

**Demographic and Genetic Status of a Reintroduced River Otter Population in
North-central New Mexico**

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SUMMARY

River otters (*Lontra canadensis*) were extirpated from New Mexico by the 1950s. A limited reintroduction occurred during 2008–2010 in which 33 otters sourced from Washington (WA) were translocated to the Upper Rio Grande Basin (URG) of New Mexico. We conducted a noninvasive genetic capture-recapture survey during the winter of 2018 by collecting fecal DNA samples from river otter scats found at latrines in the URG dendritic network of perennial waterways. Our objectives were to: 1) estimate genetic diversity and effective population size; 2) genetic divergence from the WA source population and potential connectivity with regionally proximal populations; 3) spatially explicit population density and size; and 4) population growth rate since the founder event. Between February and April 2018, we collected 1,184 fecal DNA samples from 622 individual scats at 20 latrines; genotyping was attempted at 10 otter-specific microsatellite loci for a subsample of 543 samples. A bottlenecking founder effect was strongly supported, which, combined with genetic drift, reduced genetic diversity and effective population size by 20–26% and 106–170%, respectively, compared with the WA source population. Estimated population density from spatial capture-recapture models was 0.23–0.28 otter/km of waterway, or 1 otter/3.57–4.35 km of waterway, corresponding to a total population size of 83–100 otters across 359 km of the perennial dendritic network from La Mesilla, New Mexico to Alamosa National Wildlife Refuge, Colorado. Estimated average annual population growth rate since the founder event was 1.12–1.15/year. Despite successful population establishment, the URG river otter population remains small, is genetically degraded, and does not yet meet the criteria for long-term reintroduction success. Projections suggested that the population could reach the recommended minimum viable population size of ≥ 400 otters by the years 2030–2033, though sufficient habitat may not exist in the URG Basin to support that many otters.

INTRODUCTION

The reduction of carnivores in native ecosystems has degraded biodiversity and caused disruptive landscape-scale ecological changes (Ripple et al. 2014). Although improved wildlife management, conservation policy, and habitat restoration efforts have allowed some carnivores to naturally recolonize portions of their historical ranges, irreversible habitat loss, anthropogenic development, and climate change represent considerable impediments to recolonization for many carnivore species. Reintroduction has thus become a useful tool for overcoming the barriers to natural recolonization and reestablishing carnivore populations to historical ranges, which can also restore biodiversity and top-down ecological processes (Rondinini and Visconti 2015). However, reintroduced populations are often established using small founder groups, which renders populations vulnerable to stochastic demographic and environmental processes. Consequently, reintroduced populations often exhibit deleterious demographic and genetic anomalies, including founder and Allee effects, which can elevate the probability of population extinction and reintroduction failure (Szűcs et al. 2017).

North American river otters (*Lontra canadensis*) historically inhabited most major watersheds in the conterminous United States, but overexploitation, habitat degradation, and water pollution extirpated the species from much of its native range by the mid-twentieth century (Anderson 1977). Many state wildlife management agencies and non-government organizations, concerned over the seemingly rapid and widespread disappearance of the species, conducted reintroductions to attempt to restore river otters during the latter half of the twentieth and early twenty-first centuries. River otters were extirpated from New Mexico and other arid southwestern states by the 1950s, remaining absent from the former until a small, localized reintroduction occurred during the late 2000s. Between 2008 and 2010, 33 river otters (unknown

age or sex ratios) were translocated from the Puget Sound, Washington, to the Upper Rio Grande Basin (URG), where they were released near the confluence of the Rio Grande and the Rio Pueblo de Taos in north-central New Mexico (Savage and Klingel 2015).

Although no founders were radio-monitored and, therefore, their post-release fates are unknown, reproduction was documented in the URG population in 2009 and 2013 (Long 2010, Converse et al. 2014). Opportunistic citizen-science monitoring has resulted in >150 reported sightings of individual otters, their tracks, or scats since the founder event (Savage and Klingel 2015). Most of those sightings occurred along the Rio Grande, but additional confirmations along the Chama River, Red River, Rio Hondo, and Rio Pueblo de Taos, as well as near the headwaters of the Rio Grande in southern Colorado (Long 2010, Savage and Klingel 2015, Colorado Parks and Wildlife 2018), suggest that population growth and range expansion likely have occurred. Nevertheless, because the URG reintroduction used a small founder group of otters that were released in a dendritic system of waterways that was devoid of conspecifics, founder and Allee effects could rapidly develop if immigration does not occur from other occupied waterways. However, the nearest known population of river otters to the URG population is located in the San Juan River Basin, approximately 250 Euclidean km away and separated by the Tusas Mountains. For small reintroduced carnivore populations that endure prolonged isolation, demographic and genetic monitoring at predefined time intervals is imperative to reintroduction success (Robert et al. 2015), but no such efforts have occurred for the reintroduced URG population of river otters.

