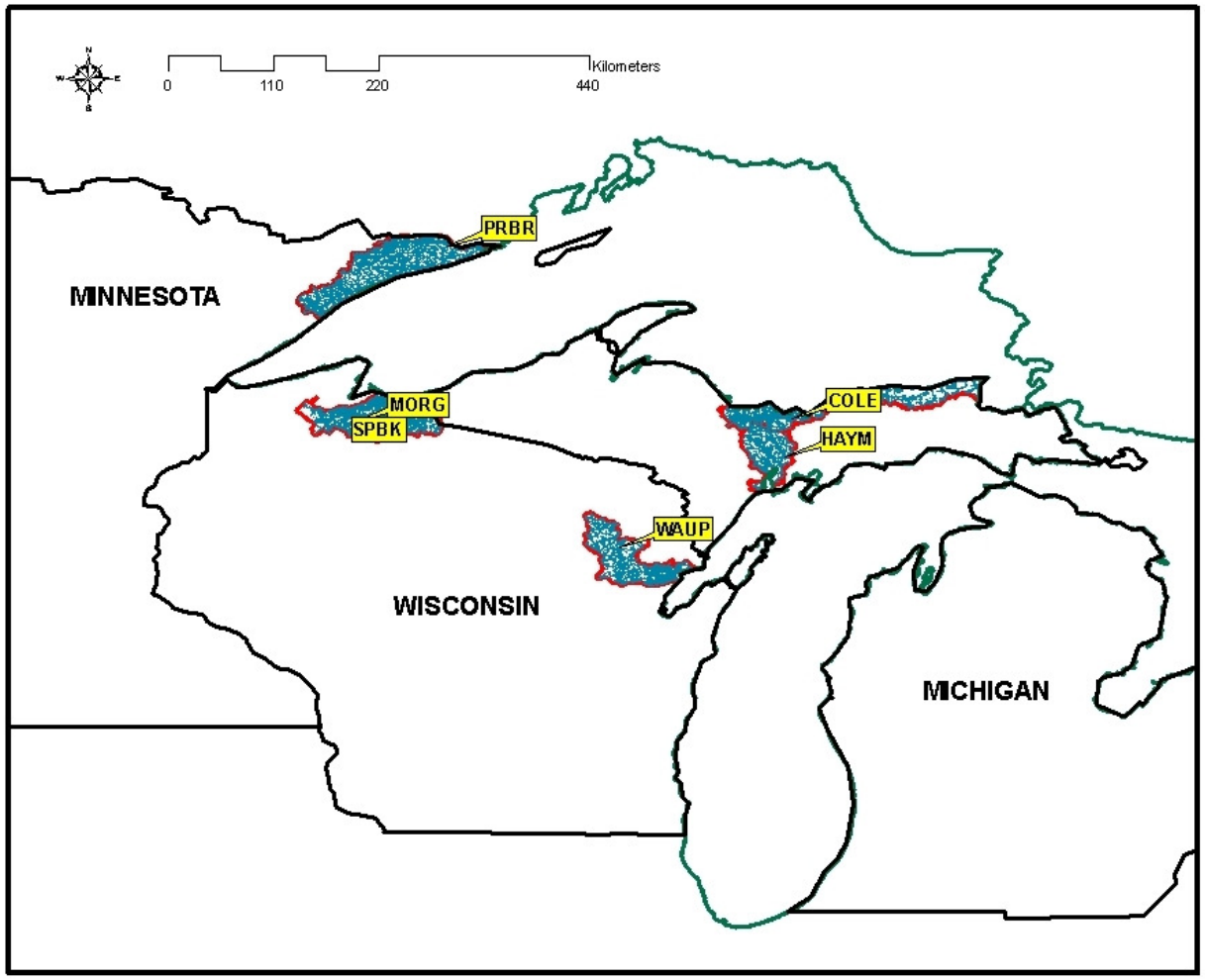


Figure 3.2. Study sites sampled in the Blue Ridge Level III Ecoregion (Bailey 2005) (15 sites) (SASS=Sassafras Falls; CBCR=Cabin Creek; BWLU=Big Wilson Creek, upper; BWLL=Big Wilson Creek, lower; NFKS=North Fork Stony Creek; CASC=Little Stony Creek, Cascades; APOR=Apple Orchard Falls; CORN=Cornelius Creek; SHCS=Shoe Creek Cascade; STAT=Staton's Creek Falls; SHOE=Shoe Creek; STMY=Saint Mary's Falls;WHRK=White Rock Falls; CRTR=Crabtree Falls; STAU=Staunton River).

