Monitoring Demographic and Genetic Responses of a Threatened Inland Trout to Habitat Reconnection

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Abstract

Freshwater fishes living in streams and rivers can be affected strongly by isolation, which causes a disproportionate degree of fragmentation in such dendritic systems. Isolation disrupts important ecological and migratory processes as well as the ability to access refuge habitats during disturbances. The restoration of habitat connectivity, then, should be a productive strategy for improving the resiliency of freshwater fish populations, but the local and broader ecological benefits of barrier removal are still poorly understood. We report on a long-term, spatially intensive effort to monitor the responses of inland trout to stream habitat reconnection at a watershed scale, using both demographic and genetic techniques. Individual-based genetic assignment uncovered clear evidence of movement into the primary tributary of interest, which had been blocked by an assumedly complete barrier, but the source population generating this movement varied over time. A linear mixed-effect model suggested trout densities in this target stream increased threefold and the population sustained more large migratory-sized individuals after habitat reconnection. Densities overall did not respond to or variable spring flows which occurred during the course of the study, but a negative parameter estimate for “year” suggested a possible decline in densities over the study period (although 95% CIs barely overlapped with 0). Population genetic metrics showed no change in population differentiation or metrics of genetic diversity in most cases, except for a significant decline in allelic richness in the target population. The effective biological reconnection documented in our study should improve population resiliency in the future, but we discuss the benefits of spatially intensive monitoring at a watershed scale and combined inference from both demographic and genetic metrics to uncover unexpectedly complex fish responses to habitat reconnection.

As with other freshwater aquatic organisms, inland trout can be particularly vulnerable to isolation because the fragmentation of dendritic aquatic systems can have disproportionately negative influences on local demographic and metapopulation dynamics (Fagan 2002; Lake et al. 2007). Additionally, trout express complex migratory life histories and reliance on different habitat characteristics at different life stages, necessitating access to diverse, complementary (Dunning et al. 1992), often geographically dispersed parts of stream networks (Rieman and Dunham 2000; Dunham et al. 2002; Fausch et al. 2002). Spatial and temporal refuge from disturbances such as floods, drought, inhospitable temperatures, and fires is also increasingly recognized as essential to their persistence (Sedell et al. 1990; Gresswell 1999; Dunham et al. 2003; Lake et al. 2007; McCullough et al. 2009; Ebersole et al. 2015). In western North America, isolation has been a significant factor in the decline of native trout, and remaining populations are often restricted to small headwater stream reaches, where long-term persistence may be tenuous and the loss of genetic diversity can be marked (Dunham et al. 2002; Taylor et al. 2003; Wofford et al. 2005; Neville et al. 2006b, 2009; Haak and Williams 2012).

The Lahontan Cutthroat Trout Oncorhynchus clarkii henshawi (LCT)—endemic to the northern part of the Great
Basin Desert of Nevada, Oregon, and California—is no exception. It was one of the first taxa listed under the Endangered Species Act, due primarily to habitat degradation, fragmentation, and nonnative species (Coffin and Cowan 1995; USFWS 2009). Range-wide, LCT is confined to only 10% of its historical stream habitat and is typically isolated in small headwater stream fragments, where populations are at risk and continue to be extirpated (Dunham et al. 1997; Elliott et al. 1997; USFWS 2009). In the few remaining larger, interconnected watersheds LCT seemingly sustain migratory life histories, where both spatial and temporal segregation of resident and migratory fish and metapopulation dynamics may be important for population persistence and the retention of genetic diversity (Neville et al. 2006b).

Compared with other types of habitat improvements, then, the removal of passage barriers may have the largest effect on population responses (Roni et al. 2008) and may be especially helpful for a desert fish like LCT in improving resiliency to a dynamic environment and climate change. However, to date there exist few examples where responses to reconnecting habitat have been monitored for any inland trout species (but see Neville and Peterson 2014; Whiteley et al. 2014; Chelgren and Dunham 2015), even though such information would provide helpful validation of the efficacy of barrier remediation to guide future efforts (Rolls et al. 2013). Furthermore, as most connectivity projects are implemented and monitored only at local scales (stream reach scales), the broader ecological effects of restoration are still poorly understood (Bernhardt et al. 2005; Lake et al. 2007; Rolls et al. 2013). The thoughtful monitoring of biological responses to reconnection “experiments” could contribute essential insight for our understanding of the population dynamics of fishes historically found in large interconnected stream networks (Lake et al. 2007). We describe the results from a long-term monitoring study of responses to watershed-scale reconnection in a native inland trout, where habitat reconnection was expected to foster movement among tributaries and boost genetic diversity and fish abundances, particularly in what was previously the most isolated tributary in the system. Our sampling design and combined inference from both demographic and genetic data allow for a unique view of responses at a watershed scale, and may provide a useful foundation for understanding the benefits of connectivity for other stream fish populations.

**METHODS**

*Background.*—Maggie Creek is one of the larger tributaries to the Humboldt River, which meanders east to west across the Lahontan basin of northern Nevada (Figure 1, inset map). This high desert basin is generally dominated by sage brush *Artemisia* spp. in the uplands, while stream corridors house a mix of herbaceous and woody riparian vegetation, including willows *Salix* spp. The watershed spans elevations from 1,500 to over 2,700 m, and drains approximately 1,600 km². Average precipitation varies from 18 cm at the lowest reaches of the main-stem river to 63 cm in the higher elevations (Horton 2000).

