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ARTICLE

## Brook Trout Distribution, Genetics, and Population Characteristics in the Driftless Area of Minnesota

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### Abstract

The Driftless Area in southeastern Minnesota is on the southwestern edge of the native range of Brook Trout *Salvelinus fontinalis*. It was assumed that native Brook Trout were extirpated from this region in the early 1900s due to degraded stream conditions and stockings of eastern-origin Brook Trout and European Brown Trout *Salmo trutta*. Our objectives were to examine Brook Trout populations in the region to determine their spatial and genetic distribution and quantify population characteristics. Information on presence or absence of Brook Trout was gathered by electrofishing 174 streams in southeastern Minnesota. Brook Trout were present in 68% of coldwater streams compared with only in 3% in the early 1970s. The increase is likely due to increasing stream discharge throughout the Driftless Area, enabling recolonization or successful establishment of stocked populations. Streams with higher base flow discharge also had higher abundance, larger size at maturity, and larger Brook Trout present. Genetic data on 74 populations were analyzed to characterize genetic variation within populations, assess genetic structure among populations, and determine possible origins. Numerous populations were not associated with known hatchery sources but were primarily composed of geographic groupings that could represent remnant lineages. Although population characteristics were similar among genetic origins, potentially remnant populations should be given conservation priority because they have proven their ability to sustain themselves in this region. Management actions that emphasize maintaining or increasing stream base flows throughout the region will likely enhance remnant Brook Trout populations in the Driftless Area.

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Populations found at the edge of a species range are important in terms of range contraction, genetic divergence, and life history variants. While edge populations are more susceptible to declines than their centrally located counterparts, the risk becomes even greater with intolerant coldwater species such as trout (family Salmonidae) (Haak et al. 2010). A relatively tolerant, nonnative trout species may be more likely to succeed along the periphery of an intolerant trout species' native range given that the environmental conditions are often suboptimal for the native trout species. Also, coldwater fish such as Brook Trout *Salvelinus fontinalis* are predicted to decline along the edge of their southern range due to a warming climate

(Meisner 1990; Flebbe et al. 2006; Lyons et al. 2010). Conversely, an increase in stream base flows resulting from increased precipitation could have a positive impact on both water quality and instream habitat for stream fishes (Hakala and Hartman 2004; Novotny and Stefan 2007). Because Minnesota represents the southwestern edge of the native range of Brook Trout, conservation measures are of added importance. Managers would benefit from a detailed distributional analysis of these populations given that they may differ from those of more central populations and may be more likely to show adverse effects from stressors such as climate change and invasive species. However, habitat characteristics do not

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necessarily follow a strict latitudinal or linear gradient. The Driftless Area of southeastern Minnesota, due to its distinct geology, may provide quality habitat that makes its Brook Trout populations more resistant to stressors than would be expected for peripheral populations.

While coarse-level modeling exercises can lend insight into species spread and declines, they need to be relevant on both a temporal and spatial scale to effectively measure the impacts of disturbance (Dauwalter et al. 2009). On a spatial scale, the knowledge of a species presence in a watershed does little to help define the factors influencing the species distribution in an individual stream. Range contraction of trout due to climate change has focused on entire watersheds or latitudes, as opposed to individual streams. Because individual streams differ in water temperature (based on groundwater inputs, shading, orientation, etc.), base flow, instream habitat, and other physical and biological conditions, it is unlikely that all streams in a watershed or latitude will respond in the same way to large stressors. Similar to downscaling global climate models, efforts are needed to gather distributional data of fishes at a finer scale that is useful to both scientists and fisheries managers. In addition to land use and environmental conditions, competition from invasive species can also alter native species distribution (Wagner et al. 2013). Brook Trout populations are negatively affected throughout their native range by the introduction of both Brown Trout *Salmo trutta* and Rainbow Trout *Oncorhynchus mykiss* (Larson and Moore 1985; Fausch 2008). Information on the distribution of trout is needed on a finer scale to better understand the factors influencing their spread or decline.

The knowledge of genetic origin and diversity is another critical need in identifying the status of a population. Because of the ubiquitous movement of trout by state and governmental agencies during the last century, attempts must first be made to determine genetic origin. Remnant populations of Brook Trout in the Driftless Area would deserve the highest conservation priority because of their locally adapted genetic characteristics. Populations that have successfully naturalized and persisted in the region also deserve conservation consideration because of the potential for rapid evolution in salmonids (Hendry et al. 2000; Pearse et al. 2009). Low genetic diversity may result from founder effects associated with colonization, isolation, and small population size, especially where competing in reaches with Brown Trout (Whiteley et al. 2013). Low genetic diversity within populations may limit a population's ability to adapt to changes. Information on genetic origin and diversity will help to prioritize conservation efforts in the Driftless Area.

Before fishes disappear from a region or stream, early indications of stress can be found in population parameters such as mortality, growth, and maturity. However, little information exists in the published literature on genetics or other population characteristics of Brook Trout in the Driftless Area or on how these populations may differ from those found in the

central part of their range. Stressed trout populations may show signs of slow growth, decreased survival, and few age-classes (Marschall and Crowder 1996). Defining the population characteristics of Brook Trout in the Driftless Area is needed to develop conservation management strategies and establish baseline information.

Our objectives were to examine Brook Trout populations in the Driftless Area of southeastern Minnesota to (1) determine the current spatial distribution of Brook Trout populations, (2) determine genetic diversity and structure and identify potential remnant lineages, and (3) quantify population characteristics (abundance, growth, size structure, annual mortality, and length at maturity) to compare with other populations in their native range.

## METHODS

*Study area and stocking history.*—In addition to being on the southwestern edge of the native Brook Trout range, southeastern Minnesota has a unique landscape of karst geology combined with agricultural land use. The Driftless Area of southeastern Minnesota consists of steep valleys dominated by hardwood forests, with a mix of agriculture and forests on the flatter uplands and valley bottoms. Agricultural practices mainly include row crops and pasture. Coldwater streams in this region are supported by groundwater inputs, often near the headwaters. Many streams warm as they reach downstream lower-gradient portions of the valleys and are further away from groundwater inputs. The western portion of the study area receives most of its groundwater from the Galena and Decorah Edge rock formations and the eastern half receives most of its groundwater from the St. Lawrence Edge and Prairie Du Chien. Groundwater from the latter two formations typically originates from deep, confined aquifers, and therefore water temperatures are very stable with minimal annual variation (Luhmann et al. 2011). Most coldwater streams in this region are very fertile with high alkalinity (Kwak and Waters 1997). Brook Trout are the only native stream-dwelling salmonid in the Driftless Area (MacCrimmon and Campbell 1969).

During the mid-1800s, logging and intense agriculture degraded many trout streams in southern Minnesota, causing a precipitous decline in Brook Trout populations throughout the region (MacCrimmon and Campbell 1969; Thorn et al. 1997). By the late 1800s, most native Brook Trout populations in southern Minnesota were presumed extirpated and introductions of eastern-strain Brook Trout, Brown Trout, and Rainbow Trout were used to provide fishable populations (Thorn et al. 1997). Brook Trout stocking began in Minnesota in the late 1800s by public and private hatcheries. The source of these Brook Trout is unknown but likely came from local stocks. Starting in the 1980s, the origins of Brook Trout were recorded when they were brought into state hatcheries. Many of the Brook Trout brought into Minnesota hatcheries were from domesticated stocks originating in the eastern United

States. Two strains were widely stocked during this time in southeastern Minnesota. The St. Croix Falls strain, obtained from the St. Croix Falls hatchery in Wisconsin but originally from Nashua fish hatchery, New Hampshire, was stocked from 1983 to 1997. The Owhi strain, obtained from the White Sulfer Springs hatchery, West Virginia, was stocked from 1986 to 1992. Other strains were used for short periods. In 1982 and 1983, fish from Rome, New York, and North Attleboro, Massachusetts, were raised and stocked from Minnesota hatcheries, as were fish from Phillips hatchery in Maine in the mid to late 1980s. The strain used in current reintroductions and supplemental stocking is referred to as Minnesota Wild (MNWILD) and has been stocked since 1995. This strain was developed by crossing Brook Trout from Spring Brook in Rice County, Minnesota, and those from Coolridge Creek in Winona County, Minnesota. Given the numerous strains that have been stocked, it is difficult to know the ancestry of Brook Trout in Minnesota streams. Often times multiple strains were stocked into the same stream. Also, connectivity among coldwater streams allows Brook Trout to move among streams. Some streams in southeastern Minnesota that have no records of being stocked have reproducing Brook Trout populations, which could represent remnant populations or could have resulted from unknown stocking (either public or private) or been established through immigration from stocked or remnant populations. Brown Trout are naturally reproducing in most of the area's coldwater streams; however, some supplemental stocking of fingerling Brown Trout continues. Rainbow Trout are stocked as fingerlings and yearlings in areas with high fishing pressure but are not known to naturally reproduce.

