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A Predictive Model for Brook Trout Restoration in the Cherokee National Forest

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To the Graduate Council:

I am submitting herewith a thesis written by Caylor Garrett Romines entitled "A Predictive Model for Brook Trout Restoration in the Cherokee National Forest." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

J. Brian Alford, Major Professor

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A Predictive Model for Brook Trout Restoration in the Cherokee National Forest

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Abstract

Over the course of the last century, Brook Trout (*Salvelinus fontinalis*) have been reduced in abundance and extirpated from many high elevation streams throughout the southern Appalachian Mountains. These fish have been threatened by anthropogenic factors that restrict their distribution across the longitudinal gradient of the streams they occupy. A large portion of Tennessee's Brook Trout streams are located within the Cherokee National Forest (CNF). Many agencies in the southern Appalachian Mountains are working to restore Brook Trout populations throughout this species' historic native range. The purpose of this research is to develop a model of important habitat variables used to characterize the suitability of a stream for Brook Trout restoration based on its predicted biomass in the CNF. Thirty streams across the CNF known to support Brook Trout were evaluated by completing a three-pass depletion fish survey, quantifying instream habitat characteristics, and examining riparian forest structure. Habitat characteristics were modeled against Brook Trout biomass (kg/ha) to determine significant variables that characterize Brook Trout abundance. Ten additional streams on the CNF were sampled to validate the accuracy and precision of the models. A Random Forest model determined the significant habitat variables (n=11), then a multi-nomial logistic regression model predicted Brook Trout biomass based on these variables. For optimal biomass, values of the important variables should be: percent riffle area <25%, >350 m to the nearest road, >13% slope, elevation ≥1,000 m, >55% boulder substrate, *Rhododendron* cover <10% or 25-40%, canopy cover 92-97% or ≥98%, dominant geologic rock type of gneiss, granite, or sandstone, <25% cobble substrate, total volume of 1 to 7.5 m^3, and total dissolved solids >12 ppm. This model provides a technique for rapid habitat assessment to

aid in the decision-making process of Brook Trout restoration site selection. Based on these selected variables, efforts to improve Brook Trout habitat should focus on four primary areas: reduction of riffle habitat (i.e., creating more pools), maintaining canopy closure, reducing *Rhododendron* cover, and preventing sediment run-off from nearby roads.

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CHAPTER I: INTRODUCTION

The clear, cold mountain streams of east Tennessee are the stronghold for Brook Trout (*Salvelinus fontinalis*), the region's only native salmonid. Also known as the "mountain trout" or "speckled trout" by local people, Brook Trout are actually members of the charr genus (*Salvelinus*). Six clades are now recognized (Habera et al. 2017): sea run, northern Atlantic Slope, St. Lawrence River/Great Lakes, upper interior basin (Ohio River), southern Atlantic Slope, and lower interior basin (Ohio River). The historic range of Brook Trout in the United States extends from New England to the headwaters of the Mississippi River in Minnesota and Wisconsin. Brook Trout distribution also extends along the Appalachian Mountains from Virginia and West Virginia into Tennessee, North Carolina, South Carolina, and northern Georgia (Hudy et al. 2008). Those strains native to Tennessee (hereinafter referred to as native Brook Trout) belong to the lower interior basin (Ohio River) clade (Habera et al. 2017). Brook Trout have been introduced widely for sport on a global scale since the early 20th century.

Many state and federal agencies in the eastern United States, particularly those in the southern Appalachian region of Georgia, North Carolina, South Carolina, Tennessee, and Virginia, are working to restore Brook Trout populations throughout this species' historic native range (Eastern Brook Trout Joint Venture 2005). Brook Trout have been exposed to numerous chemical, physical, and biological stressors that threaten the long-term viability of the species throughout its native range (Marschall and Crowder 1996; Galbreath el al. 2001). Brook Trout populations have declined in size and range due to historic and persistent anthropogenic impacts, including uncontrolled logging, acid precipitation, mine drainage, overharvesting and agonistic interactions with non-native species (Larson and Moore 1985; Marschall and Crowder 1996; Wigington et al. 1996; Hudy et al. 2008; Isaak et al. 2010). Many agencies and non-governmental organizations consider the restoration of Brook Trout an important management goal because of their recreational, cultural, and ecological values. Stream characteristics such as higher alkalinity and low amounts of fine sediments (i.e., silt and sand) have been cited as key factors for Brook Trout reproduction success and population sustainability (Petty et al. 2005; Hartman and Hakala 2006). However, many of the watersheds in the southern Appalachians have low primary productivity because of their low alkalinity, and alternative habitat factors may be important for expanding and sustaining existing Brook Trout populations (Habera and Strange 1993).

Due to the decline of Brook Trout in the eastern U.S., a diverse group of partners, including state fish and wildlife agencies, federal resource agencies, Indian tribes, academic institutions and non-governmental organizations are working to conserve Eastern Brook Trout and their habitats. This partnership – the Eastern Brook Trout Joint Venture (EBTJV) – has produced a range-wide population assessment of wild Brook Trout, completed extensive work that identifies key threats to wild Brook Trout and their habitats, and developed conservation strategies to protect, enhance, and restore wild Brook Trout. Historically, approaches to the conservation of eastern Brook Trout have been fragmented across its range (Thieling 2006). A comprehensive range-wide conservation strategy assists all partners in effectively addressing common large-scale threats to Brook Trout and their habitat. The EBTJV promotes recognition that aquatic habitat loss is a national problem and that the quality and diversity of aquatic resources depend on habitat conservation. The EBTJV demonstrates the effectiveness of broad collaborative endeavors to improve aquatic habitats and conserve valuable aquatic resources (EBTJV 2005).

Study Objectives

The Appalachian Mountains, specifically within the Cherokee National Forest (CNF), provide a unique research opportunity to expand knowledge of Brook Trout habitat at the southern end of their range. The U.S. Forest Service (USFS) has helped restore Brook Trout and their habitat in many streams and plans to restore Brook Trout in additional systems that have suitable habitat. This research relates influential instream and riparian habitat factors of headwater reaches with Brook Trout biomass in CNF streams to develop a suitable habitat model of important factors for future Brook Trout management. It will provide the USFS, Tennessee Wildlife Resources Agency (TWRA) and other entities working to restore Brook Trout with a guide for assessing the suitability of streams as prospective restoration projects in the CNF and potentially the Southern Appalachian mountain region.

Research objectives are as follows:

1) Estimate Brook Trout biomass (kg/ha) in CNF mountain streams;
2) Quantify riparian and geospatial habitat characteristics that are available to Brook Trout;
3) Assess instream habitat characteristics that are available to Brook Trout;
4) Model and validate habitat factors from objectives 2 and 3 as predictor variables of Brook Trout abundance to aid in selection of suitable stream segments for restoration in the CNF.

CHAPTER II: LITERATURE REVIEW

Brook Trout Ecology

Tennessee has 112 Brook Trout populations in 226 km of streams and one pond. Sixty-seven are putative native populations (60%), 17 are hatchery-reared (15%), and the rest (25%) have varying degrees of introgression from stocking of hatchery Brook Trout (which are typically derived from the northern Atlantic Slope clade) (Habera et al. 2017). Hatchery-reared Brook Trout were stocked extensively to replenish depleted populations of native Brook Trout (Sherrill et al. 2001).

Brook Trout generally spawn during the fall to early winter (Raleigh 1982), and can be very successful in lentic environments around spring upwelling areas, with spawning occurring at 4.5-10° C (Webster and Eiriksdottier 1976). Brook trout spawn in gravel that is small enough to move during redd excavation (Witzel and MacCrimmon 1983), but they tend to avoid fine sediments because these reduce embryo survival and emergence success (Power 1980; Alexander and Hansen 1983). Once spawning occurs and the eggs are fertilized, they are then deposited in redds. Spawning success is reduced as the amount of sedimentation increases in the stream channel and the dissolved oxygen concentration is diminished (Harshbarger 1975). Both female and male Brook Trout exhibit mate choice. Males prefer larger females, which are capable of producing more eggs, and females prefer males that are of equal or greater size, perhaps because the incidence of egg cannibalism is lower when a larger male fertilizes a redd (Blanchfield and Ridgway 1999). Males depart after fertilizing the eggs, and no parental care is provided after the female buries the eggs (Hutchings 1994; Blanchfield and Ridgway 1999).

Unlike other diadromous salmonids, Brook Trout are typically potamodromous, thus they are mostly restricted to the headwaters of river drainages, except in some of the more northern catadromous populations where they may migrate to the Atlantic Ocean; other potamodromous populations will migrate from headwaters to the Great Lakes. Brook Trout presumably exhibit some degree of site-fidelity characteristics of other salmonids (e.g., Quinn 1993). Water temperature appears to be critical in determining the timing of spawning activity, especially in the native range (Baril and Magnan 2002). Homing to natal habitat is presumed to occur at larger scales (10^2-10^3 m), but evidence for site fidelity on a microhabitat scale (1-10 m) is less clear (Baril and Magnan 2002; Bernier-Bourgault and Magnan 2002). Brook Trout are generally considered to have the shortest lifespan of all charr species (Power 1980). However, significant variation in longevity is apparent between their native and introduced ranges. Brook Trout often do not survive for more than three or four years in streams within their native range and often do not grow larger than 250 mm (McFadden 1961; McFadden et al. 1967; Flick and Webster 1975; Fausch and White 1981; Whitworth and Strange 1983).

Habitat requirements and diets often change as juvenile fish grow larger and energetic needs and the size of feeding territories typically increase (Keeley 1998, Keeley 2001). This change can lead to a thinning of density within local populations in many salmonids but is still relatively unclear for Brook Trout. It appears that juvenile fish do not move far. A study of Brook Trout in a West Virginia stream found that density of juvenile fish was correlated with the density of spawning fish in the previous fall and remained seasonally constant (Petty et al. 2005). The ability to move in search of resources is probably limited by swimming ability, which is length-dependent (Northcote 1997). Brook Trout are opportunistic sight feeders, consuming all types of aquatic macroinvertebrates and terrestrial insects (Reed and Bear 1966). Young individuals prefer small, drifting organisms, especially Diptera and Ephemeroptera. Larger and older trout often prefer late-instar Trichoptera larvae (Griffith 1974). This suggests that populations need an ecosystem supporting diverse prey types, particularly taxa that are intolerant of organic pollution in order to thrive.

On a broad scale, Brook Trout typically occur in areas with a cool temperate climate, cold spring-fed ground water, and moderate precipitation (MacCrimmon and Campbell 1969). Currently, warming temperatures in lower elevation reaches and acidic deposition in the headwaters seem to be the major contributing factors to Brook Trout extirpation in certain areas (Jackson 2015). Brook Trout require dissolved oxygen concentrations greater than 5 mg/L (Avault 1996), and concentrations of 7 mg/L or more are optimal (Raleigh 1982). Brook Trout are known to occur in waters with a wide range of alkalinity and specific conductivity, whereby high concentrations of each of these tend to indirectly increase Brook Trout production through bottom-up energy transfers (Raleigh 1982). Stream channel gradient appears to be an important correlate for Brook Trout habitat. Brook Trout can move through higher-gradient stream reaches, but they are often more abundant in low to moderate gradient stream reaches within higher-elevation mountain streams (Raleigh 1982). Canopy cover is important in mountain streams for maintaining shade that regulates stream temperatures. However, too much shade can restrict overall stream productivity by restricting light penetration. Temperatures can be regulated by controlling the amount of shade the stream receives, where 50-75% midday shade appears to be optimal for most small streams that support trout (Raleigh 1982). During summer, cover from shade, overhanging banks, and large woody debris is used mostly by trout for resting and predator avoidance. They will utilize different microhabitats in the winter than in the summer, most likely seeking cover under rocks or in crevices (Bustard and Narver 1975).

CHAPTER III: METHODS

Study Area

The study area is located on the CNF in east Tennessee along the border of North Carolina and is split into two distinct zones, north and south, with Great Smoky Mountains National Park situated between the two zones. The north zone of the CNF is approximately 150,000 ha on two different districts (Unaka and Watauga Ranger Districts) in Carter, Cocke, Greene, Johnson, Sullivan, Unicoi and Washington counties (Figure 1). The south zone of the CNF is also approximately 150,000 ha on two different districts (Tellico and Ocoee Ranger Districts) in McMinn, Monroe and Polk counties (Figure 1). All 10 of the validation streams are located on the north zone of the CNF, along with 28 of the test sites. The two remaining test sites are located on the south zone of the CNF in the Tellico Ranger District. The CNF has a multitude of high-elevation mountain streams, most meandering through mature forest (80+ years) with some passing through wilderness areas where the land has not been manipulated in almost a century. The streams on the north zone are within four separate Hydrologic Unit Code 8 (HUC 8) watershed units, which are the South Fork of the Holston River, Watauga River, Nolichucky River and the Lower French Broad River. The streams on the south zone are within a single HUC 8 watershed unit which is the Little Tennessee River. Most of the CNF is in the Blue Ridge physiographic province with only the western-most portions being in the Ridge and Valley province (Fenneman, 1938).

