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Experimental test of genetic rescue in isolated populations of brook trout

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Abstract

Genetic rescue is an increasingly considered conservation measure to address genetic erosion associated with habitat loss and fragmentation. The resulting gene flow from facilitating migration may improve fitness and adaptive potential, but is not without risks (e.g., outbreeding depression). Here, we conducted a test of genetic rescue by translocating ten (five of each sex) brook trout (*Salvelinus fontinalis*) from a single source to four nearby and isolated stream populations. To control for the demographic contribution of translocated individuals, ten resident individuals (five of each sex) were removed from each recipient population. Prior to the introduction of translocated individuals, the two smallest above-barrier populations had substantially lower genetic diversity, and all populations had reduced effective number of breeders relative to adjacent below-barrier populations. In the first reproductive bout following translocation, 31 of 40 (78%) translocated individuals reproduced successfully. Translocated individuals contributed to more families than expected under random mating and generally produced larger full-sibling families. We observed relatively high (>20%) introgression in three of the four recipient populations. The translocations increased genetic diversity of recipient populations by 45% in allelic richness and 25% in expected heterozygosity. Additionally, strong evidence of hybrid vigour was observed through significantly larger body sizes of hybrid offspring relative to resident offspring in all recipient populations. Continued monitoring of these populations will test for negative fitness effects beyond the first generation. However, these results provide much-needed experimental data to inform the potential effectiveness of genetic rescue-motivated translocations.

KEYWORDS
brook trout, extinction vortex, genetic rescue, habitat fragmentation, inbreeding

1 | INTRODUCTION

Small habitats patches generally support small populations that experience greater demographic stochasticity than larger habitats (Jamieson & Allendorf, 2012; Lande, 1993). Individuals within small stochastic populations are vulnerable to the loss of fitness associated with genetic load and inbreeding depression (Willi, Van Buskirk,

Hoffmann, Van Buskirk, & Hoffmann, 2006). This can form a positive feedback loop (termed an extinction vortex), where the loss of fitness further reduces population size thus exacerbating the negative genetic effects associated with small population size (Gilpin & Soule, 1986).These synergistic effects have been shown, in both laboratory and natural populations, to reduce probability of population persistence (Newman & Pilson, 1997; O'Grady et al., 2006; Palomares

et al., 2012). The benefit of gene flow to small, isolated populations has been widely documented in a variety of taxa and at low levels of immigration in laboratory and natural populations (Fitzpatrick et al., 2016; Hogg et al., 2006; Kronenberger et al., 2017; Madsen, Shine, Olsson, & Wittzell, 1999; Newman & Tallmon, 2001; Westemeier et al., 1998). The alleviation of detrimental inbreeding effects by gene flow, whether naturally or anthropogenically induced, is referred to as genetic rescue (GR). GR is defined as an increase in population fitness, greater than can be attributed to the demographic contribution of immigrants, due to the immigration of new alleles (Tallmon, Luikart, & Waples, 2004; Whiteley, Fitzpatrick, Funk, & Tallmon, 2015).

Until recently, GR was most often applied for the conservation of threatened and endangered species (e.g., Hedrick, 1995; Westemeier et al., 1998) or remnant populations of mega fauna (e.g., Hogg et al., 2006). However, many species of conservation concern are widely distributed, but comprised of many disconnected populations in marginal or small habitats. Although these species may not meet conservation listing requirements, each population could have high vulnerability to extirpation. These circumstances are common among species with low dispersal and species dependent on threatened habitat types (e.g., wetlands, vernal pools; Cushman, 2006). A broader application of GR might help mitigate the effects of fragmentation and isolation when the restoration of connectivity is impractical.

The potential for gene flow to affect fitness negatively in locally adapted populations, termed outbreeding depression, and the scarcity of empirically based recommendations currently limit broad application of GR (Frankham et al., 2011). Outbreeding depression is the reduction of population fitness due to immigration of new alleles, which can disrupt local adaptation, epistatic gene interactions, or lead to other intrinsic incompatibilities (Whiteley et al., 2015). Frameworks for predicting outbreeding depression following GR-motivated translocations have been proposed and are based on the outcomes of previous GR studies (Frankham, 2015; Frankham et al., 2011; Weeks et al., 2011). However, applications of GR to date have lacked controls for environmental conditions in wild populations and have otherwise not been performed in an experimental manner (but see Fitzpatrick et al., 2016). Additional studies with replicates and controls that monitor the multigenerational consequences of GR would help practitioners understand the utility of GR (Edmands, 2007; Tallmon et al., 2004; Whiteley et al., 2015).

Stream-dwelling salmonids often occupy highly fragmented habitats (e.g., Harig & Fausch, 2002; Hudy et al., 2008), and many of the barriers are absolute or only allow emigration (e.g., culverts, dams; Olden, Kennard, Lawler, & Poff, 2011). A decrease in probability of persistence has been documented for several salmonid species with the loss of contiguous habitat and decrease in patch size (Dunham, Vinyard, & Rieman, 1997; Dunham et al., 2008; Morita & Yamamoto, 2002). Patch size is also often positively correlated with genetic diversity and effective population size (N_e) (Fraser, Debes, Bernatchez, & Hutchings, 2014; Peacock & Dochtermann, 2012; Whiteley et al., 2010). In addition to habitat size, the importance of connectivity for metapopulation persistence has been demonstrated for multiple salmonid species (e.g., Koizumi, 2011; Letcher et al., 2007). In response to anthropogenic barriers that disrupt metapopulation connectivity and reduce habitat size, translocations are becoming more widely considered in aquatic ecosystems (Andrews et al., 2016; Olden et al., 2011; Pavlova et al., 2017).

In this study, we perform GR-motivated translocations in small-isolated headwater populations of stream-dwelling brook trout (*Salvelinus fontinalis*) near the southern extent of the species' distribution. The objectives of this study were to examine the genetic and fitness consequences of replicated translocations of ten reproductively mature individuals (five male, five female) from a common source following one reproductive bout. Specifically, we evaluated the reproductive success of translocated individuals and tested for an effect of GR-motivated translocations on genetic diversity and fitness surrogates. This is among few GR studies that are experimentally replicated and controlled in natural populations. To our knowledge, this is the first attempt to rigorously examine the consequences of GR in stream-dwelling salmonids, a taxon of general conservation concern, and of high ecological, social and economic value.

2 | METHODS

2.1 | Site descriptions and study organism

Brook trout habitat in the southeastern United States is severely fragmented and often isolated. From Pennsylvania to Georgia, 28% of historic brook trout occupied subwatersheds are extirpated, and many remaining populations are reduced to small headwater streams (Hudy et al., 2008). Of over 2,800 habitat patches in the same region, the median patch size (drainage area) was 855 ha with very few large (>10,000 ha) patches remaining (Whiteley, Hudy, Robinson, Coombs, & Nislow, 2014). Climate change could further exacerbate connectivity issues through altered hydrologic and thermal regimes within stream networks (Trumbo et al., 2014), increased frequency and severity of extreme events (e.g., flood and fire) and increased competition with invasive species (Wenger et al., 2011).