**Spatial distribution.**—Coldwater streams in southeastern Minnesota were sampled from 2005 through 2010 by electrofishing. We assessed 174 streams located in nine major watersheds (U.S. Geological Survey subbasins; level 4) for the presence of Brook Trout (Figure 1). There are 181 coldwater streams in southeastern Minnesota that could potentially support trout, not including the larger warmwater rivers. We did not sample the remaining seven streams due to access issues. Sampling locations were based on whether Brook Trout were reported at a location previously or on landowner and angler accounts of Brook Trout being present, if such information was available. In the absence of such information, we sampled headwater reaches and areas of known spring sources. Warmwater streams were only sampled if there were previous reports of Brook Trout in a particular stream. Although Brook Trout are occasionally collected in larger warmwater rivers, we considered these individuals as transients and not year-round residents of these larger systems.

Brook Trout were sampled with either a backpack or barge electrofisher, depending on stream size. Typically, a single pass was made upstream collecting all Brook Trout observed, while counting the numbers of adult and age-0 Brown Trout. Station length was a minimum of 35 times the mean stream width or until at least 25 Brook Trout were collected for

genetic analysis. Some streams were sampled at multiple locations if Brook Trout were not found at the initial sampling site or in order to increase sample sizes for genetic analysis. At the beginning and end of each station, GPS locations were taken to determine length. Brook Trout were measured for total length and an adipose fin was collected for genetic analysis. Brook Trout populations were classified based on numbers of fish per kilometer collected on a single pass as none (0/km), rare (<30/km), common (between 30 and 155/km) or abundant (>155/km).

**Genetics.**—We examined genetic diversity in most southeastern Minnesota Brook Trout populations to characterize genetic variation within populations, assess genetic structure among populations, and determine if lineages of the original Brook Trout in the Driftless Area may remain in the region. Tissue samples were collected during electrofishing surveys as described above. Populations not included in the genetic study typically had very low abundance or were currently being stocked with the MNWILD hatchery strain. In addition, we obtained data for six Brook Trout hatchery broodstocks derived from eastern U.S. sources (W. Stott, U.S. Geological Survey Great Lakes Science Center, unpublished data). These included samples from hatcheries in Maine (Phillips hatchery; PHP), New York (Rome hatchery; ROME), Michigan (Marquette hatchery; MARQ), Utah (Egan hatchery's Owhi strain; OWHI), Wisconsin (Bayfield hatchery's Nashua strain; NASH), and Minnesota (derived from Wisconsin's Nashua strain; SCF). Data for one Iowa Driftless Area (IOWA) population presumed to be remnant to that area was also obtained (T. King, U.S. Geological Survey, unpublished data).

Samples were prepared for polymerase chain reaction (PCR) amplification using a simple DNA extraction based on Walsh et al. (1991). A small piece of fin tissue was placed in a 1.5-mL tube with 250  $\mu$ L of a 5% solution of a chelating resin (Chelex; Sigma Chemical, St. Louis, Missouri). Samples were incubated overnight in a 56°C water bath and boiled 8 min. Microsatellite amplification was performed in 15- $\mu$ L reactions containing 1X polymerase buffer (10 mM tris-HCl, 50 mM KCl, 0.1% Triton X-100), 1.5 mM MgCl<sub>2</sub>, 0.2 mM each dNTP, 0.5  $\mu$ M of the forward and reverse primers, with the forward primer labeled with a fluorescent dye (6FAM, VIC, NED, or PET), and 0.5 units *Taq* DNA polymerase (Promega, Madison, Wisconsin). We used seven microsatellite DNA loci designed for Brook Trout: *SfoC24*, *SfoC38*, *SfoC86*, *SfoC88*, *SfoC113*, *SfoD115*, and *SfoD75* (King et al. 2012). Each set of samples included a water blank as a negative control to detect possible contamination of PCR solutions. Amplification was carried out in a thermocycler with 35 cycles at 95°C for 30 s, 50°C for 30 s, and 72°C for 1 min, followed by a 20-min extension at 72°C. We submitted PCR products to the Biomedical Genomics Center (University of Minnesota, St. Paul, Minnesota) for electrophoresis on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California).

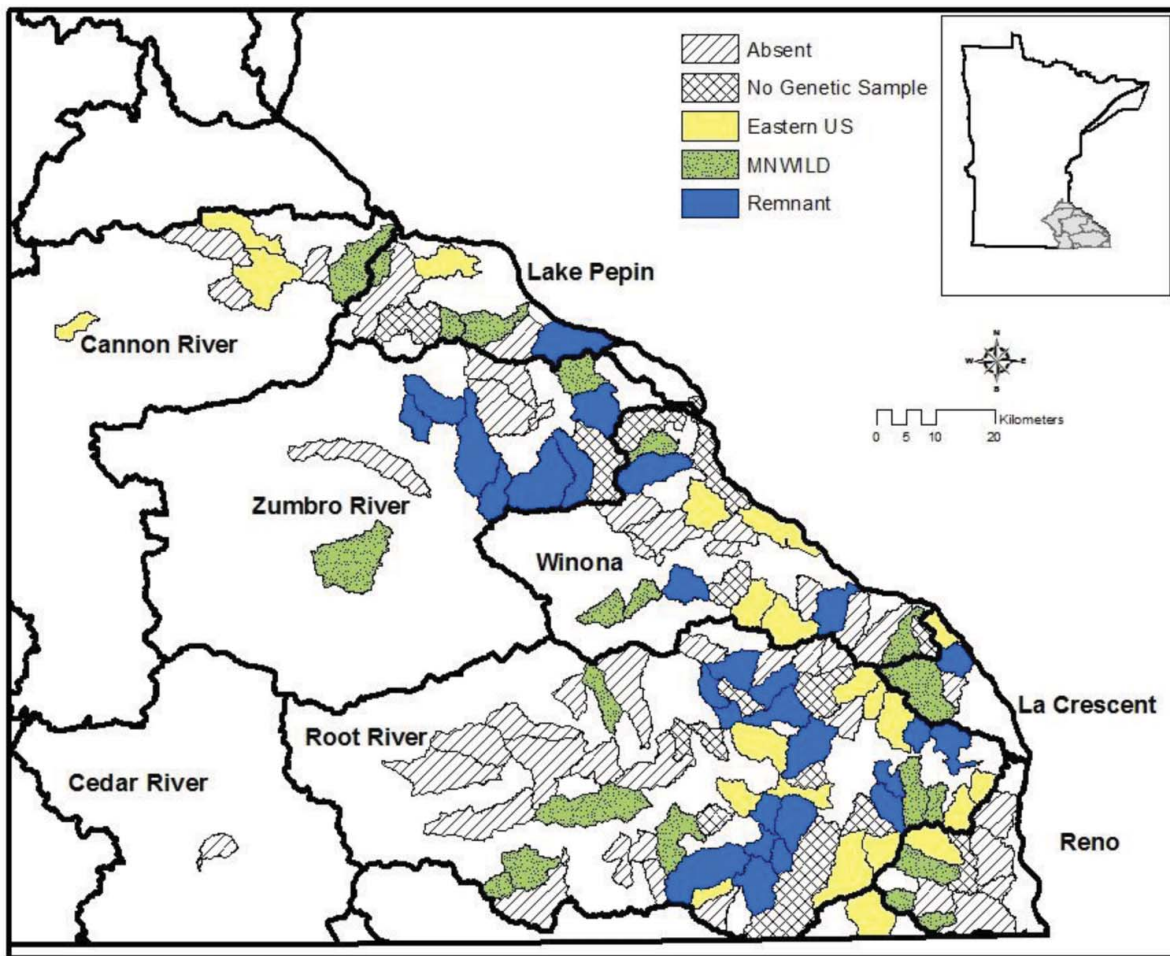


FIGURE 1. Distribution and genetic strains of Brook Trout in southeastern Minnesota delineated by major watersheds. Brook Trout populations were categorized based on genetic analysis as remnant (unique to Minnesota), MNWILD (the current strain used in Minnesota hatcheries), and eastern USA (populations associated with hatchery strains originating in the eastern USA). Absent indicates catchments where Brook Trout were not found.

Alleles were scored using the software program Genotyper 2.1 and later Genemapper 4.1 (Applied Biosystems).

We estimated measures of genetic diversity, allelic richness, and expected and observed heterozygosities and evaluated genetic equilibrium within each population. The data were tested for deviations from Hardy–Weinberg expectations and linkage equilibrium using exact tests in the software GENEPOP version 4 (Raymond and Rousset 1995). Allelic richness, the number of alleles in a sample standardized to a common sample size, was estimated using HP-RARE (Kalinowski 2005). The Garza–Williamson index ( $M$ ; the ratio of the number of alleles to the range in allele size), estimated for each population in ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010), was used as a further indicator of possible population bottlenecks. Garza and Williamson (2001) showed that, under a stepwise mutational model for microsatellites, populations with an  $M < 0.68$  can be assumed to have experienced a reduction in population size.