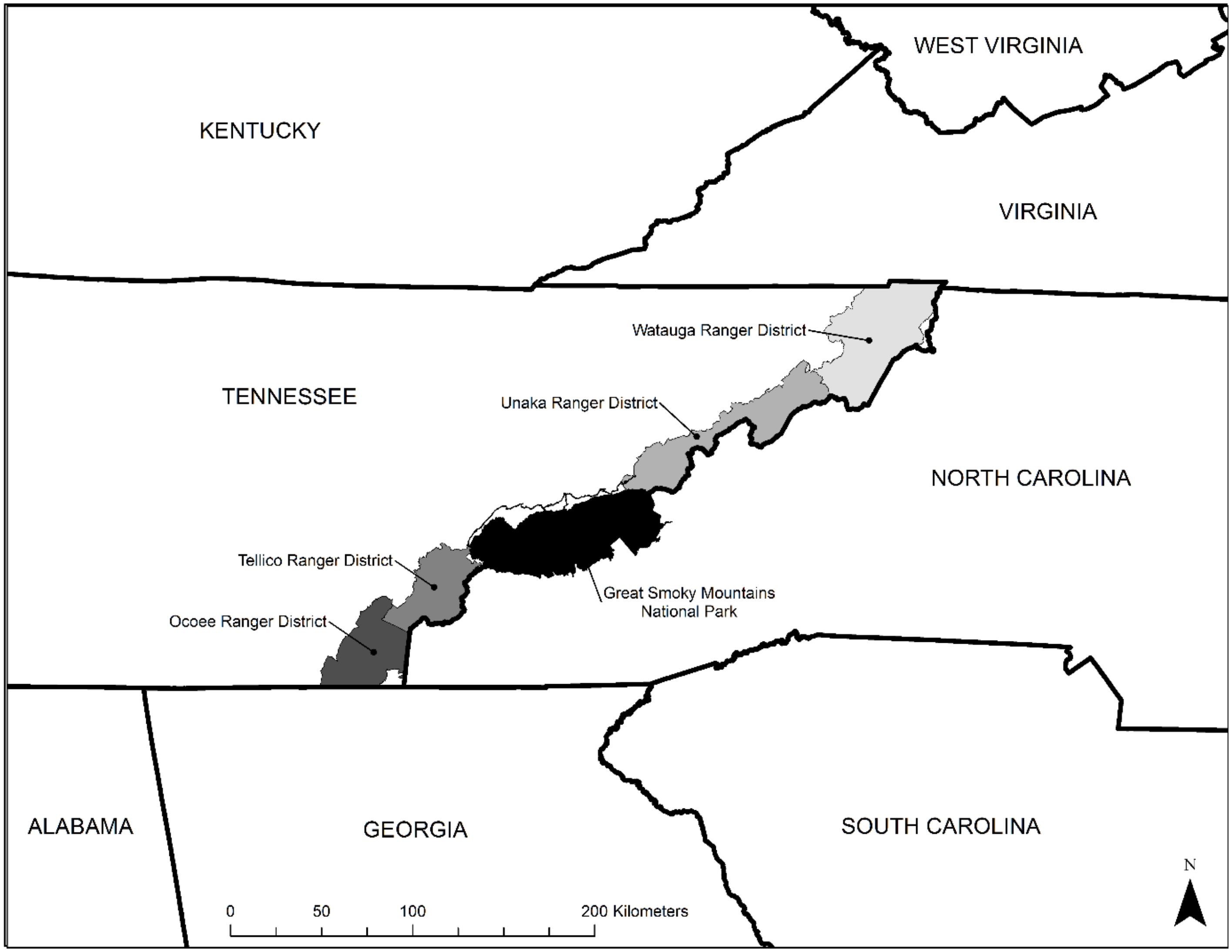

Figure 1. Study area map showing the four ranger districts on the Cherokee National Forest and the Great Smoky Mountains National Park.

Geology – The CNF consists of igneous and metamorphic rock and by deformed sedimentary rock. Most of the units range in age from Pre-Cambrian to Mississippian. The area contains extensive folding and numerous faults, which are believed to be inactive, although small tremors are occasionally felt. Anakeesta and Wilhite formations containing iron sulfide occur in many areas of the CNF. The erosion and chemical weathering of these formations when exposed produces sulfuric acid that can be harmful to aquatic ecosystems (USFS 2004).

Soils – Soils are derived from sandstone, phyllite, and shale parent materials which dominate much of the CNF. Smaller soil areas have developed from other types of rock such as limestone, granite, quartzite, gneiss, schist, and slate. The different kinds of soil have variable physical and chemical properties like texture, depth, rock content, relief, acidity, plant nutrients and available moisture. Erodibility and stability of these soils differ with steepness of slope, amount and kind of vegetation, and amount and timing of soil disturbance (USFS 2004).

Watersheds and Riparian Areas – There are approximately 4,667 km of perennial streams within the CNF. The riparian corridor associated with perennial and intermittent streams is estimated to be 51,000 ha. Most of the riparian ecosystems are largely maintained in a healthy condition and are in a later seral stage of forest development. Across the CNF, roads and dispersed recreation use are the primary impacts to the riparian areas. Total water yield is approximately 650,000 ha-m/yr. Water quality is generally good and meets criteria established by the State of Tennessee (USFS 2004).

Climate – The area is within the humid temperate domain, hot continental division, Appalachian oak forest section. The average annual temperature is 15°C, annual rainfall ranges from about 1 m at the lower elevations to over 2 m at the higher elevations. Mean annual runoff varies from 0.5 m in areas of low rainfall to over 1 m in areas of high rainfall. Snowfall contributes insignificantly to the total annual precipitation. The growing season ranges from 150 days per year at the highest elevations on the northern extent of the national forest to 230 days at the lowest elevations on the southern portion (USFS 2004).

Study Site Selection

Initially, all Brook Trout streams in the north zone were placed into a randomization assignment within a Microsoft Excel database, and 30 streams were selected as test sites for model development, whereas an additional ten sites were selected for model validation. Validation streams were sampled after all test sites were sampled. To avoid bias because of seasonality, a different randomization procedure was run through the Random Forest package in R that included a dataset with all 40 streams, whereby 10 independent streams were selected at random to validate the accuracy and precision of the training models. The updated training and validation sites are shown in Tables 1 and 2. All known wild trout streams (i.e., combination of Rainbow Trout, Brown Trout, and Brook Trout) were included to account for the vast number of stream segments that support all trout species, however the streams must have included a record of Brook Trout at some point to be considered in the randomization. The Watauga Ranger District provided 18 test sites and 6 validation sites (Figure 2), and the Unaka Ranger District hosted 10 test sites and 4 validation sites (Figure 3). The remaining two test streams were on the Tellico Ranger District on the south zone of the CNF (Figure 4). Sampling reaches were determined at each stream segment based on historical sampling conducted by the USFS and TWRA. If there were no historical surveys on a stream, the location was

Table 1. List of streams that were selected randomly and defined as test sites for this project to train the model for selection of important habitat variables.

ID	Name	District	Latitude	Longitude	Elevation (m)
1	Birch Branch	Watauga	36.555455	-81.868766	829.04
2	Camp Ten Branch	Watauga	36.226798	-82.043384	984.53
3	Dry Fork	Unaka	35.877316	-82.955682	660.99
4	Furnace Branch	Watauga	36.403052	-82.118482	603.72
5	Gentry Creek	Watauga	36.559258	-81.711131	977.65
6	Georges Creek	Watauga	36.173595	-82.118848	1036.32
7	Heaberlin Branch	Watauga	36.555010	-81.906010	875.02
8	Little Paint Creek	Unaka	35.965401	-82.812006	638.10
9	Little Stony Creek (Lake Trib)	Watauga	36.291986	-82.067031	684.26
10	Leonard Branch	Watauga	36.240545	-82.084277	883.56
11	Left Fork of Mill Creek	Watauga	36.438135	-82.078898	764.26
12	Lower Higgins Creek	Unaka	36.086764	-82.526377	775.34
13	Left Prong Hampton Creek 2	Watauga	36.145629	-82.048477	987.93
14	Little Stony Creek	Watauga	36.393313	-82.160938	549.64
15	Middle Prong Gulf Fork	Unaka	35.797789	-82.998048	910.70
16	Round Knob Branch	Unaka	36.088211	-82.681256	587.01
17	Rock Creek	Unaka	36.137214	-82.339841	707.32
18	Rocky Fork	Unaka	36.067396	-82.596084	988.41
19	Squibb Creek	Unaka	36.103734	-82.650713	584.03
20	Stony Creek	Watauga	36.468723	-81.986905	685.20
21	Toms Branch	Watauga	36.128557	-82.093233	1088.28
22	Wolf Creek	Unaka	35.861854	-82.928019	768.92
23	Camp Fifteen Branch	Watauga	36.224287	-82.052244	971.57
24	Clear Fork	Unaka	36.136608	-82.266675	922.94
25	Fagall Branch	Watauga	36.570957	-81.855616	691.37
26	Right Fork of Mill Creek	Watauga	36.438373	-82.076892	758.75
27	Roberts Hollow	Watauga	36.169521	-82.184040	951.95
28	Right Prong Middle Branch	Watauga	36.119174	-82.095846	1244.66
29	Sycamore Creek	Tellico	35.297808	-84.043283	1047.21
30	Meadow Branch	Tellico	35.320790	-84.060737	971.46

Table 2. List of streams that were selected randomly and defined as validation sites for this project to validate the model for selection of important habitat variables.

ID	Name	District	Latitude	Longitude	Elevation (m)
T1	Bill Creek	Watauga	36.163036	-82.176223	972.28
T2	Brown Gap Creek	Unaka	35.792862	-83.007219	879.59
T3	Gulf Fork Big Creek	Unaka	35.794958	-83.011499	850.46
T4	Little Laurel Fork	Watauga	36.249739	-82.084162	946.70
T5	Laurel Fork	Watauga	36.240180	-82.078417	921.15
T6	Little Jacobs Creek	Watauga	36.551226	-81.966899	672.16
T7	Rockhouse Run	Watauga	36.590769	-81.880351	864.88
T8	Sawmill Branch	Unaka	35.938089	-82.813643	783.05
T9	Briar Creek	Unaka	36.228916	-82.388289	699.28
T10	Left Prong Hampton Creek 3	Watauga	36.145629	-82.048477	987.93

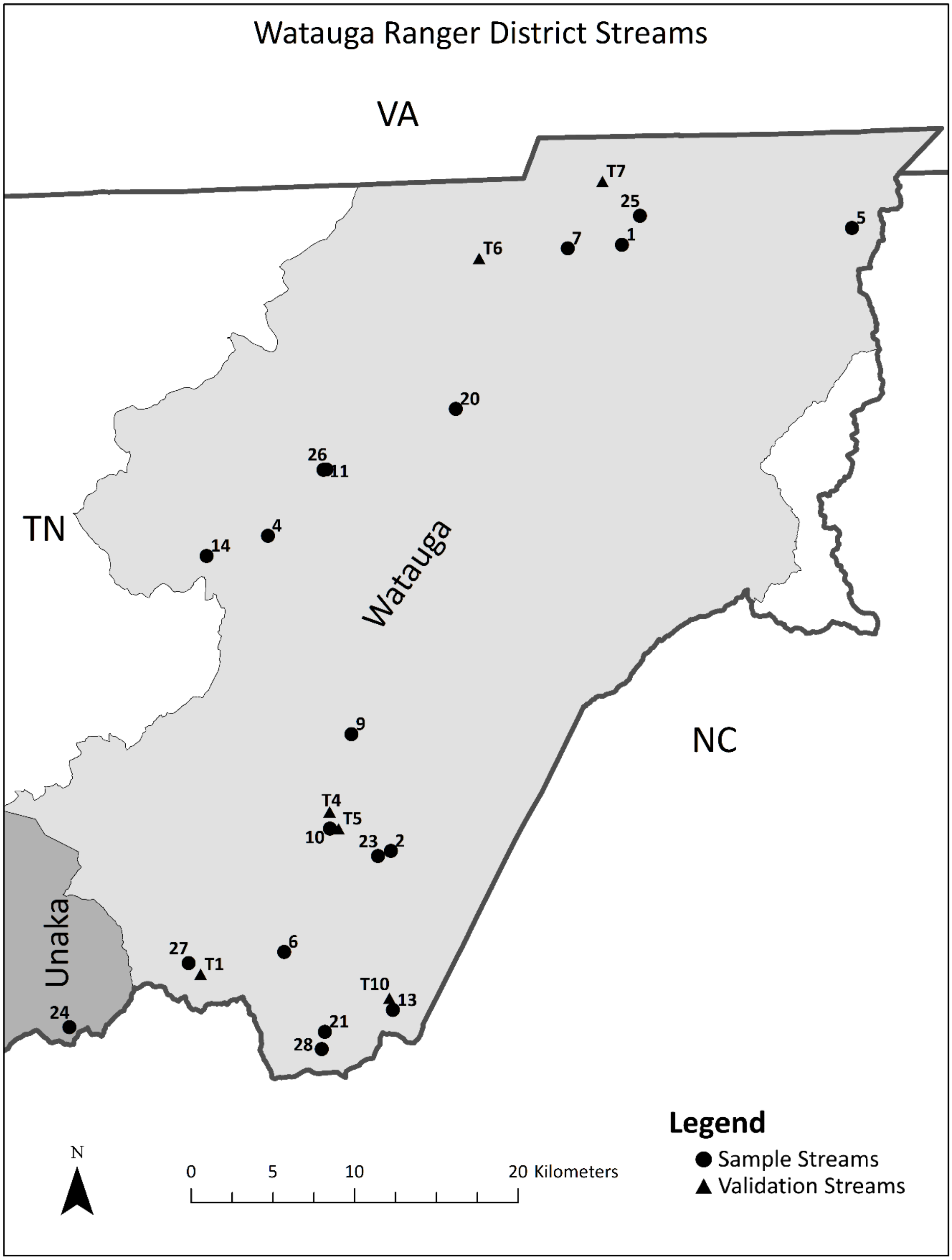

Figure 2. Map of the Watauga Ranger District of the Cherokee National Forest. Test sample sites are shown by numbered black circles. Validation sites are depicted by black triangles with the letter "T".

