Sources of coaster brook trout (*Salvelinus fontinalis*) revealed by genomic analysis of brook trout populations along Minnesota's shoreline with Lake Superior

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ARTICLE INFO

ABSTRACT

Knowledge of population-level relationships and how these relationships pertain to different life history forms is critical to developing effective management plans for native trout, char, and salmon. In the Lake Superior basin, identifying effective restoration strategies for coaster brook trout (*Salvelinus fontinalis*), a lake-inhabiting form of brook trout, is hampered by limited information on genetic connectivity and source-sink dynamics among brook trout populations. Here, we infer these relationships by surveying 8,178 single nucleotide polymorphisms in 234 brook trout from seven rivers along the Minnesota shoreline with Lake Superior, including from reaches above and below natural waterfalls that prevent upstream movement. We identified well-differentiated above-barrier populations that supply brook trout to below-barrier reaches. We also compared within-river brook trout to 26 coaster brook trout from Lake Superior. We identified at least four source populations for these coaster brook trout, three of which were located within rivers. Additionally, we estimated N_E for within-river populations and detected a decline across recent generations, with the most recent estimates approaching critical thresholds. Finally, comparisons with 94 domestic brook trout representing nine hatchery strains revealed a lack of domestic introgression into wild populations, demonstrating the importance of natural reproduction to population persistence. Our results offer novel insights into sources of coaster brook trout and highlight the role of within-river populations in supporting the coaster life history. Management efforts focused on instream restoration may be more important to rehabilitating coaster brook trout than previously thought and are urgently needed given the population-level conservation status reported here.

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Introduction

A major goal of many fisheries management programs is to conserve native species by maintaining extant populations and restoring extirpated populations. However, accomplishing this goal can be particularly challenging for native species of trout, char, and salmon. These species typically exhibit complex population structure (Rieman and Dunham, 2000; Schtickzelle and Quinn, 2007) that is heavily influenced by landscape features (Alshwairikh et al., 2021; Hecht et al., 2015; Micheletti et al., 2018; Sylvester et al., 2018), and the degree of genetic connectivity and source-sink dynamics among populations are important factors underlying the extinction risk and restoration potential of populations (Cooper and Mangel, 1999; Hoban et al., 2022; Wainwright and Waples, 1998). Further, trout, char, and salmon display complex life histories that frequently include alternative migration strategies (Quinn, 2021; Waples and Lindley, 2018), and the management efforts necessary to support each life history form are likely to be different. Knowledge of population-level relationships and how these relationships pertain to different life history strategies is thus critical to the successful management of native trout, char, and salmon. This knowledge is particularly urgent given increasing threats to these species, including rising stream temperatures and habitat loss due to a changing global climate (Battin et al., 2007; Stewart et al., 2016; Wenger et al., 2011).

In the Lake Superior basin, fisheries management efforts aimed at conserving native fishes over the last several decades have

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included a major focus on brook trout (*Salvelinus fontinalis*; Schreiner et al., 2008). Similar to elsewhere across the native species range, brook trout hold considerable social and cultural importance in the Lake Superior region, including for local indigenous tribes. Additionally, because brook trout prefer pristine coldwater habitats, this species is often considered an indicator of environmental health (Power, 1980). However, recent surveys across the United States portion of the Lake Superior basin indicate that the abundance of brook trout is below historical levels in 30% of subwatersheds, and this species has been extirpated from an additional 8% of subwatersheds (USFWS, 2016). Historical declines in the abundance of brook trout in the Lake Superior basin are primarily attributed to overharvest due to a lack of adequate fishery regulations from the late 1800s through mid-1900s, combined with heavy landscape modification associated with early logging and mining activities (Huckins et al., 2008). In comparison, contemporary declines are likely driven by habitat fragmentation resulting from man-made barriers such as dams and culverts (Newman et al., 2003; but see Zorn et al., 2020), competition with introduced non-native salmonids (Miller et al., 2019; Zorn et al., 2020), and anthropogenic climate change causing habitat loss due to large flood events and warmer water temperatures (Carlson et al., 2017).

To curb contemporary population declines, fisheries managers in the Lake Superior region established a rehabilitation plan for brook trout that emphasizes the maintenance of widely distributed, self-sustaining populations across the Lake Superior region (Newman et al., 2003). A major focus of this plan is to conserve a migratory life history form of brook trout known as coaster brook trout which occurs only in the Lake Superior basin. Compared to their stream resident counterparts, which spend their life in tributaries, coaster brook trout spend all or a portion of their life in Lake Superior (Becker, 1983), presumably to exploit resources available in lake habitats (see Huckins et al., 2008 for a review of coaster brook trout biology). Though in reality the propensity for migration likely occurs on a spectrum, for management purposes any brook trout that occurs in Lake Superior is distinguished as a coaster brook trout (Becker, 1983; Newman et al., 2003). Coaster brook trout return to natal coastal or stream habitats to spawn, therefore at least some coasters are sympatric with stream resident brook trout for a portion of the year. Coaster brook trout can reach larger sizes than stream resident brook trout, with lengths greater than 500 mm, making them a prized target of sport fisheries.

Rehabilitation of brook trout and the coaster life history is a major priority for fisheries managers across the Lake Superior basin, including the Minnesota Department of Natural Resources (MNDNR; Goldsworthy et al., 2017; Peterson, 2018). Landscape-level deforestation and intense forest fires following clearcut logging in the late 1800s caused drastic changes to tributaries across Minnesota. Along Minnesota's shoreline with Lake Superior (hereafter Minnesota North Shore), cold water tributaries once dominated by brook trout became too warm to support this species, and stocking of warm tolerant fishes was implemented to sustain popular sport fisheries (Surber, 1922). By the mid-1900s, reforestation of the landscape surrounding some tributaries, particularly in riparian corridors, provided shade to maintain water temperatures cool enough for brook trout to re-establish naturally or via hatchery stocking. Today, coaster brook trout are found in at least 12 tributaries along the Minnesota North Shore and in Minnesota waters of Lake Superior, and what was once a vast and vibrant fishery along the entire North Shore is now a series of disjunct populations.

Along the Minnesota North Shore, access to the cold water habitat preferred by brook trout is limited seasonally and by the presence of physical barriers to movement. During warmer summer months, cold water habitats are largely confined to tributary headwaters or nearshore areas of Lake Superior (Goldsworthy et al., 2017). However, for many rivers along the Minnesota North Shore, large natural waterfall barriers located near the mouth of each river function as barriers to upstream movement of below-barrier and lake residing brook trout, preventing access to cooler above-barrier reaches and adequate spawning habitat. Brook trout that occur below these waterfalls must therefore seek thermal refugia in lake habitats during summer months. Anecdotal evidence suggests that brook trout from above-barrier reaches in rivers along the Minnesota North Shore may play an important role in supplying brook trout to Lake Superior (Eddy and Underhill, 1974). However, relationships among within-river brook trout that occur above waterfall barriers and below-barrier brook trout that occur either within rivers or within Lake Superior are poorly understood. Knowledge of these relationships is critical to improving the effectiveness of rehabilitation efforts for brook trout along the Minnesota North Shore and elsewhere across the Lake Superior basin, including brook trout that exhibit the coaster life history.

In this study, we address a key information need for fisheries managers in the Lake Superior region by resolving relationships between brook trout inhabiting riverine and lake habitats, including above and below major waterfall barriers, along the Minnesota North Shore. We evaluate relationships associated with three interrelated objectives: 1) distinguish source populations for brook trout that occur within rivers below major waterfall barriers, 2) identify source populations for coaster brook trout that occur in Lake Superior, and 3) determine whether brook trout sampled within rivers and within Lake Superior are naturally produced or of domestic origin. We accomplish these objectives by surveying genome-wide variation in wild-caught brook trout and known domestic brook trout. We expected wild-caught brook trout to display substantial population structure because previous genetic studies within Lake Superior and elsewhere across the native range indicate that brook trout is a highly structured species (Burnham-Curtis, 2001; Ferchaud et al., 2020; Kazyak et al., 2021; Stott et al., 2010). Further, we expected that downstream movement of brook trout over waterfall barriers may occur but to an unknown degree given that genetic studies of other tributaries within the Lake Superior basin have identified only some above-barrier populations that function as sources of below-barrier brook trout (D'Amelio and Wilson, 2008; Scribner et al., 2012). Finally, we expected only limited evidence for domestic introgression in wild populations since results from previous genetic analysis of brook trout along the Minnesota North Shore are consistent with substantial natural production (Miller et al., 2016) and genetic studies from elsewhere across the native range report limited domestic introgression despite hatchery stocking (Annett et al., 2012; Kazyak et al., 2018; Lehnert et al., 2020; White et al., 2018).

Methods

Sample collection

We collected fin tissue samples from brook trout in seven rivers located along the Minnesota North Shore (Fig. 1; Table 1). We selected these rivers for analysis based on available sample sizes, geographic coverage, and available habitat to potentially support robust populations. Additionally, previous analyses based on thirteen microsatellite loci resolved genetically distinct groups of brook trout in the Kadunce and Onion rivers, but genetic relationships and levels of admixture in remaining rivers were unresolvable and thus targeted for analysis in this study (Loren Miller, pers. comm.). The rivers we sampled are tributaries to Lake Superior, but large waterfalls located near the mouth of each river function as barriers to upstream movement of brook trout, except for the Knife River, where a permanent catch-and-sort fish trap near
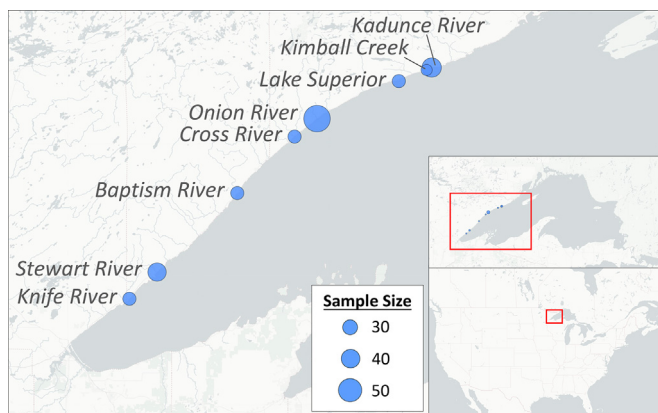
Fig. 1. Map depicting the waterways from which brook trout were sampled in this study, including seven rivers tributary to Lake Superior and a site within Lake Superior (off Grand Marais, MN). A single point per waterway is shown and is scaled according to the number of brook trout included in the final dataset. The locations of the one-way waterfall barriers located near the mouth of sampled rivers, except for the Knife River, are depicted in Supplementary Map File S1.

the mouth of the river allows brook trout to be sampled upstream of the waterfall barrier. We sampled brook trout both above and below these one-way waterfall barriers in the Kadunce River and Onion River. In the Stewart River, we collected samples of brook trout only from above the waterfall barrier. In the Baptism River, Cross River, and Kimball Creek, brook trout were sampled only below the waterfall barrier. Sampling efforts in these rivers were conducted using electrofishing. In the Knife River, brook trout were collected from the fish trap described above, which primarily captures brook trout from upstream reaches that are moving downstream over the waterfall. The locations of the one-way waterfall barriers described here, as well as the Knife River fish trap, are depicted in Electronic Supplementary Material (ESM) map file S1. Sampling above these barriers did not necessarily occur in adjacent upstream reaches, and in some instances occurred several km upstream. Sampling in above-barrier reaches occurred in late summer and in below-barrier reaches during the fall spawning season.

In addition to the within-river sampling described here, we also collected samples of brook trout from Lake Superior during June in shoreline areas of Grand Marais Harbor off Grand Marais, MN. Grand Marais Harbor is one of the few areas in Minnesota waters of Lake Superior where coaster brook trout are regularly encountered. All within-river and lake sampling efforts occurred through fishery surveys conducted by MNDNR.

RAD capture

We used tissue samples collected in this study to produce genotypes for single nucleotide polymorphisms (SNPs) located through-

out the brook trout genome. DNA was isolated from tissue samples using the magnetic bead-based protocol described by Ali et al. (2016), then quantified DNA isolations using Quant-iT PicoGreen assays (Thermo Fisher Scientific) and a BioTek FLx800 microplate reader (BioTek Instruments). We then selected DNA isolations for 334 individuals representing each waterway to prepare restriction site-associated DNA sequencing (RADseq; Baird et al., 2008) libraries. Using these DNA isolations, we prepared four RADseq libraries as described in Ali et al. (2016), except we used 100 ng of DNA for restriction enzyme digestion and we incubated ligation reactions for 12 h.

We used prepared RADseq libraries in a RAD capture reaction to target SNPs known to be effective for resolving genetically distinct populations of brook trout in the Lake Superior basin. We pooled 95 ng of each RADseq library into a single sample, then performed RAD capture following Ali et al. (2016) using Arbor BioSciences myBaits v4 chemistry (https://www.arborbiosci.com) and a capture panel recently developed for Lake Superior basin brook trout (Mariah Meek, pers. comm.). This capture panel targets 33,309 SNPs corresponding with 25,639 unique regions distributed throughout the brook trout genome. This panel was developed using wild-caught brook trout from 64 waterways across the Lake Superior basin and domestic brook trout representing nine hatchery strains. The final capture reaction product underwent 150PE sequencing on one lane of an Illumina HiSeq X next-generation sequencing platform.