The measure of population differentiation  $F_{ST}$  was estimated for all population pairs in ARLEQUIN and tested for significant deviation from 0 (i.e., no genetic differentiation) using 16,000 permutations. To assess population relationships, we calculated Cavalli-Sforza and Edwards' (1967) chord distances between each pair of populations and constructed a neighbor-joining tree, with 1,000 bootstrap replicates, using the program POPULATIONS 1.2.30 (Langella 1999). The tree was visualized using TREEVIEW 1.6.6 (Page 1996).

The program STRUCTURE 2.3.3 (Pritchard et al. 2000) was used in a stepwise fashion to further examine the genetic relationships among populations (Coulon et al. 2008). The program STRUCTURE determines the number of distinct genetic clusters ( $K$ ) and assigns ancestry of each individual to these clusters. It is useful because it can identify possible admixture between distinct populations, which was possible because multiple hatchery strains were stocked, populations were translocated between drainages, and relatively few

barriers to movement exist within some watersheds. Five replications were run for each value of  $K = 1$  through 12 using a burn-in of 20,000 iterations followed by a run of 100,000 iterations. Each simulation was performed using models with admixture and correlated allele frequencies but without prior population information. The best value of  $K$  was chosen according to an ad hoc statistic described by Evanno et al. (2005), and populations were assigned to a cluster if their average probability exceeded 0.50 (with a few exceptions noted in the results). The Evanno method often identifies high-level structure (i.e., low  $K$ ) when hierarchical structure exists (Evanno et al. 2005); thus, we repeated STRUCTURE analyses within the clusters to identify further substructure.

**Population characteristics.**—To gain a better understanding of population characteristics, we intensively examined six streams (East Indian Creek, Maple Creek, Coolridge Creek, Trout Valley Creek, Trout Brook, and Garvin Brook) over a 2-year period in both spring and fall. To encompass potential variation, we chose Brook Trout populations from different watersheds, stream sizes, and genetic origins. Populations were categorized as hatchery associated or potential remnants based on whether or not they clustered with known hatchery samples in the genetic analysis. We assessed genetic origin because both the source of a broodstock and its history of captive rearing may affect the characteristics of the population in the wild.

Brook and Brown trout population estimates were made from two-pass depletion techniques (Zippin 1956) using a backpack electrofisher in Coolridge Creek, Garvin Brook, and Trout Brook, whereas a barge electrofisher was used in Maple Creek, Trout Valley Creek, and East Indian Creek. The lengths of the sampling stations depended on mean stream width and ranged between 165 and 311 m. Sampling stations started and ended at shallow riffles to avoid trout leaving the area while sampling was conducted. Density estimates were averaged across the four time periods to account for temporal variability. Both Brook Trout and Brown Trout were measured (nearest mm) and weighed (nearest g). We removed otoliths from a subsample of Brook Trout (minimum of 40 per stream) for age determination. Otoliths were read in whole view under a dissecting microscope with reflected light on a black background.

Mean length at age was estimated using mixed distribution models developed from length frequency histograms from fall collections (both years combined) seeded with “known-age” fish. Known-age fish were those marked at age 0 and recaptured as age 1 and a subsample of fish aged with otoliths. Length frequency histograms were divided into 10-mm length-groups. We used the mixdist package (Macdonald and Du 2010) in the software program R (R Development Core Team 2009) to fit finite mixture distribution models to the length frequency histograms. Mixdist provides estimates for mixing proportions ( $\pi_a$ ), mean lengths at age ( $\mu_a$ ), and standard deviations of length-at-age distributions ( $\sigma_a$ ). Mixing proportions are described as the relative abundance of that age-group as a proportion of the entire measured sample. We

tested several different constraints and probability distributions and compared model results using minimum  $\chi^2$ -values. The best models were used for mean length-at-age estimates.

Annual mortality was derived from instantaneous mortality rates from the descending limb of the catch curve. Catch curves were generated by using mixing proportions ( $\pi_a$ ) from the mixed distribution models described above. Fall sampling data were combined for both years to help alleviate the effects of variable recruitment.

A subsample of trout was sacrificed during fall sampling for internal examination of gonads to assess maturity. The numbers of fish examined for maturity ranged from 79 (Garvin Brook) to 161 (Trout Valley). Maturation was determined by visual examination of gonads and scored as 0 for immature and 1 for mature. For each population, we then used logistic regression with length as our independent variable to calculate size at maturation for males and females (R Development Core Team 2009). Equations derived from the logistic regression model were used to determine the length at which 50% were mature. We combined data across years to increase sample size and encompass annual variability.

We tested for differences in mortality, length at maturity, length at age (age 0–3), and adult density between genetic origins (remnant versus eastern) using a  $t$ -test with streams as replicates. Because stream size can influence population characteristics, we correlated summer base flow discharge and population metrics using Pearson’s correlation coefficient. All tests were set at an alpha level of 0.05.

We measured discharge ( $\text{m}^3/\text{s}$ ) near the downstream boundary of each reach during summer base flow conditions. Velocity was measured with a Marsh-McBirney Model 2000 electromagnetic flowmeter following standard cross-sectional methods (Gallagher and Stevenson 1999). Continuous temperature loggers were placed in each of the six stream reaches where Brook Trout were collected.

## RESULTS

### Spatial Distribution

Brook Trout were present in 119 (68%) of streams sampled. Brook Trout were found in all of the major watersheds except for the Cedar River watershed, where only one stream was sampled. We sampled the most streams and Brook Trout populations in the eastern half of the Root River watershed (Figure 1). Brook Trout populations were categorized as abundant in 40 populations but rare in 21, with some samples only having one Brook Trout (Figure 2).

### Genetics

We collected genetic data for seven microsatellite DNA loci on populations in 74 southeastern Minnesota streams and 1 Minnesota hatchery broodstock (MNWILD). Average sample size was 31 and ranged from 14 to 61. All but eight



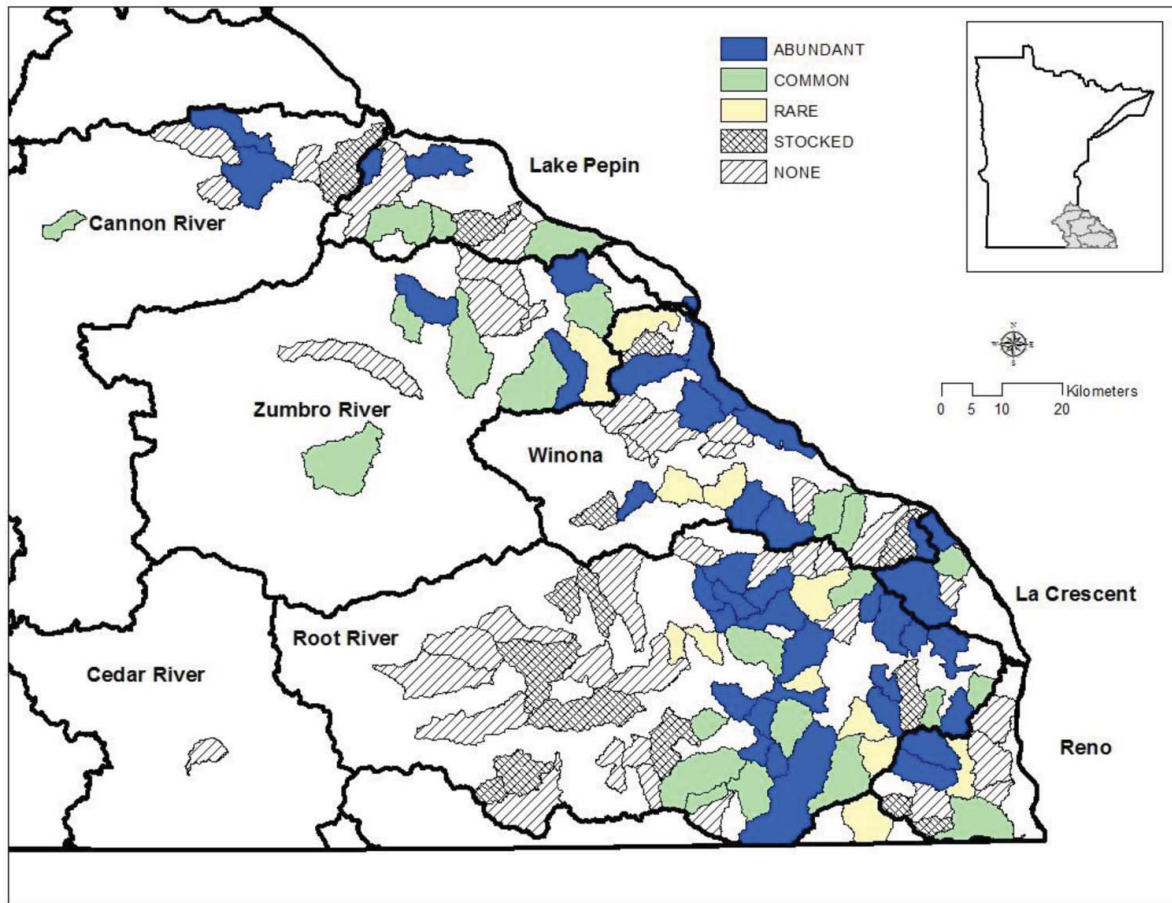


FIGURE 2. Relative Brook Trout abundance in coldwater streams in southeastern Minnesota delineated by major watersheds. Abundance was categorized with a single electrofishing pass as abundant ( $>155/\text{km}$ ), common ( $30\text{--}155/\text{km}$ ), rare ( $<30/\text{km}$ ), and none ( $0/\text{km}$ ). Streams indicated as stocked are currently being stocked with Brook Trout, and natural reproduction has not been evaluated.