Historically, the Maggie basin sustained multiple tributary populations of LCT that would have accessed the main-stem Maggie Creek and the Humboldt River as overwintering and migratory habitat (Behnke 1992; Coffin and Cowan 1995; Horton 2000). Over the 20th century, however, a combination of habitat degradation caused by intensive livestock use, water withdrawal for mining, and habitat fragmentation from road culverts isolated populations in three primary tributaries: Little Jack, Coyote, and Beaver creeks (Figure 1). These tributary streams are relatively small; wetted widths range from approximately 1–3 m, except in beaver ponded areas (H. Neville, unpublished data). Beaver Creek is the most complex of the three major tributaries, with the distribution of LCT here spanning over 30 km across several smaller tributaries (including Williams Canyon and Toro Creek; Figure 1); LCT in Little Jack Creek, in contrast, occupy less than 6 km of the stream. Beginning in the early 1990s, the Maggie Creek basin became the focus of a long-term collaborative restoration effort to offset mining impacts and to benefit LCT recovery. Restoration was initiated by a suite of partners, including the U.S. Bureau of Land Management (BLM), Newmont Mines, and several mine-owned and private ranches. Habitat restoration focused primarily on implementing a combination of seeding, grazing rest, and strategic rotational grazing schemes, largely in the main-stem river corridor and lower sections of tributaries (generally below habitat occupied by LCT in the summer). By the early to mid-2000s, the habitat conditions in restoration areas had greatly improved, showing decreased sediments, increased sinuosity and beaver ponding, and generally improved riparian cover (Simonds et al. 2009; White Horse Associates 2011; Evans and Snyder 2012), but stream isolation still remained a problem.

Accordingly, in the fall of 2005 the culverts at the base of each tributary (Beaver, Little Jack, and Coyote creeks) and an irrigation diversion below the confluence of Beaver Creek in the main-stem Maggie Creek (Figure 1, open and hatched circles) were removed and replaced with new structures intended to provide fish habitat connectivity among the three streams. Fortuitously, no nonnative trout occurred in Maggie Creek, and so the decision to reconnect these native trout populations was straightforward in comparison with the more complex considerations necessary in other trout systems, where native trout populations are often intentionally isolated to prevent invasion by nonnative trout (Novinger and Rahel 2003; Rahel 2013). Still, a permanent barrier was installed at the base of the system (off the map downstream of Figure 1) to protect the newly connected populations from invasion by nonnative fishes from the Humboldt River, such as Rainbow Trout *O. mykiss* and Smallmouth Bass *Micropterus dolomieu.*
FIGURE 1. Lahontan Cutthroat Trout sample sites in Maggie Creek, northern Nevada. Lahontan Cutthroat Trout occupy three primary tributaries outlined in blue: Little Jack, Coyote, and Beaver (including Toro Creek and Williams Canyon) creeks. Culverts in Little Jack and Coyote creeks were assumed seasonally passable (shown as open circles), while the culvert at the base of Beaver Creek and in the main-stem Maggie Creek (shown as open circles marked with an ×) were thought to be complete fish barriers except possibly in the highest flows; all four culverts were replaced in 2005 with structures designed to allow fish passage. Black dots show locations of 100-m fish sampling sites, and the different hatched perimeters outline fires which occurred during the course of the study.
Above this barrier, watershed-scale reconnection was expected to restore Maggie Creek to one of the largest interconnected systems currently available to LCT rangewide (approximately 130 km of seasonally accessible habitat). Thus, the goal was to reconnect as much habitat as possible to encourage movement and life history diversity, while protecting the whole interconnected restoration area from invasion. It is important to note, however, that despite high interannual variability, summer flows in this desert system are relatively low, with an average monthly discharge of 0.04 m$^3$/s in the main-stem Maggie Creek from July to September during the study period (based on U.S. Geological Survey [USGS] gauge 10321940 in Maggie Creek downstream of sampled tributaries), such that in drier years the lower reaches of Little Jack and Coyote creeks are typically desiccated and these tributaries often do not connect to the main-stem Maggie Creek. It was assumed, then, that even after physical reconnection was achieved, LCT were only likely to migrate among tributaries sporadically, in years of relatively high flow. Improvements observed in main-stem habitat over the past few decades may allow for limited (but unsubstantiated) overwintering use and access to growth habitat in the main-stem Maggie Creek, but this habitat is still sub-optimal in summer in terms of stream temperatures, substrate, and cover; only one LCT was ever captured here by field crews (see below). The expected benefit of the reconnection was thus primarily to allow migratory movement to and from the main-stem river and among tributaries, not necessarily to increase the year-round use of main-stem habitat.

To document LCT responses to watershed-scale reconnection, in 2001 Trout Unlimited established 44 sample sites to begin prerestoration monitoring on the populations in the three primary tributaries—Beaver, Coyote, and Little Jack creeks (Figure 1). Unfortunately, prerestoration measurements of the culverts were not taken to determine their true status as barriers (e.g., Clarkin et al. 2005), but the culverts at the base of both Little Jack and Coyote creeks were assumed to be only partial barriers, estimated to impede consistent upstream passage but likely allowing for occasional movement during times of sufficient flow. The two structures below Beaver Creek (a road culvert at the base of Beaver Creek and an irrigation diversion in the main-stem Maggie Creek; Figure 1), in contrast, were thought to be complete barriers to upstream movement into Beaver Creek, save for exceptional flow events when it may have been possible for fish to circumvent the structures (see Discussion).

Because it was the most isolated, we predicted the Beaver Creek population to have the greatest demographic and genetic response to restored connectivity, and here expected the immigration of fish from Little Jack and Coyote creeks to lead to increased LCT abundances and more complete size distributions (Neumann et al. 2012), possibly reduced genetic differentiation and increased genetic variability (see caveats below), and direct evidence of immigration captured by individual-based genetic approaches. Because the creeks were previously only partially isolated, the population attributes in Little Jack and Coyote creeks were predicted to be largely unaffected by restored connectivity, though some influence of the reconnection of Beaver Creek on these populations would not be unexpected if Beaver Creek fish now emigrated more freely and thus were more likely to move to these tributaries and affect population characteristics in all streams.

Fish sites, sampling, and abundance estimation.—Forty-four fish sampling sites were established systematically throughout habitat occupied by LCT in each of the three tributary creeks. Due to differences in stream length, these sites were distributed at 0.5- and 1-km intervals in Little Jack and Coyote creeks, respectively, and 2-km intervals in Beaver Creek. Sites were sampled during annual low flows in August and September in 2001–2007, 2009, and 2012. Beaver Creek was not sampled in 2001 due to a fire affecting much of its headwaters (Coyote Fire; Figure 1), and Little Jack Creek (the smallest and most ephemeral of the three tributaries) was not sampled in 2002 or 2004. Each sample site was 100 m in length. The upper and lower ends of sites were blocknetted, and depletion electrofishing was conducted using two to four passes. Trout were assumed to be absent from a site if no fish were captured on the first pass. If fish were captured in the first pass, a second was always completed, and a third or fourth pass was conducted if >20% of the total number of fish from the first two passes were caught in the second or third pass, respectively. Fish were counted and measured to the nearest millimeter (TL). In 2001, 2002, 2007, 2009, and 2012, for all fish excluding age-0 fish (see below) a small fin tissue sample was taken and desiccated in a coin envelope for genetic analysis. Fish were released in the sample site after sampling. In several sampling years, intensive efforts were made to capture fish in the main-stem river via electrofishing, a temporary weir, and angling, but only one LCT was captured via electrofishing in 2002.