This study includes six stream-dwelling brook trout populations in Rockingham and Augusta Counties, Virginia, in the North River watershed within the Potomac River basin. The focal brook trout populations, Dry Run (DN-a), Skidmore Fork Dry River (DV-a), Dry River (DV-b), Little River (LR-a), Skidmore Fork North River (SF-a) and Briery Branch (BB-a) all occur in heavily forested watersheds within the George Washington National Forest (Table 1; Figure 1). Five of the streams have been isolated due to flood control dams (-a suffix on the site abbreviation denotes above a dam, and -b denotes below), which were constructed from 1962 to 1970 (Table 1). Based on observations during the summer sampling periods of 2010–2013, all above-barrier streams are seasonally intermittent to a variable extent and duration. Habitat patch areas and river lengths were calculated in Whiteley et al. (2013), and habitat patches were defined as the area of contiguous catchments (seventh level, 14 digit hydrologic unit codes) of connected brook trout habitat. Patch areas in

TABLE 1 Site descriptions for the brook trout habitat patches examined

Site name	Treatment	Site code	Patch area (ha)	Stream length (km)	Patch size	Dam age
Skidmore Fork-a	Recipient	SF-a	993	5.11	5.1	1962
Dry Run-a	Recipient	DN-a	1,217	8.06	9.8	1968
Briery Branch-a	Recipient	BB-a	2,438	6.14	15.0	1966
Little River-a	Recipient	LR-a	4,121	12.7	52.3	1965
Dry River-a	Control	DV-a	3,807	27.6	105.1	1970
Dry River-b	Source	DV-b	10,880	40	435.2	–

Patch size is the product of patch area and stream length divided by 1,000. The -a or -b suffix on site name and site code denotes above or below dam, respectively.
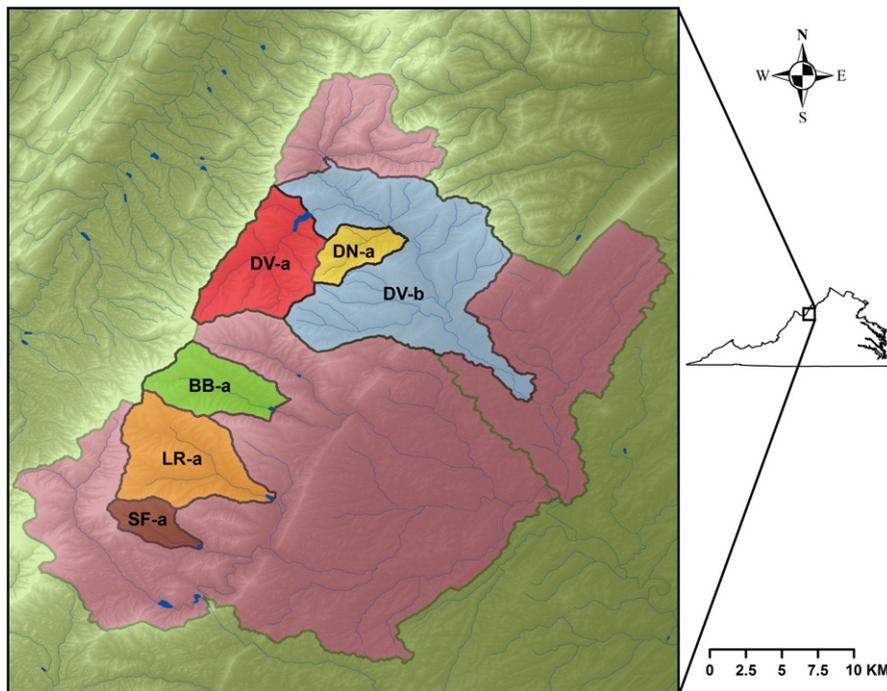

FIGURE 1 Map of study area in north-central Virginia, USA. The six brook trout habitat patches examined in this study are shown: DV, Dry River; DN, Dry Run; BB, Briery Branch; LR, Little River; SF, Skidmore Fork. Above-dam sites are denoted by -a; below-dam sites are denoted by -b. The wider boundary represents the hypothesized historical range of brook trout in this river system. DN-a, BB-a, LR-a and SF-a were recipients of translocated brook from DV-b. No brook trout were translocated trout to DV-a, and it serves as a control in this study [Colour figure can be viewed at wileyonlinelibrary.com]

the above-barrier patches range from 993 to 4,121 ha, and stream lengths in the above-barrier patches range from 5.1 to 27.4 km (Table 1). The source population for translocated brook trout was DV-b. DV-b is a perennial stream with altered hydrology due to upstream dams. DV-b is a substantially larger habitat patch than the five above-barrier populations and is among the largest connected brook trout habitats in Virginia with a patch area of 10,880 ha and a stream length of 40.0 km. Despite historical and contemporary stocking of hatchery-reared brook trout into DV-b, we tested for and did not find evidence of hatchery introgression into this population (Appendix S1).

2.2 | Brook trout sampling and translocations

To estimate population sizes and collect genetic samples, the above-barrier brook trout populations were exhaustively sampled for two consecutive years (2010 and 2011) prior to experimental translocations (which occurred in the fall of 2011). These data along with data from adjacent below-barrier populations are

presented in Whiteley et al. (2013), which describes the genetic effects of fragmentation in this watershed. Sampling in recipient habitats consisted of exhaustive single-pass electrofishing surveys of the entire habitat patch for DN-a, SF-a and BB-a during July–August prior to translocations (2010 and 2011) and following translocations (2012 and 2013). DV-a was exhaustively sampled with single-pass electrofishing surveys in July–August 2010 and 2011 and then was subsampled due to logistical constraints in 2012 and 2013. LR-a was exhaustively sampled with single-pass electrofishing surveys in July–August 2010, 2011 and 2012; time constraints only allowed for a subsample to be collected in 2013. DV-b was sampled with electrofishing surveys from a single starting location below Switzer Dam (which isolates DV-a) for young-of-year (age-0) fish during September 2010 and for all size classes in October 2011 while acquiring potential transplants. Upon capture, individual length (nearest mm, total length [TL]) and location (50-m sections from the barrier) were recorded, and a tissue sample (caudal fin clip) was taken as a source of genetic material and to serve as a mark for mark–recapture.

Each exhaustively sampled patch was resampled within 2–4 weeks of the initial capture event to estimate the proportion of marked to unmarked fish. Abundance was estimated with the Lincoln–Petersen estimator (Otis, Burnham, White, & Anderson, 1978). We calculated population size estimates for age-0 and age-1 and older (hereafter adults) separately to accommodate lower detection probability for age-0 fish. Sampling during late summer allowed age-0 brook trout to become large enough to be captured efficiently while still enabling year-class differentiation based upon length (Hudy, Downey, & Bowman, 2000). Empirical length frequency histograms, constructed for each population and sample year, were highly bimodal as expected, and age-0 fish were easily distinguished from adults (Fig. S3). Abundance estimates based on subsamples of LR-a and DV-a were calculated using ratios of previous stream reach abundances to observed reach abundances multiplied by the previous total observed abundance and then divided by the observed probability of detection. The probability of detection was estimated within each population and respective year from either 3-pass depletions or mark–recapture.

In October of 2011, brook trout were moved from the large genetically diverse DV-b to the isolated recipient habitat patches (Figure 1). The translocation coincided with the earliest expected onset of spawning to allow for immediate reproduction by translocated individuals (Jenkins & Burkhead, 1994). We used a single source population (DV-b) to control for potential source effects across replicates. Ten brook trout (five males and five females) over 145 mm TL and ostensibly sexually mature were translocated to each recipient habitat patch: DN-a, BB-a and LR-a (Figure 1). In SF-a, four males and six females were moved from DV-b (Appendix S2). The above-barrier habitat patch DV-a did not receive translocated individuals and served as a control for this study. To keep track of individuals during transfer, 12-mm PIT tags (Biomark, Boise, ID, USA) were implanted in the abdomen of intended transplants. Prior to releasing transplants, five male and five female residents were removed from each recipient stream to control for demographic contribution of translocated fish. Due to the lack of a reliable sex marker for these brook trout populations (A. Whiteley, unpublished data), sex was determined from physiological differences apparent in the field (e.g., expressing milt, gravid females with swollen abdomen and head shape). To evaluate the accuracy of visual classification of sex, twenty of the fish removed from recipient sites were euthanized and then sexed after dissection. The fish were sexed correctly prior to dissection 100% of the time. We also verified that translocated individuals were representative of the resident adult body size distribution using a randomization test approach and found no statistical support for this hypothesis (Appendix S7).

2.3 | Genotyping

Individuals were genotyped at eight microsatellite loci (*SfoC113*, *SfoD75*, *SfoC88*, *SfoD100*, *SfoC115*, *SfoC129*, *SfoC24*; King et al., 2012) and *SsaD237* (King, Eackles, & Letcher, 2005) following protocols for DNA extraction and amplification detailed in King et al.

(2005). PCR product was electrophoresed on either an ABI Prism 3100-Avant or an ABI Prism 3130xl genetic analyser (Applied Biosystems Inc., Foster City, CA, USA) and hand-scored using GENEIOUS version 7.1.2 (Biomatters Ltd.) All samples obtained in 2012 (post-translocation) were genotyped in SF-a, DN-a and BB-a. Some analyses were limited to these three populations, and these are referred to as the core recipient populations. In LR-a, due to the unexpectedly large number of brook trout captured in 2012, we took a stratified random sample of 544 age-0 fish from three equidistant in-stream locations for genotyping. In total, 3,909 individual brook trout were genotyped at eight microsatellite loci from five brook trout patches, in addition to the 2,502 genotypes previously published in Whiteley et al. (2013) for these populations.