samples had more than 20 individuals and more than half had 30 or more individuals. Most loci in most populations conformed to Hardy–Weinberg expectations. The number of tests per locus with  $P$ -values  $< 0.05$  ranged from 2 to 11 and 4 to 14 for heterozygote deficits and excesses, respectively, out of 505 tests (locus  $\times$  sample). Only five tests remained significant after sequential Bonferroni correction (Rice 1989) for multiple testing. Loci *SfoC38*, *SfoC113*, and *SfoC115* showed significant heterozygote deficits in the Rome hatchery sample, and *SfoC88* had a heterozygote deficit in the Nashua hatchery sample, while *SfoC24* had an excess in Camp Hazard Creek. Significant linkage disequilibrium was found in only 10 tests, with no locus pair significant for more than two samples.

Expected heterozygosities averaged 0.64 and ranged from 0.19 to 0.77 in samples from southeastern Minnesota and Iowa populations (Table 1). The lowest heterozygosity was found in the Pleasant Valley tributary sample (0.19), but the next lowest was substantially higher at 0.39 in the Deering Valley Creek sample, and all but two others (Spring Brook and Trout Pond tributary) had values greater than 0.50. Allelic richness

had a similarly skewed distribution with an average of 4.2 (resample size of 22 genes), a range of 1.8–5.4, and all but seven samples with values greater than 3.1. Heterozygosities in hatchery samples ranged from 0.38 to 0.73. Phillips and Rome samples had relatively low heterozygosities of 0.38 and 0.42, respectively, while other hatchery samples exceeded 0.60. Allelic richness averaged 2.7 in Phillips and Rome samples but 4.7–4.8 in the other hatchery samples.

The Garza–Williamson index  $M$  was relatively high for most populations (mean = 0.75; SD = 0.28) but was at or below the critical value of 0.68 in several populations. Low values were found in Spring Brook and populations founded by translocations from Spring Brook (Deering Valley Creek, Miller Valley Creek, Trout Valley Creek), PHP hatchery, Pleasant Valley tributary, Schad tributary, IOWA, and East Beaver Creek.

Most sample pairs were significantly differentiated ( $F_{ST} > 0$ ; following sequential Bonferroni adjustment; Supplementary Table S.1 available in the online version of this article). The majority of samples that were not differentiated involved the

MNWILD strain and populations recently founded from this strain. Most other undifferentiated pairs came from tributaries and their recipient streams. In addition, replicate samples taken 2 years apart at both the unnamed Riceford Creek tributary Number 3 and Spring Brook Creek were not significantly differentiated (data not shown), demonstrating the stability of allele frequencies over short time periods. Samples taken 8 years apart at Coolridge Creek had a significant but low  $F_{ST}$  of 0.01 (data not shown).

The clustering algorithm based on Cavalli-Sforza–Edwards chord distances (Cavalli-Sforza and Edwards 1967) grouped populations into several distinct clusters. Several of the main clusters contained hatchery samples and varying numbers of southeastern Minnesota populations (Figure 3), indicating likely contributions of these hatchery strains to the current populations. The MNWILD hatchery sample and several populations recently founded from this strain clustered together but near another cluster containing Coolridge Creek, one of the two source populations for MNWILD. Several clusters of populations were not closely associated with hatchery samples. These clusters were primarily composed of geographic groupings from Rush Creek, Zumbro River, and South Fork Root River tributaries (Figure 3). Rush Creek and Zumbro River clusters were part of a major branch separated from branches with hatchery samples (with the exception of MNWILD). The South Fork Root River cluster incorporated the Iowa sample from South Pine Creek, the last putative remnant Brook Trout population in Iowa. In all, 36 streams had Brook Trout populations that were not closely associated with known hatchery samples (Figure 3).

The STRUCTURE analyses identified multiple clusters that were mostly consistent with major groupings on the neighbor-joining tree (Figure 4). Three major clusters were initially identified using all data, and further analysis broke some clusters into distinct subclusters. The hatchery sample MNWILD and populations reintroduced with this strain showed mixed ancestry, as expected, from the two clusters containing its founding populations. These samples were removed from further analysis along with a tributary to a MNWILD-stocked stream. The three hatchery samples MARQ, SCF, and NASH did not assign with probability  $> 0.50$  to any one group but were included in the two highest clusters to which they assigned for further analysis. The first cluster contained PHP and ROME hatchery samples and several Minnesota samples. Further analysis including MARQ, SCF, and NASH formed three subclusters, one with PHP, ROME, and two Minnesota samples, a second with Spring Brook and several populations founded by translocations from Spring Brook, and a third with MARQ, SCF, and NASH hatchery samples and two Minnesota samples. The second major cluster contained OWHI hatchery, the IOWA sample, and numerous Minnesota samples from the South Fork Root River, other Root River tributaries, and three Zumbro River samples. A few samples were slightly below 0.50 but were included because of geographic proximity to

others in this cluster. Further analysis separated primarily the Iowa and South Fork Root River samples from the hatchery and other Minnesota samples. The third cluster contained numerous samples from Rush Creek tributaries and their translocated populations (Swede Bottom Creek, East Burns Valley Creek, East Indian Creek) and four samples from the Zumbro River. This differed from the tree diagram, in which all Zumbro River samples grouped together. The STRUCTURE analyses identified additional partitioning, but these subclusters isolated hatchery samples from numerous Minnesota samples and grouped many of those Minnesota samples by watershed.

It was difficult to ascertain admixture because of the many populations and relatively few loci, and these difficulties were compounded by the lack of strong assignment of several hatchery samples to any one cluster. Despite these complications, there were suggestions of admixture in some population groupings. Many samples in the upper South Fork Root River grouped with Iowa in the tree diagram, but while Iowa assigned to a STRUCTURE cluster with 0.85 probability, the South Fork Root samples assigned with an average probability of only 0.61 (range = 0.46–0.68), possibly resulting from mixture of a lineage similar to Iowa with other lineages. Consistent with admixture, many of the South Fork Root River samples had relatively high heterozygosity and allelic richness compared with most samples and considerably higher than that for Iowa. In a second instance, all Zumbro River samples grouped in the tree diagram but STRUCTURE split them into two main clusters. Four samples from the lower Zumbro River assigned with an average probability of 0.76 to cluster 3, while three from the upper Zumbro River assigned with an average probability of only 0.55 to cluster 2. This lower assignment may have resulted from admixture in the latter three samples.

### Population Characteristics

The six intensively studied populations were grouped into two categories based on genetic origin. Trout Brook, Trout Valley, and Garvin Brook were associated with eastern U.S. hatchery samples and were categorized as Eastern USA. Maple Creek, Coolridge Creek, and East Indian Creek were not associated with hatchery samples and were categorized as remnant (Figure 3). Mean summer water temperatures were warmest in East Indian Creek and Maple Creek (Table 2). These streams had the warmest summer temperatures and the coldest winter temperatures. Water temperatures did not exceed maximum limits ( $\sim 20^{\circ}\text{C}$ ) for adult Brook Trout in any of the study streams.

Brook Trout density ranged from 0.023 to 0.174/m<sup>2</sup> across streams but did not differ between genetic strains ( $t$ -test:  $P = 0.52$ ; Table 2). Brook Trout density was positively related to stream discharge ( $r = 0.98$ ;  $P < 0.001$ ). All six streams had low densities of Brown Trout, but there was no relationship between Brook Trout density and Brown Trout density ( $r = 0.40$ ;  $P = 0.42$ ).



TABLE 1. Stream identification with year of sample collection, sample size, expected and observed heterozygosities ( $H$ ), the Garza–Williamson index ( $G-W$ ), and allelic richness ( $A_R$ ). Samples include Driftless Area streams from southeastern Minnesota and Iowa and known hatchery strains. The six streams intensively studied for population characteristics are in bold italics.