The abundance of trout at each 100-m sampling site was estimated using the maximum likelihood depletion estimator in MicroFish 3.0 (Van Deventer and Platts 1985; Van Deventer 1989). Length frequencies showed a relatively clear break in size at 80-mm TL, and fish <80 mm were identified as age-0 and removed from abundance estimation as they had not yet recruited to the population (Maceina and Pereira 2007). For each stream in each year, we used abundance estimates from MicroFish to calculate the mean trout density (number per 100 m) across index sites (including dry and fishless sites). Given that we sampled the same index sites at every sampling event, this information should provide valuable insight as to the relative demographic responses to improved connectivity.

We evaluated the effect of fish passage restoration in 2005 on LCT abundance by using a linear mixed-effect model. This approach allowed us not only to evaluate the impact of passage restoration on LCT density but also to account for the potential effects of unanticipated wildfires, an expected influence of temporally variable spring flows on recruitment, and “other” influences not directly evaluated. The response
variable in the model was log-transformed age-1 and older LCT density \(\log_{10}(N/100 \text{ m}^2 + 1)\). The effect of barriers was evaluated using a categorical variable whereby “complete barrier” (Beaver Creek) and “partial barriers” (Little Jack and Coyote creeks) were compared with a baseline of “no barrier” (i.e., for years after barriers were removed), with each having its own parameter (complete = 1, no barrier = 0; partial = 1, no barrier = 0). Fire was included as a categorical variable, whereby for 3 years following a fire sample sites within a burn perimeter were classified as burned (burn = 1; no burn = 0); after relatively short-term (several years) responses to fire, trout abundances have typically been observed to rebound (Gresswell 1999; Neville et al. 2009; Rosenberger et al. 2015). Spring flow in the year prior to each sample year (spring flow \(t - 1\)) was also included as a covariate, as high flows in the previous year have been observed to influence LCT recruitment positively in other systems (Ray et al. 2007). Average stream flow for spring (March through June) was based on measurements from USGS gauge 10321940 in Maggie Creek downstream of sampled tributaries. Spring flow was treated strictly as a temporal, not spatial variable; that is, the same flow was used for all sites within a year (Figure 2a). We included year as a fixed effect to account for any monotonically trends in LCT densities across our study time frame; we anticipated this effect could potentially reflect continually improving habitat conditions in Maggie Creek as a result of long-term grazing management, but it may also capture any unmeasured broad influence affecting LCT populations (e.g., climate). Last, because sites were resampled over time, each site was included as a random effect to account for the influence of the other covariates. The model was fit using the lmer function in program R (Development Core Team 2009).

**Genetic analyses.**—We incorporated two types of genetic approaches into our monitoring study. “Indirect” population-level genetic methods require a priori definition of population structure (Manel et al. 2005), and population dynamics are inferred from observed genetic patterns via mathematical equations (see Neville et al. 2006a). Although they have widely been promoted as useful for monitoring population changes over time (e.g., Schwartz et al. 2006; Luikart et al. 2010; Saura and Faria 2011; Hoban et al. 2014), their application to monitoring responses to restoration activities may prove difficult. Response times are expected to be long (Raeymaekers et al. 2009; Langduth et al. 2010), and natural populations—especially those impacted by restoration—often inherently violate theoretical assumptions such as equilibrium population dynamics, which can complicate interpretation (Whitlock and McCauley 1999; Osborne et al. 2012; Neville and Peterson 2014). Still, we cautiously evaluated two metrics of population genetic diversity as well as genetic differentiation between pairs of populations over time because these metrics are commonly used for monitoring and may provide information complementary to demographic data as well as useful baseline insight about the overall (and possibly changing) “genetic health” of populations (e.g., Dunham et al. 1999). As a “direct” genetic measurement of movement more appropriate for the temporal scale and dynamics of our particular question, we also used information in individual genotypes to identify individuals in our samples that had likely moved between tributaries (Paetkau et al. 2004; Manel et al. 2005; Hudy et al. 2010).

In years when given population samples exceeded our budget for laboratory analyses, we subsampled genetic collections by retaining a random set of individuals from each sample site to ensure spatial representation within each population. Genotypes of retained individuals were obtained for 11 polymorphic microsatellite loci developed specifically for LCT (OCH 5, 6, 9, 13, 14, 16, 17, 21, 22, 24, 30), with primer sequences and laboratory protocols outlined in Peacock et al. (2004) and Robinson et al. (2009). We defined a “population” sample for a given year as including fish combined across all sample sites within a tributary. We used FSTAT version 2.9.3.2 (Goudet 2001) to perform population-level genetic estimations, adjusting the critical significance level for bootstrap resampling results to account for multiple tests. We first tested for Hardy–Weinberg equilibrium in each sample at each locus (Goudet 2001) as well for linkage equilibrium between each pair of loci across all samples. Changes in effective population size \(N_e\) would be the ideal measurement of genetic diversity and population genetic “health,” but the most appropriate approach to estimating \(N_e\) for our need to contrast point estimates across short time periods—the linkage disequilibrium method (LD method; Hill 1981)—would be directly compromised by the changes in movement expected in this study (Luikart et al. 2010), making predicting responses for this metric difficult. Recent “pulse” migration can actually decrease \(N_e\) as estimated by the LD method (Waples and England 2011; Neel et al. 2013), and changes in reproductive success can have similar effects (Waples and England 2011); accordingly, we chose not to evaluate \(N_e\) for our particular monitoring question. Instead, within-population genetic variability was (still cautiously) estimated each year for each tributary sample using Nei’s unbiased heterozygosity \(H_u\) and allelic richness \(R_a\), a rarefied estimate of the number of alleles that is independent of the sample size and which has been demonstrated to be particularly sensitive to population bottlenecks or isolation (El Mousadik and Petit 1996; Petit et al. 1998; Neville et al. 2009). Pairwise population genetic differentiation and statistical significance was evaluated by estimation of \(F_{ST}\) in FSTAT, and we used the fastDivpart function in the diveRsity package version 1.9.73 in program R (Development Core Team 2009) to generate 95% CIs around \(F_{ST}\) estimates by bootstrapping across loci. We also calculated...
theta in FSTAT as a metric of global population differentiation each year (with 95% CIs generated by bootstrapping across loci). We expected that, if any population-level response was observed, restored connectivity (relevant to our 2006–2012 samples) would lead to decreased differentiation (globally, in pairwise contrasts involving Beaver Creek, and possibly for all pairwise contrasts) and also may cause slight increases in $H_e$ and $R_s$ in Beaver Creek if migration led to an influx of new alleles in this population.