After obtaining genotypic data, we tested for deviations from Hardy–Weinberg (HW) proportions and linkage disequilibrium (LD). We expected to observe deviations from HW proportions and significant LD due to the introgression of genetically divergent individuals as a result of the translocations (Slate & Pemberton, 2007). The markers used here have not exhibited HW violations or LD in mixed-aged samples in other populations examined (Annett, Gerlach, King, & Whiteley, 2012; Kanno, Vokoun, & Letcher, 2011; Kazyak et al., 2015). We previously reported extensive testing of Hardy–Weinberg (HW) proportions and linkage (gametic) disequilibrium (LD) for these populations and markers in Whiteley et al. (2013). It was concluded that family structure (number and size of family groups) was most likely responsible for the signal of HW deviations and LD (Whiteley et al., 2013). As a result, we took a random sample of one full-sibling per family (estimated with COLONY v1.2, see *Pedigree reconstruction* below), to create a data set that was free of family structure (Rodriguez-Ramilo & Wang, 2012). To test for HW proportions, we used the statistical computing program R version 3.3.1 (R Development Core Team, 2016) and the R package 'HWXTEST' (Engels, 2016). We used GENEPOP version 4.0.10 (Rouseset, Rousset, De, Montpellier, & Bataillon, 2008) to test for LD. For both LD and HW tests, we used sequential Bonferroni correction ($\alpha = .05$) within each population to correct for inflated type I error rates due to multiple testing (Rice, 1989). We found no evidence for deviations from HW proportions and only mild evidence for LD. Briefly, of the 123 HW tests, no significant ($\alpha = .05$; initial nominal *p*-value was .00625) tests were found using the one sibling per family data set after sequential Bonferroni correction. Of the 463 LD tests performed, 10 were significant ($\alpha = .05$; initial nominal *p*-value was .00078) using the one sibling per family and sequential Bonferroni correction. Of the 10 significant LD tests, eight occurred in 2012 post-translocation cohort (age-0) and are likely due to introgression of translocated individuals causing elevated levels of LD (see Appendix S3).

2.4 | Genetic summary

Translocations were predicted to increase genetic diversity and reduce overall genetic divergence among populations. To evaluate this prediction, we calculated a variety of genetic summary statistics using all cohort-specific (age-0 individuals per sample year)

genotypes in each population without purging siblings (see Waples & Anderson, 2017). The R package 'HIERFSTAT' (Goudet, 2014) was used to estimate allele frequencies, mean observed (H_O) and expected (H_S) heterozygosity, mean number of alleles (A_O), and allelic richness (AR; mean number of alleles scaled to smallest sample size). We estimated effective number of breeders (N_b) for each population prior to translocation with the program LDNE version 1.31 (Waples & Do, 2008). Post-translocation LDNe-N_b estimates are not reported because migration has been shown to cause short-term spikes in LD in neutral markers, increasing both bias in LDNe-N_b and the number of significant LD tests (Slate & Pemberton, 2007; Waples & England, 2011). We used Meirmans and Hedrick's unbiased estimator G_{ST}'' (Meirmans & Hedrick, 2011) for estimates of overall and pairwise F_{ST}', and Nei's unbiased estimator of G_{ST} (Nei, 1987) for estimates of overall and pairwise F_{ST}. Both F_{ST}' and F_{ST} were calculated with the R package 'MMOD' (Winter, 2012).

2.5 | Pedigree reconstruction

We reconstructed full-sibling families within each population for the post-translocation 2012 first filial (F_1) cohort using COLONY version 1.2 (Wang, 2004). It is worth noting that previous simulation-based analyses for three of the populations examined here with the same marker panel revealed mean sibship reconstruction accuracies of 91.2% (range 87.4%–93.2%; Whiteley et al., 2012). Mean full-sibling family size was calculated as μ from a fitted negative binomial distribution using the R package 'MASS' (Venables & Ripley, 2002). We summarized the distribution of full-sibling family size by calculating family evenness (FE) for each cohort (Whiteley et al., 2013). FE is the application of Pielou (1975), originally defined for species data, to full-sibling families (FE = 1 indicates equal family sizes). FE was calculated as $FE = \frac{H'}{H'_{Max}}$, where $H' = -\sum_1^S p_i \ln(p_i)$ and $H'_{Max} = \ln(S)$. S is the number of families, and p_i is the proportion of offspring in the ith family.

Assigning offspring to possible cross types (hereafter lineage types) in the post-translocation cohort was central to this study and was determined by parentage assignment. Using PEDAPP version 1.1 (Almudevar, 2007), we assigned parents to each age-0 individual. The potential parents considered were all unique brook trout genotypes (i.e., recaptures excluded) from 2010, 2011 and adult fish captured in 2012 for each population. We then used PEDAGREE version 1 (Coombs, Letcher, & Nislow, 2010) for sibship constrained (SC) parentage assignment using the PEDAPP parentage assignments and the full-sibling family output from COLONY. The SC method was run using a minimum threshold value of 0.2501 for full-sibling families with two members and 0.1667 for full-sibling families with three or more members (Letcher, Coombs, & Nislow, 2011). Individuals in recipient patches that were assigned one transplant parent were considered to be resident-by-transplant first filial (F_1) lineage type (RT). Similarly, offspring assigned two transplant parents were considered to be transplant-by-transplant F_1 lineage type (TT). All other offspring were assigned as resident-by-resident F_1 lineage type (RR).

2.6 | Migrant reproductive success and offspring body size

Translocated individuals from the genetically diverse source population were predicted to have elevated reproductive success relative to resident individuals. To test whether reproductive success (production of families) of translocated individuals was greater than expected under the assumption of random mating, we created a null distribution of family lineage types (RR, RT, TT) for the observed number of families in each population in 2012. We refer to these as tests of neutral introgression at the family level. Deviations from random expectations create the conditions for adaptive introgression, although we did not explicitly evaluate adaptive introgression in this study. This testing was accomplished by drawing a parent for each observed family at random from a binomial distribution where the probability of drawing a transplant as a parent was equivalent to their proportional representation in the estimated 2011 adult (≥age-1) population. The second parent was not treated as independent of the first; therefore if the first parent was a translocated fish, then the probability of drawing a resident fish increased with the decrease in the proportional representation of transplants. We then calculated the proportion of families in each lineage, repeating this procedure 1000 times (see Appendix S4). Introgression into the 2012 cohort (age-0) was calculated as the proportion of parental haplotypes originating from migrants (RR = 0, RT = 1, TT = 2), with the total number of parental haplotypes being equal to two times the number of offspring.

We investigated potential differences in full-sibling family size and offspring body size among the different lineage types (RR, RT, TT) using a Bayesian modelling approach. We predicted that evidence of hybrid vigour might be reflected in these metrics. However, both metrics are potentially influenced by parental body size. Full-sibling family size is intuitively affected by parental size due to size-fecundity relationships, and sexual competition. Individual length may be influenced by maternal investment or heritability of body size (Letcher et al., 2011). To account for this potential bias, we used mid-parent length as a covariate. However, our approach was limited due to incomplete parental assignments and missing body size data for assigned parents that were not captured in 2011. In response, we drew unobserved parental body sizes from a fitted truncated normal distribution in a Bayesian modelling framework. This mitigated the missing data problem and enabled a more biologically relevant modelling of full-sibling family size and offspring body size. Briefly, within the models of full-sibling family size and offspring length (described below) observed parent body sizes were used to fit a truncated normal distribution, and unobserved parent body sizes were imputed from this fitted distribution. The mean of both parents was calculated and used as a continuous covariate. We refer to this covariate as pseudo-mid-parent length (PMP).

To evaluate the influence of lineage type (RR, RT, TT) on full-sibling family size, we used a Bayesian negative binomial family generalized linear model (GLM) with a log link function coded in JAGS version 4.0 (Plummer, 2003). Negative binomial error structure was assumed due to the nature of family size as overdispersed count

data, and the precedence for its use (e.g., Araki, Waples, Ardren, Cooper, & Blouin, 2007; Naish, Seamons, Dauer, Hauser, & Quinn, 2013). We called JAGS using the R package 'R2JAGS' (Su & Masanao, 2015). Our model predicted full-sibling family size as a function of three-category lineage, and PMP. For all parameter estimates, noninformative (flat) priors were used; however, they were constrained within biologically realistic intervals (see Appendix S5). We specified 1,000 adaptive phase iterations and 50,000 estimation iterations with a thin rate of 10.