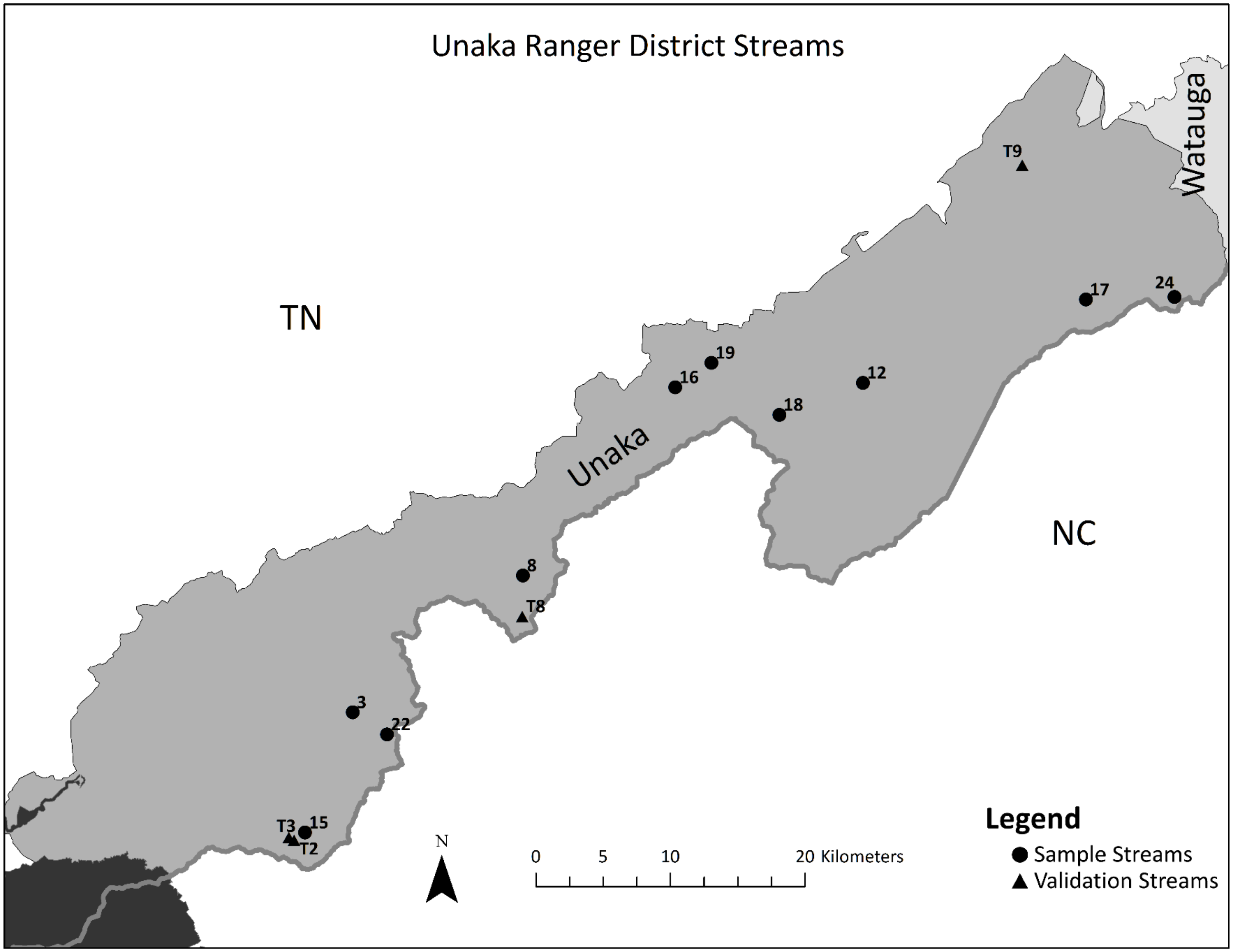

Figure 3. Map of the Unaka Ranger District of the Cherokee National Forest. Test sample sites are shown by numbered black circles. Validation sites are depicted by black triangles with the letter "T".

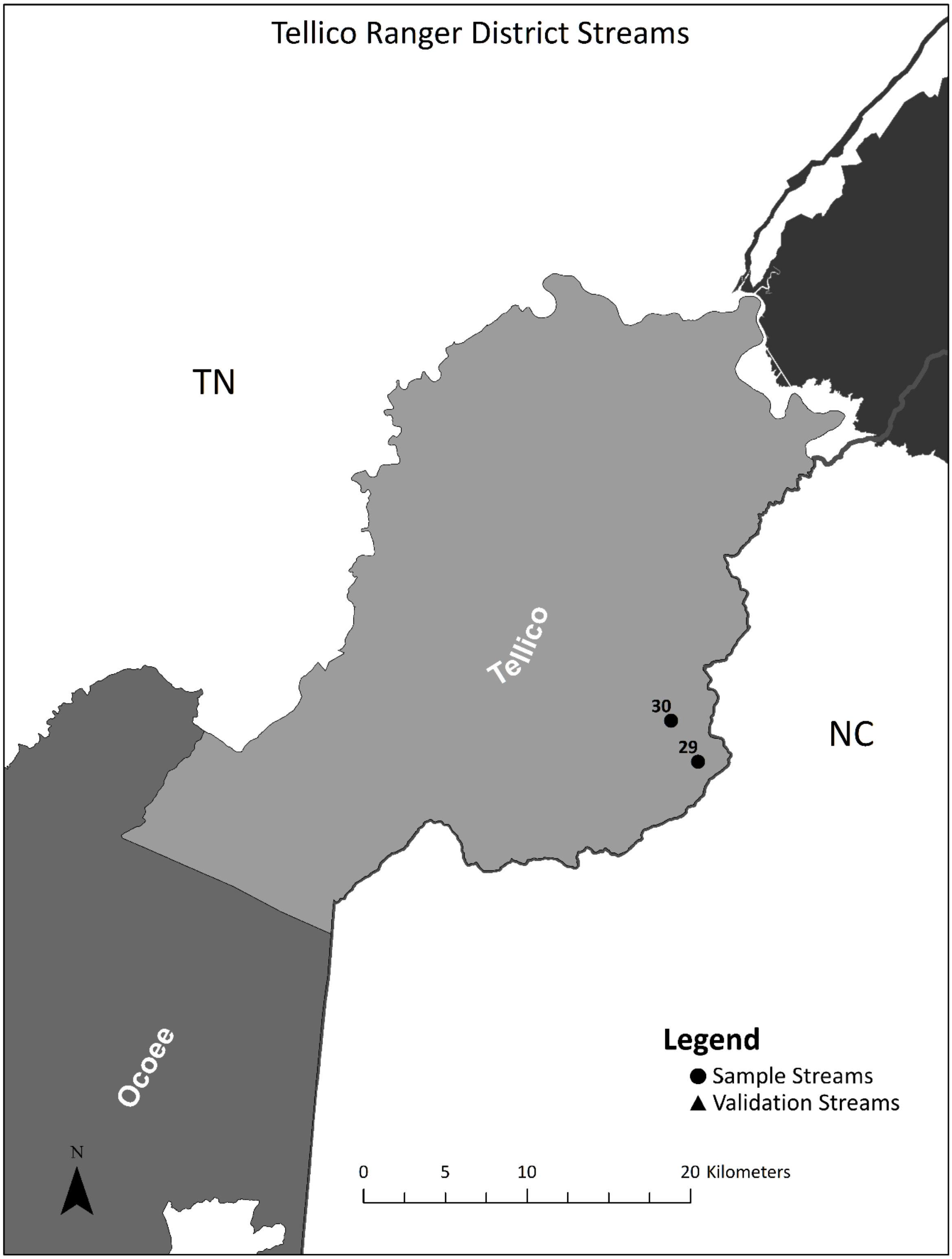

Figure 4. Map of the Tellico Ranger District of the Cherokee National Forest. Test sample sites are shown by numbered black circles.

designated based on accessibility and a portion that represents the entire stream most accurately. A sample reach was defined as a minimum of 100 m in length beginning at the start of a habitat unit (i.e., pool, riffle, or run) and ending at logical break in habitat at or beyond 100 m where fish movement is most obstructed.

For each test or validation stream site, a Global Positioning System (GPS) device was used to capture coordinates in decimal degrees format at both the upper and lower end of the site. Photographs were taken at the beginning looking upstream and at the end looking downstream with unique documentation in the photo to establish a photo point log to evaluate changes over time as well as to aid in locating the site. Aluminum tags are used at the two reach endpoints secured to a tree with an aluminum nail containing the stream name and location etched into the tag. Total reach length was measured using a calibrated hip chain following the contour of the stream channel width to determine the start and end points of the site.

Fish Surveys

Fish abundance was estimated using three-pass depletion sampling following the protocol of Temple and Pearsons (2007). Representative 100 m reaches were used as sample sites, with each site beginning at a break in habitat type, usually one limiting fish passage, and extending upstream for at least 100 m following the contour of the stream channel until the next significant break in habitat. We used an Appalachian Aquatics backpack electrofisher with output voltage adjusted for the specific conductivity of the stream (150 to 550V AC). Personnel included at least one electrofisher, one dip-netter and one person with a bucket. All habitats at each site were thoroughly electrofished during each pass and fish captured were held in buckets containing fresh water until the reach length was sampled. Upon completion of each pass, the fish were separated into buckets by species. Total length (mm) and weight (g) were recorded for each trout, which were then placed into a separate bucket to recover. Total counts, length ranges (mm), and batch weights (g) were recorded for all non-game species (sculpins, dace, etc.). All fish were placed in a holding cage outside the sampling area after all measurements were recorded to avoid capture during subsequent passes. All fish were released throughout the sample area after electrofishing was completed.

Habitat Surveys

Instream habitat structure— Instream habitat data was characterized following the protocol of Dolloff et al. (1993). A calibrated hip chain was used to measure instream habitat in each reach. The person wearing the hip chain stopped at the end of each habitat unit to record variables within that unit. Total length (m) of each habitat unit was recorded using the hip chain and average wetted width (m) was measured using a metric tape. Average depth (m) was measured using a metric depth pole with five measurements per meter in length (each meter of habitat included two measurements at the 25th and 75th quartile, then a measurement at the center of the stream, then another set of measurements at the 25th and 75th quartiles) and maximum depth (m) was measured at the deepest portion of the habitat unit. Slope (%) was measured using a clinometer to determine the slope of each individual habitat unit. Habitat types were classified as a pool, riffle, run, cascade or a complex unit. A complex unit was a mixture of multiple habitat types within one unit. Substrate was examined and categorized by the three types representing the most surface area within the unit. Substrate types are organic debris, clay, silt, sand, small gravel, large gravel, cobble, boulder and bedrock using the size classes from Dolloff et al. (1993). The diameter and length of each piece of woody debris greater than 50 cm in diameter and 1 m in length were also recorded. Large woody debris is characterized as wood that is in or crossing the stream channel that could potentially provide shade or protective cover for fish at various flow intervals. These measurements were repeated for each habitat unit along the stream reach until the endpoint of the site was reached.

Riparian forest structure— The riparian zone of each stream was considered to include trees that would reach the stream if they fell, regardless of tree diameter. Reaches were divided and flagged into five equidistant transects every 20 m. Within each transect, standing snags (i.e., dead trees) were tallied only if they would hit the stream if they were to fall. Eastern Hemlocks (*Tsuga canadensis*) were also recorded using three size categories: 5-10 cm diameter at breast height (DBH), 10-50 cm DBH, and >50 cm DBH. The percent cover of live foliage was recorded to gauge the current state of decomposition that the individual tree was in. This habitat measurement is to predict the future composition of LWD that may be present in a stream due to the invasive Hemlock Wooly Adelgid (*Adelges tsugae*) that causes hemlock mortality. An observer recorded four measurements using a forester's spherical densitometer at breast height, the observer also recorded four measurements at each transect during the growing season to determine percent canopy cover (i.e., shade potential for trout and stream temperature). The four measurements were taken facing upstream, downstream, left bank and right bank, yielding a total of 20 readings for a 100 m reach. Percent *Rhododendron* cover was recorded at the first quarter, center, and third quarter of the stream using a spherical densitometer mounted 1.6 m above the water surface on a tripod during the dormant season. *Rhododendron* was regarded as an important variable because it can easily overtake a stream and reduce the overall production of macroinvertebrates (i.e., food for trout) throughout the stream segment. Four measurements were taken facing upstream, downstream, left bank and right bank at each quarter, totaling 60 readings for the 100 m reach. These measurements were repeated at the 20 m, 40 m, 60 m, 80 m, and 100 m transects.

Water quality— Basic water chemistry parameters were measured at each site prior to sampling using various devices and test kits. An Oakton PC 450 was used to measure water temperature (°C), pH, conductivity (µS), and total dissolved solids (ppm). A Hach Dissolved Oxygen test kit was used to measure dissolved oxygen (mg/L). Alkalinity (mg/L as $CaCO_3$) was measured using a Hach Alkalinity test kit.

Landscape factors— Multiple landscape-scale factors were measured in a Geographic Information System (GIS) using the shortest radial distance from the upstream end of the stream segment, including distance to eighty-year-old mature forest (m), distance to private land (m), and distance to nearest road (m). Classification was performed with ArcMap 10.1 using historic aerial imagery and 2015 USFS remotely-sensed satellite land cover data. Forest type, soil profile, property ownership, nearest road surface type and open status, dominant rock type, and the geologic unit that surrounds the site were recorded to describe landscape factors that could directly affect stream productivity. Total number of upstream road crossings and the distance to nearest upstream road crossings were quantified to model the influence of these characteristics on Brook Trout abundance derived from current roads datasets provided by the USFS.

Analyses

All stream habitat data was placed into Microsoft Excel spreadsheets. Data were reduced to a single value for each variable, such as average, sum, or percent of the reach (Table 3). Reducing the structure of the data facilitated application of subsequent statistical methods. Three-pass depletion data were analyzed for each stream with MicroFish 3.0 for Windows (http://microfish.org). Trout ≤90 mm in length were analyzed separately from those >90 mm as trout in the smaller size group tend to have lower catchabilities (Lohr and West 1992; Thompson and Rahel 1996; Peterson et al. 2004; Habera et al. 2010), making separate analysis necessary to avoid bias. These two groups also roughly correspond to young-of-the-year (YOY) and adults. Biomass (kg/ha) estimates were added to the master datasheet as a response variable for inclusion in the analyses.

Several variables were excluded from the analyses because of data sparsity or temporal variability. For example, percent organic materials was removed because it never occurred as a dominant substrate. Temperature was removed due to its seasonality (although specific conductivity was relative to the temperature at the time of collection). Distance to mature forest and distance to private land were removed because most streams were located in or directly adjacent to mature forest and private land does not exist upstream of sites in most of the CNF. Forest type was removed from the analyses because most streams were located in the same or very similar forest type classification. This reduction of variables allowed the number of variables (n=28) to be less than the sample size (n=30), which is desirable for model development (Table 3).

Table 3. List of variables used in analysis with the unit, type, technique and variable description.