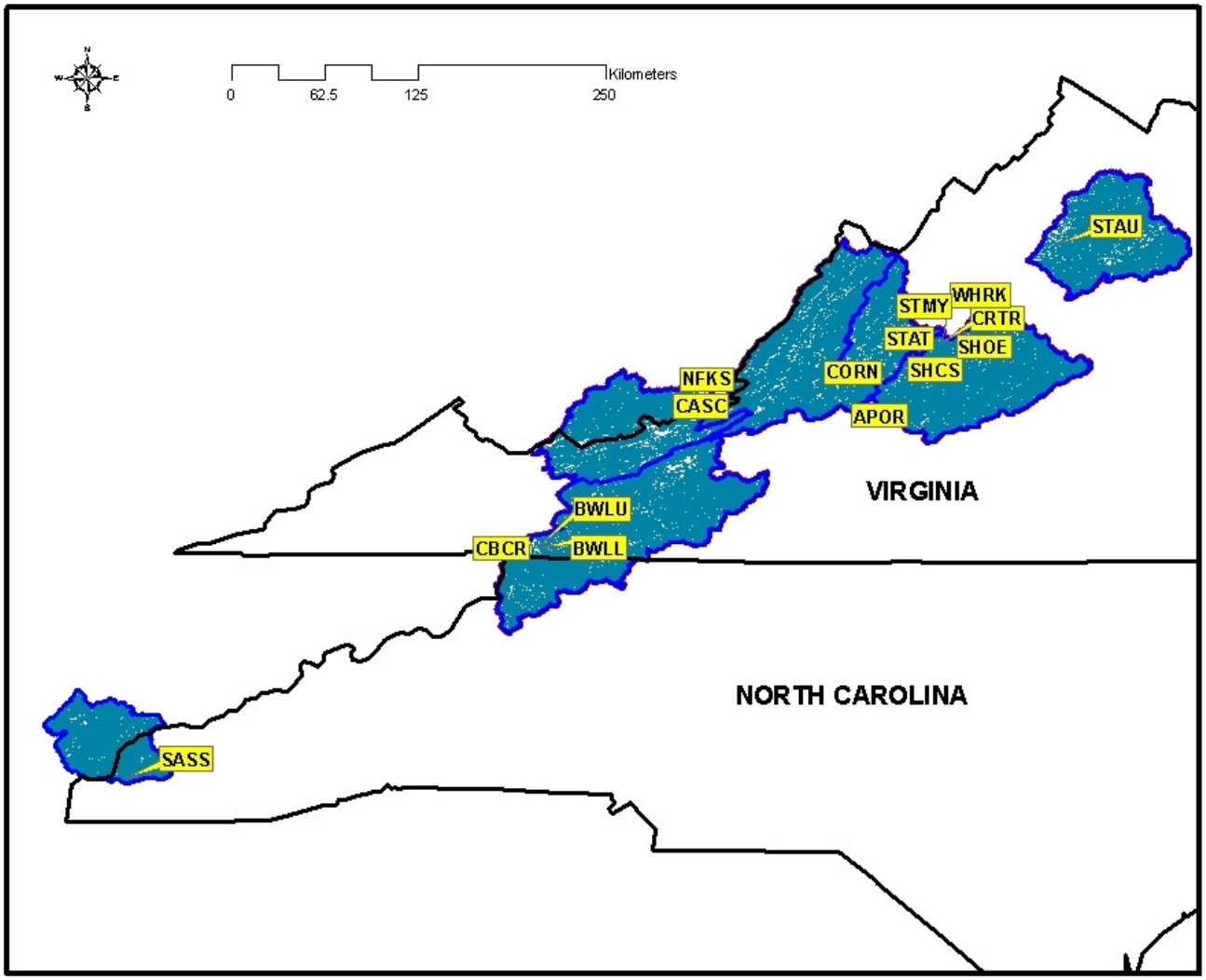
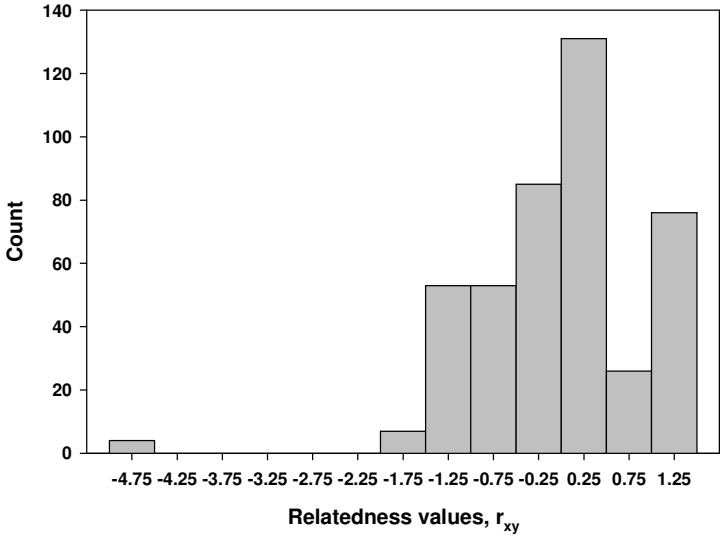


Figure 3.3. Histogram of the APOR site showing counts of relatedness values generated by applying Queller and Goodnight's (1989) pairwise relatedness estimator, r_{xy} using the IDENTIX program (Belkhir et al. 2002). Relatedness values ≥ 0.25 represent half-sibs and relatedness values ≥ 0.50 represent full-sibs. The APOR site had 54% of individuals that were related, with 24% full-sibs and 30% half-sibs.