Individual offspring length for the 2012 F_1 cohort within each recipient population was modelled as a Bayesian linear mixed model (LMM) coded in JAGS using noninformative priors. Offspring length was modelled as a function of lineage, PMP and full-sibling family membership as a random effect. Ten chains were run with 1,000 adaptive phase iterations and 50,000 estimation iterations with a thin rate of 10. For all JAGS implemented Bayesian models, we assessed chain convergence using the potential scale reduction factor (diagnostic values <1.1 indicate good chain mixing). Chain convergence was also visually inspected using the R package 'MCMCPLOT' (Curtis, 2015). Given the explicit biological hypotheses being tested, we did not compare alternative models. Model performance was assessed based on magnitude and variance of parameter estimates.

2.7 | Family-level summary statistics

We calculated a series of family-level summary statistics for the 2012 F_1 cohort including mean individual body size, median distance from barrier (family centroid), median absolute deviation (MAD) of location of family members (family dispersion) and full-sibling family size. The family centroid (median location of the individuals of a given family) was the inferred redd location (Hudy, Coombs, Nislow, & Letcher, 2010). We evaluated the assumption of Gaussian error distribution for variables using a Shapiro–Wilk test. We compared central tendencies of the RR and RT lineage types for offspring body size, family dispersion and family size using either Wilcoxon signed-rank test or Welch's t test with unequal variance. To assess sensitivity to small families that may be less accurately assigned, produce outliers and otherwise influence results (Hudy et al., 2010), we used minimum family size thresholds of 1–5. Families smaller than a given threshold were excluded from the analysis. TT families were omitted due to underrepresentation at the full-sibling family level. Sequential Bonferroni correction was applied for the five tests for each metric and population. These two-sample tests for family size and individual length are intentionally redundant with the GLMs due to the data structure required for each type of analysis.

3 | RESULTS

3.1 | Pretranslocation summary

During the two-year pretranslocation sampling period, we observed low adult and age-0 abundances and indications of loss of genetic diversity. Mean estimated adult census size $(\widehat{N}_c)$ in 2011

TABLE 2 Demographic summary of six brook trout habitat patches by sample year

Site code	Sample year	Adult $\widehat{N}_c$	$\widehat{N}_{YOY}$
SF-a	2010	268 (231–346)	47 (42–130)
SF-a	2011	90 (73–130)	70 (50–117)
SF-a	2013	374 (333–415)	1,189 (913–1,466)
SF-a	2012	84 (75–93)	929 (832–1,025)
DN-a	2010	83 (78–156)	117 (86–367)
DN-a	2011	47 (46–48)	30 (29–31)
DN-a	2012	37 (34–40)	718 (656–781)
DN-a	2013	529 (523–535)	771 (706–836)
BB-a	2010	366 (296–576)	236 (139–457)
BB-a	2011	129 (104–175)	139 (91–215)
BB-a	2012	87 (74–99)	921 (833–1,008)
BB-a	2013	474 (438–510)	1,876 (1,641–2,112)
LR-a	2010	728 (637–873)	463 (347–633)
LR-a	2011	323 (236–438)	677 (519–882)
LR-a	2012	270 (244–297)	3,978 (3,702–4,255)
LR-a	2013	1,147*	2,105*
DV-a	2010	1,982 (1,726–2,202)	1,285 (843–2,077)
DV-a	2011	616 (529–719)	1,009 (795–1,275)
DV-a	2012	555*	3,203*
DV-a	2013	1,759*	4,792*

$\widehat{N}_c$ is the estimated number of adult (age-1 and older) brook trout, and $\widehat{N}_{YOY}$ is the estimated number of age-0 brook trout. Asterisks denote population estimates based on subsamples where confidence interval calculation was unreliable.

(pretranslocation) recipient populations was 147 (range 47–323; Table 2). Over the two-year pretranslocation sampling period, we observed an average decline in $\widehat{N}_c$ of 59% across all five above-barrier populations. In 2011, SF-a and DN-a adult abundances were estimated as 90 and 47, respectively (Table 2). The two smallest populations (SF-a and DN-a) had 33% and 59% reduction in allelic richness and 15% and 49% reduction in mean heterozygosity relative to adjacent below-barrier populations sampled in Whiteley et al. (2013), respectively (Figure 2a). Mean LDNe-N_b for the five above-barrier populations from 2010 and 2011 was 37.3 (range 4.9–75.0) and was depressed relative to adjacent below-barrier populations by 58% on average (Figure 2a). Tests for genetic bottlenecks produced weak support for bottlenecks in DN-a and DV-a, but these tests likely suffered from low power (Appendix S6).

3.2 | Demographic response

Dramatic increases in abundance and habitat use of age-0 brook trout were observed in the cohort following the translocation (hatched in 2012). We observed a mean per cent change in YOY abundance (ΔN_{YOY}) of 1057.6% (range 317.4%–2393.3%) from 2011 to 2012 (Figure 2b). The large post-translocation cohorts of 2012 successfully recruited to age-1 as demonstrated by the large
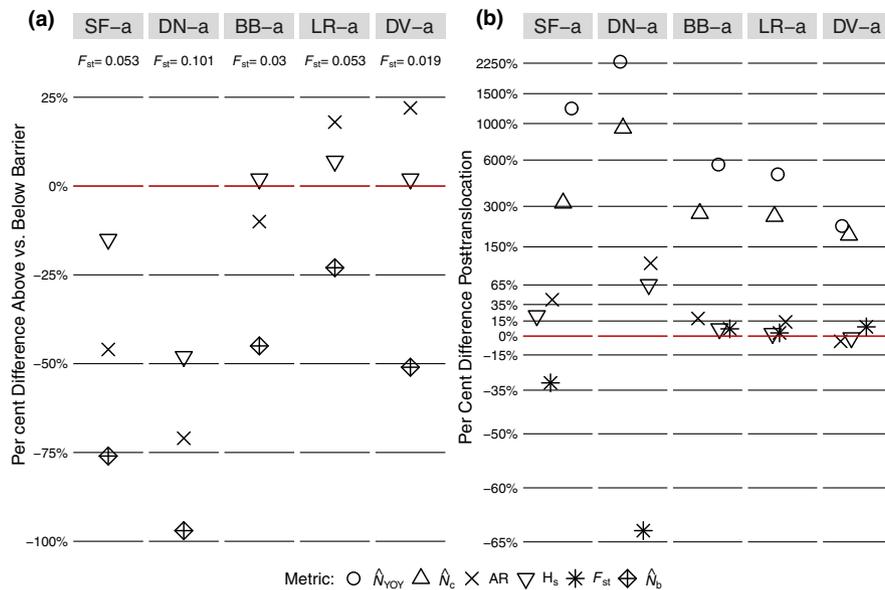
FIGURE 2 Summary of genetic and demographic metrics pretranslocation (a) and post-translocation (b). (a) Per cent difference in genetic diversity (AR and H_S) and effective number of breeders ($\widehat{N}_b$) between adjacent above and below-dam populations before translocation (July–August 2011). Below-barrier data are presented in Whiteley et al. (2013). Initial F_{ST} between adjacent below- and above-barrier populations are reported. (b) Per cent difference following the translocations that occurred in October of 2011 for the following metrics estimated in 2012: estimated abundance of young-of-year ($\widehat{N}_{YOY}$), genetic diversity (AR and H_S) and F_{ST} relative to adjacent below-barrier populations. Per cent difference in adult ($\widehat{N}_c$) abundance was calculated comparing 2011 and 2013 and reflects the recruitment of 2012 age-0 individuals [Colour figure can be viewed at wileyonlinelibrary.com]

increases in adult abundance that were observed from 2011 to 2013 with a mean per cent change of $\Delta\widehat{N}_c = 392.2\%$ (range 185.6%–937.3%; Figure 2b). However, the control site (DV-a) also exhibited an increase in abundance ($\Delta N_{YOY} = 317.4\%$ and $\Delta\widehat{N}_c = 185.6\%$; Figure 2b). The total number of occupied 50 m stream sections by brook trout of all age classes increased by 54.6% (range 10.0%–141.6%) on average from 2011 to 2012 in recipient populations. The number of occupied 50 m stream sections by age-0 individuals increased by 88.0% (range 29.4%–237.5%) on average from 2011 to 2012 in recipient populations. Increase in occupied sections was not calculated for the control due to incomplete sampling, but similar expansions in habitat use were likely (see Discussion below). Using Kendall's τ, per cent change in abundance ($\Delta\widehat{N}_c$ and $\Delta\widehat{N}_{YOY}$) of recipient populations (n = 4) was correlated with increase in occupied 50 m sections (τ = 0.33, p = .8), habitat patch area (τ = −0.67, p = .2) and most strong correlated (inversely) with pretranslocation genetic diversity (τ = −1, p = .04; Table S4).