Before performing these and further genetic analyses, however, we first evaluated family structure in our samples, as inclusion of siblings can bias both population-level and individual-based genetic metrics (Hansen et al. 1997; Anderson and Dunham 2008; Whiteley et al. 2013; Neville and Peterson 2014). We used the maximum likelihood estimation in the program COLONY (Jones and Wang 2010) to identify full-sibling families in each of our temporal collections; COLONY analyses were implemented using the full-likelihood
algorithm, updated allele frequencies, an assumption of male polygamy, and “no prior” for family relationships. We did separate analyses for each collection year, combining individuals from all three streams in each temporal analysis (e.g., Beaver, Little Jack, and Coyote Creek samples from 2002 were all run together but separately from 2007 collections). Because COLONY may not accurately estimate very small full-sibling families (Wang and Santure 2009; Hudy et al. 2010; Neville and Peterson 2014), we ignored information from defined “dyads” and made inferences only from full-sibling groups of three or more. Where full-sibling families of three or more were identified within a stream, all but one sibling were removed from the data set. Additionally, though family structure may bias interpretation of genetic analyses, information about siblings and their locations can also be used as an effective “direct” method of capturing movement (Hudy et al. 2010). In our study, collection of full siblings in different streams would indicate movement between tributaries, i.e., based on the “sib-split” approach of Whiteley et al. (2014; see also Neville and Peterson 2014). Accordingly, for full-sibling families with offspring found in different tributaries, we noted the occurrence of this sib-split as indicative of movement and retained one individual from each tributary in the final data set for further genetic analyses; for example, if three siblings were found in one stream and one in another, we removed only two of the three in the first stream to retain one sibling in each stream. In this manner, our genetic collections were not biased by siblings, but the genetic information reflecting movement was retained.

Once the influence of family structure is accounted for and removed, individual clustering and assignment approaches can effectively provide further “direct” information about movement (Paetkau et al. 2004). We used the Bayesian clustering algorithm in Structure version 2.3.3 (Pritchard et al. 2000), which determines the proportion of individual ancestry, or $Q$, in each of a given number of genetic clusters, $k$. Where individuals captured in a location (stream) characterized by a certain genetic cluster have strong ancestry (here we used $Q > 90\%$) in a different genetic cluster, movement can be inferred (Manel et al. 2003; Paetkau et al. 2004; Manel et al. 2005). We assumed an admixture model with correlated allele frequencies. As our focus was not to evaluate all hierarchical levels of genetic structure (e.g., Vaha et al. 2007) but rather to capture movement among streams, we evaluated three genetic clusters, or $k = 3$, to represent the three tributaries of interest. Separate analyses were run for each sample year. Based on preliminary analyses showing a relatively stable determination of $Q$ for individuals, for each analysis we used a burn-in length of 100,000 followed by 100,000 Markov chain–Monte Carlo replicates for each of five runs. We used the program Structure Harvester (Earl et al. 2012) to compile results. To evaluate the degree of consistency among runs quantitatively, we calculated the pairwise similarity statistic ($H^r$, with maximum similarity reflected by a value of 1) in CLUMPP version 1.1.2 (Jackobsson and Rosenberg 2007) using the Greedy algorithm with 10,000 random inputs. For the final evaluation of clustering and individual proportional ancestries, we relied on the output from CLUMPP, which resolves any situations where clusters may have been labeled differently across runs and provides one results file using information consolidated from the five different runs.

**RESULTS**

**Demographic Data**

Average capture probabilities estimated by MicroFish were >0.86 for age-1 and older fish for each stream in our study, slightly higher than efficiencies estimated by mark–recapture in similar LCT habitats (Miller 2011; J. Dunham, USGS, unpublished data). The distribution and density of LCT in Maggie Creek was highly variable across sample streams and across years (Figure 2b), a result that was not unexpected given the harsh desert environment these fish occupy and extreme fluctuating population dynamics relative to other salmonid species (Platts and Nelson 1988). The average density across sites in Coyote and Little Jack creeks (with partial barriers) declined from 2001 to 2005 (before culvert removal), while estimates in Beaver Creek (complete barrier) were consistently low during this period (Figure 2b). After passage was restored, from 2006 to 2012 the density of fish in Little Jack Creek was quite variable, increasing dramatically in 2007 but in 2009 and 2012 reducing to numbers similar to earlier years (Figure 2). Mean density across index sites in Coyote Creek also varied during this period, never fully recovering to the numbers from the first several sampling years. Beaver Creek density increased in 2006 and remained remarkably stable across the four post-culvert removal years (Figure 2b).