Noninvasive genetic sampling conducted in a capture-recapture survey framework can achieve simultaneous demographic and genetic monitoring. Such methods typically result in larger sample sizes compared with live-capture methods, which can improve estimation of

demographic parameters and allow a more thorough evaluation of population genetics.

Noninvasive genetic sampling to collect fecal DNA from scats has proven reliable for obtaining large sample sizes and high detection rates of river otters elsewhere in the species' occupied range (Mowry et al. 2011, Brzeski et al. 2013). Density is an invaluable demographic parameter because it can be compared among populations of varying size and distribution to provide insight into population-environment relationships and inform conservation and management. Traditional non-spatial capture-recapture models (e.g., models available in Program MARK; White and Burnham 1999) are incapable of estimating density and instead estimate population size for an undefined geographical area. Density must then be derived by applying estimated population size to an *ad hoc* effective sampling area, which has limited ecological or statistical basis and consequently tends to result in positively biased density (Obbard et al. 2010). Relatively recently developed hierarchical spatial capture-recapture models overcome these issues, among other weaknesses of traditional models (e.g., unaccounted for spatial heterogeneity in detection), to estimate the probability of detection as a function of distance between detectors and animal activity centers, and directly estimate density for a defined geographical area (Efford 2004, Royle et al. 2014).

We conducted a study during 2018 in which we collected fecal DNA from river otter scats in a capture-recapture framework to determine the demographic and genetic status of the reintroduced URG population. Our objectives were to: 1) estimate population genetic metrics that are important to conservation of reintroduced and small populations; 2) estimate genetic divergence from the source population and potential connectivity with regionally proximal populations; 3) estimate spatially explicit population density and size using spatial capture-recapture models; and 4) estimate population growth rate since the founder event. We note that

our efforts to obtain genetic samples from otters in nearby populations (e.g., URG in Colorado and San Juan River Basin) from state wildlife agencies were unsuccessful; thus, we could not address the population connectivity portion of objective #2. Nevertheless, the results of this study provide critical baseline ecological information that is required for conservation and management of river otters in the URG Basin (New Mexico Department of Game & Fish [NMDGF] 2016).

METHODS

Study area

Our study occurred along perennial rivers and streams in the URG Basin, primarily in the semiarid Taos Plateau Level IV Ecoregion and the San Luis Shrublands and Hills Level IV Ecoregion (Griffith et al. 2006). Predominant woody vegetation cover along perennial waterways includes cottonwood (*Populus deltoides*), desert willow (*Chilopsis linearis*), and salt cedar (*Tamarix* spp.). Outside of riparian areas, vegetation on the Taos Plateau is sparsely distributed and primarily comprised of semiarid grasslands and desert shrub-scrub flora; higher elevations are covered by pinyon (*Pinus* spp.)-juniper (*Juniperus* spp.) and mixed forests of ponderosa pine (*Pinus ponderosa*), Douglas fir (*Pseudotsuga menziesii*), and Gambel oak (*Quercus gambelii*; Ruhlman et al. 2012). Elevations in the portions of the two ecoregions where our study occurred range from 1,830 m a.s.l. at the lowest stretch of the Rio Grande to 4,013 m a.s.l. on Wheeler Peak; the lowest and highest elevations of surveyed otter latrines in our study were 1,831 m a.s.l. and 2,261 m a.s.l., respectively. The study area receives an average of just 15–25 cm of precipitation per year, though higher elevations in the Sangre de Cristo Mountains receive more; temperatures vary considerably by season and elevation, ranging from a low of -17°C during winter to a high of 36°C during summer (Ruhlman et al. 2012).