SNP genotyping

We used resulting sequence data to produce a dataset comprising high quality SNP genotypes. First, we demultiplexed sequence data using the *process_radtags* module of Stacks v2.4 (Catchen et al., 2011; Rochette et al., 2019) and options to allow a barcode mismatch of 1 bp, remove reads with uncalled bases, and discard reads with low quality scores. Next, we trimmed demultiplexed reads using Trimmomatic v0.39 (Bolger et al., 2014) where reads with an average quality score of <15 within a sliding window of 10 bp were trimmed. We also removed reads with lengths < 30 bp. We then aligned trimmed reads to the *Salvelinus* sp. reference genome (Christensen et al., 2018; but see Christensen et al., 2021) using the BWA-MEM algorithm implemented in BWA v0.7.17 (Li, 2013). Finally, we used SAMtools v1.9 (Li et al., 2009) to remove unmapped reads, secondary and supplementary alignments, and alignments with quality scores < 30.

We combined quality filtered read alignments generated in this study with alignments for nine domestic strains of brook trout used in historical or contemporary stocking efforts in the Lake Superior basin (Table 2). Four of these strains were originally derived from source populations within the Lake Superior basin,

Table 1
Sampling details for wild-caught brook trout comprising quality filtered SNP dataset. Brook trout sampled from Lake Superior were collected in nearshore areas off Grand Marais, MN or in Grand Marais Harbor.

Waterway	Sampling Location Relative to Waterfall Barrier	Year	No. Individuals	No. Unrelated Individuals
Kadunce River	Above-barrier	2019	17	17
	Below-barrier	2013	23	22
Kimball Creek	Below-barrier	2013	22	22
Lake Superior	Lake	2019	26	25
Onion River	Above-barrier	2019	30	26
	Below-barrier	2018	24	24
Cross River	Below-barrier	2018	27	26
Baptism River	Below-barrier	2018	26	26
Stewart River	Above-barrier	2019	37	33
Knife River	At barrier	2019	28	27
		Total:	260	248

and one strain was derived from the Lake Michigan basin. Remaining strains were derived from populations outside of the Great Lakes basin. Data for domestic brook trout were produced using the same methodology employed here and were included to enable comparisons of the wild brook trout sampled in this study with brook trout of known domestic origin.

We used alignments for wild-caught and domestic brook trout to identify SNPs and perform quality filtering of SNP genotypes. First, we built a catalog of SNPs and produced a VCF file comprising individual-based genotypes using the *gstacks* and *populations* modules of Stacks, respectively. Next, we used VCFtools v0.1.15 (Danecek et al., 2011) to perform quality filtering of the dataset by removing SNPs and individuals missing large proportions of genotypes, genotypes with low quality scores and read depths, and SNPs exhibiting low minor allele counts (ESM Table S1). We also used JVarkit (Lindenbaum, 2015) to remove genotypes exhibiting excessively low or high allele balance, which compares the number of reads between reference and alternate alleles in heterozygotes. Additionally, we used HDplot (McKinney et al., 2017) to identify SNPs corresponding with paralogous regions of the brook trout genome. We excluded SNPs associated with >50% heterozygotes or read ratio deviations of >20 or < -9. We then used dartR (Gruber et al., 2018) to remove SNPs rendered monomorphic by the quality filtering process. To reduce the probability of linkage disequilibrium among loci, we next used a custom R script to retain a single SNP per RAD locus. We retained the SNP exhibiting the largest minor allele frequency. Finally, we used VCFtools to identify and exclude individuals exhibiting excessive levels of relatedness using the method described by Manichaikul et al. (2010); we removed individuals exhibiting relatedness values ≥ 0.2 (e.g., the average kinship coefficient of half-siblings or closer relatives). The resulting dataset comprised high quality genotypes for both wild-caught and domestic individuals and was used in the analyses described below.

Population-level relationships

We analyzed the genomic dataset produced here to identify source populations for brook trout sampled downstream of waterfall barriers and in Lake Superior. First, we performed principal component analysis (PCA) to infer overall genetic variation among individuals. We then performed discriminant analysis of principal components (DAPC) to identify genetically distinct populations and hierarchical structure among populations. PCA and DAPC are ideal for datasets comprising genome-wide SNPs because they efficiently summarize complex genetic information without assuming an underlying model of population structure (Jombart et al., 2009, 2010). We performed PCA in adegenet v2.1.6 (Jombart, 2008) using centered and non-scaled allele frequencies. DAPC was also performed in adegenet, using groups defined via sequential K-means

clustering. We assessed scenarios with K = 2–20 and identified the most likely values for K by evaluating Bayesian information criterion (BIC) and by comparing DAPC results with those from PCA. To determine the number of PCs to retain in DAPC analyses, we used the optim.a.score function of adegenet, which identifies the number of PCs that will optimize the discriminatory power and stability of DAPC results. We performed PCA and DAPC with and without the most divergent individuals included to improve resolution of comparatively less divergent relationships. Additionally, we analyzed datasets that were limited to wild-caught individuals and that included both wild-caught and domestic individuals. Wild-caught individuals identified as previously stocked domestic fish in results from PCA and DAPC were excluded from the analyses described below.

To further assess relationships of brook trout sampled in downstream reaches and in Lake Superior, we used network theory to depict relationships among individuals as a network topology. Network-based approaches facilitate exploration of genetic connectivity without making assumptions about an underlying population genetic model. We inferred networks using netviewr v2.1.0.9 (Steinig et al., 2016). We explored topologies using a range of values for the number of mutual nearest neighbors (k). This approach allowed us to compare relationships among individuals at differing levels of genetic similarity, where smaller values for k retained connections among more closely related individuals and larger values for k introduced connections among more distantly related individuals. Although selecting an optimal k can be difficult (Neuditschko et al., 2012), we used the infomap and fast-greedy cluster detection algorithms implemented in netviewr to identify values for k that captured the major relationships apparent in our data.

We used results from PCA, DAPC, and network analysis to organize individuals into groups corresponding with genetically distinct populations. These groups comprised all of the individuals assigned to a population, regardless of sampling location. We then further characterized the populations of brook trout identified in this study by calculating the pairwise genetic distance between each population pair. We calculated F_{ST} (Weir and Cockerham, 1984) in StAMPP v1.6.3 (Pembleton et al., 2013) and assessed the statistical significance of F_{ST} values by performing 10,000 bootstrap iterations.

Population conservation status

We inferred the contemporary conservation status of genetically distinct populations by calculating effective population size (N_E). N_E is the evolutionary analog of population census size and reflects the rates at which evolutionary processes, including loss of genetic diversity due to genetic drift and inbreeding as well as the relative effectiveness of selection and gene flow, are expected

Table 2
Sampling details for hatchery sourced domestic brook trout comprising quality filtered SNP dataset.

Strain	Basin Origin	Waterway Origin	No. Individuals
Jumbo	Lake Superior basin	Jumbo River, MI, USA	8
Nipigon		Lake Nipigon, ON, Canada	8
Siskiwit		Siskiwit Bay, Isle Royale, MI, USA	11
Tobin		Tobin Harbor, Isle Royale, MI, USA	15
Iron	Lake Michigan basin	Iron River, MI, USA	12
Minnesota (MN) Wild	Outside of Great Lakes basin	Spring Brook and Coolridge Creek, MN, USA	10
Assinica		Lac Assinica, QC, Canada	11
Nashua/St. Croix		Nashua National Fish Hatchery, Nashua, NH, USA; St. Croix Falls State Fish Hatchery, WI, USA	14
Temiscamie × Domestic		Temiscamie River, QC, Canada; Rome Hatchery, Rome, NY, USA	5
		Total:	94

to occur (Caballero, 1994; Waples, 2022; Waples et al., 2014; Wright, 1931). N_E has been widely used to assess the genetic health and conservation status of wild populations, including in salmonid species (Lehnert et al., 2019; Lemopoulos et al, 2019; Layton et al., 2021; Luikart et al., 2021; Whiteley et al., 2010, 2013). We estimated N_E using the linkage disequilibrium method (Waples and Do, 2010) implemented in N_EEstimator (Do et al., 2014). Confidence intervals for N_E estimates were calculated using the jackknife method described by Jones et al. (2016). To minimize bias due to linkage in N_E calculations, we limited comparisons to loci on different chromosomes (Waples, 2006). To minimize bias from small sample sizes, we estimated N_E only for populations comprising ≥20 individuals. Additionally, we evaluated the impact of low frequency alleles on estimates of N_E by comparing results from analyses performed without a minor allele frequency filter and with filters to remove loci with minor allele frequencies less than 1%, 2%, and 5%. We performed N_E calculations twice, once using a dataset that comprised all of the SNPs in our quality filtered dataset, and a second time using a dataset limited to neutral SNPs. To create a dataset limited to neutral SNPs, we used pcadapt v4.3.3 (Luu et al., 2017) to identify putatively adaptive SNPs for exclusion from our dataset. Our goal was to produce a dataset where SNPs were conservatively identified as neutral, so we used an FDR of 20% to identify SNPs that appeared as outliers with respect to population structure. We then excluded outlier SNPs from our dataset prior to calculating N_E.

We also assessed the stability of conservation status across generations by inferring N_E for each population at various times in the recent past. We reconstructed N_E over time using LinkNe (Hollenbeck et al, 2016). LinkNe estimates N_E from linkage disequilibrium between unlinked pairs of SNPs, where pairs of loci with similar recombination rates are binned together for estimating N_E. The mean recombination rate (c) of each bin is then used to estimate the number of generations in the past (t) to which N_E estimates correspond based on the relationship $t = 1/2c$ (Hayes et al., 2003). We performed LinkNe analyses on populations comprising ≥20 individuals using default settings, including a minor allele frequency threshold of 5% and a correction for unequal numbers of locus pairs in each bin, plus the timebin option to enable estimates based on generations. We used the average length of linkage groups comprising the *Salvelinus* sp. reference genome (2,724 cM; Christensen et al., 2018) to bin loci. All of the SNPs in our quality filtered dataset were used for the LinkNe analysis since datasets based on all SNPs and neutral SNPs yielded equivalent results in N_EEstimator. We approximated the number of generations associated with each estimate of N_E by assuming a generation time of two years (Letcher et al., 2007). In reality, generation time for the populations analyzed in this study may vary among populations and also differ from the populations analyzed by Letcher et al. (2007; based on inland populations in western Massachusetts). However, the values we used for generation time do not influence the estimates of N_E produced by LinkNe and only inform the identification of the time period associated with each estimate.

Results

SNP dataset

We used the unfiltered dataset exported from Stacks to produce a dataset comprising high quality genotypes for the individuals analyzed here. The unfiltered dataset contained genotypes for 834,762 SNPs in 463 individuals (ESM Table S1). After performing quality filtering gand retaining a single SNP per RAD locus, our dataset comprised genotypes for 8,178 SNPs in 354 individuals. These

individuals included 260 wild-caught brook trout that comprised 22–54 individuals (average = 33 individuals) from each river and 26 individuals from within Lake Superior (Table 1; Fig. 1). Additionally, the quality filtered dataset included 94 hatchery sourced brook trout that comprised 5–15 individuals (average = 10 individuals) per domestic strain (Table 2). Results from analyses to infer relatedness among brook trout in the quality filtered dataset revealed sets of individuals that exhibited kinship coefficients greater than 0.2. We retained only a single representative from each set by excluding 12 individuals from the analyses described below (Table 1).

Relationships of above-barrier populations

Results from PCA and DAPC revealed the presence of several genetically distinct populations, including upstream populations that serve as sources of brook trout below major waterfall barriers within rivers and within Lake Superior. PCA performed using all wild-caught individuals resolved distinct groups corresponding with the Kadunce, Onion, Stewart, and Knife rivers (Fig. 2, ESM Fig. S1, S2). These groups were also apparent in results from DAPC (Fig. 2, ESM Fig. S3), for which BIC scores indicated that the most likely values for K were two through six. DAPC most clearly differentiated the groups apparent in results from PCA at a K of five (Fig. 2). For the Kadunce and Onion rivers, the groups resolved by PCA and DAPC comprised all of the brook trout sampled above the waterfall barrier and a subset of the brook trout sampled below this barrier. The group associated with the Stewart River comprised all of the brook trout from this river, which were only sampled above the waterfall barrier. Finally, the group associated with the Knife River, for which sampling occurred in a trap at the waterfall barrier, comprised all except two of the brook trout from this waterway. Collectively, these results are consistent with the presence of genetically distinct populations in the Kadunce, Onion, Stewart, and Knife rivers, where the primary source population for below-barrier brook trout in the Kadunce and Onion rivers is located above the major waterfall barrier within each river. This scenario may also apply to the Stewart and Knife rivers; however, analyses of below-barrier fish in these rivers are necessary to confirm these relationships.