3.3 | Genetic response

The translocations (fall 2011) of brook trout dramatically increased the genetic diversity within and reduced genetic divergence among recipient populations in the following cohort (hatched in 2012). Mean per cent increase in AR (standardized to n = 27) in the post-translocation cohorts was 45.4% (range 14.0%–108.2%), and mean per cent increase in H_S was 24.1% (range 2.5%–65.7%) in recipient populations (Figure 2b). Across all populations, mean AR (standardized to n = 27) was 6.3 (range 2.8–8.8), and mean H_S was 0.68 (range 0.39–0.80)

(Table 3). Genetic differentiation among populations declined following the experimental translocation. From 2011 to 2012, there was a decrease in pairwise F_{ST} (mean = 35.0%) and F'_{ST} (mean = 32.9%) following the translocation from DV-b to above-barrier populations. F_{ST} between recipient populations relative to adjacent below-barrier populations sampled in Whiteley et al. (2013) was greatly reduced in the two smallest habitat patches, SF-a (31.5%) and DN-a (64.2%), while increasing slightly in BB-a (6.8%) and LR-a (2.9%) (Figure 2b). Principal component analysis also revealed a reduction in genetic divergence among populations following the translocation (Fig. S4).

3.4 | Pedigree reconstruction and migrant reproductive success

In recipient populations, mean reconstructed full-sibling family size was 4.50 (range 1.42–13.15), mean number of full-sibling families per population was 59 (range 12–145), and mean FE was 0.917 (range 0.834–0.975) (Table 3). The mean proportion of successfully assigned parents in recipient populations was 65.4% (range 49.1%–85.4%) (Appendix S8). The RT hybrid lineage type consistently had larger mean full-sibling family sizes across all populations. Mean full-sibling family size in each lineage was RR = 10.0, RT = 14.1, TT = 13.0 across the core populations (Figure 3a). The mean percentage of offspring in each lineage type in the 2012 cohort for the core recipient populations was RR = 55.7%, RT = 39.8% and TT = 4.5% (Table S5). We observed a mean introgression (proportion of alleles of transplant origin) in the core populations of 24.4% (range 20.9%–30.9%) and 4.8% in the subsampled LR-a (Table 4).

TABLE 3 Genetic summary statistics of brook trout cohorts (age-0) from six populations by sample year

Site code	Sample year	N_G	A_O	AR	H_S	$\widehat{N}_{Fam}$	Mean FS	FE	$\widehat{N}_b$
SF-a	2010	41	5.4	4.8	0.542	12	4.2	0.852	10.1 (5.2–15.1)
SF-a	2011	50	4.1	3.9	0.519	14	3.6	0.924	17.1 (10.5–26.5)
SF-a	2012	543	7.9	5.5	0.629	47	13.1	0.914	
DN-a	2010	84	3.4	3.4	0.565	15	3.1	0.925	4.9 (3.8–8.7)
DN-a	2011	27	2.8	2.8	0.392	13	2.1	0.941	40.2 (12.6–∞)
DN-a	2012	546	6.9	5.7	0.649	46	11.9	0.907	
BB-a	2010	129	7.4	6.7	0.731	25	2.9	0.866	26.2 (20.7–33.0)
BB-a	2011	91	7.9	6.6	0.703	30	3.0	0.834	32.6 (26.1–40.6)
BB-a	2012	572	10.0	7.7	0.752	71	8.9	0.897	
LR-a	2010	313	9.5	6.8	0.712	89	3.4	0.886	46.0 (39.6–53.2)
LR-a	2011	383	8.8	6.8	0.717	90	4.2	0.923	53.9 (44.0–65.2)
LR-a	2012	2,333	10.6	7.8	0.735	145	3.8	0.918	
DV-a	2010	403	10.9	8.2	0.780	106	3.6	0.899	66.6 (57.8–76.5)
DV-a	2011	524	11.8	8.8	0.796	139	3.7	0.900	75.0 (60.9–91.4)
DV-a	2012	303	10.5	8.4	0.784	60	1.4	0.975	166.2 (125.6–232.6)
DV-b	2010	99	8.8	7.8	0.777	57	1.7	0.949	191.2 (140.3–279.8)
DV-b	2011	67	9.4	8.0	0.771	41	1.6	0.970	152.8 (111.5–227.2)

Measures are as follows: N_G is the number of genetic samples, A_O is the number of observed alleles, AR is allelic richness (scaled to smallest sample size; $n = 27$), H_S is the mean expected heterozygosity, $\widehat{N}_{Fam}$ is the number of sampled full-sibling families, FE is a family evenness (see Methods for equation), and $\widehat{N}_b$ is the LDNe-based single-sample estimate of the effective number of breeders that gave rise to that year's cohort. The -a or -b suffix on site name and site code denotes above or below dam, respectively.

The majority of translocated individuals reproduced (78%) in the reproductive bout immediately following translocation with observed reproduction from 31 of 40 individuals. In many cases, it appears that translocated individuals moved from their release location within the stream system to spawn (Figure 4). In SF-a, DN-a and BB-a combined, three of the male migrants and four of the female migrants were recaptured in 2012, with a mean recapture rate of 23.3% for these three sites. All five female transplants produced offspring in BB-a and DN-a. Five of the six females reproduced in SF-a. Three of five males reproduced in BB-a and DN-a, while three of the four males reproduced in SF-a. In LR-a, although not comprehensively genotyped, we observed reproduction from four females and three males. There was a general pattern of females producing more offspring in the two smaller populations and males producing more offspring in larger habitat patches. In DN-a and SF-a, translocated females produced 69.1% and 66.4% of offspring with at least one transplant parent, respectively. In BB-a and LR-a, translocated males produced 57.9% and 67.3% of offspring with at least one transplant parent, respectively.

3.5 | Migrant reproductive success and offspring body size

We found evidence for an excess production of hybrid (RT) offspring relative to neutral expectations and consistently larger (but not statistically significant) full-sibling families produced by RT crosses. Results from the randomization test of neutral introgression revealed that there was consistently an excess of RT families and a deficit of RR families in all recipient populations. This pattern was significant ($\alpha = .05$) in BB-a, DN-a and SF-a (Table 4). The fitted Bayesian negative binomial family GLMs provided little support for the influence of lineage on full-sibling family size (Table 5). All slope coefficients for the indicator variable representing RT families were positive, but 95% credible intervals contained zero in SF-a, DN-a and LR-a. However, in BB-a credible intervals for the RT slope coefficient did not include zero, which was consistent with larger hybrid (RT) families relative to RR families. In one site (LR-a), PMP had a positive slope coefficient with credible intervals not containing zero (Table 5).

We consistently observed larger hybrid offspring body size across populations (Figure 3b). The fitted Bayesian LMMs with individual length (F_1 2012 Cohort) as a function of lineage, PMP and family membership (random effect) supported this finding. Mean slope coefficients for hybrid lineage as an indicator variable were positive and had credible intervals that did not contain zero across all populations (Table 5). Mean slope coefficients for TT as an indicator variable were positive in SF-a and DN-a, and weakly negative in BB-a, but all credible intervals contained zero. Mean slope coefficients for PMP were positive in DN-a, BB-a and LR-a and slightly negative in SF-a. Credible intervals of PMP did not contain zero in one site (BB-a).