All founder river otters were released in the Rio Pueblo de Taos in Taos County, New Mexico, approximately 6 km east of the confluence with the Rio Grande. Since the founder event, direct and indirect occurrences of river otters have been documented in ~325 km of perennial rivers and streams in the New Mexico portion of the URG dendritic network (Long 2010, Savage and Klingel 2015). However, logistical constraints in this study, primarily due to limited manpower and accessibility of portions of perennial waterways, precluded capture-recapture surveying of the entirety of waterways where otter occurrence has been documented. Therefore, we restricted our surveys to the Rio Grande and its perennial tributaries within Taos County only, which collectively had the highest densities of documented river otter occurrence (observations, photos, tracks, and scat) and likely represented the core of the population (Savage and Klingel 2015). Specifically, surveyed waterways included the entirety of the Rio Grande in Taos County, as well as the lower reaches of the Red River, Rio Hondo, and Rio Pueblo de Taos (Fig. 1).

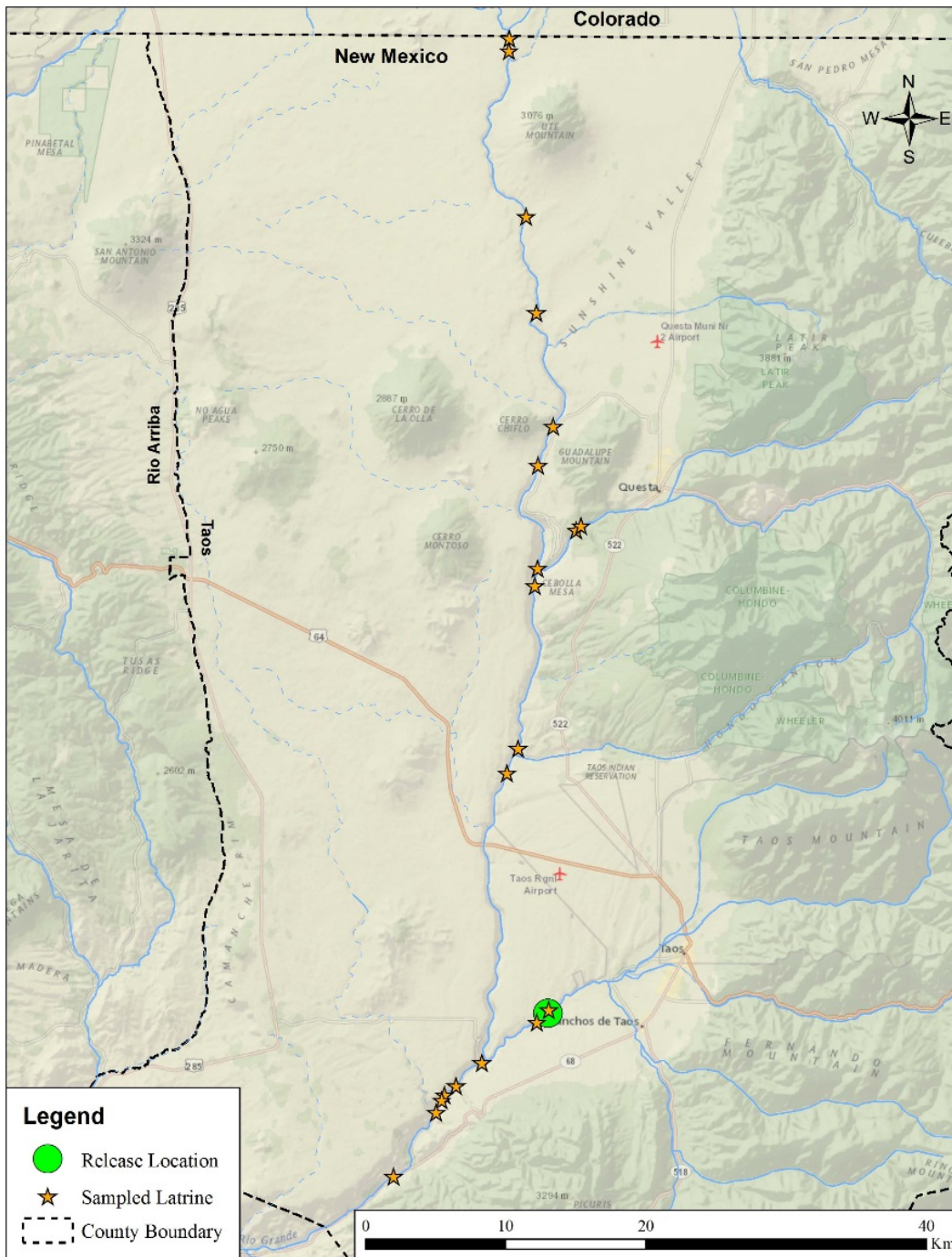


Figure 1. Study area in Taos County, New Mexico, where river otter latrines were found along perennial waterways (solid blue lines) and subsequently surveyed in a capture-recapture framework for 8 consecutive sampling occasions to collect otter fecal DNA samples.