Relationships of below-barrier populations

After excluding genetically distinct above-barrier populations, results from PCA and DAPC clarified relationships among remaining brook trout, which were sampled below major waterfall barriers. PCA revealed the presence of at least four distinct groups (Fig. 3, ESM Figs. S4, S5). BIC scores associated with DAPC were equivocal across values for K but generally indicated that the most likely K was less than ten. We defined genetically distinct populations using K equal to five because at this value for K, the groups resolved by DAPC appeared stable and were consistent with results from PCA (Fig. 3, ESM Fig. S6). Both PCA and DAPC resolved a genetically distinct group in Kimball Creek. We also identified a group comprising the majority of brook trout sampled from the Baptism River. However, this group exhibited a close relationship with below-barrier brook trout from multiple waterways in results from PCA, and was not resolved by DAPC until a K of five. Two additional groups apparent in results from PCA and DAPC comprised large proportions of brook trout from the Cross River; both of these groups also included individuals sampled within Lake Superior. A fifth group resolved by PCA and DAPC comprised remaining below-barrier brook trout, which were sampled from multiple waterways, including the Kadunce, Onion, Cross, Baptism, and Knife rivers. This group also included the majority of individuals sampled from Lake Superior. DAPC results for K greater than five
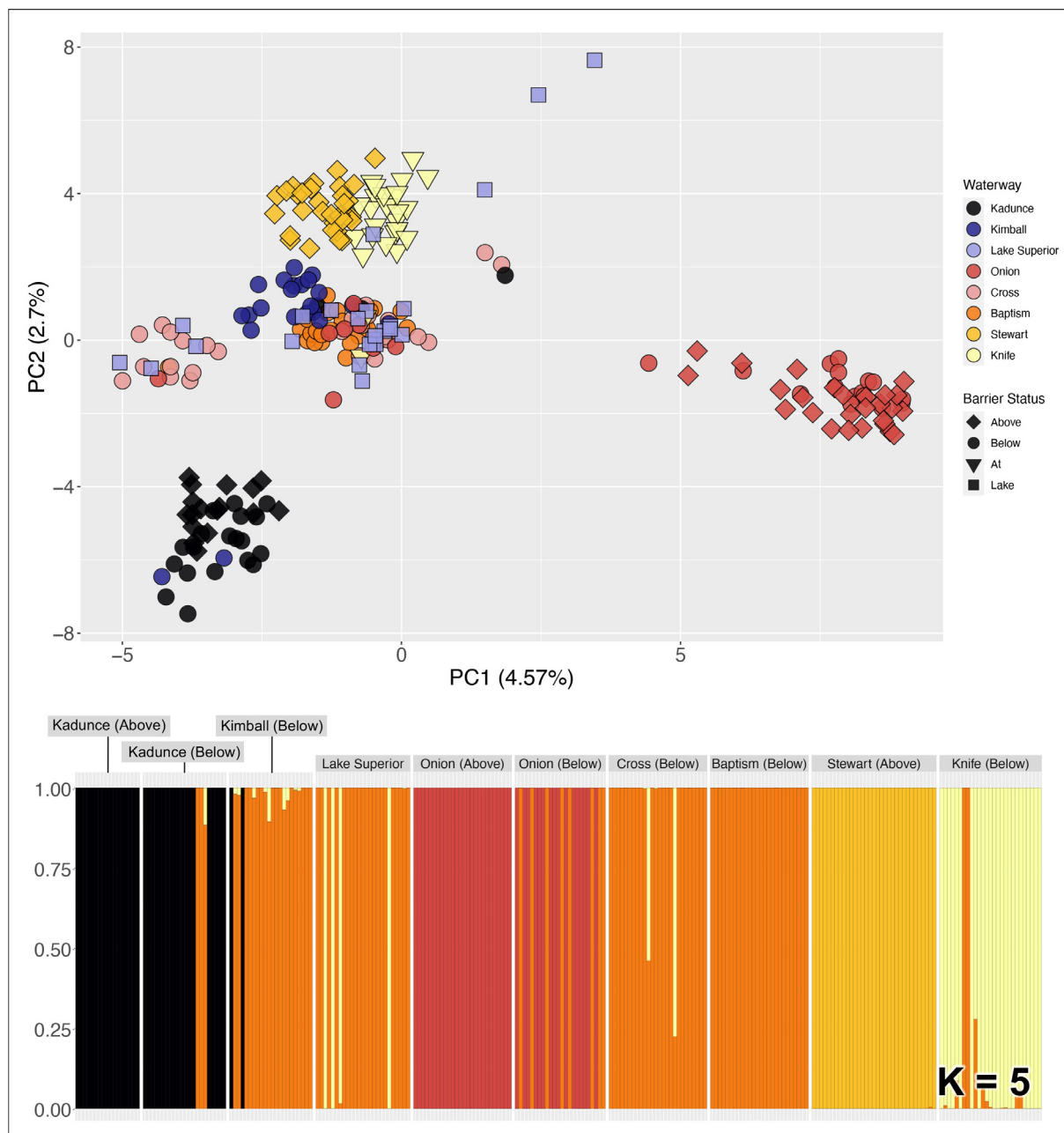
Fig. 2. Results from PCA (*top panel*) and DAPC (*bottom panel*) of the wild-caught brook trout analyzed in this study. *Top panel:* Axes corresponding with PCs one and two. The proportion of variation explained by each axis is shown. Individuals are represented by symbols that reflect whether sampling occurred within Lake Superior (squares) or within rivers above (diamonds), below (circles), or at (inverted triangles) a large natural waterfall barrier located near the mouth of each river. Individuals are color-coded by the waterway from which they were sampled. *Bottom panel:* Results for *K* equal to five. Vertical bars correspond with individuals and are color-coded to reflect the proportion of membership to a particular DAPC group. Individuals are arranged by waterway and, for individuals sampled within rivers, whether sampling occurred above or below the waterfall barrier.

indicated the possibility of substructure within this group (ESM Fig. S6); however, analyses of individuals from additional waterways are necessary to reliably distinguish these relationships.

Collectively, the results described here are consistent with the presence of genetically distinct populations in Kimball Creek and the Baptism River, and two genetically distinct populations in the Cross River. Microsatellite-based analyses indicate that one of the groups we identified from the Cross River may correspond with migrants from a neighboring waterway rather than substructure within the Cross River (Loren Miller, pers. comm.). Additionally, our results indicate that brook trout sampled within Lake Superior originated from the Kimball Creek and Cross River populations, and

at least one additional source. This source comprises at least one genetically distinct population and included most of the brook trout sampled within Lake Superior. This source also exhibits a close relationship with the Baptism River population identified here.

Relationships among all populations

Analyses to resolve genetically distinct populations corresponding with the brook trout evaluated in this study also revealed the presence of migrants reflecting movement between waterways (Figs. 2, 3, ESM Figs. S3, S6). These migrants included brook trout
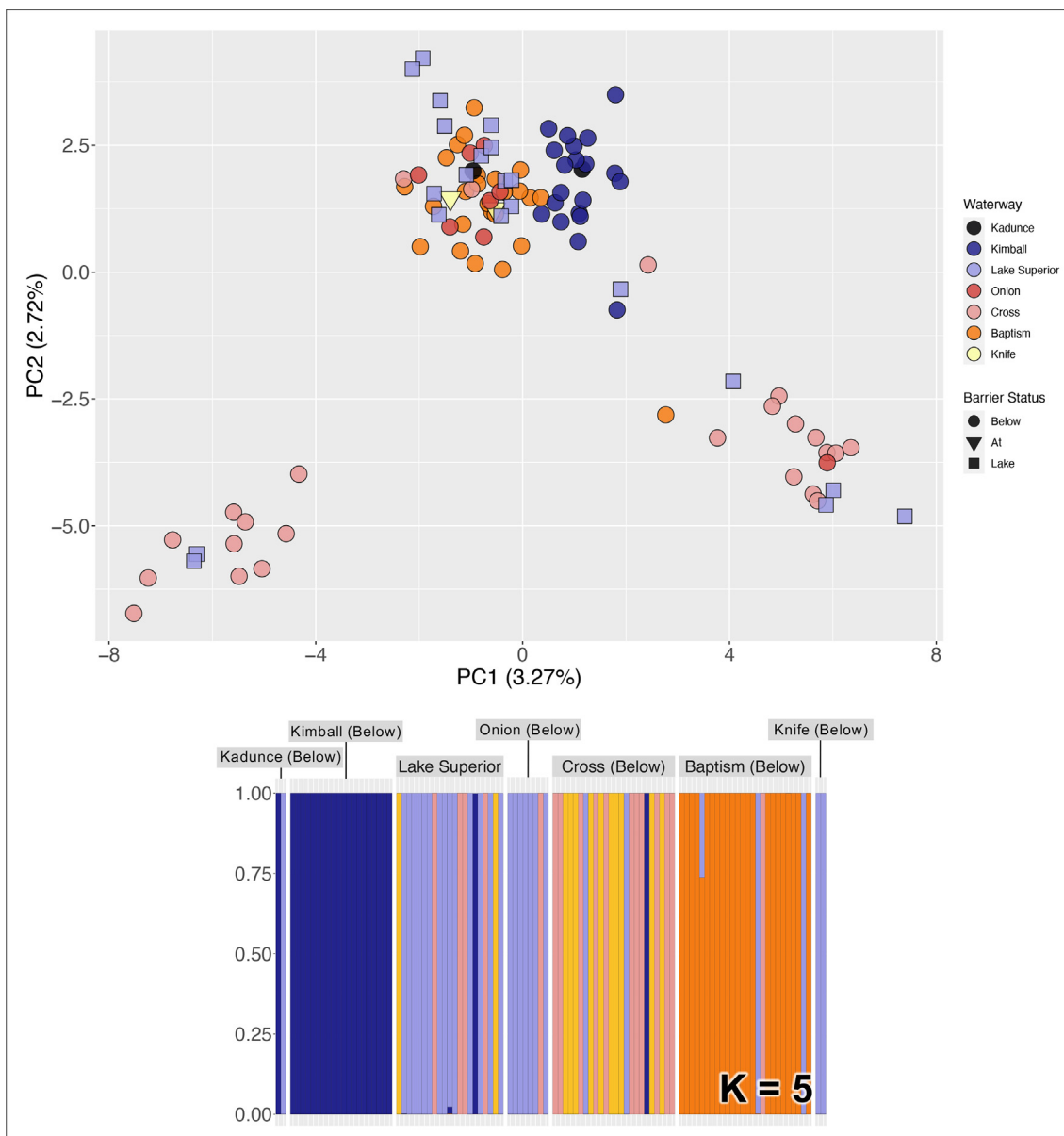
Fig. 3. Results from PCA (*top panel*) and DAPC (*bottom panel*) of wild-caught brook trout analyzed in this study, where genetically distinct populations associated with the Kadunce, Onion, Stewart, and Knife rivers were excluded. Wild-caught domestic individuals were also excluded. *Top panel:* Axes corresponding with PCs one and two. The proportion of variation explained by each axis is shown. Individuals are represented by symbols that reflect whether sampling occurred within Lake Superior (squares) or within rivers below (circles) or at (inverted triangles) a large natural waterfall barrier located near the mouth of each river. Individuals are color-coded by the waterway from which they were sampled. *Bottom panel:* Results for *K* equal to five. Vertical bars correspond with individuals and are color-coded to reflect the proportion of membership to a particular DAPC group. Individuals are arranged by waterway.

from the following populations: 1) the Kadunce River population that were sampled in Kimball Creek, 2) the Kimball Creek population that were sampled in the Kadunce and Cross rivers and in Lake Superior, 3) one of the Cross River populations that were sampled in the Onion River and in Lake Superior, and 4) the remaining Cross River population that were sampled in Lake Superior. Additionally, the below-barrier population that comprised the majority of brook trout sampled within Lake Superior included individuals sampled from every waterway surveyed in this study except Kimball Creek and the Stewart River. DAPC results for the individuals analyzed here reflected limited degrees of admixture, indicating that movement among populations is largely unaccompanied by gene flow.

Network analysis of wild-caught brook trout revealed relationships consistent with those apparent from PCA and DAPC. We found that netviewr topologies with *k* equal to 15–20 captured the major relationships within our dataset (Fig. 4, ESM Figs. S7,

S8). Across these topologies, populations in the Kadunce, Onion, and Stewart rivers that comprised individuals sampled above major waterfall barriers exhibited few or no connections with remaining individuals. These results are indicative of a comparatively large degree of genetic differentiation associated with above-barrier populations. Conversely, brook trout from the population in the Baptism River exhibited many connections with the population comprising the majority of individuals from Lake Superior. This result is consistent with results from DAPC, where analyses of below-barrier individuals grouped the Baptism River population with the majority of Lake Superior fish until $K \geq 5$. The netviewr topologies also positioned most of the remaining brook trout sampled from Lake Superior within groups corresponding with the two genetically distinct populations in the Cross River.