Summary and Conclusions

The overall goal of this study was to investigate applications of population genetic analysis tools as biological indicators of barrier effects on brook trout *Salvelinus fontinalis* populations in two different Level III Ecoregions. I applied statistical analyses to multilocus microsatellite marker genotype data from 1,257 brook trout from 15 sites in the Blue Ridge (abbreviated BR) Level III Ecoregion and six sites in the Northern Lakes and Forests (abbreviated NLF) Level III Ecoregion to test effects of barriers on population genetic diversity and differentiation (Chapter 1), effective population size (N_e) (Chapter 2), and kinship relationships (Chapter 3).

Chapter 1

This study was the first to investigate how barriers of different heights and gradients drive patterns in resident brook trout population genetic diversity, differentiation, and population structuring in two Level III Ecoregions of the eastern United States. The objective this study was to test the hypothesis that brook trout population genetic diversity (*A*, *H*) and population differentiation (F_{ST} and R_{ST}) differed in relation to barrier height (m) and gradient. There was no significant difference between above and below samples for the population genetic diversity measures, but barrier height and F_{ST} values were positively related (r^2=0.38, *F*=8.48, *P*=0.011) (Figure 1.3). Correlations between height and gradient were significant (Spearman's rho=0.562, *P*=0.02) and barrier height was identified as the preferred predictive variable (MLR height, *P*=0.011, gradient, *P*=0.443). There were no significant relationships identified between R_{ST} and barrier height (m) or gradient.

Further evidence to support an effect of barriers on population differentiation included genetic distance-based unrooted NJ consensus trees and individual assignment analysis. Samples from this study that stand out as genetically isolated include the APOR above-barrier sample from the BR Level III Ecoregion, which is genetically divergent according to the NJ consensus tree, 100% bootstrap support (Figure 1.4). The MORG in the NLF Level III Ecoregion above-barrier sample is also genetically divergent according to the NJ consensus tree with 100%

bootstrap support (Figure 1.5).

Individual assignment analysis for all sites included in this study showed that Q values identified a threshold height of 4 meters that resulted in population differentiation between the above and below sample. These Q values were supported by a 97% correct assignment of individuals to their source population for the BR Level III Ecoregion and a 91% correct assignment of individuals to their source population for the NLF Level III Ecoregion (Figure 1.6).

This study provides evidence that supports a management strategy for brook trout populations at the subwatershed scale based on NJ consensus tree clustering of populations that show genetic similarity and mixing of individuals at the subwatershed scale. The results of this study further emphasize the need to develop management strategies according to ecoregion as well, as indicated by the significantly higher difference in genetic diversity for the NLF Level III Ecoregion as compared to the BR Level III Ecoregion.

Chapter 2

This study was the first to investigate habitat fragmentation factors affecting N_e of resident Southern Appalachian brook trout populations associated with natural barriers. It presents estimates of N_e from one generation of brook trout collected from a stream reach adjacent to a natural barrier within a unique subwatershed.

These results support the importance of the relationship among habitat connectivity, reproductive success, and N_e, as estimated N_e values were most significantly related to total stream length isolated above each barrier and road density (adjusted $r^2 = 0.50$, F=5.46, $P = 0.04$). The total potential stream length isolated above the barrier represents the potential habitat that is inaccessible to breeding adults. The distribution of roads within subwatersheds can also affect breeding success due to direct disturbance to spawning habitat from road construction and indirect effects due to increased sedimentation (Wood and Armitage 1997; Jones et al. 2000; Wheeler et al. 2005).

Despite the significant correlation between N_e, total potential stream length isolated

above the barrier, and road density, this model still only explains 50% of the variability (adjusted $r^2 = 0.50$). Investigated variables in this study had limited ranges of variability and other parameters may be more detectable at other spatial scales. Demographic parameters that affect N_e of brook trout need to be incorporated into future study as well to target other sources of variability. Such parameters include: sex ratio, number of breeders, and variance in family size (Arden and Kapuscinski 2003; Shrimpton and Heath 2003; Araki et al. 2007).