Genetic sampling

Our survey approach generally followed the methods described by Mowry et al. (2011) for noninvasively sampling river otters via scat deposited at latrine sites. Latrines are communal defecation sites that river otters primarily use for intraspecific communication (Melquist and Hornocker 1983, MacDonald and Mason 1987, Swimley et al. 1998). In established river otter populations, multiple lone individuals and romps use and reuse latrines regularly throughout a given year, or possibly lifetime (Melquist and Hornocker 1983, MacDonald and Mason 1987, Gallant et al. 2007). Latrine visitation and defecation rates by river otters vary among individuals and seasons, with the highest visitation rates typically occurring during the winter breeding season (December–April; Stevens and Serfass 2008, Mowry et al. 2011); therefore, to maximize detection rates, we sampled latrines during February–April, 2018. This period is also characterized by colder weather, which typically corresponds to higher genotyping success rates for otter fecal DNA samples (Arrendal et al. 2007, Mowry et al. 2011, Aristizábal Duque et al. 2018).

A team of two technicians conducted an initial two-week (14-day) scouting period to find active latrine sites along perennial rivers and streams in the URG by a combination of walking the banks of waterways on foot and floating waterways via self-propelled watercraft. The UTM coordinates of each latrine were recorded and all scats were cleared from all latrines; clearing scats from latrines during this scouting period was necessary to ensure that only extant individuals would be detected during the capture-recapture survey and to equalize baseline detection rates across survey occasions (Morin et al. 2016, Murphy et al. 2018). Immediately following this scouting period, we initiated the capture-recapture survey in which each latrine was revisited at 7- to 10-day intervals for 8 consecutive survey occasions to collect fecal DNA

samples from scats and anal jellies (anal sac secretions). Based on spatial capture-recapture model analysis of simulated detection data for 6, 8, 10, and 12 survey occasions, we found that surveying latrines for 8 consecutive occasions represented the best compromise between obtaining unbiased estimates of river otter density and survey effort/costs.

During each occasion, we collected two fecal DNA samples from every scat and anal jelly present at a latrine by extracting a $\sim 0.5\text{-cm}^3$ portion of the outside of a scat using tweezers and ~ 0.5 mL of jelly using a metal spoon (Stenglein et al. 2010, Morin et al. 2016, Murphy et al. 2018). We placed each collected fecal sample in an individually labeled vial that contained 1.4 mL of DETS buffer to mitigate DNA degradation (Stenglein et al. 2010). To prevent cross-contamination among samples, we sterilized tweezers and spoons between sample collections using flame from a lighter. We then removed all sampled scat and anal jellies from each latrine during each occasion to prevent double sampling in subsequent survey occasions; all sampled scat were collected and placed in individually labeled Ziploc bags that we stored at -20°C for use in a separate river otter food habits study (not presented in this report).

In addition to collecting fecal DNA samples from the reintroduced URG river otter population, we acquired tissue samples from river otters in the source population located in the Puget Sound, Washington (WA). Tissue samples were collected by Washington Department of Fish and Game biologists from river otters that were legally harvested by trappers during the 2017–2018 and 2018–2019 seasons. As noted earlier, we also attempted to acquire genetic samples from river otters in the San Juan River Basin and the Colorado portion of the URG, but we never received any samples from the biologists that we contacted.

Laboratory genotyping

Following the conclusion of our 8-occasion capture-recapture survey, we sent all collected fecal DNA samples and all WA tissue samples to the Laboratory for Ecological, Evolutionary and Conservation Genetics at the University of Idaho (Moscow, ID) for DNA extraction, PCR amplification, and microsatellite genotyping. The costs for genotyping all fecal DNA samples that we collected from the URG population would have totaled ~\$59,000, which was financially prohibitive. Therefore, we instructed laboratory personnel to conduct subsampling by first randomizing all samples collected at each latrine within a given occasion (i.e., latrine-occasion pairing), and then randomly selecting 3–4 samples from each latrine during each occasion for genotyping. Murphy et al. (2016) demonstrated that such randomized subsampling has a negligible influence on spatial capture-recapture model parameter estimates.