Sample	ID	Year	$N$	Expected $H$	Observed $H$	$G-W$	$A_R$
<b>Southeastern Minnesota</b>							
Badger Creek tributary	BCT	2003	29	0.67	0.71	0.80	5.0
Bee Creek	BEE	2008	16	0.64	0.64	0.76	3.7
Blagsvedt Creek	BLAG	2003	26	0.66	0.62	0.75	4.6
Borson Spring Creek	BRS	2003	30	0.69	0.73	0.69	4.3
Bridge	BDG	2008	24	0.61	0.62	0.78	4.3
Brush Valley	BRV	2009	24	0.65	0.70	0.72	4.3
Bullard Creek	BUL	2006	30	0.57	0.52	0.83	3.7
Butterfield	BUT	2006	24	0.66	0.64	0.70	4.2
Camp Hazard Creek	CHC	2003	31	0.66	0.82	0.71	3.9
Campbell	CAM	2008	26	0.74	0.75	0.77	5.1
Chickentown Creek	CKC	2003	29	0.61	0.62	0.84	4.0
Cold Spring Brook	CSB	2003	25	0.68	0.74	0.78	4.2
<b>Coolridge Creek</b>	CC	2001	46	0.68	0.69	0.78	4.3
Corey	COR	2006	25	0.75	0.71	0.75	5.2
Crooked Creek	CRK	2006	39	0.70	0.73	0.64	4.9
Crooked Creek, South Fork	CRC	2008	28	0.65	0.63	0.82	4.5
Dakota Creek	DKC	2003	32	0.63	0.60	0.65	3.9
Deering Valley Creek	DVC	2009	32	0.39	0.39	0.80	2.4
Diamond	DIA	2006	40	0.66	0.65	0.83	4.6
East Beaver Creek	EBC	2008	16	0.55	0.58	0.71	3.0
East Burns Valley Creek	EBVC	2003	27	0.56	0.59	0.73	3.4
<b>East Indian Creek</b>	EIC	2006	56	0.70	0.69	0.70	4.7
Ferguson Creek	FER	2007	26	0.64	0.77	0.79	3.7
Ferndale	FDL	2008	30	0.65	0.60	0.78	4.5
<b>Garvin Brook</b>	GAR	2006	27	0.64	0.66	0.78	3.7
Girl Scout Camp Creek	GSCC	2003	26	0.71	0.69	0.77	4.8
Gribben	GRB	2010	30	0.63	0.65	0.74	4.1
Hallum	HAL	2009	25	0.72	0.70	0.79	5.3
Hammond Creek	HMC	2003	30	0.69	0.63	0.78	4.9
Helbig Creek	HEL	2009	42	0.69	0.72	0.75	4.1
Hemmingway Creek	HEM	2001	34	0.69	0.64	0.79	4.4
Larson Creek	LAR	2006	24	0.70	0.69	0.81	4.5
Long	LNG	2006	30	0.67	0.70	0.75	4.5
Looney Creek	LVC	2003	30	0.65	0.67	0.80	4.4
<b>Maple Creek</b>	MAP	2006	32	0.70	0.67	0.74	4.8
Mazeppa Creek	MAZ	2006	34	0.68	0.71	0.62	4.2
Middle Branch Whitewater	MBW	2007	22	0.67	0.67	0.76	4.9
Middle Creek	MDC	2003	27	0.62	0.60	0.80	4.2
Miller Valley Creek	MVC	2009	37	0.55	0.54	0.76	3.3
Nepstad Creek	NEP	2003	30	0.73	0.73	0.75	4.8
Newburg	NEW	2006	15	0.70	0.63	0.75	4.6
Peterson Creek	PTC	2003	31	0.60	0.61	0.70	3.5
Pine Creek	PIC	2010	21	0.58	0.56	0.77	3.6
Pine New Hartford	PCR	2007	25	0.70	0.69	0.78	4.8
Pine New Hartford, south fork	PINE	2008	28	0.77	0.76	0.78	5.3

TABLE 1. Continued.

Sample	ID	Year	N	Expected <i>H</i>	Observed <i>H</i>	G–W	<i>A<sub>R</sub></i>
<b>Southeastern Minnesota</b>							
Pleasant Valley tributary	PVT	2009	14	0.19	0.18	0.62	1.8
Rush tributary–Fillmore	URUF	2008	29	0.69	0.70	0.75	4.3
Rush tributary–Winona	URUW	2008	26	0.62	0.59	0.80	3.9
South Branch Whitewater tributary	SBWTr	2003	29	0.68	0.68	0.73	2.9
Schad tributary–Boynton	SCD	2008	16	0.56	0.69	0.58	4.5
Schueler Creek	SHC	2003	26	0.70	0.69	0.78	4.1
Second Creek	SEC	2003	26	0.65	0.70	0.69	4.8
Shamrock	SHA	2007	25	0.72	0.71	0.82	5.1
Silver	SIL	2009	25	0.71	0.72	0.83	4.4
Silver Springs	SSP	2010	15	0.67	0.77	0.72	3.2
Sorenson	SRN	2008	20	0.54	0.61	0.69	2.8
Spring Brook	SBC	2001	32	0.47	0.46	0.60	3.5
Stockton Valley Creek	SVC	2008	19	0.59	0.56	0.70	4.5
Storer	STR	2006	29	0.72	0.72	0.76	4.2
Sullivan Creek	SUL	2006	27	0.68	0.71	0.77	4.5
Swede Bottom Creek	SWB	2003	26	0.68	0.69	0.78	4.7
Thompson Creek	THM	2006	30	0.70	0.73	0.75	3.7
Trail Run	TRN	2009	61	0.60	0.59	0.73	4.5
<b>Trout Brook</b>	TRB	2007	33	0.68	0.69	0.78	4.6
Trout Brook	TBW	2009	21	0.63	0.69	0.77	2.7
Trout Pond tributary	TPT	2008	27	0.46	0.56	0.70	4.5
Trout Run	TTR	2009	27	0.71	0.79	0.78	3.7
<b>Trout Valley Creek</b>	TVC	2007	25	0.57	0.56	0.68	4.4
Unnamed Riceford3	3UN	2006	29	0.73	0.73	0.81	4.1
Unnamed Riceford4	4UN	2006	26	0.61	0.59	0.81	4.6
Vesta	VES	2009	27	0.60	0.65	0.78	4.2
Voelker Brook	VKB	2003	28	0.64	0.72	0.79	4.6
West Beaver Creek	BCW	2006	25	0.71	0.71	0.80	5.2
Wisel Creek	WIS	2009	24	0.71	0.69	0.80	5.0
<b>Iowa<sup>a</sup></b>							
South Pine Creek	IOWA	1999	54	0.62	0.60	0.65	3.1
<b>Hatcheries<sup>b</sup></b>							
Phillips	PHP	1998	51	0.38	0.41	0.63	2.7
Marquette	MARQ	2003	73	0.61	0.59	0.87	4.8
MN Wild	MNW	1998	60	0.67	0.70	0.78	4.7
Nashua	NASH	1997	37	0.72	0.68	0.85	4.8
Owhi	OWHI	1998	52	0.73	0.73	0.80	4.7
Rome	ROME	1998	50	0.42	0.33	0.74	2.7
St. Croix Falls	SCF	1998	48	0.67	0.63	0.81	4.5

<sup>a</sup>Data from King, unpublished data.<sup>b</sup>Data from Stott, unpublished data.

Growth was variable across streams, with Coolridge Creek, Trout Brook, and Garvin Brook having the slowest growth rates to age 3 (Table 3). These three streams also had the lowest summer water temperatures. Maple Creek and Trout Valley Creek had larger Brook Trout than the other populations as a result of having older fish but not necessarily

faster growth rates (Table 3; Figure 5). Length at age 3 was positively related to stream discharge ( $r = 0.89$ ;  $P = 0.017$ ), but discharge was not related to mean length at age for younger Brook Trout (ages 0–2, all  $P > 0.05$ ). Mean length at age did not differ by genetic origin for any age-class ( $t$ -test:  $P > 0.05$ ).