F_{ST} calculated pairwise between the genetically distinct populations identified here revealed comparatively close relationships
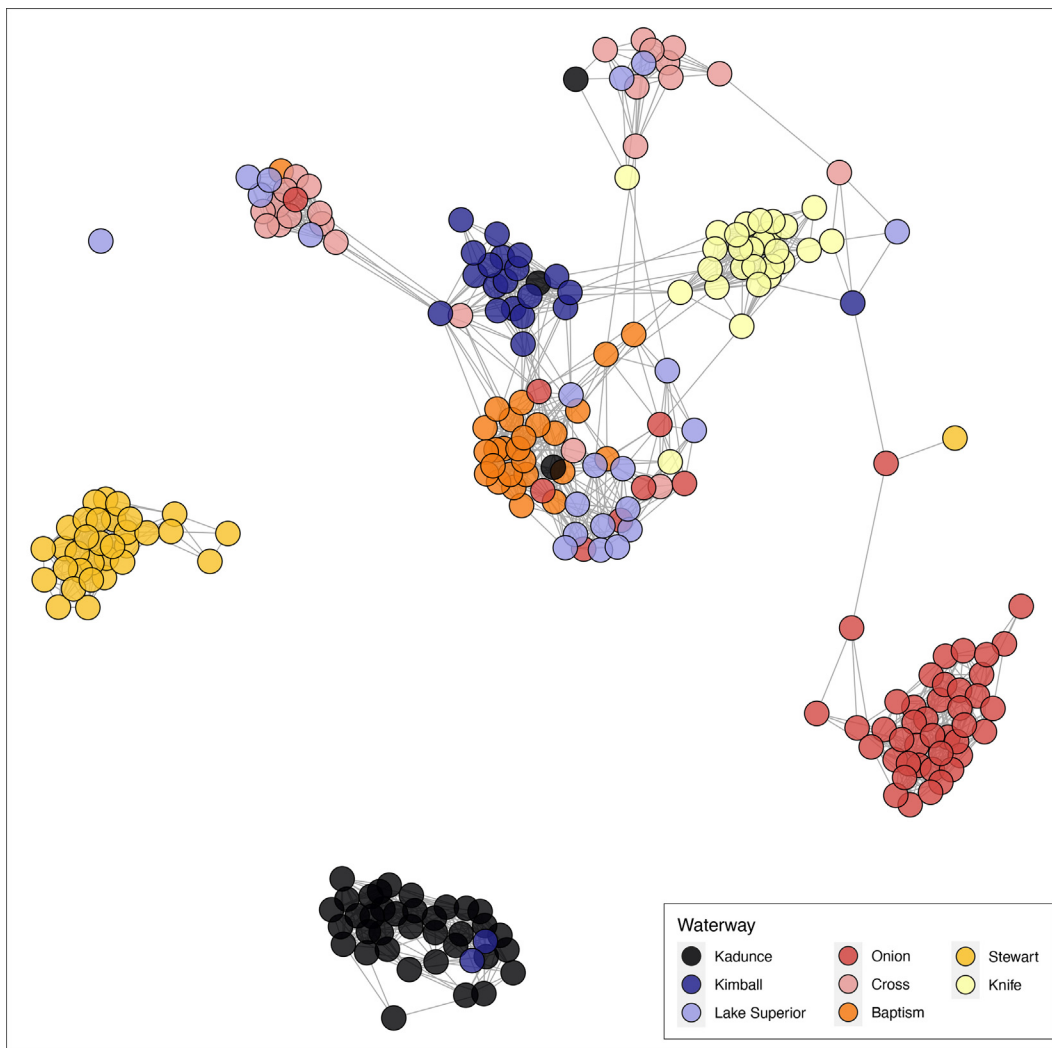
Fig. 4. Network topology for the wild-caught brook trout analyzed in this study, where wild-caught domestic individuals were excluded. Results for *k* equal to 15 are shown. Connections between individuals are depicted by gray edges. Nodes represent individuals and are color-coded by the waterway from which they were sampled.

between the population identified in Lake Superior and some of the within-river populations. These results also reflected the comparatively distant relationship of above-barrier populations. F_{ST} ranged from 0.018 to 0.135 and these values were all statistically significant at $p < 0.0001$ (Table 3). The largest F_{ST} corresponded with the comparison between the Onion River population and one of the Cross River populations. The four largest F_{ST} values observed here were all associated with the Onion River population, highlighting the genetic distinctiveness of this population relative to remaining populations. In contrast, the lowest observed F_{ST} corresponded with the comparison between the Baptism River population and the population that comprised the majority of brook trout sampled from Lake Superior, again reflecting a close relationship between these populations. Remaining comparisons between within-river populations and the Lake Superior population ranged from 0.025 to 0.072. The lowest of these comparisons corresponded with the Kimball River population, and the highest of these comparisons corresponded with the Onion River population.

Relationship between wild-caught and domestic brook trout

In comparisons of wild-caught brook trout and known domestic brook trout, evidence for widespread introgression of domestic alleles into wild populations was lacking. However, we identified a small number of brook trout that represent either previously stocked domestic individuals sampled in wild collections, or migrants from populations used to develop broodstock for the domestic strains analyzed here. Initial results from PCA and DAPC based on a dataset that included all wild-caught and known domestic brook trout revealed that domestic strains sourced from outside of the Great Lakes basin were very divergent from remaining brook trout, making it difficult to infer less divergent relationships. Therefore, we repeated these analyses where only strains derived from within the Great Lakes basin (Table 2) were retained for comparisons to wild-caught fish. We also performed PCA and DAPC where genetically distinct populations identified in the Kadunce, Onion, Stewart, and Knife rivers were excluded to improve the clarity and assess the stability of comparatively less divergent relationships between wild-caught and known domestic individuals. Results from PCA of these datasets revealed four brook trout from Lake Superior that were positioned among the Nipigon, Tobin, and Siskiwit domestic strains (Fig. 5, ESM S9-S12). These domestic strains comprise the coaster brook trout strains stocked within Lake Superior. DAPC resolved these individuals as belonging to either the Nipigon or Siskiwit domestic strains (ESM Fig. S13), though some scenarios identified the Nipigon strain as the source, except for one individual where DAPC indicated a non-domestic source. Additionally, PCA positioned one brook trout from the

Table 3

F_{ST} calculated pairwise between the genetically distinct populations identified in this study. F_{ST} values are shown below diagonal and associated p-values are shown above diagonal (all p-values < 0.0001). F_{ST} corresponding with comparisons between within-river populations and the Lake Superior population are highlighted using a color-scale, where the lowest F_{ST} is shown in dark blue and the highest F_{ST} is shown in white.

	Lake Superior	Kadunce	Kimball	Onion	Cross1	Cross2	Baptism	Stewart	Knife
Lake Superior	--	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Kadunce	0.049	--	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Kimball	0.025	0.059	--	0.000	0.000	0.000	0.000	0.000	0.000
Onion	0.072	0.121	0.100	--	0.000	0.000	0.000	0.000	0.000
Cross1	0.045	0.071	0.045	0.135	--	0.000	0.000	0.000	0.000
Cross2	0.052	0.097	0.069	0.111	0.085	--	0.000	0.000	0.000
Baptism	0.018	0.057	0.032	0.083	0.045	0.057	--	0.000	0.000
Stewart	0.042	0.080	0.051	0.103	0.072	0.084	0.046	--	0.000
Knife	0.040	0.085	0.042	0.097	0.081	0.087	0.047	0.058	--

Kadunce River and two brook trout from the Cross River near the Jumbo domestic strain (Fig. 5, ESM S9-S12). These results were consistent with those from DAPC (ESM Fig. S13). For the remaining wild-caught brook trout we analyzed, wild and domestic individuals were non-overlapping in results from PCA, and DAPC revealed little to no admixture associated with domestic ancestry.

Population conservation status

N_E calculated for a subset of the populations resolved in this study offers valuable insights into the genetic health of brook trout along the Minnesota North shore. We excluded the Cross River populations because of small sample sizes and the Lake Superior population because of the possibility of substructure within this group. In results from N_EEstimator, N_E calculated using all of the SNPs in our quality filtered dataset were nearly identical to results from a dataset limited to neutral SNPs. Therefore, the results we describe for N_E are based on calculations performed using all SNPs. In results from N_EEstimator, the Baptism River population exhibited a wide range in N_E across the minor allele frequency thresholds tested here (range = 473–1,533; ESM Fig. S14). The Stewart River population exhibited a moderate range in N_E across thresholds (range = 256–385). Remaining populations displayed small ranges in N_E across minor allele frequency thresholds (difference between smallest and largest N_E across thresholds per population was ≤ 54), reflecting estimates that are less dependent on the presence of minor alleles and thus more stable compared to estimates for the Baptism and Stewart river populations. We used a minor allele frequency threshold of 5% to assess contemporary conservation status. N_E based on this threshold was lowest in the Onion River population (N_E = 133; Fig. 6) and highest in the Baptism River population (N_E = 473). Upper bounds on the 95% jackknife confidence intervals associated with each estimate were infinite for every population except in the Onion River. In the Onion River, Knife River, and Kimball Creek populations, the lower bounds of these intervals were below thresholds frequently used to identify the most at-risk populations (N_E = 50 and 100; Frankham et al., 2014a, 2014b; Franklin, 1980; Franklin et al., 2014; Jamieson and Allendorf, 2012), though point estimates for every population were above these thresholds.

Historical N_E reconstructed using LinkNe revealed declining N_E for each population over recent history (Fig. 7), which corresponded with a period spanning at least 20 generations. The 95% confidence intervals were non-overlapping between the oldest (at least 20 generations ago) and most recent (approximately one to two generations ago) estimates of N_E for each population, indicating that declines over the entire time period are statistically significant. Declines corresponding with the most recent time interval (between approximately four generations and one to two generations ago) were also statistically significant. The number of unlinked loci used to estimate N_E at each time point was largest for the most recent estimate (ESM Fig. S15); however, this difference was accounted for by the sample-size bias correction option used in the analysis. It is likely that the oldest estimates of N_E produced by LinkNe are influenced by even earlier generations than what is approximated here and, given the declining trend observed for populations, estimates of N_E may be underestimates of the true N_E (Hollenbeck et al., 2016). Additionally, overlap among generations, an assumption that is typically violated in natural populations, frequently produces underestimates of N_E. Our results thus likely represent conservative estimates of N_E over time, though the number of generations to which the observed declines correspond may be much longer than what is indicated here.

Discussion

The primary goal of this study was to address a key information need for fisheries managers in the Lake Superior basin by resolving relationships among brook trout inhabiting riverine and lake habitats along the Minnesota North Shore. We found that brook trout above major waterfall barriers within rivers comprise well-differentiated populations that supply brook trout to below-barrier reaches. We also found that, for at least some rivers, brook trout from multiple populations occur in below-barrier reaches, but admixture among populations is limited. Additionally, we observed that the coaster brook trout we analyzed from one location within Lake Superior were produced by multiple source populations, including riverine populations. Finally, though we identified previously stocked domestic brook trout in wild collections, introgression of domestic alleles into wild populations was
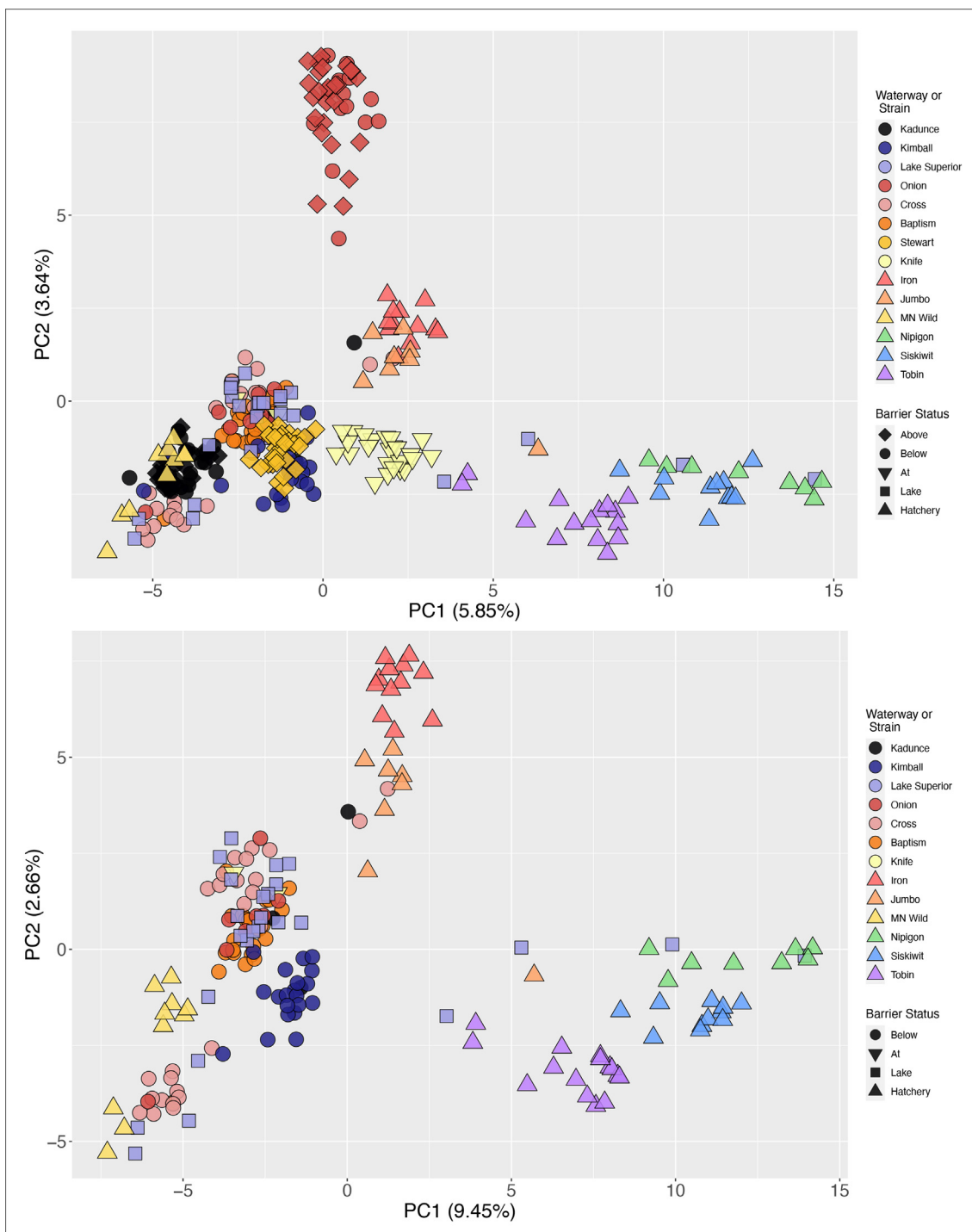
Fig. 5. Results from PCA of the wild-caught and domestic brook trout analyzed in this study. PC axes one and two and the proportion of variation explained by each axis are shown. Wild-caught individuals are represented by symbols that reflect whether sampling occurred within Lake Superior (squares) or within rivers above (diamonds), below (circles), or at (inverted triangles) a large natural waterfall barrier located near the mouth of each river. Wild-caught individuals are color-coded by the waterway from which they were sampled. Domestic individuals (triangles) are color-coded by hatchery strain. *Top panel:* Results from analyses performed with all wild-caught brook trout. *Bottom panel:* Results from analyses performed with genetically distinct populations associated with the Kadunce, Onion, Stewart, and Knife rivers excluded.