Variable	Unit	Type	Simplification	Description
PercentPool	%	Instream	Percent	Percent of Pools
PercentRiffle	%	Instream	Percent	Percent of Riffles
PercentCascade	%	Instream	Percent	Percent of Cascades
PercentRun	%	Instream	Percent	Percent of Runs
TotalVolume	%	Instream	Sum	Mean length*width*depth
MaxDepth	m	Instream	Maximum	Reach maximum depth
PercentSand	%	Instream	Percent	Percent of Sand as Dominant Substrate
PercentSilt	%	Instream	Percent	Percent of Silt as Dominant Substrate
PercentSmallGravel	%	Instream	Percent	Percent of Small Gravel as Dominant Substrate
PercentLargeGravel	%	Instream	Percent	Percent of Large Gravel as Dominant Substrate
PercentCobble	%	Instream	Percent	Percent of Cobble as Dominant Substrate
PercentBoulder	%	Instream	Percent	Percent of Boulder as Dominant Substrate
PercentBedrock	%	Instream	Percent	Percent of Bedrock as Dominant Substrate
PercentSlope	%	Instream	Mean	Average Percent Slope/Unit
TotalWood	-	Instream	Sum	Total count of all wood located in or across stream
CanopyCover	%	Riparian	Mean	Mean canopy cover of the entire reach
RhodoCover	%	Riparian	Mean	Mean *Rhododendron* cover of the entire reach
pH	-	Water Quality	-	pH
DissolvedOxygen	mg/L	Water Quality	-	Dissolved Oxygen
Conductivity	µS	Water Quality	-	Specific Conductivity
TotalDissolvedSolids	ppm	Water Quality	-	Total Dissolved Solids
Alkalinity	mg/L as $CaCO_3$	Water Quality	-	Alkalinity
DistanceRoad	m	Landscape	-	Distance to nearest Road
RoadStatus	-	Landscape	-	Closed, Open or Seasonal
DominantRock	-	Landscape	-	Dominant Geology
CulvertDistance	m	Landscape	-	Distance to upstream culvert
UpstreamCulvert	-	Landscape	Sum	Sum of culverts upstream
Elevation	m	Landscape	-	Elevation

A principal components analysis (PCA) was performed on the revised variable set. PCA is a standard tool in multivariate data analysis used to reduce the number of dimensions, while retaining much the data's overall variation. Rather than investigating many variables, the first few components should contain the majority of the data's variation and be explored. In the case of this study, the PCA revealed that 14 axes were explaining ≥70% of the data's variation, proving that this method is unsuitable for determining important habitat variables.

Therefore, a separate analysis was performed that creates a classification tree through machine-learning methods to construct prediction models from the data. A classification tree is beneficial because of its interpretability; however, they suffer from reduced predictability. Classification trees are designed for dependent variables that take a finite number of values to be used as the predictor variable, with prediction error measured in terms of misclassification. In R software (R Core Team 2016; Liaw and Wiener 2002), the *tree* package was used to predict the quality classification for each site based on the habitat predictor variables. The data featured a training dataset of 30 observations with Brook Trout biomass as the class variable and 28 habitat predictor variables. Four Brook Trout biomass categories were used to rank the streams in order of quality (i.e., where higher values represent higher quality) and to serve as the class variable. The categories are low, moderate, high and very high in increments of 10 kg/ha. Low biomass was characterized as ≤10 kg/ha, moderate was characterized by 10-19.99 kg/ha, high was characterized as 20-29.99 kg/ha, and very high was characterized as ≥30 kg/ha. The goal was to find a model for predicting the values of biomass from new predictor values. After executing the classification tree method, it proved to have a high misclassification rate (i.e. low predictability), thus it was necessary to perform further analysis using other modeling techniques (Loh 2011).

The Random Forests classification method (R Core Team 2016) was next used to predict the quality classification for each site based on the input habitat variables (Brieman 2001; Cutler et al. 2007). Random Forests is a classification tree-based bootstrap method that corrects many of the known issues in classification and regression trees (CART), like over-fitting (Brieman 2001; Cutler et al. 2007), and provides well-supported predictions for models that incorporate larger numbers of independent variables (Cutler et al. 2007). Rather than using the construction of a single classification tree, Random Forests grows many classification trees. For example, to classify a new object from input vector, the input vector was used on each of the trees in the forest and each tree gives a classification (i.e., vote) for that class. Random Forests chooses the classification having the most votes over all trees in the forest and was used because it tends to yield greater accuracy compared to other methods (thus greater predictive capabilities) and it can run efficiently on large sets of mixed data types. One thousand bootstrap replicates (k) were run without replacement using a 25% data-withhold [out-of-bag (OOB)] sample. The ten validation streams (25%) that were withheld (Table 2) and the remaining thirty streams (75%) were characterized as test streams also known as the training data (Table 1). In the analysis, this OOB error stabilization occurs close to k = 1,000. This number of trees was sufficient, so I decided that k = 1,000 was an adequate number to account for both error and interaction stabilization. There is no need for cross-validation in Random Forests to get an unbiased estimate of the test set error as it is estimated internally during the run. The m parameter, the number of variables tested at each node, was defined as m =

√(number of variables). Most statistical procedures for classification measure variable importance by selecting them using criteria like statistical significance and Akaike's Information Criterion, but Random Forests takes an entirely different approach. To determine the importance of a predictor variable, the values are randomly permuted for the OOB observations and are the applied to the tree to obtain new predictions. Differences between the misclassification rates are divided by the standard error to measure the variable importance. Gini is a measure of node impurity in this classification. A low Gini (i.e. higher decrease in Gini) indicates that the predictor variable is more important in partitioning the data into classes. For this analysis, important variables with a mean decrease Gini ≥0.8 (n = 11) were included in further analysis.

A multinomial logistic regression (MLR) model was utilized to create a usable model for the predictor variables from the Random Forests model. Multinomial logistical regression is used to predict categorical placement on a dependent variable with multiple independent variables. The independent variables can be either dichotomous or continuous, thus it was necessary to drop Dominant Rock variable as it is a categorical variable. Multinomial logistic regression is an extension of binary logistic regression in that it allows for more than two categories of the dependent variable. It uses maximum likelihood estimation to evaluate the probability of categorical placement (Schwab 2002). The *multinom* function from the *nnet* package in R was used to estimate a MLR model (R Core Team 2016). I decided to use this function because unlike other functions, it does not require the data to be reshaped. Before running the model, the data was releveled through the *relevel* function with "Low" as the baseline outcome. A model was created and executed using 10 variables related to the categorical Brook Trout biomass variable. The *nnet* package does not include p-value calculations with the coefficients, so p-values were calculated using Wald tests. The MLR models relate the probabilities of the three other categories to the baseline category as shown in these formulas:

$$Y1 = ln\left[\frac{P(Moderate)}{P(Low)}\right] = \beta_{01} + \beta_{11}X_1 + \beta_{21}X_2 + \cdots + \beta_{P1}X_P$$

$$Y2 = ln\left[\frac{P(High)}{P(Low)}\right] = \beta_{02} + \beta_{12}X_1 + \beta_{22}X_2 + \cdots + \beta_{P2}X_P$$

$$Y3 = ln\left[\frac{P(VeryHigh)}{P(Low)}\right] = \beta_{03} + \beta_{13}X_1 + \beta_{23}X_2 + \cdots + \beta_{P3}X_P$$

Where β_{01}, β_{02} and β_{03} are the intercept coefficients and the remaining β coefficients are multiplied by the given independent variable values (X). Based on the probabilities from the three models, I then solved for the probability of low, moderate, high and very high by:

$$P(Low) = \frac{1}{1 + e^{Y1} + e^{Y2} + e^{Y3}}$$

$$P(Moderate) = \frac{e^{Y1}}{1 + e^{Y1} + e^{Y2} + e^{Y3}}$$

$$P(High) = \frac{e^{Y2}}{1 + e^{Y1} + e^{Y2} + e^{Y3}}$$

$$P(VeryHigh) = \frac{e^{Y3}}{1 + e^{Y1} + e^{Y2} + e^{Y3}}$$

CHAPTER IV: RESULTS AND DISCUSSION

Fisheries Analysis

In the test streams, Brook Trout biomass ranged from 0.0-54.5 kg/ha and in the validation streams it ranged from 0.0-36.3 kg/ha. Individual stream biomass estimates were plotted for Brook Trout, Rainbow Trout and Brown Trout (Figure 5). Streams with varying biomass estimates were included in the analysis to have each biomass category represented in the model to boost the predicting power. Several streams (i.e., Little Laurel Fork, Little Paint Creek, Meadow Branch, and Rock Creek) had zero fish in the sampled reach, however these streams had historical records of trout residing in them. The streams were still included to discern habitat variables affecting the low biomass estimates on these streams.

Classification Tree

The *tree* package was used to predict the quality classification for each site based on the habitat predictor variables (R Core Team 2016). The data featured a training sample of 30 observations with Brook Trout biomass as the class variable with 28 predictor variables. The goal was to find a model for predicting the values of biomass from new predictor values. The classification tree is a method that is excellent at its interpretability as it is practically a step-wise decision tree that measures node purity at each step indicating predictability (De'ath and Fabricius 2000). The classification tree in this study proved to be valuable with an error rate of 0.22 in the test data set, however it would be best used as a factor leading into the Random Forest. The classification tree in Figure 6 describes elevation to be the leading split factor with a threshold of 975 m, but the tree cannot be split further.

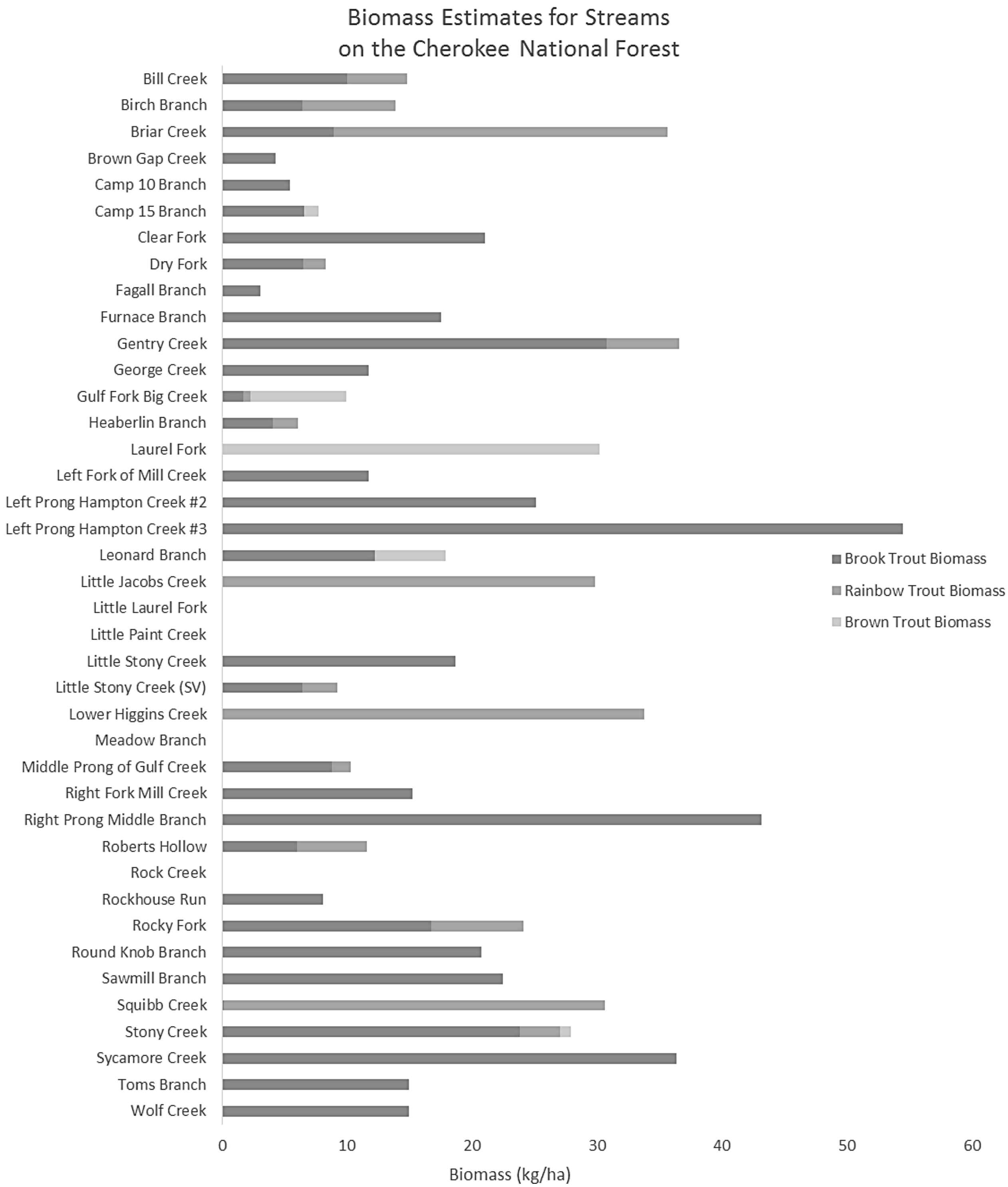

Figure 5. Estimated biomass (kg/ha) depicted for Brook Trout, Rainbow Trout and Brown Trout for all 40 study sites.

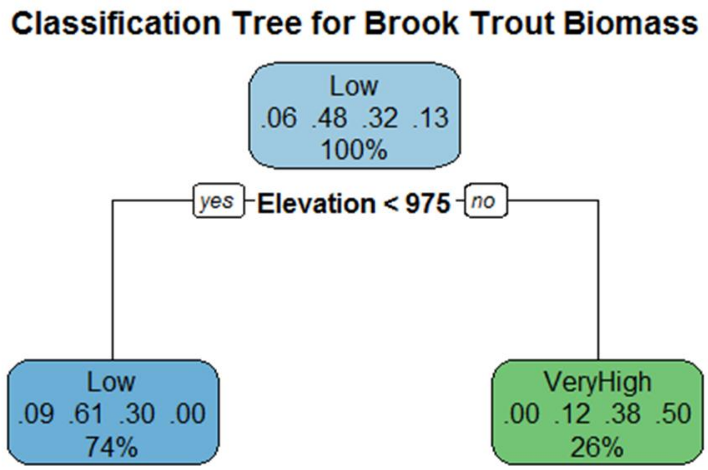

Figure 6. Classification tree for predicting Brook Trout biomass with only one split of the elevation variable. Streams greater than 975 m elevation are "Very High" biomass predictions.