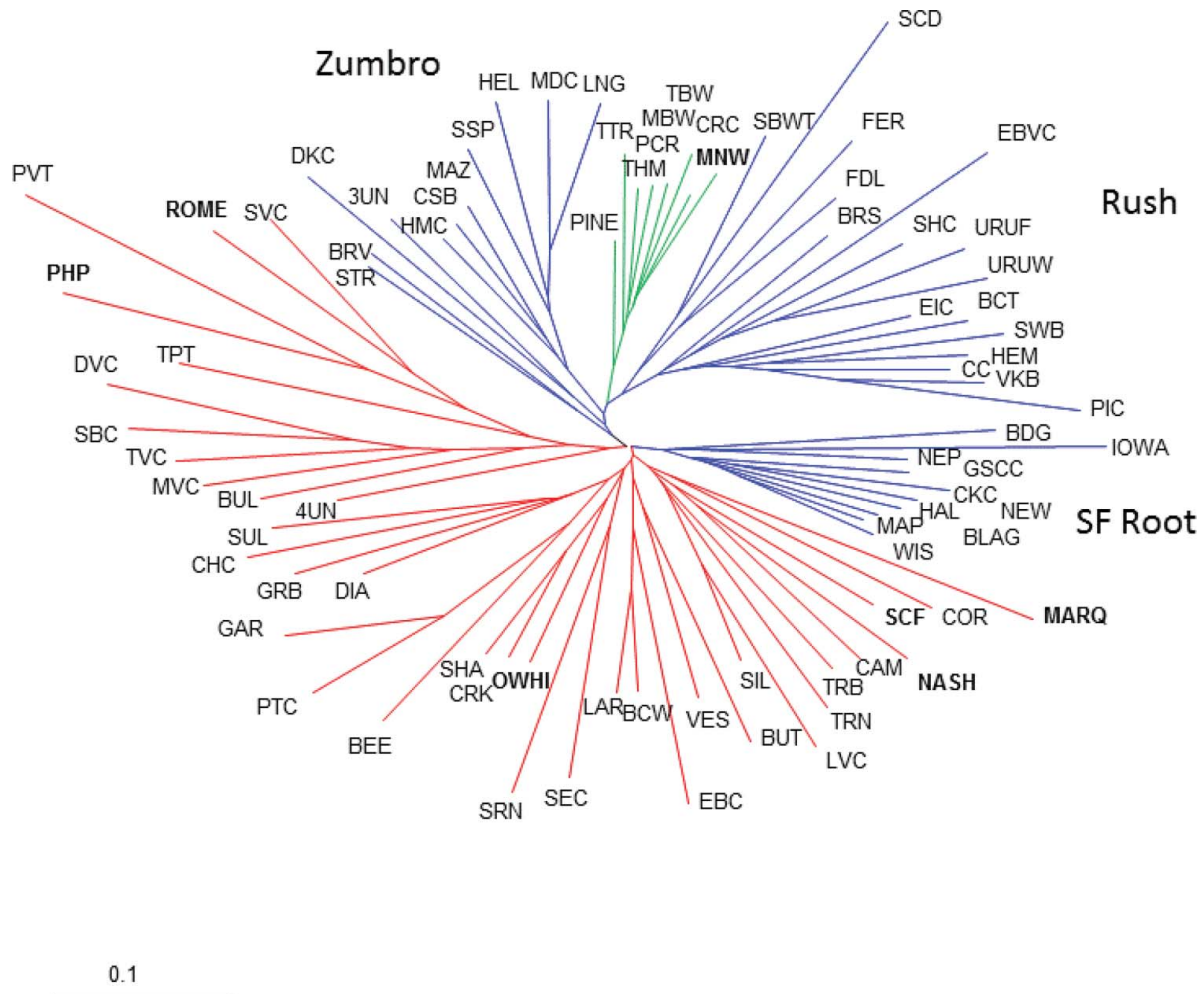


FIGURE 3. Radial neighbor-joining tree diagram, based on chord distances, showing the genetic relationships among Brook Trout populations in southeastern Minnesota streams and known hatchery strains ( $n = 102$ ). Potential remnant Driftless Area Brook Trout in southeastern Minnesota are in blue and cluster according to watersheds (Zumbro River, Rush Creek, South Fork Root River). Hatchery strains are in bold and include MNWILD (green lines) and those with eastern U.S. origins (red lines). Stream abbreviations are defined in Table 1.

Annual mortality rates were similar across three of the six study streams ( $\sim 63\%$ ; Table 2). The high mortality rate in East Indian Creek may have resulted from predation on Brook Trout by North American river otter *Lontra canadensis* (R. J. H. Hoxmeier, personal observation). Mortality did not differ between genetic origin ( $t$ -test:  $P = 0.94$ ) nor was it related with either stream discharge ( $r = 0.22$ ;  $P = 0.67$ ) or mean summer water temperature ( $r = 0.59$ ;  $P = 0.22$ ).

Brook Trout matured at a small size across all streams (Table 2). Males matured at a smaller size than females, except in Maple Creek. Males often matured in their first year of life. Maple Creek and Trout Valley Creek had the largest size at maturation for both males and females, with Coolridge Creek and Trout Brook having the smallest. Size at maturity did not differ by genetic origin ( $t$ -test: males,  $P = 0.47$ ; females,  $P = 0.81$ ), but it increased with increasing stream discharge ( $P < 0.01$ ; Figure 6).

## DISCUSSION

Intolerant coldwater fishes, such as trout, are predicted to decline given changes in both climate and land use (Flebbe et al. 2006; Hudy et al. 2008; Lyons et al. 2010); however, our study documented an increase in the distribution of a coldwater native trout during the last several decades. We found robust, remnant Brook Trout populations increasing in number in the Driftless Area of Minnesota, which is likely the result of the cumulative effects of land-use conservation practices, improved fisheries management strategies, and the unique landscape of the Driftless Area.

Previous reports suggested that native Brook Trout were extirpated in southeastern Minnesota around 1900 and that only hatchery-supported stocks remained (MacCrimmon and Campbell 1969; Thorn et al. 1997). Thorn and Ebberts (1997) reported that 3% of coldwater streams in southeastern Minnesota contained Brook Trout in the early 1970s compared with

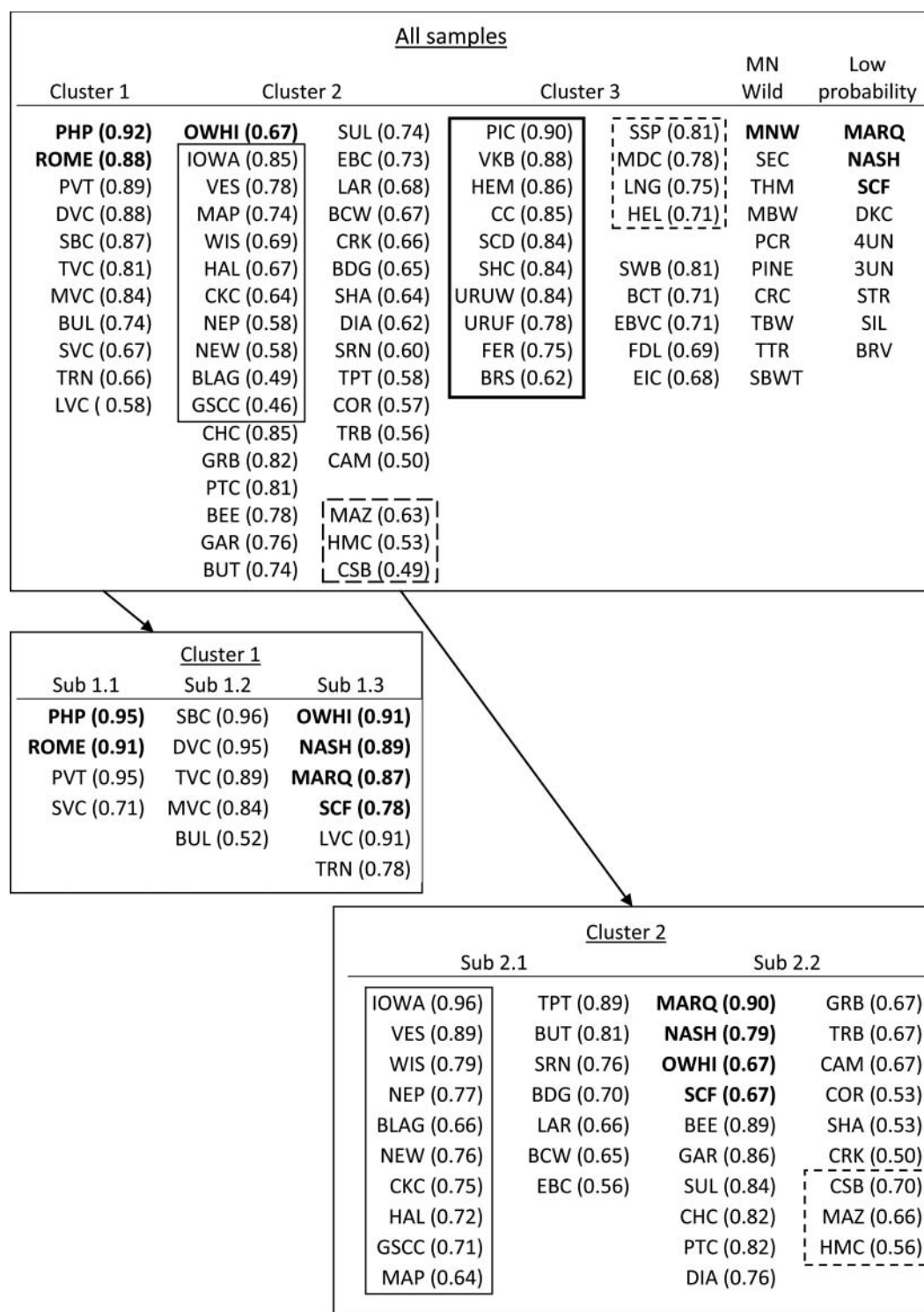


FIGURE 4. Assignment of populations to genetic clusters using a stepwise STRUCTURE analysis (Pritchard et al. 2000). Populations were initially assigned to one of three distinct genetic clusters if their average assignment exceeded 0.45 (top box). Hatchery populations are in bold. Populations derived from recent Minnesota Wild stocking (MNWILD) showed similar assignments to clusters 2 and 3, which contained their source populations, and are listed separately. Several other populations did not assign strongly to any one cluster (low probability, <0.45). Populations assigning to clusters 1 and 2 were then analyzed in separate STRUCTURE runs to identify subclusters (lower two boxes). Geographic groupings described in the text are outlined: South Fork Root River (thin solid line), Rush Creek (thick solid line), and Zumbro River (dashed line). Stream abbreviations are defined in Table 1.