lacking. Collectively, these findings provide important baseline information on the population structure and source-sink dynamics of brook trout along the Minnesota North Shore. This information is necessary to support a management goal of maintaining widely distributed and self-sustaining populations of brook trout in the Lake Superior basin, including for brook trout that exhibit the coaster life history.

Sources of coaster brook trout

At least some within-river populations along the Minnesota North Shore supply brook trout to Lake Superior, and thus play an important role in maintaining extant populations of coaster brook trout. In this study, brook trout sampled within Lake Superior off Grand Marais, MN were produced by source populations
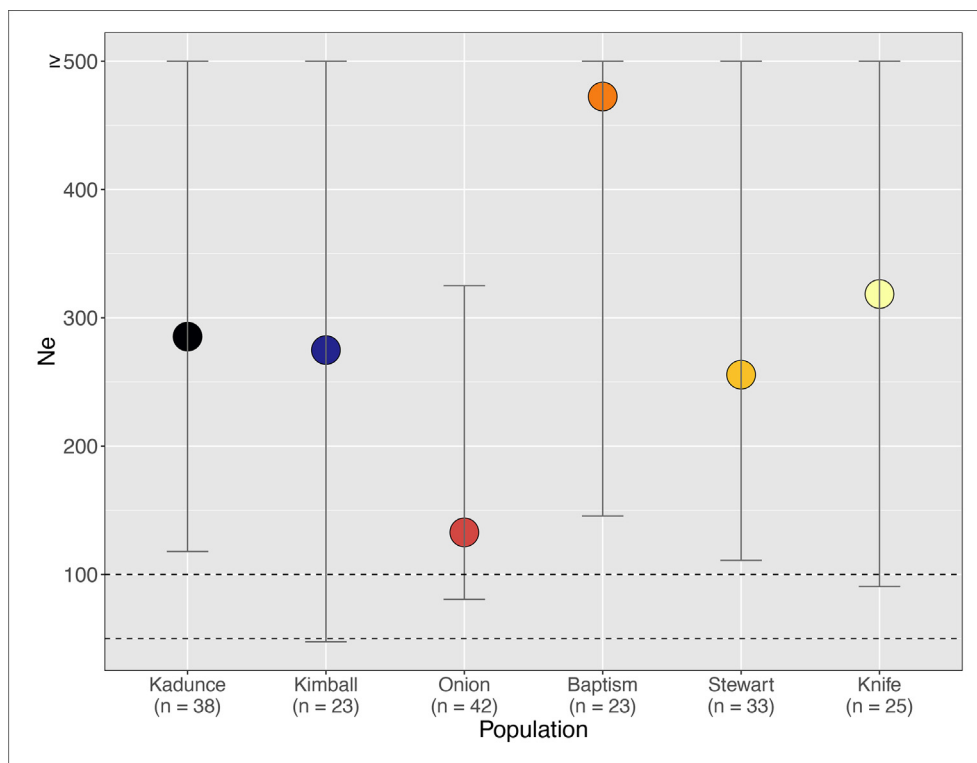
Fig. 6. Plot depicting estimates of contemporary N_E for the genetically distinct populations resolved in this study, where wild-caught domestic individuals were excluded. Populations and their sample size are shown on the bottom axis. The 95% jackknife confidence intervals calculated following Jones et al. (2016) are also shown for each estimate. Infinite estimates for the upper confidence intervals of every population except the Onion River population were converted to 500 for this figure. Results are based on a minor allele frequency of 5% and are only shown for populations with ≥20 individuals. Horizontal dashed lines depict thresholds frequently used to identify the most at-risk populations (N_E = 50 and 100).

in the Cross River, Kimball Creek, and at least one additional source (24%, 4%, and 56% of the 25 brook trout analyzed from Lake Superior, respectively). This latter source exhibited a close relationship with brook trout from the Baptism River, and potentially reflects within-river or lake-spawning populations that were unsampled in this study. It is also possible that brook trout in the Baptism River historically supplied a founding population of individuals to Lake Superior, or vice versa. Collectively, these findings offer insights into the identity of source populations for coaster brook trout within Lake Superior. Inferences about the relative contributions of these source populations are difficult due to spatially heterogeneous sampling effort and varying susceptibility to the sampling gears employed in this study, as well as differences in the seasonal availability of brook trout. Genetic studies of additional waterways within the Lake Superior basin have also revealed riverine sources for coaster brook trout (Burnham-Curtis, 2001; D'Amelio et al., 2008; D'Amelio and Wilson, 2008; Elias et al., 2018; Scribner et al, 2012).

Fisheries managers and biologists across the Lake Superior basin define coaster brook trout as brook trout spending any portion of their life in Lake Superior (Becker, 1983; Newman et al., 2003). The coaster brook trout we analyzed from Lake Superior were sampled from nearshore lake habitats off Grand Marais, MN and from within Grand Marais Harbor. Data from angler tagging efforts for coaster brook trout encountered off Grand Marias suggest a large degree of mobility for at least a subset of these fish (MNDNR, unpublished data), but it is unclear whether these fish spend the majority of their time in Lake Superior. In reality, the propensity for migration and degree to which brook trout utilize lake habitats is likely variable (Huckins et al., 2008). For example, some studies report brook trout that use Lake Superior as a corridor between tributaries (Huckins et al., 2008; Kusnierz et al.,

2009, 2014). Regardless, our findings provide new insights into sources of brook trout that spend at least a portion of their time within Lake Superior. Future work that expands sampling effort to include additional Lake Superior tributaries and locations within the lake are necessary to identify other sources of coaster brook trout, build an understanding of the relative contribution of each source, and better characterize the role of brook trout in nearshore ecosystems of Lake Superior.

In addition to natural reproduction, it is possible that some of the coaster brook trout in Lake Superior are supplied by domestic stocking. We identified three or four individuals among the coaster brook trout sampled off Grand Marais, MN that represent either previously stocked domestic fish from the three coaster strains stocked into Lake Superior (Nipigon, Tobin, and Siskiwit strains) or migrants from source populations used to maintain these strains. Source populations for the Tobin and Siskiwit strains are located on Isle Royale, MI, only 40 km from the Minnesota North Shore. These strains have been widely stocked across the U.S. portion of Lake Superior, including in jurisdictions neighboring our study region. For example, both the Tobin and Siskiwit strains have been periodically stocked in waters adjacent to our study region. The Siskiwit Bay strain has also been stocked along Isle Royale. If the putatively domestic fish we identified are truly of domestic origin, they are likely derived from stocking efforts in neighboring jurisdictions given that MNDNR ceased stocking domestic brook trout in below-barrier reaches of the Lake Superior basin in 1987 (Schreiner et al., 2006).

Relationships of above-barrier populations

Large natural waterfall barriers that prevent upstream movement of brook trout play a key role in shaping the population structure and conservation status of brook trout within the rivers
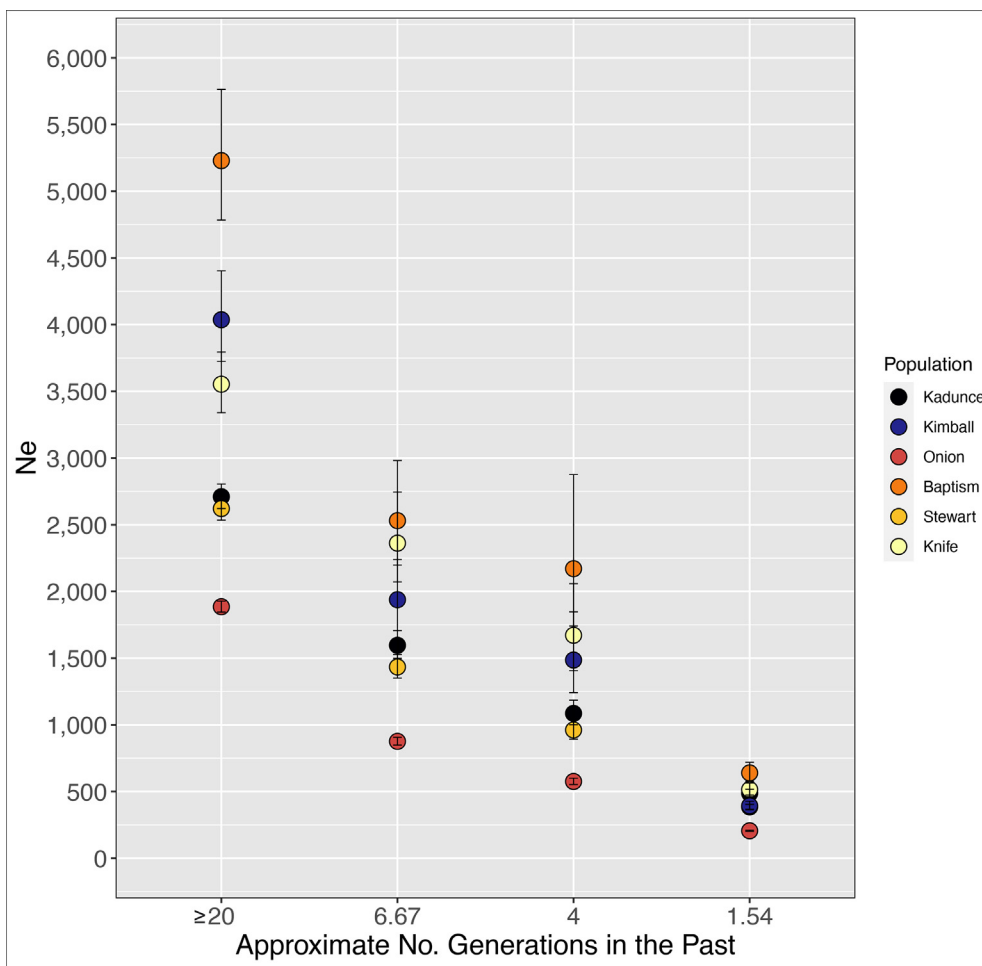
Fig. 7. Plot depicting estimates of historical N_E from LinkNe for the genetically distinct populations resolved in this study, where wild-caught domestic individuals were excluded. The number of generations in the past associated with each estimate of N_E are shown on the bottom axis and were approximated by assuming a generation time of two years. The 95% confidence intervals for each estimate are also shown. Results are based on a minor allele frequency of 5% and are only shown for populations with ≥20 individuals.

surveyed here. These waterfalls result in comparatively isolated above-barrier populations that lose individuals to downstream movement over the waterfall barrier. We identified above-barrier populations in the Kadunce, Onion, and Stewart rivers, two of which we identified as a source of brook trout to downstream reaches (the Stewart River was not sampled below the downstream waterfall). Above-barrier sources of below-barrier fish have been reported in other genetic studies of native brook trout (D'Amelio and Wilson, 2008; Kelson et al., 2015; Scribner et al., 2012). We expect that this relationship applies to additional rivers along the Minnesota North Shore that have analogous waterfall structures, including for the genetically distinct populations we identified in below-barrier reaches where above-barrier individuals were not analyzed.