Three hundred fifty observations across 44 unique sites (the random effect) over the 9 years in which sampling occurred were used to fit the model. The complete isolation of Beaver Creek prior to 2005 affected the density of LCT demographics most, as revealed by the negative parameter estimate from the linear mixed model. Isolation due to complete barriers was estimated to suppress LCT densities in Beaver Creek nearly threefold ($e^\beta = e^{-1.09} = 0.34$; lower 95% CI = 0.18, upper 95% CI = 0.62; “complete barrier,” Table 1; Figure 3). Removal of the partial barriers in Little Jack and Coyote Creeks did not have any effect on LCT densities; the estimated effect was slightly positive, but the 95% CIs were wide and encompassed zero (“partial barrier,” Table 1; Figure 3). Fire had no apparent influence on LCT densities (slight positive parameter estimate but with wide CIs that encompassed zero; Table 1; Figure 3). Springs flows the year prior, a factor which has been observed to have positive effects on LCT in previous work (Ray et al. 2007), also had a positive parameter estimate but with a narrow 95% CI that barely included zero (Table 1; Figure 3). The parameter estimate for year was negative, but
TABLE 1. Parameter estimates (±SE) and 95% CIs from a linear mixed model evaluating factors influencing age-1 and older LCT density (log(N/100 m + 1)) in Maggie Creek, 2001–2012.

<table>
<thead>
<tr>
<th>Effect</th>
<th>β</th>
<th>SEβ</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>137.36</td>
<td>70.46</td>
<td>−1.19</td>
<td>275.90</td>
</tr>
<tr>
<td>Complete barrier (barrier = 1, no barrier = 0)</td>
<td>−1.09</td>
<td>0.31</td>
<td>−1.70</td>
<td>−0.49</td>
</tr>
<tr>
<td>Partial barrier (barrier = 1, no barrier = 0)</td>
<td>0.11</td>
<td>0.32</td>
<td>−0.52</td>
<td>0.74</td>
</tr>
<tr>
<td>Fire (burn = 1, no burn = 0)</td>
<td>0.30</td>
<td>0.26</td>
<td>−0.21</td>
<td>0.82</td>
</tr>
<tr>
<td>Spring flow (1-year lag; m³/s)</td>
<td>0.07</td>
<td>0.04</td>
<td>−0.01</td>
<td>0.14</td>
</tr>
<tr>
<td>Year</td>
<td>−0.07</td>
<td>0.04</td>
<td>−0.14</td>
<td>0.01</td>
</tr>
</tbody>
</table>

also with a narrow 95% CI barely encompassing zero. The fixed effects in the model explained 13% of the variance in age-1 and older LCT density (marginal $R^2 = 0.13$; Nakagawa and Schielzeth 2013), whereas the fixed effects and random effect (site) together explained 33% of the variance (conditional $R^2 = 0.33$).

Not only are changes in density informative, but the distribution of size-classes in each stream can also provide insight into recovery, with a robust and stable population generating young of year fish indicating successful spawning as well as recruitment of older, reproductive-sized fish (Neumann et al. 2012). Size distributions in Little Jack and Coyote creeks did not show clear patterns in response to connectivity. The age distribution in Little Jack Creek was relatively flat for most of the preculvert remediation years except for a pulse of age-0 fish observed in 2005 (Figure 4a). In 2006 (the first year after culvert remediation) and 2007, this population demonstrated successful spawning and recruitment to larger size-classes with a particularly large spike in 2007, but showed little evidence of successful spawning and less representation across larger size-classes in 2009 and 2012 (Figure 4d). Coyote Creek exhibited evidence of both spawning and recruitment to larger size-classes in both time periods, with a relatively large pulse of age-0 fish collected in 2012 (Figure 4b, e). Beaver Creek maintained small but consistent age-0 size distributions yet relatively few larger individuals in 2002–2005 (Figure 4c), while the numbers of fish observed across all sizes increased and showed consistent maintenance of larger individuals from 2006 to 2012 (Figure 4f).

Genetic Data

Because only Coyote and Little Jack creeks were sampled in 2001 and Beaver and Coyote creeks in 2002, we combined the (small) Coyote Creek temporal genetic samples for 2001 and 2002 and refer to all of these samples, which represent our preculvert remediation collection, as “2002” samples. Additional genetic samples were collected after the culverts were remediated in 2007, 2009, and 2012, for a total of 1,018 tissue samples analyzed from assumedly age-1 and older fish after subsampling (Table 2); this includes one fish captured in the main-stem Maggie Creek in 2002, which was not included in population-level analyses (or Table 2) but was retained in the individual sibling and clustering or assignment analyses.

Even though we purposefully avoided age-0 fish in our genetic collections, COLONY identified at least one, and often many more, full-sibling families in the assumedly age-1 and older fish (>79 mm) in each creek across the years, with one 12-member family collected in Beaver Creek in 2009 (Table 2). We observed four families of three or more full siblings where siblings were captured in different streams, suggesting movement of at least one sibling between tributaries: two families in 2002 (one with two siblings captured in Beaver Creek and a third in Coyote Creek, the other with two siblings captured in Beaver Creek and a third in Little Jack Creek), one family in 2007 (with three siblings collected in Little Jack Creek and one in Coyote Creek), and one in 2009 (with one sibling collected in Beaver Creek and two in Coyote Creek). Across all years, a total of 416 assumedly age-1 and older siblings were
removed from the data set; the effect on particular sample sizes was significant, with a need to remove almost half of the individuals collected for several samples and 71% of individuals in one case (Little Jack Creek 2012; Table 2).

With siblings removed, all loci met expectations of Hardy–Weinberg equilibrium (based on an adjusted P-value for a 5% nominal level of 0.0004 for 132 locus × sample tests), and there were no locus pairs out of linkage equilibrium in any population (adjusted P-value of 0.00008 for 660 locus pair × sample tests). Gene diversity (H_e) averaged across loci ranged from 0.70 (Little Jack Creek 2009) to 0.81 (Beaver Creek 2002) but showed no significant change in any stream between 2002 and 2012 (Table 3; two-tailed paired t-test for each population sample in 2002 versus 2012 with a 5% P-value adjusted for three multiple tests of 0.02). Values for R_s ranged from 4.78 (Little Jack 2012) to 7.30 (Beaver Creek 2002); there was no change in allelic diversity in Little Jack or Coyote creeks between 2002 and 2012, but a significant decline in allelic richness occurred in Beaver Creek (two-tailed paired t-test: P = 0.60, 0.66, and 0.00, respectively, with a 5% P-value adjusted for multiple tests of 0.02). All pairwise F_{ST} contrasts between streams each year suggested

FIGURE 4. Length frequency distributions of LCT in Little Jack Creek, Coyote Creek, and Beaver Creek for years (a–c) before and (d–f) after culverts were replaced with structures allowing passage. Note the different y-axes and that individuals <80 mm are displayed in the histogram but are not included in the "age 1+ = N" summary for each year.
significant differentiation based on an adjusted P-value of 0.0008; despite a slight apparent increase in these estimates for each contrast over the years, the wide CIs suggest no significant trend (Figure 5). Global differentiation, as evaluated by theta, ranged from 0.05 in 2007 to 0.06 in 2012 but showed no clear trend pattern and had wide and overlapping CIs across years (Figure 6).