TABLE 2. Habitat and Brook Trout population characteristics of six streams in southeastern Minnesota. Temperature ( $^{\circ}\text{C}$ ) is the mean (SD in parentheses) summer (June–August) temperature for 2008 and 2009. Genetic origin is categorized as either remnant (R) or eastern USA (E). Length (mm) at maturity for males (M) and females (F) was determined from logistic regression. Annual mortality was estimated from catch curves combined across years to account for variable recruitment. Proportional stock density (PSD) is the number of Brook Trout over 200 mm divided by the number of Brook Trout over 130 mm. Brook Trout (BKT) and Brown Trout (BNT) density is the mean adult (age 1 and older) density across four sampling periods.

Stream	Station length (m)	Discharge ( $\text{m}^3/\text{s}$ )	Mean width (m)	Mean temperature	Genetic origin	Length at maturity		Annual mortality	PSD	BKT density ( $\text{N}/\text{m}^2$ )	BNT density ( $\text{N}/\text{m}^2$ )
						M	F				
Coolridge Creek	515	0.03	2.7	10.9(0.7)	R	118	132	54.5	41	0.023	0.020
Maple Creek	180	0.19	5.3	14.1(1.1)	R	179	172	63.2	24	0.174	0.023
East Indian Creek	209	0.11	5.7	14.1(1.0)	R	133	157	76.0	40	0.102	0.016
Garvin Brook	311	0.08	5.6	11.2(0.6)	E	127	149	62.4	36	0.064	0.017
Trout Valley Creek	278	0.11	4.8	12.4(0.9)	E	138	164	63.0	26	0.080	0.011
Trout Brook	165	0.05	3.4	11.6(1.0)	E	119	137	69.9	20	0.052	0.001

54% in the mid-1990s. Our study found that 68% of coldwater streams in southeastern Minnesota contained Brook Trout. We attribute this recent increase to successful fisheries management and a regionwide increase in stream base flows. Since the late 1990s, numerous successful Brook Trout reintroductions have been implemented by the Minnesota Department of Natural Resources (MN DNR) using the MNWILD strain. This strain was developed with wild founders from a potential remnant population and a naturalized population associated with eastern hatchery strains but with no known recent stocking. The MNWILD strain has been successful in streams where earlier stocking attempts with other strains has failed (MN DNR, file data). Wisconsin has had similar success developing self-sustaining populations of naturalized Brown Trout using wild populations to found broodstocks (Mitro 2004).

Streams in the Driftless Area offer quality habitat due to groundwater inputs from deep, confined aquifers, which contrasts with streams north and east of the region in which temperatures are regulated primarily by atmospheric conditions. Base flows have increased in Driftless Area streams as a result of earlier land-use changes and increased precipitation (Gebert

and Krug 1996; Trimble 1999; Juckem et al. 2008; Lenhart et al. 2011). An increase in base flow can benefit trout populations by providing protection from avian and mammalian predation, increasing available habitat, and increasing growth rates (Hakala and Hartman 2004; Harvey et al. 2006; Sotiropoulos et al. 2006). In our study, base flow discharge was positively related to Brook Trout density, length at age 3, and size at maturation. Size structure and size at maturity for trout is positively related to stream size in other regions (Jonsson et al. 2001; Meyer et al. 2003; Petty et al. 2005; Schill et al. 2010). Interestingly, our large streams with robust Brook Trout populations also had the highest summer water temperatures. Monitoring and protecting base flows in the Driftless Area may be as important as monitoring water temperatures in a changing climate.

Our study indicates that considerable genetic diversity exists within and among Brook Trout populations in southeastern Minnesota, which could have facilitated the recent expansion of Brook Trout and could help the species adapt and persist in a changing environment. In many instances diversity is likely sustained because of connectivity within portions of watersheds, forming larger metapopulations that incorporate

TABLE 3. Mean total length at capture (mm; SE in parentheses) for Brook Trout caught in six southeastern Minnesota streams in fall 2008 and 2009. Genetic strain is categorized as either remnant (R) or eastern U.S. origins (E). Mean length at age was determined from a subsample of aged fish, and length frequency data was analyzed with mixed-distribution models.

Stream	Strain	Length at age				
		Age 0	Age 1	Age 2	Age 3	Age 4
Coolridge Creek	R	97.1 (1.1)	169.7 (2.6)	209.2 (4.1)	217.0 (10.1)	
Maple Creek	R	107.8 (0.7)	165.2 (1.5)	208.3 (4.7)	272.1 (9.0)	307.6 (16.9)
East Indian Creek	R	116.7 (0.6)	203.8 (1.6)	238.1 (4.1)	261.6 (9.9)	
Garvin Brook	E	108.7 (0.8)	185.1 (3.9)	216.8 (13.0)	246.9 (9.3)	
Trout Valley Creek	E	128.4 (0.6)	196.5 (2.5)	222.9 (3.1)	257.5 (8.5)	300.5 (7.7)
Trout Brook	E	115.5 (0.9)	166.0 (5.5)	209.7 (7.7)	245.7 (12.4)	

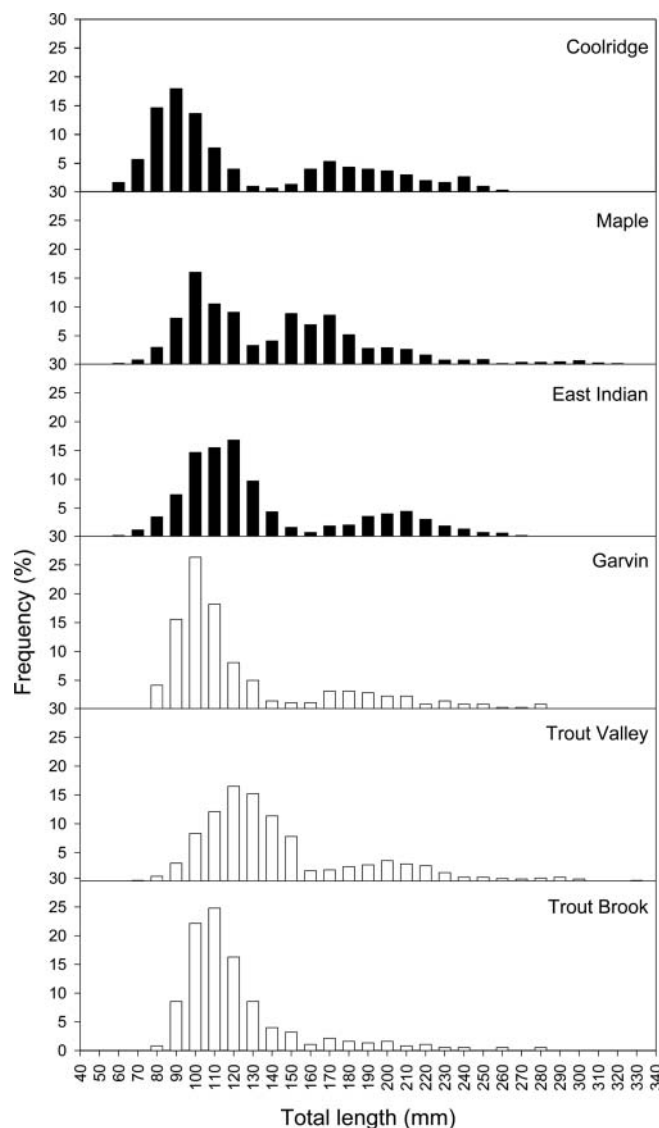


FIGURE 5. Length frequency distributions of Brook Trout collected in fall 2008 and 2009 for six streams (Coolridge Creek, Maple Creek, East Indian Creek, Garvin Brook, Trout Valley Creek, and Trout Brook) in southeastern Minnesota. Brook Trout populations were characterized as either remnant (solid bars) or hatchery origin (open bars).

the relatively small populations in individual tributaries. However, the Garza–Williamson index identified several probable bottlenecked populations with genetic diversity that was lower than other populations in the region. Population origins were linked to eastern U.S. hatchery strains, translocations within the region, current MNWILD broodstock, and potential remnant Driftless Area Brook Trout.