The large natural waterfalls located within the rivers surveyed here have presumably isolated above-barrier populations of brook trout for a long time. Most of the waterfalls along the Minnesota North Shore were formed by the erosion of sediments deposited by the last ice sheet during the Pleistocene and subsequent exposure of underlying basalt flows formed during the Proterozoic (Smith and Moyle, 1944). Given their isolation, above-barrier populations of brook trout may serve as reservoirs of unique genetic variation, including due to local adaptation (Letcher et al., 2007). It is also possible that these populations harbor native genetic diversity. However, for brook trout along the Minnesota North

Shore and elsewhere across the Lake Superior basin, domestic ancestry is possible given an extensive history of hatchery stocking in this region. For example, it is hypothesized that brook trout did not occur above waterfall barriers in Minnesota rivers tributary to Lake Superior prior to European settlement, and only occurred afterwards via relocation of below-barrier fish or stocking of hatchery strains (Smith and Moyle, 1944; Surber, 1922; Waters, 1980). The wild brook trout analyzed in this study exhibited little to no introgression from the nine hatchery strains available for comparison. But we expect that the hatchery strains evaluated here provide only a limited representation of earlier generations within each strain, and of different hatchery strains altogether, that were used in early stocking efforts. Genetic analysis of historical broodstock combined with data on historical stocking effort are necessary to confirm whether contemporary above-barrier populations of brook trout along the Minnesota North Shore harbor native genetic diversity; however, the genetic samples and stocking records required for such analyses may be unavailable or incomplete.

Below-barrier reaches

A key question central to the rehabilitation of coaster brook trout is understanding whether source populations are primarily located within Lake Superior, in above- or below-barrier reaches

within rivers, or in a combination of these habitats. In most rivers along the Minnesota North Shore, large natural waterfalls near the mouth of each river restrict coaster brook trout to below-barrier habitats that are also spatially limited. Fisheries survey efforts in 29 rivers along the Minnesota North Shore indicate that only 3% of the total mainstem length in these rivers is accessible to brook trout from Lake Superior (Goldsworthy et al., 2017). In general, the below-barrier habitat in these rivers is thermally inadequate for year-round habitation by brook trout. Below-barrier reaches are also frequently characterized by low quality spawning habitat and the presence of introduced salmonids. It is thus suspected by fisheries managers that spawning of brook trout in below-barrier reaches is limited. In comparison, habitat in above-barrier reaches is typically much more conducive to the thermal and spawning requirements of brook trout, regardless of variation in habitat quality among the above-barrier reaches of some waterways.

The wild-caught brook trout analyzed in this study exhibited limited admixture, and instead displayed large assignment probabilities to the genetically distinct populations identified here, including for brook trout sampled from below-barrier reaches. An earlier analysis of brook trout from below-barrier reaches of the Onion, Cross, and Kadunce rivers showed several potentially admixed individuals (Stott et al., 2010). However, this result could reflect the weaker power of microsatellites to resolve population-level relationships compared to the genome-wide SNPs analyzed here. In the Nipigon Bay region of Lake Superior, analyses of genome-wide SNPs in brook trout from below-barrier reaches of several waterways revealed large degrees of admixture (Elias et al., 2018). Nipigon Bay and associated tributaries support several remnant populations of coaster brook trout (Huckins et al., 2008; Newman and Dubois, 1996; Robillard et al., 2011), which may facilitate greater gene flow among populations in this region. Greater availability of adequate spawning and thermal habitat in tributaries to Nipigon Bay compared to streams along the Minnesota North Shore may also contribute to the increased admixture observed by Elias et al. (2018), particularly given sampling that occurred during the summer months, a season where low flow and high water temperatures on the Minnesota North Shore likely prohibit the presence of brook trout.

The lack of admixture observed in this study, particularly in below-barrier reaches where we detected individuals representing more than one population during the spawning season, may be indicative of limited spawning in below-barrier reaches along the Minnesota North Shore. However, it is also possible that spawning in below-barrier reaches does occur and is accompanied by strong site fidelity, although this may be less likely given the lack of quality spawning habitat in below-barrier reaches of North Shore tributaries (Goldsworthy et al., 2017). Our results may also reflect limited spawning in Minnesota waters of Lake Superior, although the presence of unsampled source populations within the lake is possible. If spawning of brook trout in below-barrier reaches and lake habitats along the Minnesota North Shore is indeed limited, then conserving above-barrier populations will be key to rehabilitating coaster brook trout across the Minnesota North Shore. However, more studies are needed to identify additional source populations for brook trout in this and neighboring regions, and to clarify the spawning ecology of brook trout in below-barrier reaches and lake habitats. Further, discerning the present-day vs. historical role of above-barrier populations in supplying brook trout to below-barrier reaches and to Lake Superior will be important for predicting the future trajectory of this system.

Population conservation status

Although N_E estimates for most of the genetically distinct populations identified in this study exhibit wide confidence intervals,

values for the upper and lower bounds of these intervals relative to thresholds typically used to gauge population conservation status are still informative for prioritizing monitoring efforts. For example, it is possible that the Onion River, Knife River, and Kimball Creek populations exhibit $N_E < 100$, given that the lower bounds of N_E estimates for these populations fall below this value. The Onion River population also exhibits the lowest N_E observed in this study, and the upper confidence interval for this estimate is below 500, a threshold frequently regarded as necessary to maintain evolutionary potential (Franklin, 1980; Franklin et al., 2014; Jamieson and Allendorf, 2012). Estimates of N_E for every other population are also below 500, though the upper confidence intervals for these estimates are infinite. Similarly, N_E for the population in the Baptism River approached 500; this result is consistent with a close relationship to the population that comprised the majority of individuals from Lake Superior. Though analysis of additional individuals from each population is needed to increase the precision of the N_E estimates presented here, our findings offer preliminary insights to guide future assessments of genetic conservation status in brook trout along the Minnesota North Shore.

The low values of N_E we observed for above-barrier populations likely reflects the relative isolation of these populations. It is also possible that at least some of the below-barrier populations we identified originated above the waterfall in each river, resulting in N_E values comparable to known above-barrier populations. This is especially likely for the Knife River population, for which sampling occurred at the waterfall and primarily targeted individuals migrating downstream from above-barrier reaches. Isolated populations are more prone to loss of genetic diversity through genetic drift, inbreeding, and allelic fixation and therefore often exhibit reduced N_E compared to non-isolated populations (Frankham, 2005; Frankham et al., 2017). Isolated above-barrier populations of brook trout and other native trout have been shown to exhibit higher extinction risks than adjacent below-barrier populations (Torterotot et al., 2014; Weathers et al., 2019; Whiteley et al., 2010); however, this effect may be less pronounced in above-barrier populations that have access to larger habitat areas (Whiteley et al., 2013). In this study, N_E generally corresponds with the relative size of the watershed inhabited by each population, except for the population in the Kadunce River. For example, the Onion River watershed is the smallest watershed in our study region, and the population we identified in the Onion River also exhibited the lowest N_E. Future studies that explore the relationship between N_E and landscape features including available above-barrier habitat size and quality are likely to provide important insights into factors shaping the conservation status of brook trout along the Minnesota North Shore.

Our reconstruction of historical N_E revealed declining N_E over recent history for each population. If we assume a generation time of two years (Letcher et al., 2007) and a starting year of 2018 (the mean sampling year for the individuals analyzed here), then our LinkNe estimates may correspond with a period spanning at least 40 years, though in reality this period is likely much longer (Hollenbeck et al., 2016). Analyses of historical N_E in other salmonid species have revealed similar declines, as well as their underlying causes. For example, Lehnert et al. (2019) reported declines in N_E over an approximately thirty year period for at least 60% of the 172 populations of Atlantic salmon they surveyed across North America and Europe. Variables for temperature, precipitation, and human population density were significantly associated with observed declines. Additionally, Layton et al. (2021) detected declines in N_E over an approximately eleven year period for all 28 populations of Arctic char (*S. alpinus*) they analyzed from northeastern Canada. Temperature was again significantly associated with observed declines. Our results point to potentially large declines in N_E for brook trout along the Minnesota North Shore, highlighting

the need for continued conservation efforts in this region. Future work that incorporates information on relevant climate and landscape variables is necessary to identify underlying causes of the declines reported here.

Hatchery stocking efforts

A lack of evidence for widespread domestic ancestry in the wild brook trout analyzed in this study indicates that domestic brook trout do not make a meaningful contribution to wild production in the populations evaluated here. This result is consistent with previous studies within the Lake Superior basin (Burnham-Curtis, 2001; Miller et al., 2016) and elsewhere across the native species range (Annett et al., 2012; Beer et al., 2019; Kazyak et al., 2018; Kelson et al., 2015; Lehnert et al., 2020; Morgan et al., 2021; White et al., 2018; but see Erdman et al., 2022). Regardless, it is possible that introgression of domestic alleles occurs in wild populations that were not surveyed in this study. It is also possible that stocked domestic brook trout impact their wild counterparts in other ways, for example through competition for finite food and habitat resources, which may be especially limited in below-barrier reaches along the Minnesota North Shore. Understanding potential impacts of hatchery stocking is important given that domestic brook trout stocked in one location may be capable of dispersing to additional locations and interacting with local populations, as evidenced by the wild-caught brook trout we putatively identified as previously stocked domestic fish.

Management and conservation implications

This study offers important insights to inform coaster brook trout restoration along the Minnesota North Shore. In 1997, Minnesota increased the stringency of harvest regulations in below-barrier reaches accessible from Lake Superior by implementing a restrictive possession limit of one fish over 20 in. This measure was implemented to protect larger brook trout, which are more likely to exhibit the coaster life history. However, this regulation may not achieve the intended result of rehabilitating coaster brook trout if spawning in below-barrier reaches is limited. Likewise, stocking domestic individuals below barriers will be ineffective if they fail to reproduce. If, instead, coaster brook trout are primarily produced via above-barrier spawning, then rehabilitation efforts will need to focus more on restoring instream habitat, rehabilitating riparian forest, protecting cold water seeps and springs, and removing man-made fish passage barriers in above-barrier reaches.

This study also includes conservation and management implications for brook trout beyond efforts focused on the coaster life history. The riverine brook trout evaluated in this study exhibited substantial population structure, with large degrees of genetic differentiation possible across small spatial extents. Highly structured populations have been reported in genetic studies of brook trout from across the native species range (Ferchaud et al., 2020; Kazyak et al., 2021). The large degrees of population structure exhibited by brook trout potentially result from extensive local adaptation and the impact of increasingly fragmented landscapes on genetic connectivity. Regardless of the underlying factors, these findings indicate that efforts to conserve the genetic diversity characteristic of local populations may need to occur on equally localized scales, presenting unique challenges to fisheries managers. Despite these challenges, such efforts are increasingly important to maintain a portfolio of genetic diversity (e.g., Hilborn et al., 2003) critical for supporting the long-term persistence of native brook trout populations despite changing environmental, ecological, and climatic conditions.

Concluding remarks

Our study comprises the first survey of genome-wide variation in brook trout along the Minnesota North Shore. Results from this study support the restoration of brook trout in this region by supplying baseline information on the population structure and source-sink dynamics of brook trout inhabiting riverine and lake habitats. In particular, results from this work offer novel insights into sources of coaster brook trout within Lake Superior and the conservation status of brook trout populations along the Minnesota North Shore. Our results also indicate that the most effective way to conserve and restore coaster brook trout populations in Lake Superior may be to renew focus on watershed health and resilience, thus providing fisheries managers with insight into the importance of protecting, enhancing, and rehabilitating the physical and thermal habitat of brook trout within tributary watersheds. Future work to survey brook trout from more waterways along the Minnesota North Shore and locations within Lake Superior will be important for identifying additional sources of coaster brook trout. Analyses to explore ecological and environmental drivers of population-level conservation status will also be critical for guiding future mitigation efforts. Importantly, our use of a RAD capture panel, which targets a standardized set of SNPs distributed throughout the brook trout genome, will enable additional individuals to be easily incorporated into the dataset produced here. Such standardized datasets are critical for directly integrating results across studies and building datasets with increasing spatiotemporal coverage. Our goal is for the results presented here to equip fisheries managers in Minnesota and elsewhere across the Lake Superior region with information to support healthy brook trout populations and ensure the long-term sustainability of this ecologically, economically, and culturally important species.

Data accessibility

Raw sequence data produced in this study are publicly accessible from the NCBI Sequence Read Archive (BioProject PRJNA895104). Metadata for the brook trout analyzed in this study and custom scripts are available via Dryad (https://doi.org/10.5061/dryad.w9ghx3ftq).