Random Forests

The Random Forests model proved to be the most effective model for predicting the accuracy of habitat variables in comparison to Brook Trout biomass. With this method, there is no need for pruning the trees and there is minimal tuning required. This is due in part to the number of trees that are created, and the package compares all trees to create the most precise tree. The total class error rate for the Random Forests analysis was 30% representing a predictability accuracy of 70%. After the model was created, a variable importance plot was drawn to show the purity (i.e., importance to the model) of the variables used. The model included 11 variables that were ≥0.8 mean decrease Gini, signifying the variables' importance to the model (Figure 7). Variables that were classified as important were: percent riffle, distance to nearest road, average slope per habitat unit, elevation, percent of the reach where boulder was the dominant substrate type, average *Rhododendron* cover across the reach, average canopy cover across the reach, dominant geologic rock type, percent of the reach where cobble was the dominant substrate type, total volume of the sampled reach, as well as total dissolved solids. Partial dependence plots are shown in Figures 8-18, with the y-axis representing the dependence of the model on the given variable. Values farther from zero signify a greater importance that variable has on the model (Breiman 2001).

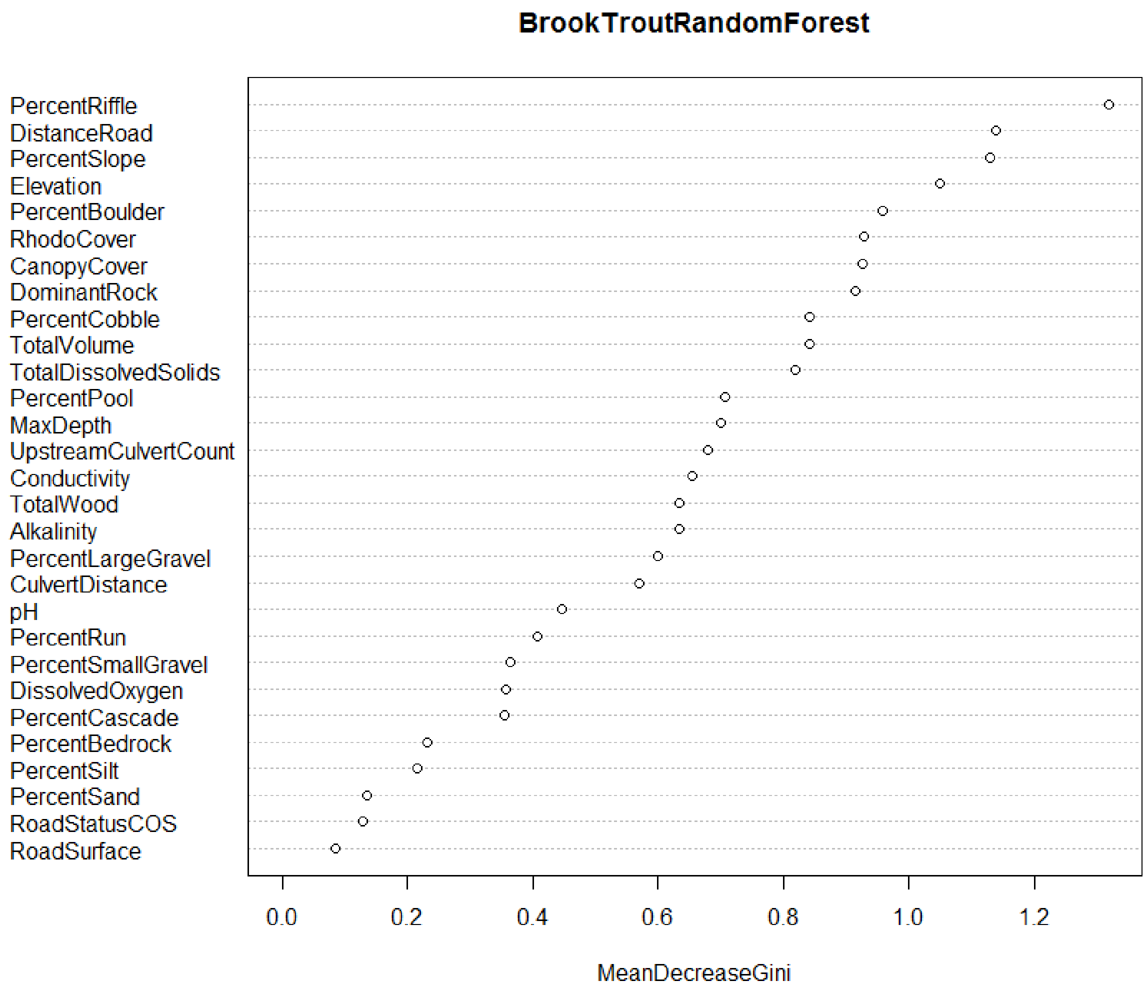

Figure 7. Variable Importance Plot showing that the first 11 habitat variables (from top of graph) are statistically significant (≥0.8 mean decrease Gini) predictors of Brook Trout biomass (kg/ha) in Cherokee National Forest streams.

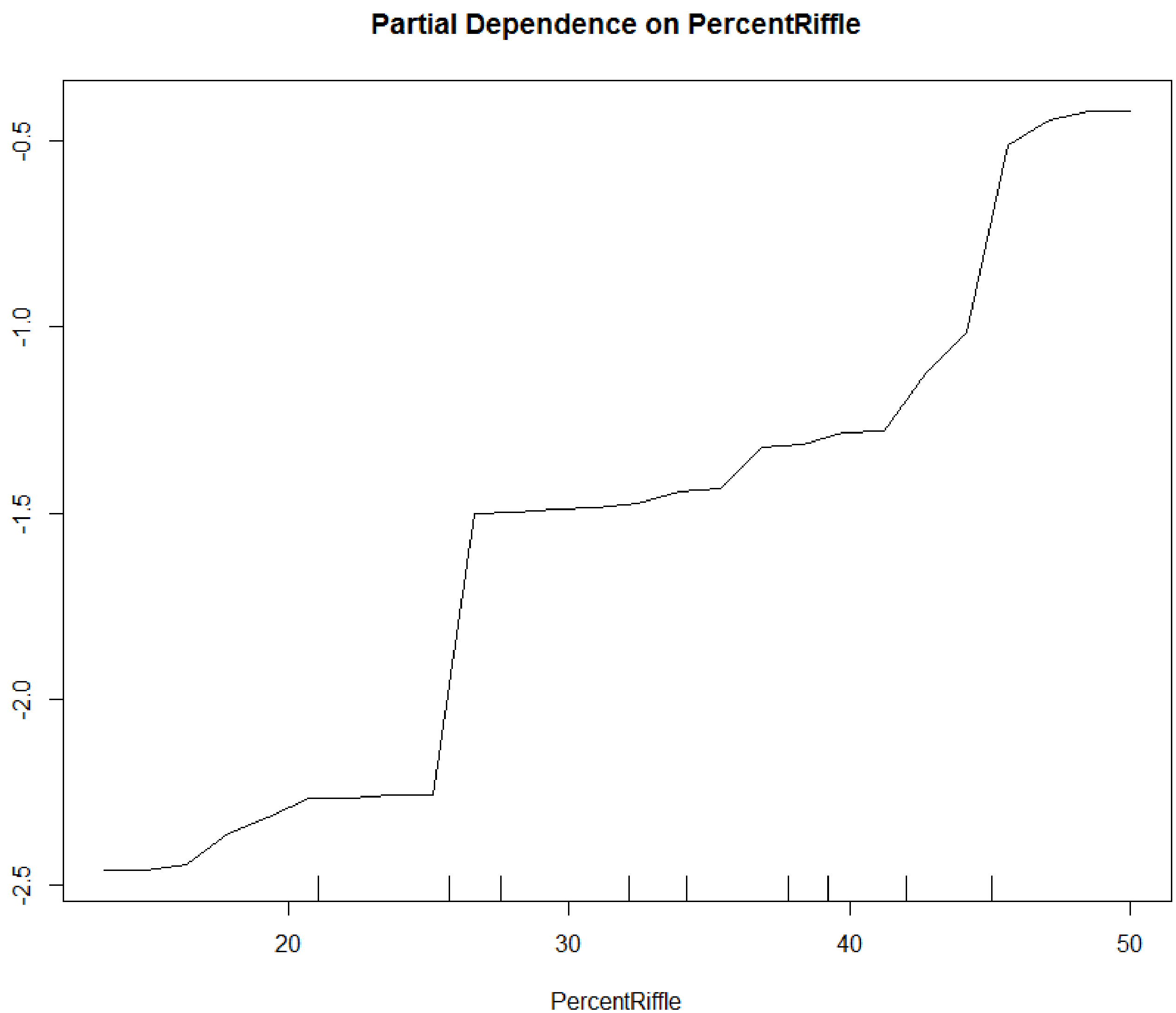

Figure 8. Partial dependence plot of Percent Riffle, where <25% riffle area within a 100-m reach provides optimum Brook Trout biomass (kg/ha).

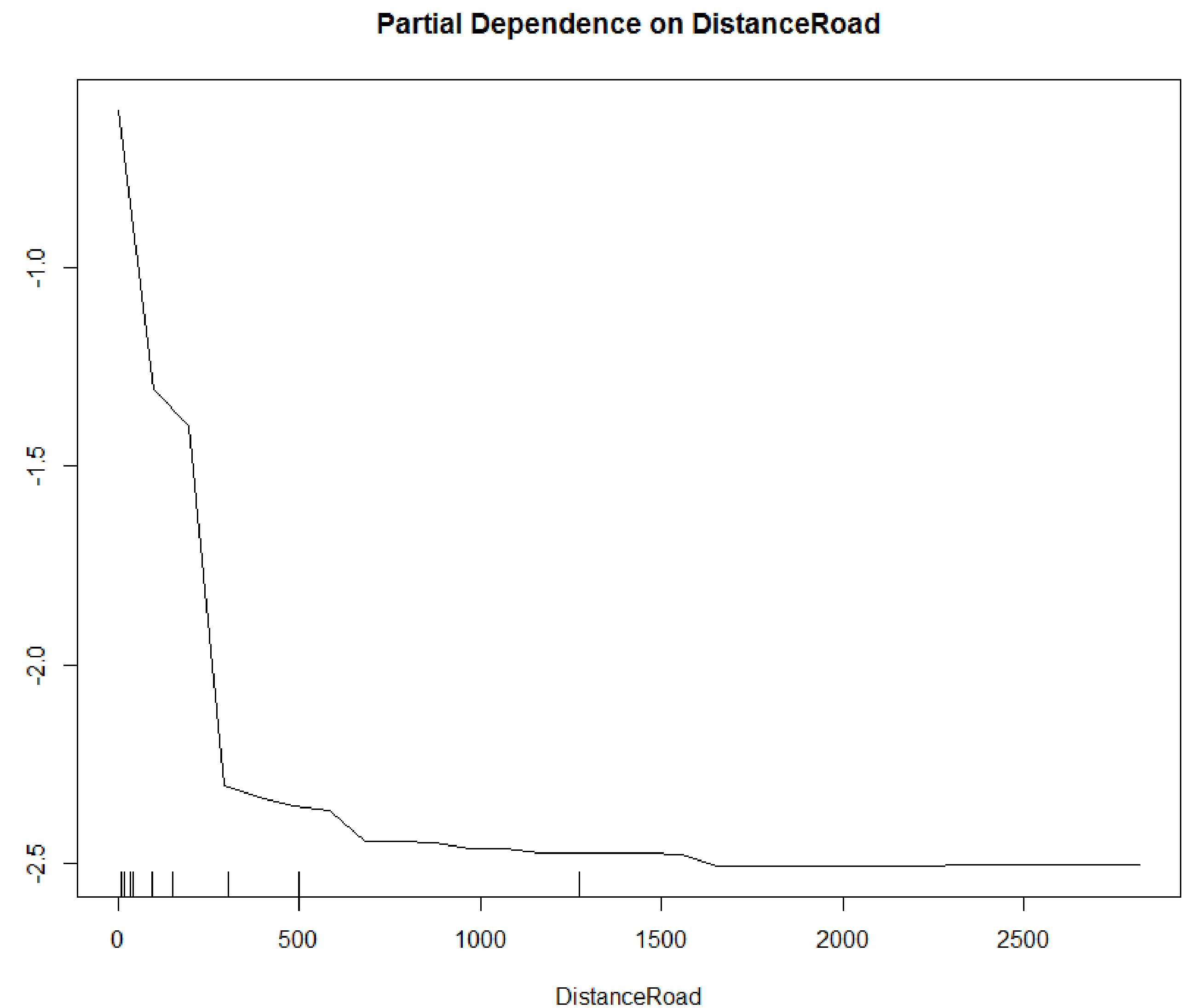

Figure 9. Partial dependence plot of distance to the nearest road where streams located ≥350 m from a road provides for optimum Brook Trout biomass (kg/ha).