Several population clusters were not associated with known hatchery sources from the past three decades, and these could represent remnant Driftless Area Brook Trout lineages. One consistent cluster in both the neighbor-joining tree and STRUCTURE analyses incorporated all populations in the

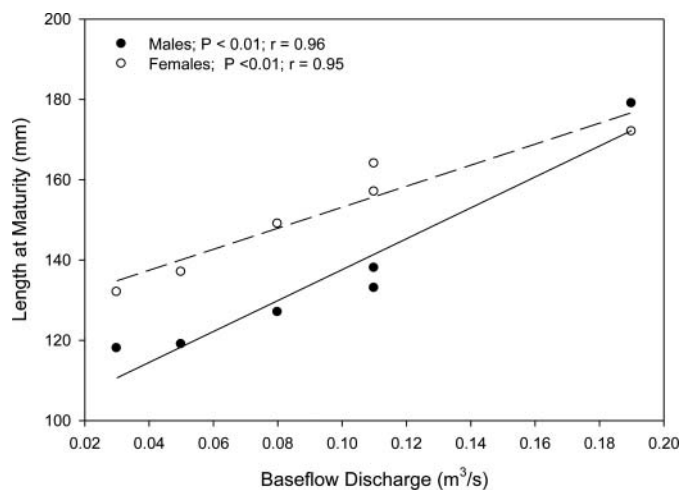


FIGURE 6. Length at maturity (mm) for male (solid line) and female (dashed line) Brook Trout related to stream discharge from six southeastern Minnesota streams. Stream discharge was measured during summer base flow conditions.

Rush Creek subbasin of the Root River. An adjacent cluster in the tree diagram incorporated the populations from Zumbro River tributaries, although some populations are split off in STRUCTURE analyses. A third group is separated from the previous groups but includes tributaries of the South Fork Root River along with an Iowa sample from what is thought to be that state's last remnant population of Brook Trout (B. Kalishek, Iowa Department of Natural Resources, personal communication). Several associations of populations were inconsistent with geography but can be accounted for by recorded translocations rather than stocking of hatchery strains (MN DNR, file data). While these populations do not associate closely with known recent stocking sources, establishing their histories will require further genetic data and potential source population samples. Whether these lineages prove to be descendants of remnant Driftless Area populations or other unknown exogenous populations stocked in the past, they have proven their ability to persist in southeastern Minnesota and deserve extra conservation attention.

The persistence of native genotypes despite the stocking of exogenous sources would not be unprecedented for Brook Trout. It has been reported throughout their range, including in Ontario (Danzmann and Ihssen 1995), Maryland (Hall et al. 2002), Massachusetts (Annett et al. 2012), Wisconsin (Krueger and Menzel 1979), and the southern Appalachians (Galbreath et al. 2001; Habera and Moore 2005). Similar to Minnesota, streams in the southern Appalachians experienced extreme habitat degradation in the early 1900s from logging, causing a decrease in Brook Trout distribution and yet native and mixed-ancestry stocks persist (Habera and Moore 2005). In neighboring Wisconsin, Krueger and Menzel (1979) argued that natural patterns of genetic variation existed among populations despite years of stocking hatchery fingerlings and catchable-sized fish.



Although we propose that remnant Driftless Area lineages may persist, we do not imply that Brook Trout populations survived in all of the streams with potential remnant ancestry. Habitat conditions, especially water quality and flow, were degraded enough that Brook Trout were likely extirpated from many streams (Thorn et al. 1997). Remnant lineages may have persisted near some remaining headwater springs with suitable habitat or in hatchery broodstocks or trout ponds maintained in the region. Human assistance or recolonization could then have spread lineages after base flows increased in the region. Potential remnant populations in East Indian Creek, Swede Bottom Creek, and East Burns Valley Creek can all be traced to recorded translocations from Hemmingway Creek in the 1970s. Considerable Brook Trout movement between nearby tributaries has been documented in this region (Hoxmeier and Dieterman 2013) and connectivity is high, at least within many subbasins. The remnant population in Badger Creek tributary was not directly stocked with Hemmingway Creek fish but meets the South Fork Root River only 0.4 km away from the translocated Swede Bottom Creek population.

Few demographic characteristics distinguished potential remnant from hatchery-associated populations; rather, Brook Trout appear to exhibit phenotypic plasticity. For example, the population characteristics in the potential-remnant East Indian Creek were much more similar to those in Trout Valley Creek, with an identical base flow, than to those in Coolridge Creek, another remnant population that grouped closely in genetic analyses but had a much lower base flow. Demographic characteristics may have been similar among populations due to strong environmental effects coupled with the potential for rapid evolution in naturalized populations (Hendry et al. 2000; Pearse et al. 2009). Native southern Appalachian and naturalized northeastern U.S. hatchery populations have similar growth and life spans in the wild, but significant differences have been observed in laboratory experiments (Habera and Moore 2005; Wesner et al. 2011). A common garden experiment, in which potential remnant and eastern hatchery strains are monitored in the same stream, may be needed to determine if there are genetically based differences in characteristics between strains.

Adult Brook Trout densities in our six intensively studied streams were similar to those found in centrally located populations in Michigan, Massachusetts, and Pennsylvania (Carlson and Letcher 2003; Kocovsky and Carline 2006; Grossman et al. 2012). While temporal variation in trout abundance is often high and may mask differences across streams located in the center versus the edge of their range (Dauwalter et al. 2009), it would appear that some Driftless Area streams can maintain high densities. Length at age for Brook Trout in the Driftless Area was higher than that reported for several Michigan, New York, Pennsylvania, and Tennessee streams (Cooper 1967; Flick and Webster 1975; Whitworth and Strange 1983; Alexander and Nuhfer 1993). In a comprehensive age and

growth study in the southern Appalachians, only 3 out of 28 streams had Brook Trout over 200 mm (Konopacky and Estes 1988), whereas all of our six study streams had Brook Trout this size. The potential of Driftless Area streams to maintain Brook Trout populations with high densities and large size structures should provide a recreational benefit to anglers, as well as conservation benefits.

While Brook Trout distribution has expanded in the Driftless Area in the last 40 years, so has the abundance and distribution of Brown Trout (Thorn et al. 1997). The same reasons for Brook Trout expansion can be given for the success of Brown Trout in the Driftless Area. Even though we targeted stream reaches towards Brook Trout, Brown Trout were present in 78% of our samples. Brown Trout density in our intensively studied streams did not influence Brook Trout population characteristics; however, Brown Trout densities in these six streams were on the lower end of the range found in this region (Kwak and Waters 1997). Higher Brown Trout densities have been shown to have negative effects on Brook Trout in the Driftless Area (Hoxmeier and Dieterman 2013).

### Management Implications

This study represents one of the most comprehensive evaluations of Brook Trout in the Driftless Area and provides a useful baseline for developing conservation strategies and monitoring future impacts of stressors, such as climate change. Brook Trout populations in this region did not conform to the expectations of peripheral populations, likely because of the quality stream habitat conditions found in the region. The Driftless Area acted as a refugium during the last glaciation period for numerous flora and fauna that still persist in the region today (Rowe et al. 2004; Li et al. 2013). Because of the thermally stable groundwater and increased base flows, this region may again act as a refugium for Brook Trout in a warming climate. However, fisheries managers must ensure the protection of base flows through enhancing groundwater infiltration and reducing groundwater withdrawals. Although Brook Trout were found in the majority of streams, and in more than previously recorded, the number of populations where they were abundant was still low. Also, even though we found Brook Trout in 68% of Driftless Area streams in Minnesota, we presume the remaining 32% had Brook Trout prior to European settlement. This study should be repeated in the future at a similar spatial scale to determine whether the Driftless Area continues to offer a refugium or whether Brook Trout distribution declines as predicted for this region due to warming air temperatures (Lyons et al. 2010).

Only 21% of Driftless Area streams potentially have remnant Driftless Area Brook Trout lineages. These unique populations should be given conservation priority because they have proven their ability to sustain themselves in this region. In addition, their potential for high density and growth should

be of value to fisheries managers. In particular, we recommend ceasing management attempts to increase Brown Trout in these remnant streams and treating major watersheds as distinct management units to conserve genetic diversity among populations within the Driftless Area. Although we found few differences in population characteristics associated with potential remnant versus introduced Brook Trout populations, the influences of stream size and other environmental variables, along with a relatively small number of studied populations, limited our ability to detect differences. A more controlled study is needed to determine whether differences in population characteristics, and particularly fitness, exist among genetic origins. The persistence of these distinct genetic groups despite stocking of many sources suggests they may be well adapted to their local environment.

Most populations in the region have moderately high genetic diversity. To sustain this diversity, management should focus on preventing drastic population declines and maintaining the existing connectivity of tributary networks by preventing the placement of barriers and assuring habitats are suitable for the movement of Brook Trout within subwatersheds (Petty et al. 2012). Several probable bottlenecked populations were identified, and their genetic diversity was low compared with other populations in the region. Many of these populations are isolated and may be candidates for “genetic rescue,” the addition of individuals from other populations to enhance diversity and alleviate possible inbreeding depression (Tallmon et al. 2004). The use of source populations we have identified as genetically similar could help alleviate short-term inbreeding risks while lessening the concern about outbreeding depression (Edmands 2007).

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