Clustering and assignment results suggested temporally changing patterns of movement among tributaries. Structure runs demonstrated a high degree of pairwise similarity among replicates within each yearly analysis, suggesting little discrepancy in estimating individual Q or “cluster switching” in assignments among runs: H’ statistics in CLUMPP were accordingly high, ranging from 0.98 (2007) to 1.00 (2002 and 2012). In 2002, before the culvert removals (Figure 7a), one individual captured in Beaver Creek (generally represented by the gray cluster) strongly assigned to the cluster, which was generally representative of Little Jack Creek (green, with an individual Q of 0.98 for this cluster). In 2002, the only individual captured in the main-stem Maggie Creek in any year (MS, the farthest right individual in Figure 7a) assigned strongly to the gray cluster generally representative of Beaver Creek (Q = 0.96 for this cluster). The first postculvert remediation analysis in 2007 (Figure 7b) suggested a relatively high degree of movement from Coyote Creek into Beaver Creek. As with all four Structure analyses, most individuals collected in Coyote Creek demonstrated relatively high ancestry in one cluster (dark purple, Figure 7); in 2007, eight individuals captured in Beaver Creek had an

<table>
<thead>
<tr>
<th>Year</th>
<th>Stream</th>
<th>Number removed</th>
<th>Number remaining (N)</th>
<th>Percent removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>Beaver</td>
<td>9</td>
<td>44</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Coyote</td>
<td>2</td>
<td>47</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Little Jack</td>
<td>11</td>
<td>23</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Beaver</td>
<td>17</td>
<td>54</td>
<td>24</td>
</tr>
<tr>
<td>2007</td>
<td>Coyote</td>
<td>2</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Little Jack</td>
<td>4</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Beaver</td>
<td>41</td>
<td>94</td>
<td>30</td>
</tr>
<tr>
<td>2009</td>
<td>Coyote</td>
<td>48</td>
<td>59</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Little Jack</td>
<td>18</td>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td>2012</td>
<td>Coyote</td>
<td>95</td>
<td>123</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Little Jack</td>
<td>50</td>
<td>20</td>
<td>71</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>416</td>
<td>601</td>
<td></td>
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TABLE 2. Year of sample, stream where sample was collected, number of full-sibling families of different sizes (across observed family sizes of 3–9 and 12 members), number of siblings removed from each stream × year sample (number removed), number of individuals remaining in the sample or the ultimate sample size (number remaining [N]), and percentage of each initial sample that was removed from analyses (percent removed).

<table>
<thead>
<tr>
<th>Year</th>
<th>Stream</th>
<th>Number of full-sibling families of each size (by number of members)</th>
<th>Number removed</th>
<th>Number remaining (N)</th>
<th>Percent removed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>Beaver</td>
<td>2 1</td>
<td>9</td>
<td>44</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Coyote</td>
<td>1</td>
<td>2</td>
<td>47</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Little Jack</td>
<td>4 1</td>
<td>11</td>
<td>23</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Beaver</td>
<td>4 1 1</td>
<td>17</td>
<td>54</td>
<td>24</td>
</tr>
<tr>
<td>2007</td>
<td>Coyote</td>
<td>1</td>
<td>2</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Little Jack</td>
<td>1</td>
<td>4</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Beaver</td>
<td>10 2 1</td>
<td>41</td>
<td>94</td>
<td>30</td>
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<tr>
<td>2009</td>
<td>Coyote</td>
<td>12 3 1 1</td>
<td>48</td>
<td>59</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Little Jack</td>
<td>9</td>
<td>18</td>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td>2012</td>
<td>Coyote</td>
<td>16 2 5 6 1</td>
<td>95</td>
<td>123</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Little Jack</td>
<td>2</td>
<td>50</td>
<td>20</td>
<td>71</td>
</tr>
</tbody>
</table>

TABLE 3. For each stream and each year, Nei’s gene diversity (H_e) and allelic richness (R_s) averaged across loci, after removal of siblings; R_s estimation was based on a minimum sample size of 10 individuals.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Little Jack</td>
<td>Average H_e</td>
<td>0.72</td>
<td>0.73</td>
<td>0.70</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Average R_s</td>
<td>4.90</td>
<td>5.07</td>
<td>4.91</td>
<td>4.78</td>
</tr>
<tr>
<td>Coyote</td>
<td>Average H_e</td>
<td>0.79</td>
<td>0.77</td>
<td>0.78</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Average R_s</td>
<td>6.30</td>
<td>6.20</td>
<td>6.30</td>
<td>6.30</td>
</tr>
<tr>
<td>Beaver</td>
<td>Average H_e</td>
<td>0.81</td>
<td>0.77</td>
<td>0.78</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Average R_s</td>
<td>7.30</td>
<td>6.40</td>
<td>6.50</td>
<td>6.30</td>
</tr>
</tbody>
</table>

FIGURE 5. Pairwise FST values between streams from population samples from 2002, 2007, 2009, and 2012 (Beav = Beaver Creek, Coy = Coyote Creek, and LJ = Little Jack Creek), with associated 95% CIs based on bootstrapping over loci. Where groups of three or more full siblings were observed within a stream, all but one was removed prior to analysis (see text). All contrasts were significant after correction for multiple comparisons.
average $Q$ of 0.94 for this dark purple cluster generally representative of Coyote Creek, suggesting these individuals moved to Beaver Creek prior to our sampling. A similar pattern of influx to Beaver Creek was shown in 2009 (Figure 7c), but this time from individuals originating in Little Jack Creek. Little Jack Creek was generally characterized by high ancestry in one (green) cluster each year, with an average $Q$ of 0.95 in the green cluster in 2009 for individuals collected there, yet seven individuals collected in Beaver Creek assigned to this green cluster with a $Q > 0.9$ (average for these individuals = 0.94). The results for 2012 (Figure 7d) suggested less movement, with only one individual estimated to have moved into Beaver Creek (i.e., captured in Beaver Creek but with 0.96 ancestry in the green cluster representative of Little Jack Creek).