3.6 | Family-level summary statistics

Family-level t tests were consistent with the trend of larger offspring body size in families with one transplant parent relative to families with two resident parents. All t tests between RR-RT lineage types
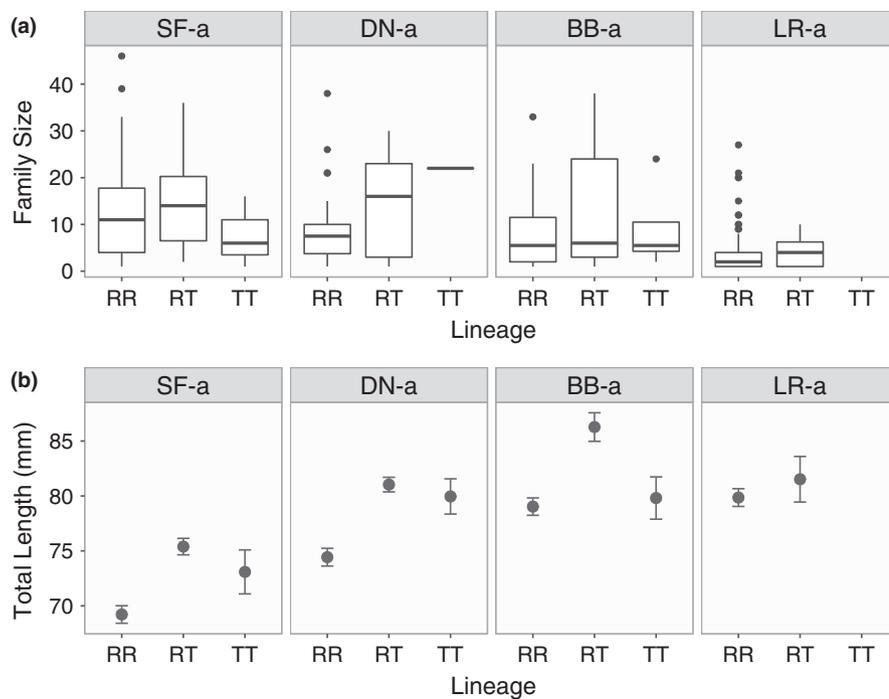
FIGURE 3 Full-sibling family size (a) and body size (b) in the initial cohort (2012) produced following GR translocations. (a) Box plots of full-sibling family sizes from COLONY v. 1.2 sibship assignments by lineage type (RR = resident × resident, RT = resident × transplant, TT = transplant × transplant). (b) Mean and 95% confidence intervals of total length (TL; nearest millimetre) by lineage type. These values are at the individual level and have not been aggregated to the family level

TABLE 4 Results of randomization tests of neutral introgression of translocated brook trout at the family level in the 2012 F_1 cohort

Site code	Transplant representation	Observed introgression	Observed (exp) full-sibling families		
			RR	RT	TT
SF-a	0.1111	0.209	30 (37) $p = .013$	14 (9) $p = .035$	3 (0) $p = .002$
DN-a	0.1961	0.309	24 (20) $p = .058$	21 (15) $p = .022$	1 (1) $p = .492$
BB-a	0.0775	0.216	52 (61) $p = .006$	15 (10) $p = .039$	4 (0) $p = .0$
LR-a	0.0310	0.048	133 (136) $p = .176$	12 (9) $p = .088$	0 (0) $p = .131$

Transplant representation is the proportional representation of translocated individuals in the 2011 $\hat{N}_c$ for each population, and observed introgression is the proportion of migrant alleles in the population. Within each lineage type (RR = resident × resident, RT = resident × transplant, TT = transplant × transplant) and population, the observed and expected (based on binomial expectations) numbers of families are reported. The one-sided p-values from the estimated null distributions are reported (lower-tail probability for RR and upper-tail for RT and TT).

were significant following sequential Bonferroni correction ($\alpha = .05$; initial nominal p-value was .01) in DN-a and SF-a with RT offspring being larger (Table 6). In BB-a, all five t tests for individual length were significant ($\alpha = .05$), with RT lineage type being consistently larger in body size. Following sequential Bonferroni correction (initial nominal p-value = .01), two tests remained significant in BB-a at family size thresholds one and three (Table 6). In LR-a, two t tests for individual length were significant ($\alpha = .05$) at family size thresholds one and two, with RT offspring consistently larger in body size. Following sequential Bonferroni correction (initial nominal p-value = .01), one tests remained significant in LR-a at a family size threshold of one (Table 6).

Family-level tests did not support the general pattern of larger hybrid family sizes and supported greater spatial dispersion of hybrid families in one population (DN-a). Family size and family dispersion were considered non-normally distributed, as supported by a Shapiro–Wilk test, and therefore, Wilcoxon signed-rank tests were used. We found no significant differences between RR-RT lineage types for full-sibling family size or family dispersion in SF-a, LR-a and BB-a. In LR-a and SF-a, dropping small families resulted in larger resident mean full-sibling family sizes relative to hybrid families in a few cases. Interestingly, four tests for family dispersion and full-sibling family size were significant in DN-a at ($\alpha = .05$) with larger family size and greater dispersion in RT families. Insignificant tests occurred at family size threshold of one. Following sequential Bonferroni correction (initial nominal p-value = .01), no significant family size tests and two family dispersion tests remained significant in DN-a (Table 6).
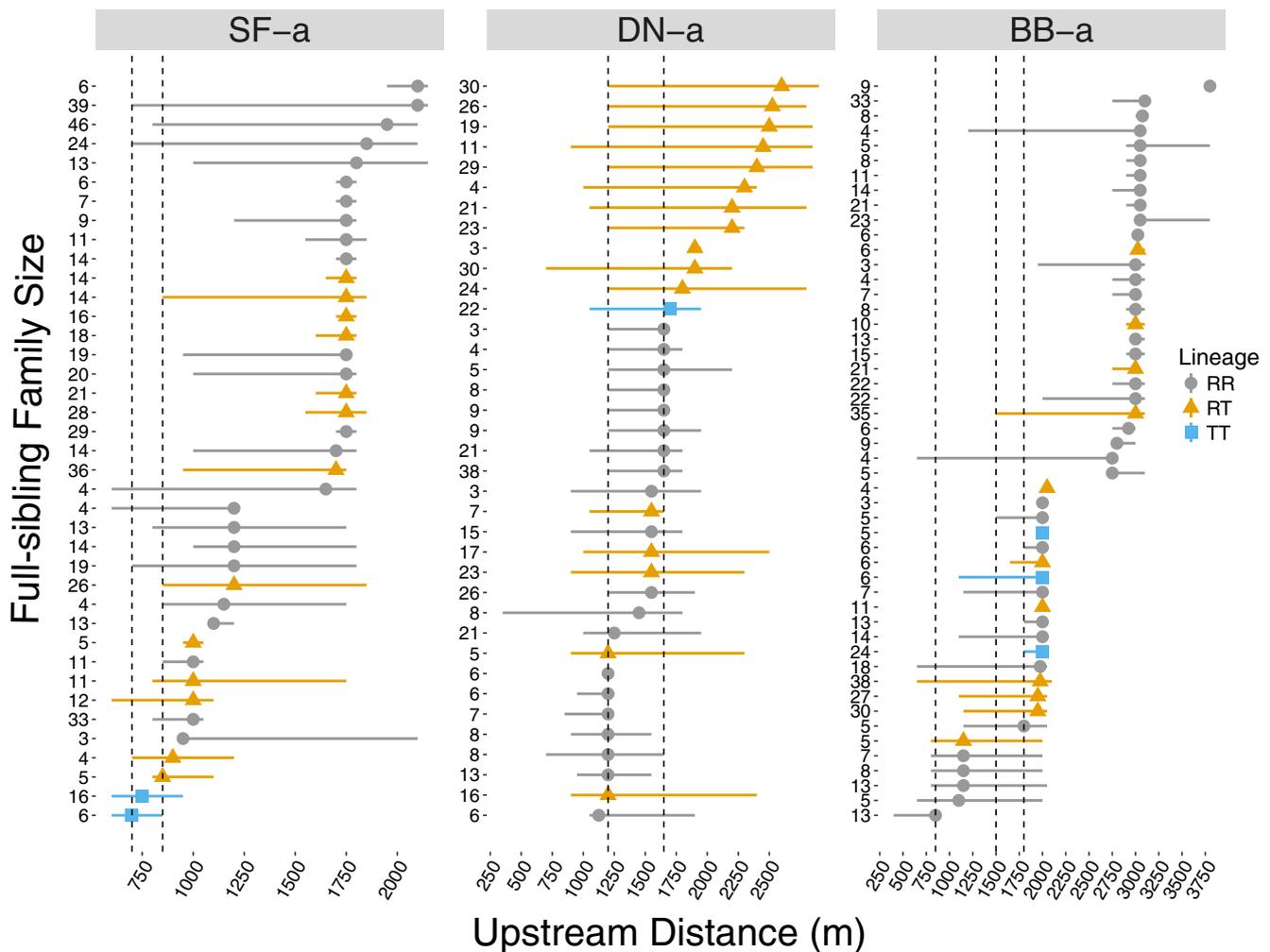
FIGURE 4 Dispersion patterns and inferred redd locations (centroids) of full-sibling brook trout families within three recipient populations (2012 cohort; only full-sibling families with three or more individuals). Each point represents an inferred redd location, and the accompanying horizontal line is range of spatial dispersion from that location at approximately 4 months postemergence. The y-axis (unranked) shows the number of individuals within each full-sibling family. Shape of centroids denotes corresponding lineage type (RR = resident × resident, RT = resident × transplant, TT = transplant × transplant) of each family. The release locations of translocated individuals are presented as dashed lines [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