All sampled brook trout populations from the BR Level III Ecoregion had N_e values below recommended values for long-term maintenance of genetic variation (N_e=500), however, N_e is naturally variable and samples from this study only represent one sample of 60 individuals from the previous generation. In addition, sampled brook trout populations affected by barriers are unlikely to be in migration-drift equilibrium, which is assumed when estimating N_e, so results should be interpreted with caution. Tallmon et al. (2010) recommend sampling at least 60 individuals ≥ five generations apart over the long-term to detect long-term trends in N_e that may be of conservation concern.

Chapter 3

This was the first study to apply microsatellite markers to assess kinship in resident brook trout populations associated with natural barriers. This study investigated applications of microsatellite multilocus genotype kinship relationships, which can be used to infer the amount of dispersal occurring at a site. The study objective was to identify sites with increased inbreeding to identify populations genetically isolated above the barrier.

I tested the null hypothesis that mean pairwise relatedness coefficients, r_{xy} (Queller and Goodnight 1989) will not differ between above- and below-barrier samples. I tested a second null hypothesis that barrier height will not affect brook trout mean pairwise relatedness coefficients, r_{xy} (Queller and Goodnight 1989) or numbers of full-sibs per family group (Jones and Wang 2009) for each site. I also tested for the presence of parent-offspring relationships between above- and below-barrier individuals at each site to investigate dispersal across the barrier. There was no significant difference detected between mean pairwise relatedness values

for above- and below-barrier samples of brook trout and there was no significant relationship between mean pairwise relatedness or number of full-sibs per family and barrier height.

Maximum likelihood analysis of pairwise kinship relationships between above- and below-barrier individuals indicated the presence of parent-offspring relationships between above- and below-barrier individuals at six sites in the BR Level III Ecoregion and five sites in the NLF Level III Ecoregion. This finding indicates the movement of individuals between the above- and below-barrier location for natural barriers ranging in height from 0.9 to 42.7 meters. This study was not able to identify whether individuals moved upstream above a barrier or downstream over a barrier to establish a family. Further longitudinal sampling throughout all available habitat above and below barriers of all age classes would eliminate the bias of only sampling a limited number of families from specific redd locations.

Synthesis

Population genetics is an essential tool for developing large-scale long-term conservation management plans for the brook trout and other fish species. This study documented brook trout population structuring at the subwatershed scale based on genetic distance analysis and showed that brook trout allelic diversity can vary significantly by Level III Ecoregion. The study also documented the value of combining population genetic markers, as the combination of brook trout diagnostic allozyme loci and microsatellite loci documented a network of connected streams in adjacent subwatersheds with Southern Appalachian strain brook trout that are of conservation concern.

Brook trout population genetic structuring results that show genetic similarity due to interbreeding suggest that the minimum scale of management is at the subwatershed scale. Managing at the stream reach scale, for this species would not consider the evolutionary paradigm definition of a population, which considers interbreeding individuals as members of the same population (Waples and Gaggiotti 2006).

Management implications.- Results from this study can be used to identify potential source populations and populations at risk for the BR and NLF Level III Ecoregions. The

following samples from this study have the highest representative allelic diversity and represent populations that can function as source populations for conservation in the NLF Level III Ecoregion include: HAYM (98 alleles) and COLE (87). For the BR Level III Ecorgion, the following sites have the highest allelic diversity and can function as source populations for conservation: BWLL (75), non-barrier site, NFKS (73), and non-barrier site, SHOE (70). Sites that showed differentiation above the barrier according to the NJ consensus tree included the APOR, CRTR, and MORG sites as well.

The following sites have low allelic diversity and represent a site that may be at risk due to genetic isolation: APOR, STMY, SASS, and WHRK. The APOR site has the lowest allelic richness of all 21 sites in the study, had the lowest N_e value of all 21 sites, had no mixed full-sib families, and had the highest relatedness above the barrier, all indicating genetic isolation above the barrier (Table 1.4, Table 2.5, Table 3.5, Figure 3.3). The STMY site also had the second lowest N_e of this study and a high relatedness value (Table 2.5, Table 3.5). The SASS site also had a high relatedness and inbreeding value (Table 3.5, Table 3.8).

Further research. - This study was based on one sample at one above- and one below-barrier location for each site at the spatial scale of 50 meters to 585 meters. Collecting more samples longitudinally throughout a particular stream network where barriers occur could help to single out particular population genetic effects at a barrier location relative to population genetic relationships found throughout the stream network as well.

Long-term research with repeated sampling from multiple generations is needed to document movement of fish above a barrier (upstream movement) versus fish below the barrier (downstream movement) and to document long-term changes in effective population size. Long-term research also could investigate further barrier physical characteristics and flow that are related to dispersal events. Permanent pit-tag readers and mark-recapture data combined with N_e and gene flow estimates could be used to further investigate long-term effects of asymmetric gene flow and parent-offspring dispersal as well.

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