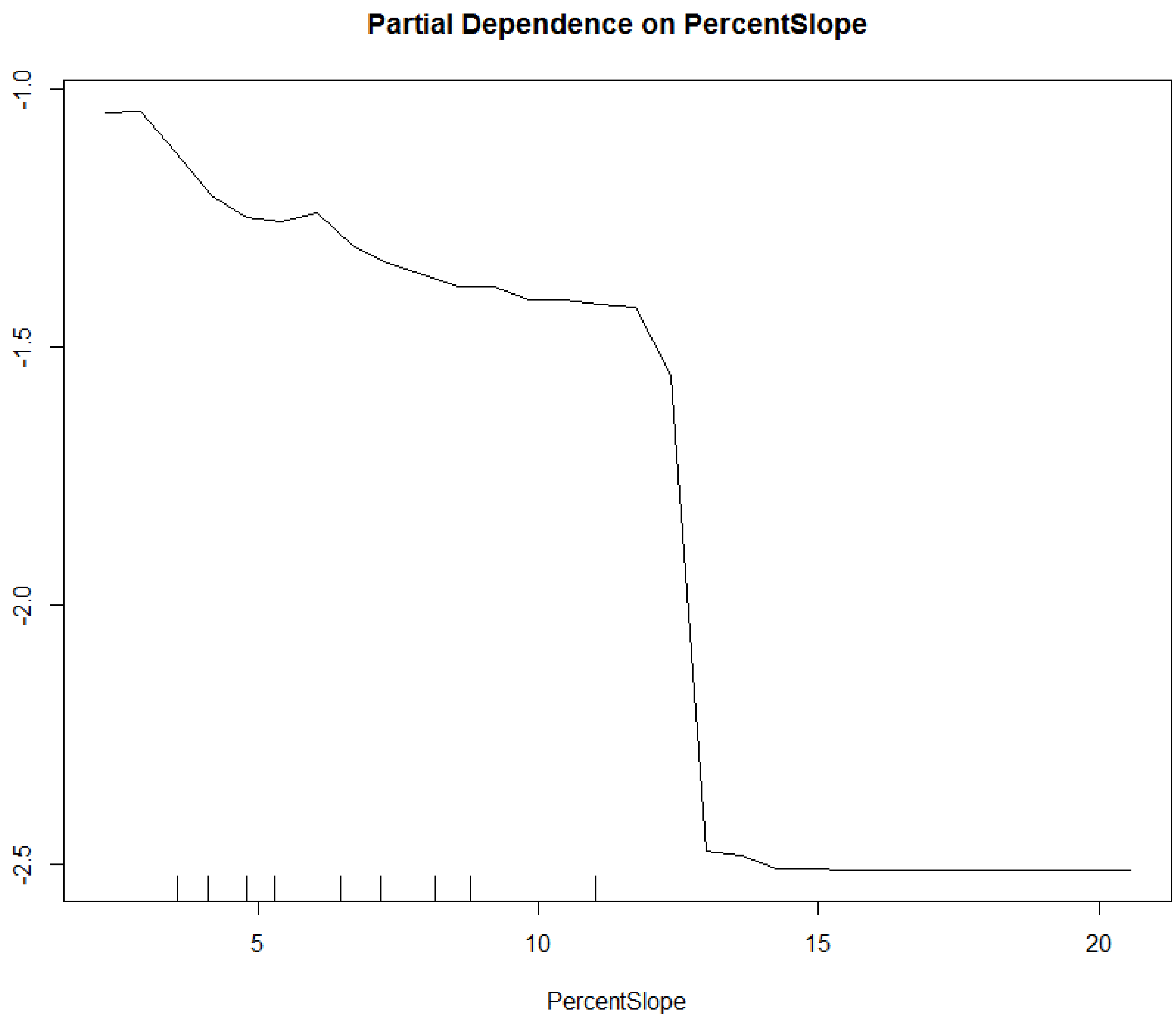

Figure 10. Partial dependence plot of Percent Slope where >13% average slope per habitat unit provides for optimum Brook Trout biomass (kg/ha).

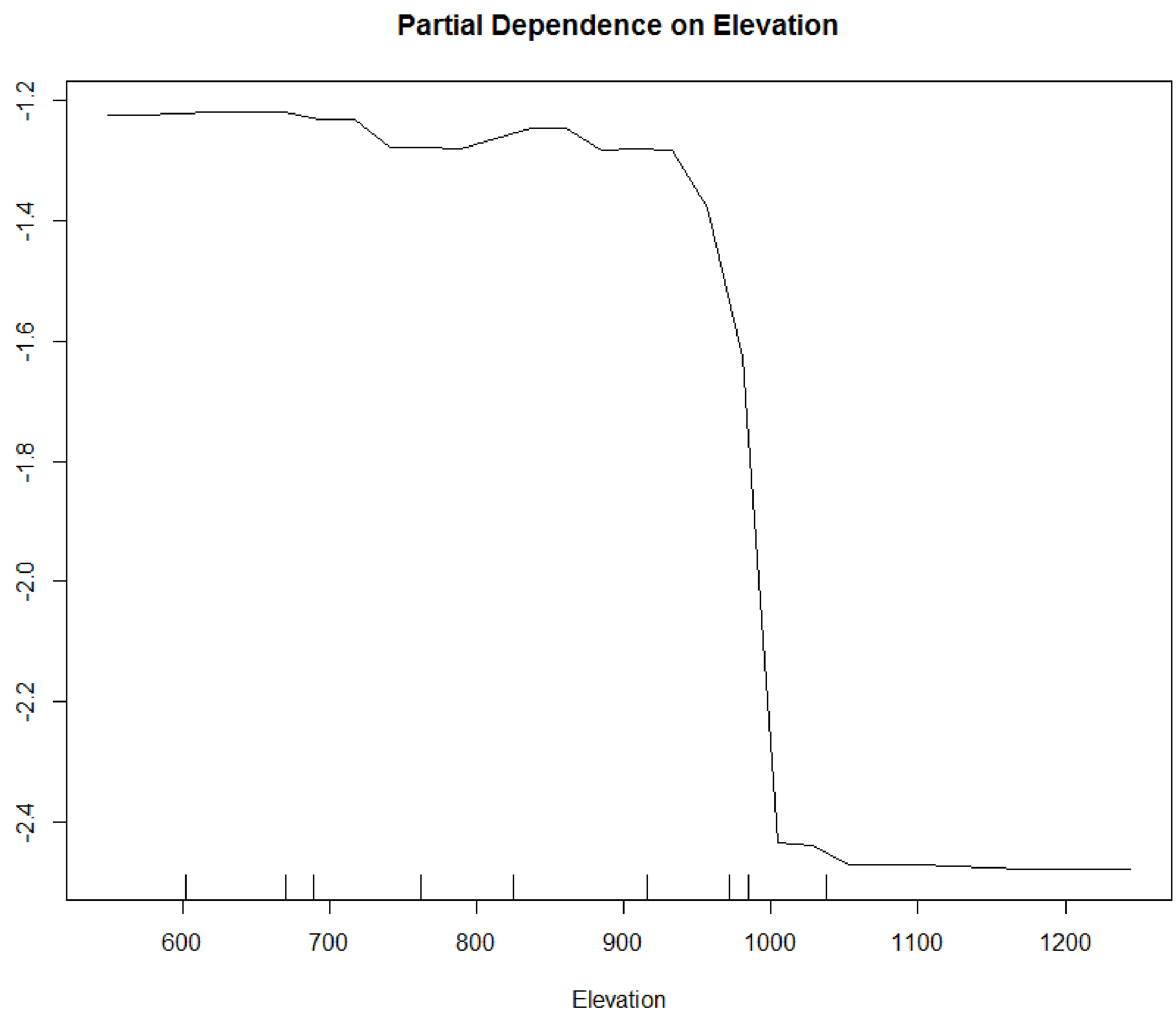

Figure 11. Partial dependence plot of Elevation where ≥1,000 m elevation provides for optimum Brook Trout biomass (kg/ha).

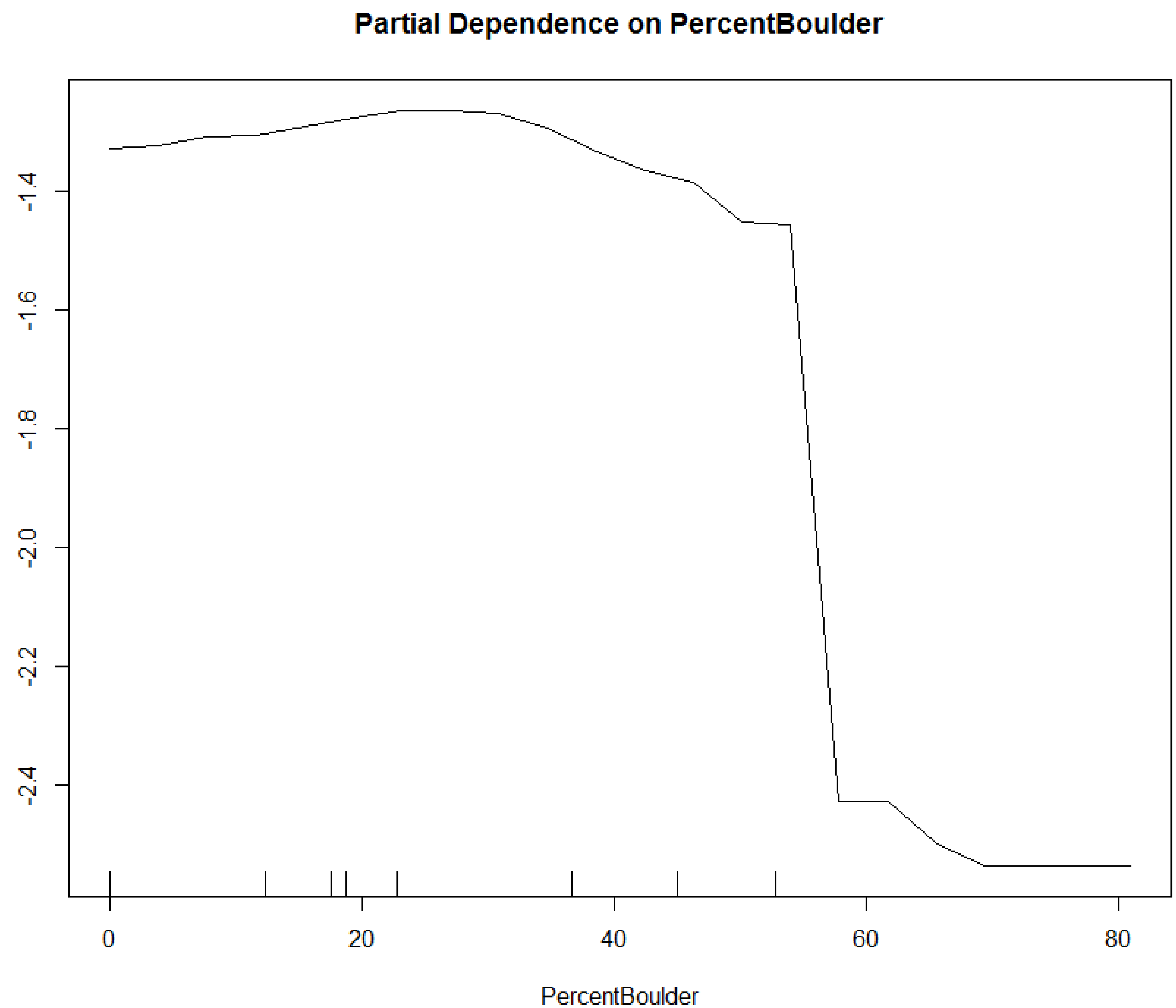

Figure 12. Partial dependence plot of Percent Boulder where dominant substrate across the reach is >55% boulder provides for optimum Brook Trout biomass (kg/ha).

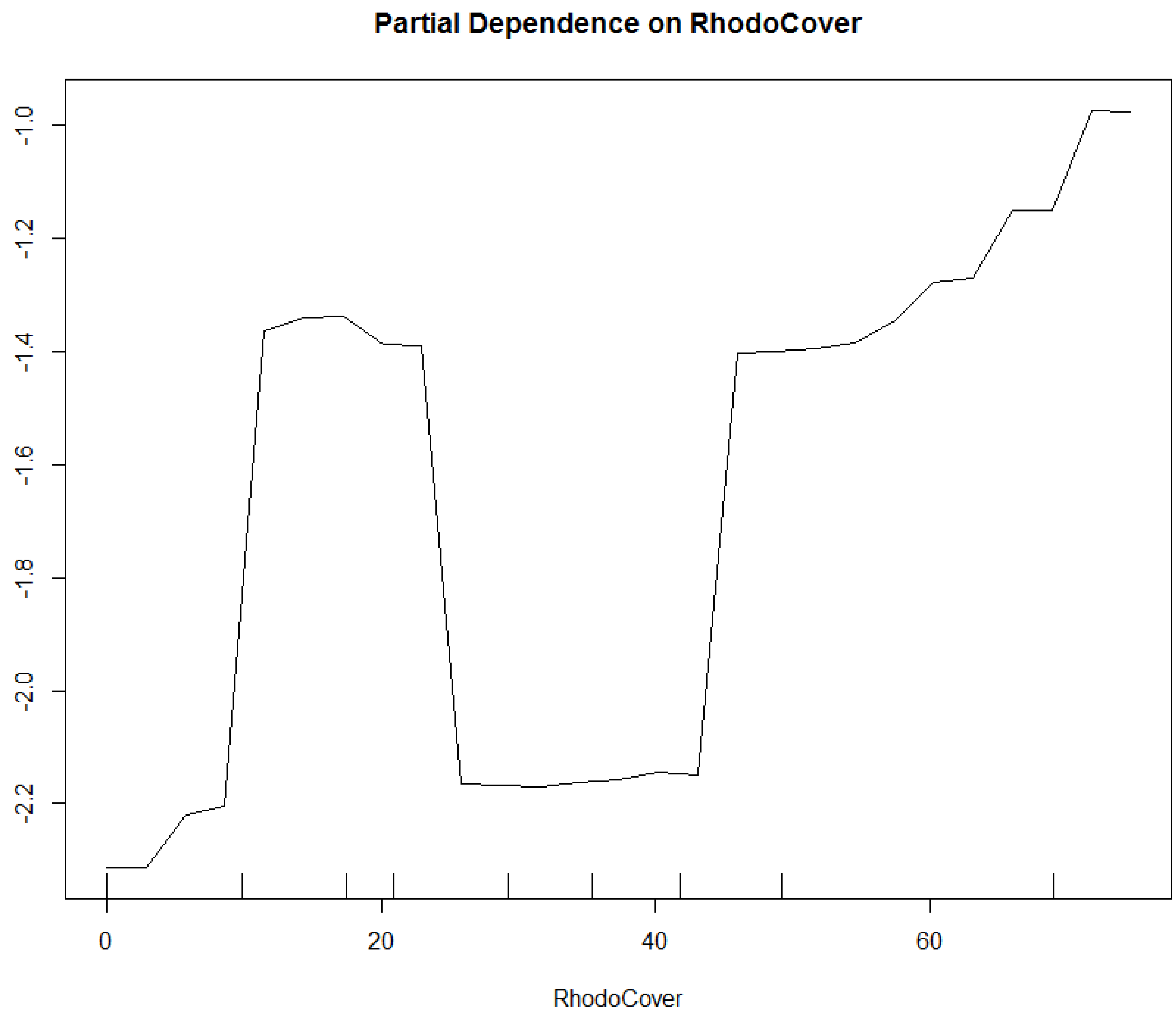

Figure 13. Partial dependence plot of average *Rhododendron* Cover where <10% or 25-45% *Rhododendron* cover across the reach provides for optimum Brook Trout biomass (kg/ha).