**DISCUSSION**

Our study, monitoring both demographic and genetic responses to reconnection of what is now one of the largest interconnected stream systems available to LCT (Dupham et al. 2002; USFWS 2009), provides valuable insight regarding the benefits of such watershed-scale reconnection. The harsh desert environment of northern Nevada and the relatively volatile population dynamics of LCT (e.g., Platts and Nelson 1988) make this a particularly difficult fish in which to document clear population responses; yet it is this dynamic nature of desert trout streams, our study suggests, that makes reconnecting habitats so important for ensuring LCT resilience. Combined inference from both demographic and genetic data emphasize the general importance of watershed connectivity that enables movement among diverse habitats and allows for population responses to both intrinsic demographic factors as well as extrinsic environmental factors that vary in space and time. It is generally known that access to variable habitats throughout large, interconnected habitat patches is necessary for the viability of salmonid and other fish species (Schlosser 1995; Schlosser and Angermeier 1995; Dunham et al. 2002; Isaak and Thurow 2006), but rarely have the benefits of reconnection efforts been documented at broad spatial and temporal scales. An important factor to consider, however, is that in this case we had the ability to reconnect and protect (with a permanent barrier) a large habitat network free of nonnative trout to restore migratory movement in a native trout. In other situations where nonnative salmonids are more widely distributed, this opportunity may not be afforded and the complicated risks and benefits of reconnecting versus isolating habitats to protect against nonnative populations need to be weighed carefully (e.g., Novinger and Rahel 2003; Fausch et al. 2009; Peterson et al. 2013; Rahel 2013).

Our demographic model allowed us to parse out the effects of barrier removal from other environmental factors affecting LCT, including possible influences affecting Maggie Creek as a whole that were not directly evaluated (e.g., Scheuerell et al. 2015) through the inclusion of “year” as a fixed effect to capture any potential monotonic change in abundance across the study period. As expected, the removal of what were thought to be only partial barriers below two creeks (Little Jack and Coyote creeks) had no effect on population densities upstream. In contrast, the removal of two barriers which effectively isolated the largest and most spatially complex stream in the watershed led to an almost threefold increase in LCT densities in this stream, with densities in this reconnected habitat remaining remarkably stable in the four post-reconnection sample years.

Somewhat surprisingly, the other factors considered here showed little impact on LCT densities. We had anticipated that high spring flows in the previous year would have a positive influence on density, as was observed in age-0 and age-1 LCT by Ray et al. (2007) using age-structured recruitment models based on annual sampling. In our study, this parameter estimate was indeed positive but any effect was not, from a conservative interpretation, statistically supported given the overlap of the 95% CI with zero. Flows were relatively static (and low) during the first few years of our study (Figure 3a), which may have hampered an ability to link variability to trout dynamics. Further investigation of this effect using age-based recruitment may provide a better understanding of population responses related to spring flow in this and other populations. Similarly, the parameter estimate for fire was positive, but again the 95% CI barely included zero. Fires are typically internally patchy in nature, and where habitat complexity provides for refuge and connectivity allows for rapid recolonization of affected sites (even if internally, in a given tributary), trout can typically recover rapidly after fire (Gresswell 1999; Neville et al. 2009). In Maggie Creek, each stream had at least some LCT habitat outside the periphery of each of the fires during the course of our study, which may have allowed each population to avoid impacts even during the years when streams were not connected to each other. Recent work also
suggests that simply evaluating densities may not capture more complex fish responses to fire, such as shifts in life history strategies in response to opportunities for rapid growth in burned habitats (Rosenberger et al. 2015). Thus, our simple metrics of fish densities may not have captured all of the complex responses to fire (and flow) in Maggie Creek, but this complexity merits attention in future monitoring of this and similar systems. Finally, it is notable that our “year” effect had a negative parameter estimate, even if a statistically conservative interpretation of CIs would reject its importance. It is unclear what influences would possibly negatively affect the trajectories of all populations, especially assuming the
improvements in riparian habitat affected over the last few decades will continue to benefit LCT. Climate change, however, is expected to impact LCT negatively rangewide (Wenger et al. 2011). The uncertain influence of the year effect in our model suggests the need for further long-term population monitoring as well as monitoring of streamflow and stream temperature to ensure the viability of these populations even given their renewed connectivity.

Individual-based genetic approaches demonstrated both temporally and spatially varying movement patterns, with Beaver Creek being the clear recipient of immigrants in years of movement. Assignment of individuals to genetic clusters representing each stream suggested little movement in 2002. However, the one individual captured in 2002 in the main-stem Maggie Creek clearly assigned to Beaver Creek, and one fish collected in Beaver Creek was estimated to have originated in Little Jack Creek. Previous captures at a weir at the base of Beaver Creek had confirmed movement out of Beaver Creek (A. Harig, Trout Unlimited, unpublished data), but this latter genetic assignment suggests occasional passage into Beaver Creek even before culvert remediation (likely during high flows via several overflow culverts). As noted above, physical measurements of the culverts at the initiation of this study would have been useful in characterizing the likelihood of passage, but our data suggest the assumption that Beaver Creek was completely isolated from upstream movement may not have been true. In 2007 genetic assignments captured movement from Coyote Creek into Beaver Creek, while in 2009 there was little evidence of movement from Coyote Creek but substantial movement was observed from Little Jack Creek into Beaver Creek. The gaps in our temporal sampling (e.g., no sampling in 2008) make it difficult to pinpoint what factors influenced these patterns, but certainly the higher flows preceding each of these years allowed for a somewhat rare opportunity to move. Additionally, the unusually high abundances in Little Jack Creek in 2007 may have led to the density-dependent emigration of these fish to Beaver Creek (observed in 2009 genetic collections), as has been observed in other connected LCT populations (Ray et al. 2007), although no such demographic pattern was apparent in Coyote Creek in previous years to explain the large emigration captured by assignment in 2007. By 2012, northern Nevada had fallen into the beginning of a prolonged drought and only one obvious migrant was observed that fall, having moved from Little Jack Creek into Beaver Creek.