In this study, we documented consistent evidence of migrant advantage in both translocated individuals and their offspring across four populations. The translocated individuals contributed to more families than expected based on their proportional representation in the population, they produced larger families on average, and hybrid resident-by-transplant offspring were significantly larger in body size than nonhybridized resident offspring. Our rare approach of removing the same number of resident individuals as were translocated isolated genetic effects and allowed us to rule out demographic rescue (see Hufbauer et al., 2015). This study is among few replicated and controlled genetic rescue (GR) attempts in natural populations and to our knowledge the first in salmonids. These findings lend further support to potential effectiveness of genetic rescue as a conservation tool, although further examination will be needed to assess

the fitness consequences of these GR attempts beyond the first generation.

4.1 | Demographic and genetic responses to GR

We observed a dramatic positive response in abundance of age-0 fish following translocations across all populations (including the control), which was likely the result of favourable environmental conditions. We also observed recruitment of these fish into the adult populations producing high abundances of adult fish in 2013 (Table 2; Figure 2b). The low abundances prior to the translocations (in 2010 and 2011) might have been due to regionally low cumulative annual rainfall (Fig. S5). Other studies also documented low brook trout abundance in 2010 in nearby populations (Huntsman & Petty, 2014; Kanno et al., 2015). Further, reports of large year-classes (cohorts) in 2012 (the year following translocations) were

TABLE 5 Parameter estimates for Bayesian negative binomial generalized linear model of full-sibling family size and linear mixed model of 2012 offspring total length (nearest millimetre) as a function of lineage type (RR = resident × resident, RT = resident × transplant, TT = transplant × transplant) and pseudo-mid-parent length (PMP)

Parameters	Family size			Total length		
	Mean	95% Credible interval	$\hat{r}$	Mean	95% Credible interval	$\hat{r}$
	SF-a			SF-a		
Intercept	0.010	−2.847 to 2.866	1.01	75.67	57.90–92.50	1.03
RT	0.189	−0.365 to 0.762	1.00	**5.34**	**1.75–8.88**	1.00
TT	−0.421	−1.470 to 0.813	1.00	3.28	−3.39 to 9.91	1.00
PMP	0.017	−0.002 to 0.037	1.01	−0.03	−0.11 to 0.06	1.03
Deviance	338	330–347	1.11	9,886	9,847–9,913	1.02
	DN-a			DN-a		
Intercept	−0.077	−2.856 to 2.832	1.00	37.79	23.64–75.50	1.06
RT	0.370	−0.175 to 0.912	1.00	**5.29**	**2.59–8.05**	1.06
TT	1.030	−0.532 to 2.718	1.00	5.79	−2.49 to 13.84	1.03
PMP	0.016	−0.004 to 0.035	1.00	0.19	−0.03 to 0.26	1.10
Deviance	321	304–329	1.16	7,382	7,242–7,535	1.05
	BB-a			BB-a		
Intercept	−0.046	−2.861 to 2.857	1.01	59.95	50.65–69.87	1.01
RT	**0.563**	**0.026–1.127**	1.00	**4.04**	**0.43–7.74**	1.00
TT	0.264	−0.668 to 1.341	1.00	−0.61	−6.74 to 5.55	1.00
PMP	0.014	−0.006 to 0.033	1.01	**0.10**	**0.05–0.15**	1.01
Deviance	455	384–466	1.10	13,753	13,714–13,791	1.00
	LR-a			LR-a		
Intercept	−1.944	−2.948 to −0.853	1.03	72.45	63.72–81.92	1.01
RT	0.210	−0.334 to 0.739	1.00	**5.21**	**0.93–9.53**	1.00
PMP	**0.020**	**0.012–0.028**	1.03	0.03	−0.02 to 0.07	1.01
Deviance	565	525–686	1.02	8,757	8,710–8,803	1.00

Mean estimate, 95% credible interval and potential scale reduction factor ($\hat{r}$) are reported. Total length was modelled with family membership as a random effect. Parameters with credible intervals not overlapping zero are shown in bold.

widespread throughout the US native brook trout range (J. Coombs, unpublished data). This suggests that favourable environmental conditions are the mostly likely explanation for the observed increases in abundance across our study populations. The prominent role of regional environmental conditions influencing abundance highlights the importance of our incorporation of a control population in our experimental design, something that has been lacking in other GR studies (Fredrickson, Siminski, Woolf, & Hedrick, 2007; Hedrick, 1995; Hogg et al., 2006; Vilà et al., 2003; Westemeier et al., 1998). It is worth noting that the proportional increases in abundance were greater in all recipient populations than the control, which suggests that the demographic response was greater than it might have been in the recipient populations had we not induced gene flow.

The experimental pulse of gene flow from 10 translocated individuals into each of the four GR populations led to a strong increase in within-population genetic diversity and a reduction of genetic divergence among populations. After one round of reproduction of the translocated fish, H_S and AR greatly increased (Figure 2b) and approached below below-barrier levels. Yamamoto et al. (2006)

documented a similar increase in H_S and AR following translocations into above-dam populations of whitespotted char (*Salvelinus leucomaenis*) after one year. This is the only other study in salmonids where below-barrier fish were moved to above-barrier populations, and a response in genetic diversity was measured, but the lack of analysis of effects on fitness characters or abundance prevents evaluation of this study in the context of GR. We also observed reduced population differentiation among recipient populations compared to the pretranslocation baseline, as expected from restoring (albeit artificially and temporarily) connectivity within a former metapopulation. Although genetic differentiation between recipient populations and adjacent below-barrier populations sampled in Whiteley et al. (2013) was greatly reduced in the two smallest populations (SF-a and DN-a), it increased modestly in BB-a and LR-a. This increase is most likely due to low initial genetic differentiation between adjacent above and below-barrier populations in BB-a and LR-a and introducing new alleles as a result of translocations.

In this study, we found that the observed increases in abundance of adults and young-of-year were correlated most strongly with

TABLE 6 Two-sample tests of total length (nearest millimetre), full-sibling family size (FS), and family dispersion by between RR (resident × resident) and RT (resident by transplant) lineage types at the family level

Site code	Total length $\bar{x}_D$	Significant tests
SF-a	−4.9 (−5.6 to −4.5)	5
DN-a	−5.6 (−6.3 to −4.6)	5
BB-a	−5.3 (−7 to −4.3)	2[1,3]
LR-a	−3.6 (−6.1 to −1.3)	1[1]
	Full-sibling FS $\bar{x}_D$	
SF-a	−0.5 (−2.4 to 0.8)	0
DN-a	−6.7 (−8 to −4.3)	0
BB-a	−6.4 (−7.3 to −5.5)	0
LR-a	0.2 (−1.1 to 1.8)	0
	Family dispersion $\bar{x}_D$	
SF-a	29.8 (15.7 to 38.3)	0
DN-a	−182.6 (−242.2 to −94.6)	2[4,5]
BB-a	12.4 (3.7 to 31.7)	0
LR-a	12.2 (−62.4 to 100.9)	0

For each population, there were five tests per metric with one test at each FS threshold (1–5). Sequential Bonferroni correction was applied across the five FS thresholds within each metric and population. The numbers of significant tests are reported with the threshold FS at which each occurred (given as a superscript). The means of mean differences ($\bar{x}_D$) and range between RR–RT lineage types across all FS thresholds are reported. Welch's *t* tests were used for total length, and Wilcoxon rank-sum tests were used for family size and family dispersion.

initial genetic diversity, rather than metrics reflecting habitat amount or availability (Table S4). Power limitations ($n = 4$) demand a cautious interpretation of this result, but it does support our a priori prediction that GR effect should be strongest in the most genetically depauperate populations. This provides support for the general importance of maintaining genetic diversity in small populations and adds to the growing understanding of the importance of genetic diversity in small salmonid populations. For example, a recent study of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) that translocated embryos from five sources were translocated into a vacant habitat found that the juveniles from the most genetically diverse source populations had higher survival (Andrews et al., 2016).