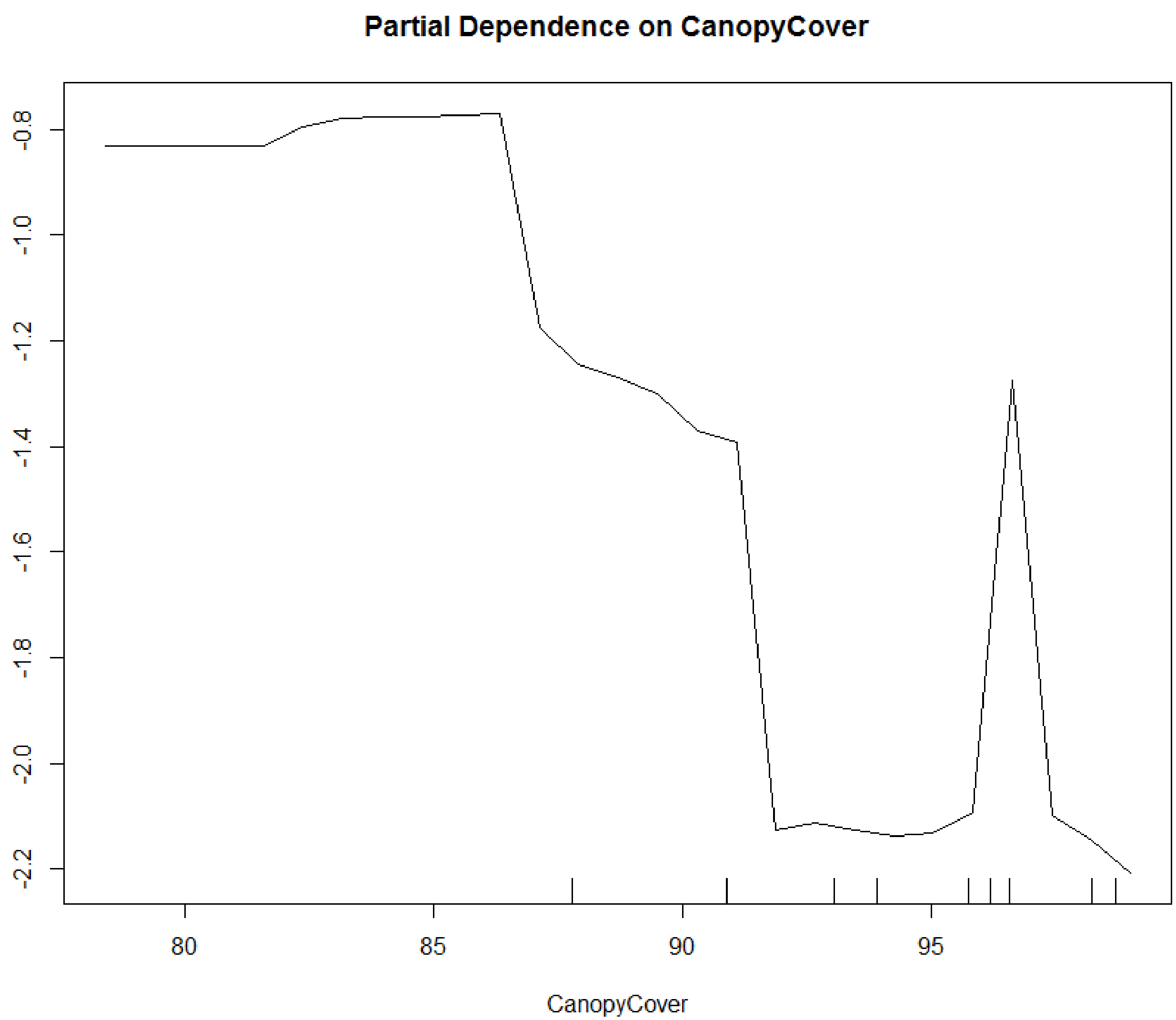

Figure 14. Partial dependence plot of average Canopy Cover where 92-97% or ≥98% canopy cover across the reach provides for optimum Brook Trout biomass (kg/ha).

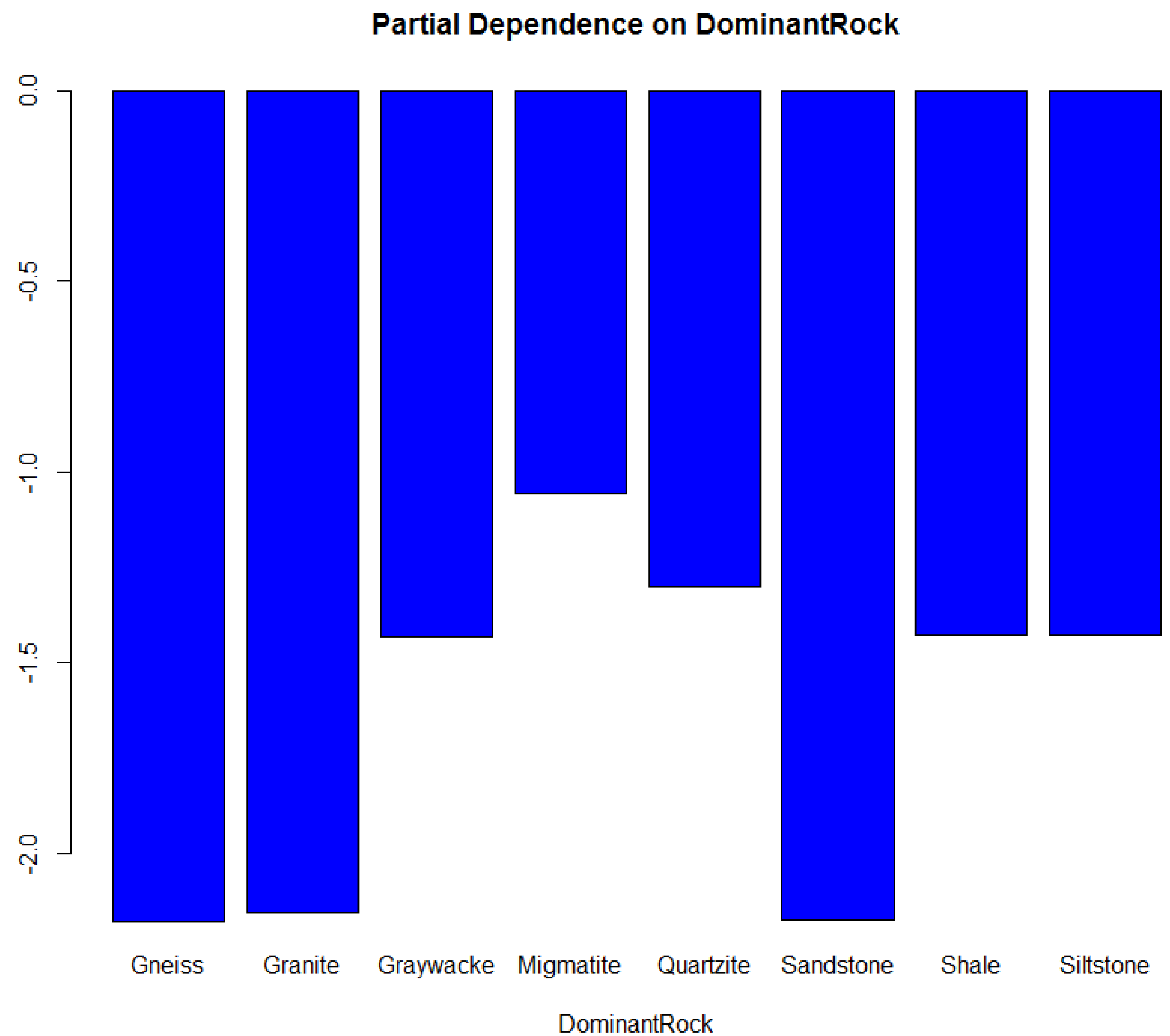

Figure 15. Partial dependence graph of Dominant Rock type where streams feature Gneiss, Granite or Sandstone provides for optimum Brook Trout biomass (kg/ha), whereas Migmatite minimizes Brook Trout biomass (kg/ha).

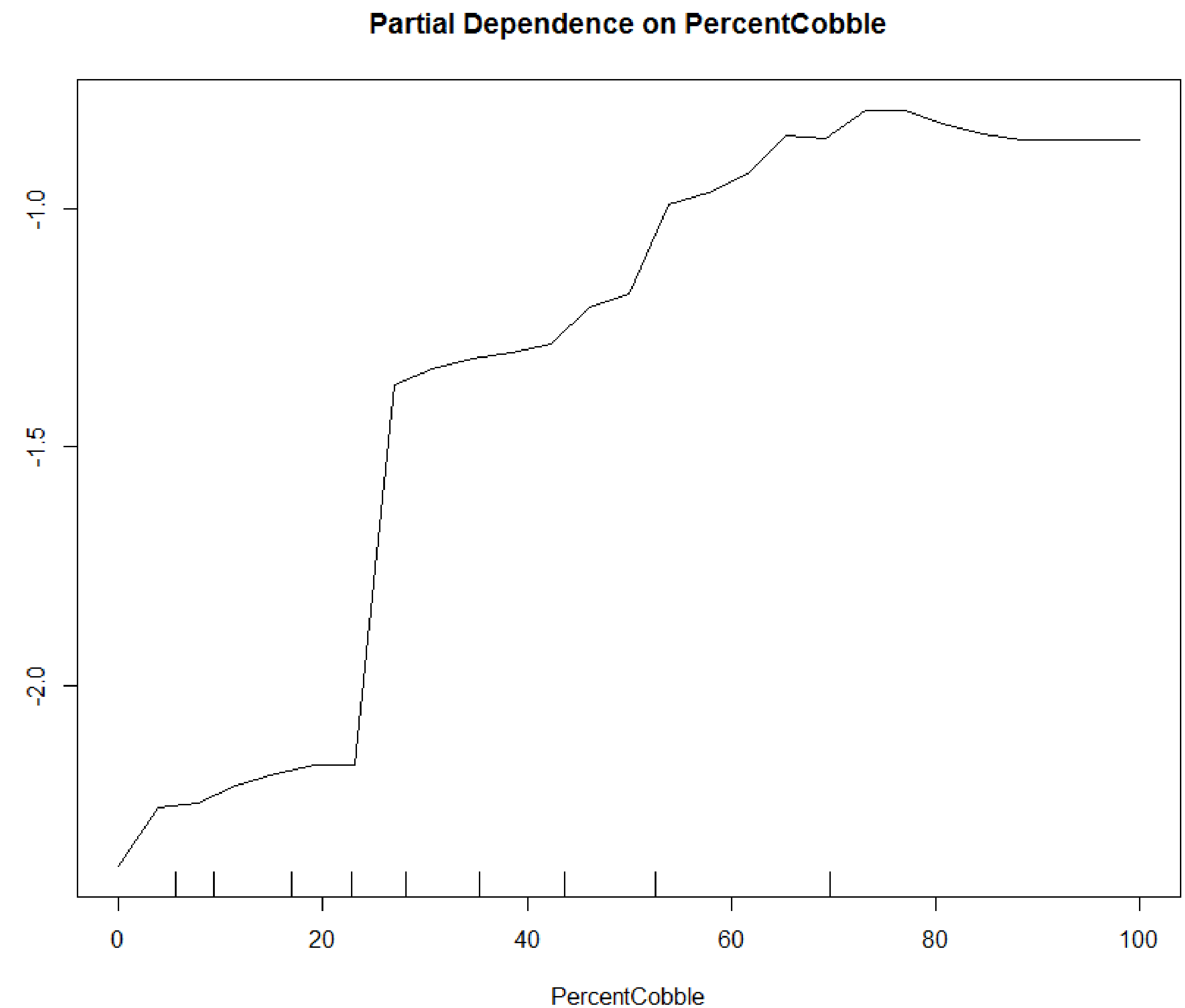

Figure 16. Partial dependence plot of Percent Cobble where dominant substrate across the reach is <25% cobble provides for optimum Brook Trout biomass (kg/ha).

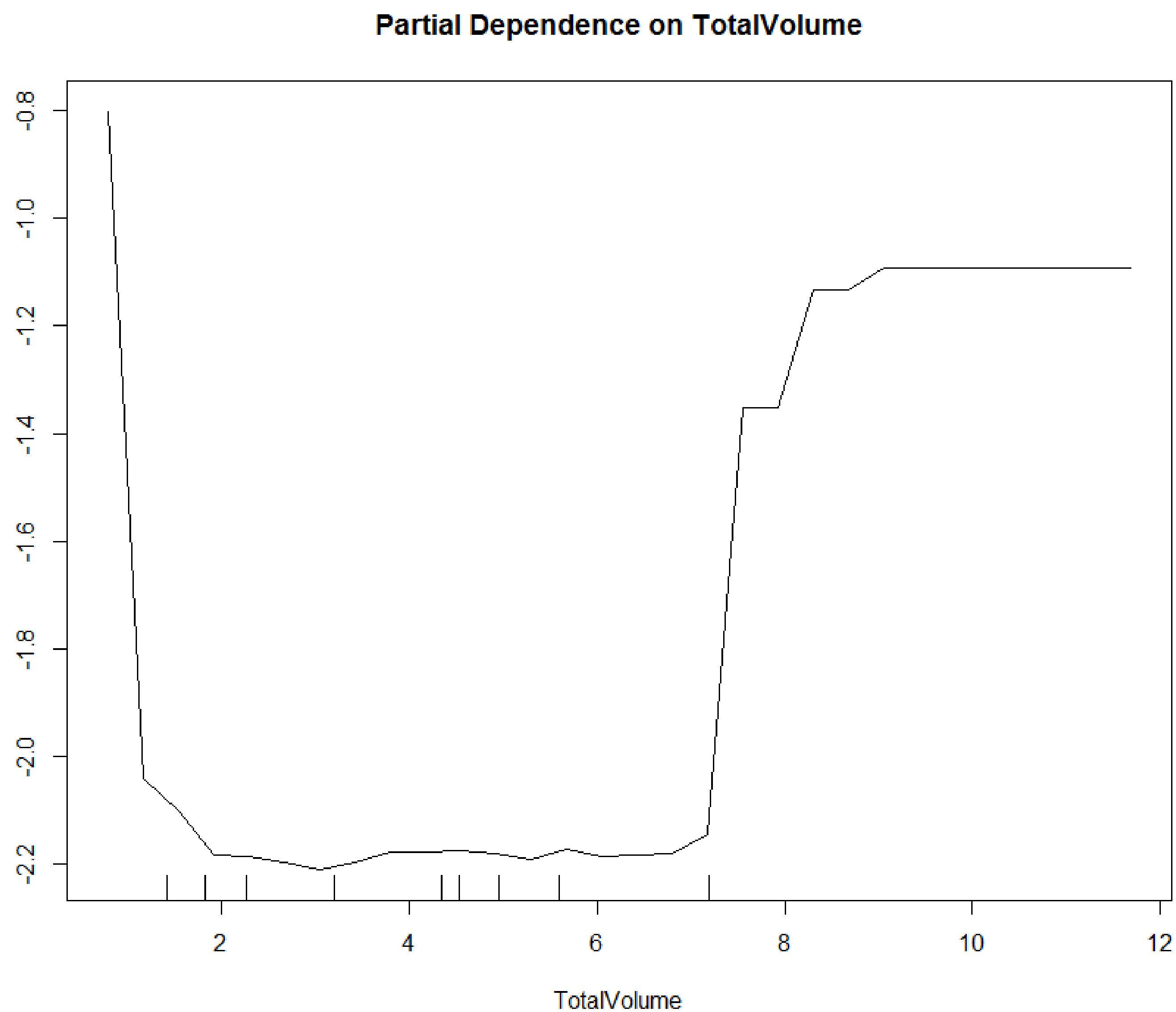

Figure 17. Partial dependence plot of Total Volume where 1-7.5 m^3 provides for optimum Brook Trout biomass (kg/ha).