Most evaluations of the efficacy of genetic monitoring have focused on the ability to detect the effects of isolation and declines in genetic variation due to negative anthropogenic impacts (e.g., Schwartz et al. 2006; Antao et al. 2010). There is less guidance on the appropriate expectations for capturing population recovery with genetic data, and ultimately the possibilities will often depend on the characteristics of a given system. Population-level genetic metrics in our study generally showed little response to restored connectivity, and in one case actually responded contrary to our expectations: both heterozygosity and allelic richness were relatively stable over the years in Coyote and Little Jack creeks, but Beaver Creek actually suffered a loss of diversity with a significant decline in allelic richness. For a closed system such as the Maggie basin, we would not expect an influx of new alleles from outside this system but rather simply a shift of variation among tributaries. Interestingly, despite its greater isolation, Beaver Creek—the largest of the three tributaries—had the highest levels of genetic diversity initially, and the influx of individuals from the other two streams with less genetic variation may have actually reduced genetic diversity in the Beaver Creek population after connectivity was restored.

All pairwise $F_{ST}$ contrasts remained significant across years, and levels of global differentiation did not change. Previous simulations have suggested that response times for genetic differentiation are on the order of a decade or longer, depending on the life history and dispersal capacity of the species (Raeymaekers et al. 2009; Langduth et al. 2010), but the use of $F_{ST}$ as an indicator of trout movement has shown the predicted decrease in differentiation after culvert restoration in other trout systems (Neville and Peterson 2014; Whiteley et al. 2014). Compared with other trout, however, LCT are prone to large demographic fluctuations (Platts and Nelson 1988) in their volatile desert environment, and such fluctuations were observed in our study even after culvert removal (e.g., for Little Jack Creek from 2007 to 2009). As noted above, our question at hand (the restoration of habitat connectivity) inherently assumes nonequilibrium population dynamics, which makes relating $F_{ST}$ to movement difficult (Whitlock and McCauley 1999); the large fluctuations in abundance observed across our sampling years—and potentially in years we did not sample—may mean that even the relatively high number of movers we observed may not have translated to enough “effective dispersers” to counter the strong effects of genetic drift on $F_{ST}$ values (Wright 1969; Mills and Allendorf 1996). Overall, Maggie Creek represents an interesting example of where restoration activities may lead to a conflicting interpretation of “benefits” in terms of genetic versus demographic assessments, at least in the short term. It will be interesting to determine if, over the long term, genetic responses begin to track the clear demographic responses (movement captured via genetic assignment and increased abundances) to restored connectivity in Beaver Creek.

One other aspect of using genetic approaches to assess the effectiveness of restoration activities that bears mentioning is the need to remove siblings to improve adherence to theoretical assumptions of these methods (i.e., that the genetic collection represents a random sample of the population; see Hansen et al. 1997). In our study, even though we sampled the entire distribution of LCT over large reaches in each stream (ranging from 5.8 km in Little Jack Creek to over 30 km in Beaver Creek) and removed recently emerged and presumably spatially clumped age-0 fish from our genetic
samples to avoid this issue, we still collected a substantial number of siblings each year (Table 2). This required the removal of almost half of the individuals in many samples, and a marked 71% for one collection. Because family structure generally cannot be known in natural field populations until genotypes are procured and analyzed, this represents a significant expense (see also Neville and Peterson 2014).

CONCLUSIONS

Our study emphasizes the value of a long-term, watershed-scale monitoring program to document the complex responses of fish populations to habitat reconnection and other unexpected events or influences that can occur over a decade of monitoring (e.g., wildfire). Rigorous monitoring is an often neglected aspect of stream and river restoration. For example, Hassett et al. (2005) found that only 5% of restoration projects in the Chesapeake Bay watershed had an effectiveness monitoring component. Where monitoring is undertaken, often it is focused on individual restoration projects or sites (but see Pierce et al. 2013), even though the ability to document even moderate changes in fish populations requires monitoring responses across multiple sites distributed across a watershed (or geographic area of interest; Dauwalter et al. 2009; Dauwalter et al. 2010; Roni et al. 2010). Our monitoring work was cost and time intensive (with an estimated total of US$150,000 for 8 years of field sampling and 4 years of genetic laboratory work, excluding time for professional expertise for subsequent demographic and genetic analyses), but it was still only a fraction of the cost of the culvert removals (which was approximately $540,000). Importantly, the incorporation of both demographic and genetic techniques uncovered complexities that we may otherwise not have captured. Our data showed clear evidence of temporally variable movement of fish, and a demographic boost, to the most isolated population (Beaver Creek) following habitat reconnection, thus verifying success.

Table 2

RESPONSES OF THREATENED TROUT TO HABITAT RECONNECTION

ACKNOWLEDGMENTS

This study was thoughtfully initiated in 2001 by Amy Harig (Trout Unlimited) and Carol Evans (BLM, Elko District Office). We thank Warren Colyer, Dana DeGraaf, Aaron Cushing, Jim Hearsey, John Rodstrom, Diana Gould, Michael Patton, Richard Walker, and John Zablocki for assisting with field sampling, and appreciate permission to sample on private property owned by the Maggie Creek Ranch, TS Ranch, and 26 Ranch and Newmont Mining Corporation. Veronica Kirchoff and Kristina Kruse completed laboratory analyses; Figure 1 was created by Matt Mayfield, and Sean McFall helped with R scripts. This manuscript benefited greatly from comments by two anonymous reviewers and the Associate Editor. This work was funded by Trout Unlimited via support from the National Fish and Wildlife Foundation, with additional support for H.N and D.D. provided by a BLM–Trout Unlimited Cooperative Agreement and National Aeronautics and Space Administration grant NNX14AC91G.

REFERENCES