Author contributions

All authors contributed substantially to the conceptualization, design, and execution of this study. Additionally, C.G., L.M., and N.P. coordinated sample collection efforts, M.M. acquired project funding and provided overall project supervision, N.M. performed the formal analyses and wrote the original draft of the manuscript, and all authors contributed to the review and editing of the original manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

the anonymous reviewers who provided feedback on an earlier version of this manuscript. This conservation work was funded by Minnesota anglers through the purchase of fishing licenses (SWIFT Contract No. 176002).

Appendix A. Supplementary data

References

Ali, O., O'Rourke, S.M., Amish, S.J., Meek, M.H., Luikart, G., Jeffres, C., Miller, M.R., 2016. RAD capture (Rapture): flexible and efficient sequence-based genotyping. Genetics 202. https://doi.org/10.1101/028837.

Alshwairikh, Y.A., Kroeze, S.L., Olsson, J., Stephens-Cardenas, S.A., Swain, W.L., Waits, L.P., Horn, R.L., Narum, S.R., Seaborn, T., 2021. Influence of environmental conditions at spawning sites and migration routes on adaptive variation and population connectivity in chinook salmon. Ecol. Evol. 11 (23), 16890–16908.

Annett, B., Gerlach, G., King, T.L., Whiteley, A.R., 2012. Conservation genetics of remnant coastal brook trout populations at the southern limit of their distribution: population structure and effects of stocking. Trans. Am. Fish. Soc. 141 (5), 1399–1410. https://doi.org/10.1080/00028487.2012.694831.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson, E.A., Fay, J.C., 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3 (10), e3376.

Battin, J., Wiley, M.W., Ruckelshaus, M.H., Palmer, R.N., Korb, E., Bartz, K.K., Imaki, H., 2007. Projected impacts of climate change on salmon habitat restoration. Proc. Natl. Acad. Sci. 104 (16), 6720–6725. https://doi.org/10.1073/pnas.0701685104.

Becker, G.C., 1983. Fishes of Wisconsin. University of Wisconsin Press, Madison, WI.

Beer, S.D., Cornett, S., Austerman, P., Trometer, B., Hoffman, T., Bartron, M.L., 2019. Genetic diversity, admixture, and hatchery influence in brook trout (*Salvelinus fontinalis*) throughout western New York State. Ecol. Evol. 9 (13), 7455–7479. https://doi.org/10.1002/ece3.5237.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Burnham-Curtis, M., 2001. Genetic profiles of selected brook trout *Salvelinus fontinalis* populations from Lake Superior. Research completion report prepared for U.S. Fish and Wildlife Service, Ashland Fisheries Resource Office, Ashland, WI.

Caballero, A., 1994. Developments in the prediction of effective population size. Heredity 73, 657–679. https://doi.org/10.1038/hdy.1994.174.

Carlson, A.K., Taylor, W.W., Schlee, K.M., Zorn, T.G., Infante, D.M., 2017. Projected impacts of climate change on stream salmonids with implications for resilience-based management. Ecol. Freshw. Fish 26 (2), 190–204. https://doi.org/10.1111/eff.12267.

Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W., Postlethwait, J.H., 2011. Stacks: building and genotyping loci de novo from short-read sequences. G3: Genes, Genomes, Genetics 1 (3), 171–182. https://doi.org/10.1534/g3.111.000240.

Christensen, K.A., Rondeau, E.B., Minkley, D.R., Leong, J.S., Nugent, C.M., Danzmann, R.G., Ferguson, M.M., Stadnik, A., Devlin, R.H., Muzzerall, R., Edwards, M., Davidson, W.S., Koop, B.F., Palsson, A., 2018. The Arctic charr (*Salvelinus alpinus*) genome and transcriptome assembly. PLoS One 13 (9), e0204076.

Christensen, K.A., Rondeau, E.B., Minkley, D.R., Leong, J.S., Nugent, C.M., Danzmann, R.G., Ferguson, M.M., Stadnik, A., Devlin, R.H., Muzzerall, R., Edwards, M., Davidson, W.S., Koop, B.F., 2021. Retraction: The Arctic charr (*Salvelinus alpinus*) genome and transcriptome assembly. PLoS One 16 (2), e0247083.

Cooper, A., Mangel, M., 1999. The dangers of ignoring metapopulation structure for the conservation of salmonids. Fish. Bull. 97, 213–226.

D'Amelio, S., Mucha, J.M., Mackereth, R.W., Wilson, C.C., 2008. Tracking coaster brook trout to their sources: combining telemetry and genetic profiles to determine source populations. N. Am. J. Fish Manag. 28, 1343–1349. https://doi.org/10.1577/M05-193.1.

D'Amelio, S., Wilson, C.C., 2008. Genetic population structure among source populations for coaster brook trout in Nipigon Bay, Lake Superior. Trans. Am. Fish. Soc. 137, 1213–1228. https://doi.org/10.1577/T05-275.1.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., et al., 2011. The variant call format and VCFtools. Bioinformatics 27 (15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., Ovenden, J.R., 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol. Ecol. Resour. 14 (1), 209–214. https://doi.org/10.1111/1755-0998.12157.

Eddy, S., Underhill, J.C., 1974. Northern Fishes. University of Minnesota Press, Minneapolis, MN.

Elias, A., McLaughlin, R., Mackereth, R., Wilson, C., Nichols, K.M., 2018. Population structure and genomic variation of ecological life history diversity in wild-caught Lake Superior brook trout, *Salvelinus fontinalis*. J. Great Lakes Res. 44 (6), 1373–1382. https://doi.org/10.1016/j.jglr.2018.08.006.

Erdman, B., Mitro, M.G., Griffin, J.D.T., Rowe, D., Kazyak, D.C., Turnquist, K., Siepker, M., Miller, L., Stott, W., Hughes, M., Sloss, B., Kinnison, M.T., Wilson, C.C., Larson, W., 2022. Broadscale population structure and hatchery introgression of midwestern brook trout. Trans. Am. Fish. Soc. 151 (1), 81–99.

Ferchaud, A.-L., Leitwein, M., Laporte, M., Boivin-Delisle, D., Bougas, B., Hernandez, C., Bernatchez, L., 2020. Adaptive and maladaptive genetic diversity in small populations; insights from the brook charr (*Salvelinus fontinalis*) case study. Mol. Ecol. 29 (18), 3429–3445. https://doi.org/10.1101/660621.

Frankham, R., 2005. Genetics and extinction. Biol. Conserv. 126, 131–140. https://doi.org/10.1016/j.biocon.2005.05.002.

Frankham, R., Bradshaw, C.J.A., Brook, B.W., 2014a. Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biol. Conserv. 170, 56–63. https://doi.org/10.1016/j.biocon.2013.12.036.

Frankham, R., Bradshaw, C.J.A., Brook, B.W., 2014b. 50/500 rules need upward revision to 100, 1000 – response to Franklin et al. Biol. Conserv. 176, 286. https://doi.org/10.1016/j.biocon.2014.05.006.

Frankham, R., Ballou, J.D., Ralls, K., Eldridge, M.D.B., Dudash, M.R., Fenster, C.B., Sunnucks, P., 2017. Genetic Management of Fragmented Animal and Plant Populations. Oxford University Press.

Franklin, I.R., 1980. Evolutionary change in small populations. In: Soulé, M.E., Wilcox, B.A. (Eds.), Conservation Biology: An Evolutionary-Ecological Perspective. Sinauer Associates, Sunderland, MA.

Franklin, I.R., Allendorf, F.W., Jamieson, I.G., 2014. The 50/500 rule is still valid – Reply to Frankham et al. Biol. Conserv. 176, 284–285. https://doi.org/10.1016/j.biocon.2014.05.004.

Goldsworthy, C.A., Reeves, K.A., Blankenheim, J.E., Peterson, N.R., 2017. Fisheries management plan for the Minnesota waters of Lake Superior. Special Publication 181, Minnesota Department of Natural Resources, St. Paul, MN.

Gruber, B., Unmack, P.J., Berry, O.F., Georges, A., 2018. DARTR: An R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. Mol. Ecol. Resour. 18, 1–9. https://doi.org/10.1111/1755-0998.12745.

Hayes, B.J., Visscher, P.M., McPartlan, H.C., Goddard, M.E., 2003. Novel multilocus measure of linkage disequilibrium to estimate past effective population size. Genome Res. 13 (4), 635–643.

Hecht, B.C., Matala, A.P., Hess, J.E., Narum, S.R., 2015. Environmental adaptation in chinook salmon (Oncorhynchus tshawytscha) throughout their North American range. Mol. Ecol. 24 (22), 5573–5595. https://doi.org/10.1111/mec.13409.

Hilborn, R., Quinn, T.P., Schindler, D.E., Rogers, D.E., 2003. Biocomplexity and fisheries sustainability. PNAS 100 (11), 6564–6568. https://doi.org/10.1073/pnas.1037274100.

Hoban, S., Archer, F.I., Bertola, L.D., Bragg, J.G., Breed, M.F., Bruford, M.W., Hunter, M. E., 2022. Global genetic diversity status and trends: towards a suite of Essential Biodiversity Variables (EBVs) for genetic composition. Biol. Rev. 97, 1511–1538. https://doi.org/10.1111/brv.12852.

Hollenbeck, C.M., Portnoy, D.S., Gold, J.R., 2016. A method for detecting recent changes in contemporary effective population size from linkage disequilibrium at linked and unlinked loci. Heredity 117 (4), 207–216. https://doi.org/10.1038/hdy.2016.30.

Huckins, C.J., Baker, E.A., Fausch, K.D., Leonard, J.B.K., 2008. Ecology and life history of coaster brook trout and potential bottlenecks in their rehabilitation. N. Am. J. Fish Manag. 28 (4), 1321–1342. https://doi.org/10.1577/M05-191.1.

Jamieson, I.G., Allendorf, F.W., 2012. How does the 50/500 rule apply to MVPs? Trends Ecol. Evol. 27 (10), 578–584. https://doi.org/10.1016/j.tree.2012.07.001.

Jombart, T., 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24 (11), 1403–1405.

Jombart, T., Pontier, D., Dufour, A.-B., 2009. Genetic markers in the playground of multivariate analysis. Heredity 102, 330–341. https://doi.org/10.1038/hdy.2008.130.

Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. BMC Genet. 11 (1), 94. https://doi.org/10.1186/1471-2156-11-94.

Jones, A.T., Ovenden, J.R., Wang, Y.-G., 2016. Improved confidence intervals for the linkage disequilibrium method for estimating effective population size. Heredity 117 (4), 217–223. https://doi.org/10.1038/hdy.2016.19.

Kazyak, D.C., Rash, J., Lubinski, B.A., King, T.L., 2018. Assessing the impact of stocking northern-origin hatchery brook trout on the genetics of wild populations in North Carolina. Conserv. Genet. 19 (1), 207–219. https://doi.org/10.1007/s10592-017-1037-4.

Kazyak, D.C., Lubinski, B.A., Rash, J.M., Johnson, T.C., King, T.L., 2021. Development of genetic baseline information to support the conservation and management of wild brook trout in North Carolina. N. Am. J. Fish Manag. 41 (3), 626–638. https://doi.org/10.1002/nafm.10569.

Kelson, S.J., Kapuscinski, A.R., Timmins, D., Ardren, W.R., 2015. Fine-scale genetic structure of brook trout in a dendritic stream network. Conserv. Genet. 16 (1), 31–42. https://doi.org/10.1007/s10592-014-0637-5.

Kusnierz, P.C., Stimmell, S.P., Leonard, J.B.K., 2009. Movement and growth indicators in resident and adfluvial coaster brook trout (*Salvelinus fontinalis*) in the Hurricane River, Lake Superior, Michigan, USA. JGLR 35 (3), 385–391. https://doi.org/10.1016/j.jglr.2009.04.004.

Kusnierz, P.C., Stimmell, S.P., Leonard, J.B.K., 2014. Migration, size, and age structure of brook trout (*Salvelinus fontinalis*) from two Lake Superior tributaries. Am. Midl. Nat. 172 (1), 119–130. https://doi.org/10.1674/0003-0031-172.1.119.

Layton, K.K.S., Snelgrove, P.V.R., Dempson, J.B., Kess, T., Lehnert, S.J., Bentzen, P., Duffy, S.J., Messmer, A.M., Stanley, R.R.E., DiBacco, C., Salisbury, S.J., Ruzzante, D. E., Nugent, C.M., Ferguson, M.M., Leong, J.S., Koop, B.F., Bradbury, I.R., 2021.

Genomic evidence of past and future climate-linked loss in a migratory Arctic fish. Nat. Clim. Chang. 11 (2), 158–165.

Lehnert, S.J., Kess, T., Bentzen, P., Kent, M.P., Lien, S., Gilbey, J., Clément, M., Jeffery, N.W., Waples, R.S., Bradbury, I.R., 2019. Genomic signatures and correlates of widespread population declines in salmon. Nat. Commun. 10 (1). https://doi.org/10.1038/s41467-019-10972-w.