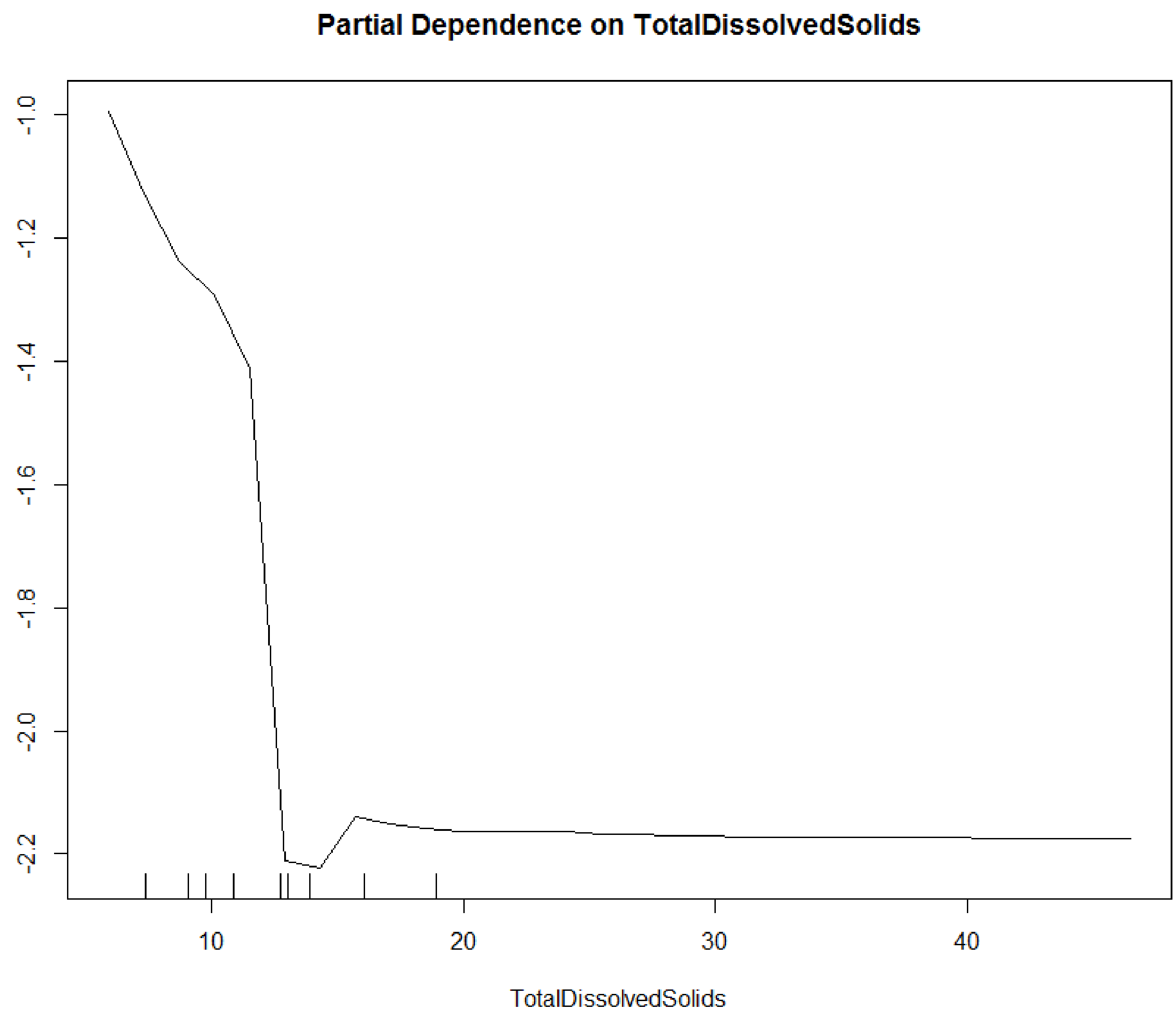

Figure 18. Partial dependence plot of Total Dissolved Solids where TDS >12 ppm provides for optimum Brook Trout biomass (kg/ha).

Multinomial Logistic Regression

Multinomial logistical regression models were formed from the important variables of the Random Forest analysis. One variable, DominantRock, had to be removed from the data set because MLR requires the predictor variables to be quantitative. The 10 variables were found to have no statistically significant multicollinearity from examining the correlation matrix of the variables. Multinomial logistic regression returns the residual deviance which is most related to the residual sum of squares in ordinary multiple regression (ter Braak et al., 1986). The residual deviance is defined by -2 log-likelihood and in this predictive model the residual deviance was 49.71. The misclassification rate for this model was 30% (11 streams) and is described in the confusion matrix (Table 4). This means that the model correctly predicted Brook Trout biomass 70% of the time from the validation dataset.

Table 4. Confusion matrix with 30% misclassification in the MLR model of Brook Trout Biomass.

	Low	Moderate	High	Very High
Low	19	7	2	0
Moderate	3	3	0	0
High	0	0	2	0
Very High	0	0	0	4

All streams were then tested against the model using the measured variables (Table 4). There were three streams that were actually low biomass that were misclassified as moderate. Six moderate streams were misclassified as low biomass. Only two streams that were actually high were misclassified as low and all of the very high streams were accurately classified in this model.

Table 5. Predicted versus observed Brook Trout biomass class for all 40 streams.

Stream	Model Type	Observed Class	Predicted Class
Birch Branch	Training	Low	Low
Camp 15 Branch	Training	Low	Low
Dry Fork	Training	Low	Low
Fagall Branch	Training	Low	Low
Heaberlin Branch	Training	Low	Low
Little Paint Creek	Training	Low	Low
Little Stony Creek	Training	Low	Low
Meadow Branch	Training	Low	Low
Middle Prong of Gulf Creek	Training	Low	Low
Roberts Hollow	Training	Low	Low
Rock Creek	Training	Low	Low
Squibb Creek	Training	Low	Low
Camp 10 Branch	Training	Low	Moderate
Lower Higgins Creek	Training	Low	Moderate
Right Fork Mill Creek	Training	Moderate	Low
Toms Branch	Training	Moderate	Low
Wolf Creek	Training	Moderate	Low
Furnace Branch	Training	Moderate	Low
George Creek	Training	Moderate	Low
Leonard Branch	Training	Moderate	Low
Little Stony Creek (Lake Trib)	Training	Moderate	Low
Rocky Fork	Training	Moderate	Moderate
Round Knob Branch	Training	Moderate	Moderate
Left Fork of Mill Creek	Training	Moderate	Moderate

Table 5 continued. Predicted versus observed Brook Trout biomass class for all 40 streams.

Stream	Model Type	Observed Class	Predicted Class
Stony Creek	Training	High	Low
Clear Fork	Training	High	Low
Left Prong Hampton Creek #2	Training	High	High
Gentry Creek	Training	Very High	Very High
Right Prong Middle Branch	Training	Very High	Very High
Sycamore Creek	Training	Very High	Very High
Gulf Fork Big Creek	Validation	Low	Low
Rockhouse Run	Validation	Low	Low
Bill Creek	Validation	Low	Low
Briar Creek	Validation	Low	Low
Laurel Fork	Validation	Low	Low
Little Jacobs Creek	Validation	Low	Low
Little Laurel Fork	Validation	Low	Low
Brown Gap Creek	Validation	Low	Moderate
Sawmill Branch	Validation	High	High
Left Prong Hampton Creek #3	Validation	Very High	Very High

CHAPTER V: MANAGEMENT RECOMMENDATIONS

Habitat mensuration can be time consuming often taking one to two days to complete a 100 m reach. Initially, there were over 30 habitat variables being evaluated in order to classify a stream. Given the results of these analyses, managers in the CNF and TWRA need to measure only 10 instream and riparian variables and a landscape-scale spatial variable in order to determine a stream's potential suitability for Brook Trout restoration. The only equipment needed to complete a stream evaluation using these variables is a measuring tape, clinometer, Forester's spherical densitometer, and a depth pole. Streams can be measured more rapidly using only 10 variables and data could be extrapolated to survey a larger portion of the stream to gauge where restoration should occur. A Microsoft Excel spreadsheet has been developed that can be distributed to fishery managers in the CNF that incorporates the MLR model formulas to characterize the suitability of a stream for Brook Trout restoration based on its predicted biomass. A 30% misclassification rate should be expected, although the model over-predicted biomass for only 7.5% (18 streams) of the 40 streams. Stream measurements can be input into the Microsoft Excel spreadsheet to generate the probability of the stream's potential to support a low (≤10 kg/ha), moderate (10-19.99 kg/ha), high (20-29.99 kg/ha) or very high (≥30 kg/ha) Brook Trout biomass.

Predictive models do have restrictions such that they can only be applied to streams that fall within the range of the values from the streams in this study (i.e., streams with total dissolved solids >46.5 cannot be applied to this model). This should be considered for all variables (Table 6), although many of the streams in east Tennessee will fit within the ranges.

Table 6. Summary statistics of variables included in analyses.

	Percent Riffle	Distance to Road (m)	Percent Slope	Elevation (m)	Percent Boulder
Min	13.46	1.25	2.00	549.60	0.00
Mean	37.28	367.09	6.67	842.70	25.62
Standard Deviation	11.60	593.62	3.91	161.36	22.90
Max	61.11	2820.56	20.57	1244.70	80.95
	Canopy Cover	***Rhododendron* Cover**	**Percent Cobble**	**Total Volume**	**TDS**
Min	78.40	0.00	0.00	0.80	5.90
Mean	93.72	29.34	34.16	6.21	15.27
Standard Deviation	4.71	21.25	25.91	11.28	8.76
Max	99.00	74.63	100.00	73.27	46.50

Many models have been established across the East (i.e., Hudy et al. 2008 and TU Conservation Success Index) that examine watersheds as a whole to determine suitability for Brook Trout. Few studies have been conducted using on-the-ground habitat variables at the stream segment level. This model would be most beneficial if used subsequently with aforementioned spatial scale models to recognize the suitability of a watershed before investigating the streams within to determine specific suitability. Techniques such as this model would be useful to examine streams without an existing wild trout population to determine where to focus restoration efforts.

Based on these selected variables, efforts to improve Brook Trout habitat should focus on four primary areas: reduction of riffle habitat (i.e., create more pools), maintaining canopy closure, reducing *Rhododendron* cover, and preventing sediment run-off from nearby roads. Other habitat types (i.e. pools and runs) can be created by installing fish habitat structures to alter the morphology of the stream to reduce the overall riffle area in the stream. This can be implemented by using rock vanes or wood habitat structures to create pools. Of the 81 studies that examined the response of trout to wood structures, 68 reported a positive response in fish abundance and biomass (Solazzi et al. 2000). Carter and Carter (2001) found that the development of pools created low velocity holding areas, cover, and provided thermal refuges during drought conditions with pool habitat being readily colonized by trout, while supporting larger trout in these areas. Optimum percentage should be less than 25% riffles according to this data. It should be mentioned however, riffles are still an important habitat type for the reproductive stages of Brook Trout and that should still be taken into consideration when altering the habitat of the stream.

Canopy cover is an important consideration for stream dwelling organisms because of its ability to regulate stream temperature and increase macroinvertebrate abundance, as well as riparian plant biodiversity. Best management practices of riparian zones should be followed, leaving many of the riparian forest trees during harvest. Even streams within a heavily forested watershed with vegetated riparian buffers cannot tolerate disruption of riparian zone trees over much more than one km in length. Riparian buffer length and area should be given strong consideration to protect streams (Jones et al. 1999). Canopy cover should remain >92% across the restoration area to provide for optimum Brook Trout biomass.

Thinning of *Rhododendron* cover to an average of 25-45% across the reach of the stream allows more light to the stream to increase macroinvertebrate abundance and increase the biodiversity of plants surrounding the stream. *Rhododendron* is readily replacing the void from the loss of Eastern Hemlock across much of the eastern U.S. Competition from shrubs (i.e., *Rhododendron* and *Kalmia*) may hinder stand regeneration after disturbance by the Hemlock Wooly Adelgid (Evans et al. 2011). This loss could affect the overall productivity of the stream and riparian ecosystems.

Streams that are more isolated and located greater distances from roads are expected to be of higher quality, however many streams that encompass the necessary habitat requirements of Brook Trout are located in relatively close proximity to roads. A study by Brown et al. (2014) discusses how gravelling nearby roads can reduce the amount of fine sediments that are transported to the stream from surface run-off. Roads with no gravel showed results of increased total suspended solids (TSS) as compared to roads with increased amounts of gravel on the road progressively decreased the amount of TSS.

In summary, management strategies should focus on examining streams according to this model to varify locations suitable for restoration efforts. Reaches can be extrapolated to larger segments of the stream at the managers' discretion. Habitat can be improved based on mitigating issues with roads, canopy cover, *Rhododendron* cover and the total riffle habitat area across the restoration area, thus increasing the likelihood of supporting higher biomass of Brook Trout over time.

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VITA

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