4.2 | Migrant success

We found a consistent pattern of high relative reproductive success for translocated individuals across sites, the most dramatic of which was in DN-a, where 30.9% of alleles were of transplant origin in the 2012 cohort. The randomization test of neutral introgression provides statistical support for this finding, by demonstrating a surplus of transplant-produced families compared to expectations from random mating (Table 4). Mean full-sibling family size was greater in hybrid families relative to resident families across all sites (Figure 3a); however, statistical tests and modelling did not support this pattern

in most cases. A post hoc power analysis revealed that these tests suffered from low power (Table S6). This power analysis revealed that the high variance in full-sibling family sizes made statistical validation of potentially biologically significant effect sizes quite difficult. Although family size is of great biological interest, attempts to statistically validate patterns will suffer from low power when appropriate probability distribution (negative binomial, see Araki et al., 2007) or nonparametric approaches are applied.

We found that hybrid offspring captured in 2012 were significantly larger than nonhybrids across all recipient populations. This is consistent with heterosis in somatic juvenile growth in hybrid (transplant-by-resident) offspring. Heterosis may be evidence that inbreeding depression and genetic load was alleviated through masking deleterious recessive alleles or restoring heterozygosity at overdominant loci (Charlesworth & Willis, 2009). Additionally, apparent heterosis demonstrates that a severe mismatch in local adaptation (as would be expected under F_1 outbreeding depression; Tallmon et al., 2004) between donor and recipient populations is unlikely. Body size is correlated with key demographic processes in brook trout such as growth, survival, reproduction and movement (Letcher et al., 2011). However, the selection regime on body size differs greatly across multiple temporal, spatial and environmental gradients (Xu, Letcher, & Nislow, 2010), thus making it difficult to infer the fitness of a given body size. During the first year of life, body size may be more directly related to fitness due to the influence of swimming ability on survival and resource acquisition (Nislow & Armstrong, 2012; Tetzlaff, Soulsby, Gibbins, Bacon, & Youngson, 2005). The ability of a brook trout juveniles to generate rapid somatic growth is likely closely related to survival (Elliott, 1993; Milner et al., 2003). Stream-dwelling brook trout can maintain their juvenile size differences throughout life, which may have cascading fitness consequences by affecting dominance hierarchies, sexual selection and resource competition (Letcher et al., 2011).

Family dispersion from putative redd sites may be related to survival, particularly in an intermittent stream (Davey & Kelly, 2007), and may also influence somatic growth through density-dependent interactions. Our results suggest that offspring produced by translocated individuals used habitat differently in DN-a compared to the other sites. In DN-a, families with transplant parents were more spatially dispersed relative to offspring of resident-by-resident lineage (Table 6; Figure 4). In all other sites, we found nonsignificantly greater family dispersion in resident families. Future work on these populations will continue to investigate movement and habitat use of translocated individuals and their offspring.

4.3 | Caveats

A concerted effort was made to demonstrate that characteristics of translocated individuals (body size and maternal effects) are unlikely sources for the observed patterns. We demonstrated that selected transplants were not larger than the adults in resident populations (Fig. S3), and based on length–fecundity relationships, it is unlikely that the translocation resulted in a biologically significant increase in

the number of eggs within recipient populations (Appendix S7, Fig. S8). We also attempted to control for parental body size by including an estimate of mid-parent length (PMP) as a covariate in our models of full-sibling family size and offspring body size. Further, sex-specific effects of migrants (e.g., maternal investment) do not appear to explain the observed pattern of hybrid vigour (Table S7). Despite these caveats, our results indicate a clear reproductive advantage of translocated individuals and are consistent with a fitness advantage (somatic growth) in hybrid offspring relative to non-hybridized resident offspring.

4.4 | Broader implications and conclusions

The magnitude of GR effect and the risks of outbreeding depression depend on characteristics of the populations receiving translocated individuals (those being rescued) and the source population of translocated individuals. Proposed guidelines to avoid outbreeding depression focus on reducing the environmental differences and genetic divergence between recipient and donor populations (Frankham et al., 2011; Weeks et al., 2011).The populations examined have been isolated for 50 years on average, which is approximately 25 brook trout generations (Letcher et al., 2007). Further, we moved fish a maximum of 100 stream kilometres within two adjacent subwatersheds with very similar environmental conditions, which likely represent a formerly interconnected system of populations (Whiteley et al., 2013). Given these conditions, we predict minimal effects of outbreeding depression and predominately positive effects of gene flow.

The 10 translocated individuals in our experimental pulse of gene flow were more successful than our initial expectations and thus raised concerns of genetic swamping. Genetic swamping is the process by which extensive hybridization leads to the substantial reduction in frequency of locally adapted genotypes (Hedrick, 1995). Although widely recognized as undesirable, genetic swamping is poorly operationally defined in the literature. One proposed threshold is >50% of alleles are of migrant origin in the population (Frankham, 2015). Hedrick (1995) suggests targeting ≤20% gene flow to avoid swamping local adaptation in Florida panthers. The proportion of transplant alleles in SF-a, DN-a and BB-a ranged from 20.9% and 30.9% and therefore falls below the 50% threshold proposed by Frankham (2015) and modestly exceeds the 20% target gene flow in Hedrick (1995). The reproductive success we observed emphasizes the need for caution in GR-motivated translocations to avoid genetic swamping. This may be particularly important in dynamic systems with high fecundity and variance in reproductive success such as these populations.

Many organisms of conservation concern exist in reduced or severely fragmented habitats where there are logistical limitations to increasing available habitat or to the construction of migration corridors to address problems of reduced connectivity and isolation (Fahrig, 2003; Jamieson & Allendorf, 2012). Habitat patches in these circumstances may be inadequate in size and connectivity to support self-sustaining populations of certain taxa (e.g., Harig & Fausch,

2002), particularly in changing environments (Aitken & Whitlock, 2013). If habitat constraints are not alleviated, active management facilitating migration may be critical for long-term demographic and genetic maintenance of these populations. Regardless of the motivation for translocations (genetic or demographic; Hufbauer et al., 2015), an empirically based understanding of their consequences across taxa, life history and extent of divergence is necessary to increase confidence in conservation applications.

Here, we have demonstrated tremendous reproductive success of translocated individuals and consistently larger body size of hybrid offspring in their first summer. We found that, as predicted, demographic increases over the study period scale to patch size and most strongly with initial genetic diversity. This suggests that the documented fragmentation-mediated genetic erosion (Whiteley et al., 2013) was reducing fitness of resident brook trout. Until future cohorts are examined we cannot rule out the potential for outbreeding depression during subsequent generations. However, results to date are consistent with genetic rescue and are highly promising for this underused management option. Monitoring will continue to examine the multigenerational consequences of these GR-motivated translocations.

DATA ACCESSIBILITY

Sampling locations, morphological measurements and microsatellite genotypes are available at Dryad https://doi.org/10.5061/dryad.656jr.

AUTHOR CONTRIBUTIONS

Z.L.R. conducted field and laboratory work, data analysis and wrote article. J.A.C. assisted in study design, field work, data analysis and manuscript revisions. M.H. assisted in study design, field work and manuscript revisions. K.H.N. assisted in study design and manuscript

revisions. B.H.L. assisted in study design and manuscript revisions. A.R.W. assisted in study design, field work, data analysis and manuscript revisions.

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