Lehnert, S.J., Baillie, S.M., MacMillan, J., Paterson, I.G., Buhariwalla, C.F., Bradbury, I.R., Bentzen, P., 2020. Multiple decades of stocking has resulted in limited hatchery introgression in wild brook trout (*Salvelinus fontinalis*) populations of Nova Scotia. Evol. Appl. 13 (5), 1069–1089. https://doi.org/10.1111/eva.12923.

Lemopoulos, A., Prokkola, J.M., Uusi-Heikkilä, S., Vasemägi, A., Huusko, A., Hyvärinen, P., Koljonen, M.-L., Koskiniemi, J., Vainikka, A., 2019. Comparing RADseq and microsatellites for estimating genetic diversity and relatedness—implications for brown trout conservation. Ecol. Evol. 9 (4), 2106–2120.

Letcher, B.H., Nislow, K.H., Coombs, J.A., O'Donnell, M.J., Dubreuil, T.L., Svensson, E., 2007. Population response to habitat fragmentation in a stream-dwelling brook trout population. PLoS One 2 (11), e1139.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al., 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25 (16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. ArXiv Preprint ArXiv, 00(00), 3. https://doi.org/arXiv:1303.3997 [q-bio.GN].

Lindenbaum, P., 2015. JVarkit: Java-based utilities for bioinformatics. Figshare 2–5. https://doi.org/10.6084/m9.figshare.1425030.v1.

Luikart, G., Antao, T., Hand, B.K., Muhlfeld, C.C., Boyer, M.C., Cosart, T., Trethewey, B., Al-Chockhachy, R., Waples, R.S., 2021. Detecting population declines via monitoring the effective number of breeders (N_b). Mol. Ecol. Resour. 21 (2), 379–393.

Luu, K., Bazin, E., Blum, M.G.B., 2017. Pcadapt: An R package to perform genome scans for selection based on principal component analysis. Mol. Ecol. Resour. 17, 67–77. https://doi.org/10.1111/1755-0998.12592.

Manichaikul, A., Mychaleckyj, J.C., Rich, S.S., Daly, K., Sale, M., Chen, W., 2010. Robust relationship inference in genome-wide association studies. Bioinformatics 26 (22), 2867–2873. https://doi.org/10.1093/bioinformatics/btq559.

McKinney, G.J., Waples, R.K., Seeb, L.W., Seeb, J.E., 2017. Paralogs are revealed by proportion of heterozygotes and deviations in read ratios in genotyping-by-sequencing data from natural populations. Mol. Ecol. Resour. 17 (4), 656–669. https://doi.org/10.1111/1755-0998.12613.

Micheletti, S.J., Matala, A.R., Matala, A.P., Narum, S.R., 2018. Landscape features along migratory routes influence adaptive genomic variation in anadromous steelhead (*Oncorhynchus mykiss*). Mol. Ecol. 27 (1), 128–145. https://doi.org/10.1111/mec.14407.

Miller, L.M., Schreiner, D.R., Blankenheim, J.E., Ward, M.C., Quinlan, H.R., Moore, S., 2016. Effects of restrictive harvest regulations on rehabilitation of coaster brook trout in Minnesota's portion of Lake Superior. J. Great Lakes Res. 42 (4), 883–892. https://doi.org/10.1016/j.jglr.2016.05.006.

Miller, L.M., Dieterman, D.J., Hoxmeier, R.J.H., 2019. Reproductive dynamics of a native brook trout population following removal of non-native brown trout from a stream in Minnesota, north-central USA. Hydrobiologia 840 (1), 49–61. https://doi.org/10.1007/s10750-019-3952-5.

Morgan II, R.P., Kazyak, D.C., King, T.L., Lubinski, B.A., Sell, M.T., Heft, A.A., Jones, J.W., 2021. Genetic structure of Maryland brook trout *Salvelinus fontinalis* populations: Management implications for a threatened species. N. Am. J. Fish Manag. 41 (4), 1097–1119. https://doi.org/10.1002/nafm.10618.

Neuditschko, M., Khatkar, M.S., Raadsma, H.W., Timpson, N.J., 2012. NetView: A high-definition network-visualization approach to detect fine-scale population structures from genome-wide patterns of variation. PLoS One 7 (10), e48375.

Newman, L.E., Dubois, R.B., 1996. Status of brook trout in Lake Superior. Prepared for the Lake Superior Technical Committee by the Brook Trout Subcommittee, Great Lakes FIshery Commission.

Newman, L.E., DuBois, R.B., Halpern, T.N., 2003. A brook trout rehabilitation plan for Lake Superior. Miscellaneous Publication 2003-03, Great Lakes Fishery Commission.

Pembleton, L.W., Cogan, N.O.I., Forster, J.W., 2013. StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations. Mol. Ecol. Resour. 13 (5), 946–952. https://doi.org/10.1111/1755-0998.12129.

Peterson, N. R. (2018). Status of coaster brook trout in Minnesota Lake Superior tributaries. F17A00190, F-29-RP-33(Year 2), Study 3, Minnesota Department of Natural Resources.

Power, G., 1980. The brook charr, *Salvelinus fontinalis*. In: Balon, E.K. (Ed.), Charrs: Salmonid Fishes of the Genus Salvelinus. Dr. W. Junk Publishers, The Hague.

Quinn, T.P., 2021. Differential migration in Pacific salmon and trout: patterns and hypotheses. Animal Migration 8 (1), 1–18. https://doi.org/10.1515/ami-2021-0001.

Rieman, B.E., Dunham, J.B., 2000. Metapopulations and salmonids: a synthesis of life history patterns and empirical observations. Ecol. Freshw. Fish 9 (1–2), 51–64. https://doi.org/10.1034/j.1600-0633.2000.90106.x.

Robillard, M.M., McLaughlin, R.L., Mackereth, R.W., 2011. Diversity in habitat use and trophic ecology of brook trout in Lake Superior and tributary streams revealed through stable isotopes. Trans. Am. Fish. Soc. 140, 943–953. https://doi.org/10.1080/00028487.2011.601219.

Rochette, N.C., Rivera-Colón, A.G., Catchen, J.M., 2019. Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Mol. Ecol. 28 (21), 4737–4754. https://doi.org/10.1111/mec.15253.

Schreiner, D.R., Cullis, K.I., Donofrio, M.C., Fischer, G.J., Hewitt, L., Mumford, K.G., Pratt, D.M., Quinlan, H.R., Scott, S.J., 2008. Management perspectives on coaster brook trout rehabilitation in the Lake Superior basin. N. Am. J. Fish Manag. 28, 1350–1364. https://doi.org/10.1577/M05-173.1.

Schreiner, D.R., Ostazeski, J.J., Halpern, T.N., Geving, S.A., 2006. Fisheries management plan for the Minnesota waters of Lake Superior. Special Publication 163, Minnesota Department of Natural Resources.

Schtickzelle, N., Quinn, T.P., 2007. A metapopulation perspective for salmon and other anadromous fish. Fish Fish. 8 (4), 297–314. https://doi.org/10.1111/j.1467-2979.2007.00256.x.

Scribner, K., Huckins, C., Baker, E., Kanefsky, J., 2012. Genetic relationships and gene flow between resident and migratory brook trout in the Salmon Trout River. J. Great Lakes Res. 38 (1), 152–158. https://doi.org/10.1016/j.jglr.2011.11.009.

Smith Jr., L.L., Moyle, J.B., 1944. A biological survey and fishery management plan for the streams of the Lake Superior North Shore watershed. Technical Bulletin No. 1, Minnesota Department of Conservation Division of Game and Fish, St. Paul, MN.

Steinig, E.J., Neuditschko, M., Khatkar, M.S., Raadsma, H.W., 2016. NETVIEW P: a network visualization tool to unravel complex population structure using genome-wide SNPs. Mol. Ecol. Resour. 16 (1), 216–227. https://doi.org/10.1111/1755-0998.12442.

Stewart, J.S., Covert, S.A., Estes, N.J., Westenbroek, S.M., Krueger, D., Wieferich, D.J., et al., 2016. FishVis, A Regional Decision Support Tool for Identifying Vulnerabilities of Riverine Habitat and Fishes to Climate Change in the Great Lakes Region. Scientific Investigations Report 2016-5124, U.S. Geological Survey.

Stott, W., Quinlan, H.R., Gorman, O.T., King, T.L., 2010. Genetic structure and diversity among brook trout from Isle Royale, Lake Nipigon, and three Minnesota tributaries of Lake Superior. N. Am. J. Fish Manag. 30 (2), 400–411. https://doi.org/10.1577/M08-213.1.

Surber, T., 1922. A Biological Reconnaissance of Streams Tributary to Lake Superior, Baptism River to Devils Track River. Typescript at Minnesota Department of Natural Resources Library.

Sylvester, E.V.A., Beiko, R.G., Bentzen, P., Paterson, I., Horne, J.B., Watson, B., Lehnert, S., Duffy, S., Clément, M., Robertson, M.J., Bradbury, I.R., 2018. Environmental extremes drive population structure at the northern range limit of Atlantic salmon in North America. Mol. Ecol. 27 (20), 4026–4040.

Torterotot, J., Perrier, C., Bergeron, N.E., Bernatchez, L., 2014. Influence of forest road culverts and waterfalls on the fine-scale distribution of brook trout genetic diversity in a boreal watershed. Trans. Am. Fish. Soc. 143 (6), 1577–1591. https://doi.org/10.1080/00028487.2014.952449.

U.S. Fish and Wildlife Service (USFWS). (2016). *Status and distribution of Lake Superior basin brook trout*. National Fish and Wildlife Foundation Project ID: 0901.11.027081, U.S. Fish and Wildlife Service, Ashland Fish and Wildlife Conservation Office, Ashland, WI.

Wainwright, T.C., Waples, R.S., 1998. Prioritizing Pacific salmon stocks for conservation: response to Allendorf et al. Conserv. Biol. 12 (5), 1144–1147. https://doi.org/10.1046/j.1523-1739.1998.97385.x.

Waples, R.S., 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv. Genet. 7 (2), 167–184. https://doi.org/10.1007/s10592-005-9100-y.

Waples, R.S., 2022. What is NE, anyway? J. Heredity 113 (4), 371–379. https://doi.org/10.1093/jhered/esac023.

Waples, R.S., Antao, T., Luikart, G., 2014. Effect of overlapping generations on linkage disequilibrium estimates of effective population size. Genetics 197 (2), 769–780. https://doi.org/10.1534/genetics.114.164822.

Waples, R.S., Do, C., 2010. Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evol. Appl. 3, 244–262. https://doi.org/10.1111/j.1752-4571.2009.00104.x.

Waples, R.S., Lindley, S.T., 2018. Genomics and conservation units: the genetic basis of adult migration timing in Pacific salmonids. Evol. Appl. 11 (9), 1518–1526. https://doi.org/10.1111/eva.12687.

Waters, T., 1980. The Streams and Rivers of Minnesota. University of Minnesota Press, Minneapolis, MN.

Weathers, T.C., Kazyak, D.C., Stauffer, J.R., Kulp, M.A., Moore, S.E., King, T.L., Carlson, J.E., 2019. Neutral genetic and phenotypic variation within and among isolated headwater populations of brook trout. Trans. Am. Fish. Soc. 148 (1), 58–72. https://doi.org/10.1002/tafs.10115.

Weir, B., Cockerham, C.C., 1984. Estimating f-statistics for the analysis of population structure. Evolution 38 (6), 1358–1370.

Wenger, S.J., Isaak, D.J., Luce, C.H., Neville, H.M., Fausch, K.D., Dunham, J.B., Dauwalter, D.C., Young, M.K., Elsner, M.M., Rieman, B.E., Hamlet, A.F., Williams, J.E., 2011. Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. Proc. Natl. Acad. Sci. 108 (34), 14175–14180.

White, S.L., Miller, W.L., Dowell, S.A., Bartron, M.L., Wagner, T., 2018. Limited hatchery introgression into wild brook trout (*Salvelinus fontinalis*) populations despite reoccurring stocking. Evol. Appl. 11 (9), 1567–1581. https://doi.org/10.1111/eva.12646.

Whiteley, A.R., Hastings, K., Wenburg, J.K., Frissell, C.A., Martin, J.C., Allendorf, F.W., 2010. Genetic variation and effective population size in isolated populations of

coastal cutthroat trout. Conserv. Genet. 11 (5), 1929–1943. https://doi.org/10.1007/s10592-010-0083-y.

Whiteley, A.R., Coombs, J.A., Hudy, M., Robinson, Z., Colton, A.R., Nislow, K. H., Letcher, B.H., Taylor, E., 2013. Fragmentation and patch size shape genetic structure of brook trout populations. Can. J. Fish. Aquat. Sci. 70 (5), 678–688.

Wright, S., 1931. Evolution in Mendelian populations. Genetics 16, 97–159. https://doi.org/10.1093/genetics/16.2.97.

Zorn, T.G., Hessenauer, J., Wills, T.C., 2020. Increasing connectivity of Great Lakes tributaries: interspecific and intraspecific effects on resident brook trout and brown trout populations. Ecol. Freshw. Fish 29 (4), 519–532. https://doi.org/10.1111/eff.12563.