Laboratory technicians extracted DNA from tissue and fecal samples using the DNeasy Blood and Tissue Kit protocol and QIAmp Fast DNA Stool Mini Kit (Qiagen, Inc.), respectively, in a laboratory dedicated to low quality, low quantity DNA sources. One negative control was included in each extraction to monitor for contamination of reagents. Multi-locus microsatellite genotypes were generated using 10 loci developed in otters (Dallas and Piertney 1998, Beheler et al. 2005, Mowry et al. 2011); the multiplex contained 0.01 μM of RIO02, 0.03 μM of RIO08 and RIO12, 0.04 μM of RIO13 and RIO16, 0.06 μM of RIO01, 0.07 μM of Lut453, 0.13 μM of RIO06, 0.23 μM of RIO07, 0.24 μM of RIO04, 0.26 μM of the SRY2 sex marker, 1X Qiagen Multiplex PCR Kit Master Mix, 0.5X Q solution, and 2 μl of DNA extract in a 7 μl reaction. Up to six replicate microsatellite PCRs were performed for the fecal samples if they consistently amplified after an initial screening step of two amplifications; the tissue samples were amplified in duplicate. PCR products were visualized using a 3130xl DNA Sequencer (Applied

Biosystems) and allele sizes were scored using Genemapper 5.0 (Applied Biosystems).

Assessment of sample quality and genotype screening methods followed those described by Stenglein et al. (2010).

Probability of identity for siblings (PI_{sibs}) was calculated for genotypes obtained from the tissue samples using GENALEX (Peakall and Smouse 2006, 2012). A PI_{sibs} threshold of 0.01 was used as a cutoff for the number of loci required to distinguish among unique genotypes (individual otters). To determine the number of unique genotypes contained in the dataset, matching analysis was conducted using GENALEX (Stenglein et al. 2010). Two samples were considered as originating from the same individual if their locus-specific alleles matched across all 10 loci, and also if two samples matched at 9 or 8 loci and the mismatches were likely due to allelic dropout.

Population genetics analysis

We used the genepop package available in the R statistical program (Rousset 2008, R Core Team 2019) to test for Hardy-Weinberg equilibrium and quantify linkage disequilibrium; we ran 1,000 Markov chain iterations for each of 100 batches. We used the diveRsity package (Keenan et al. 2013) in R to estimate allelic richness (A_R) via rarefaction, observed (H_O) and expected (H_E) heterozygosity, and the inbreeding coefficient (F_{IS}); we calculated 95% confidence intervals for each metric using 1,000 bootstrap iterations. We used the methods described by Waples et al. (2014) for iteroparous species (overlapping generations) to estimate the genetic effective number of breeders (N_B) and genetic effective population size (N_E) via the linkage disequilibrium method. For iteroparous species, such as river otters, N_B represents the number of parents that produced the sampled cohort of individuals, whereas N_E represents the size of an ideal population that loses genetic diversity at the same rate as the sampled population.

First, we used program N_E ESTIMATOR v2.01 (Do et al. 2014) to estimate raw uncorrected N_B . Second, to correct for bias caused by iteroparity, we applied a two-vital rate adjustment formula to N_B . Third, we used a separate two-vital rate adjustment formula to estimate N_E from the corrected N_B (Waples et al. 2014). The two vital rates used in the adjustment formulae are age at maturity and adult life span ($AL = \text{maximum age} - \text{age at maturity} + 1$), which collectively explain most variation in N_B and N_E (Waples et al. 2013, 2014; Waples 2016). Data on these two vital rates have not been collected from river otters in the URG population; therefore, we used averaged vital rate estimates among river otter populations throughout the species' occupied range as surrogates (Larivière and Walton 1998).

We tested for a genetic bottleneck in the URG population, which could have been caused by the small founder group size and subsequent isolation (i.e., founder effect), using BOTTLENECK v1.2.02 (Piry et al. 1999). Specifically, we evaluated departure from mutation-drift equilibrium via a two-phase model that incorporated 30% of multi-step mutations to account for uncertainty in the mutation process of microsatellites (Luikart et al. 1998, Peery et al. 2012). We ran 10,000 replicates and assessed support for a bottleneck using Wilcoxon sign-rank tests (Peery et al. 2012). We also estimated pairwise genetic differentiation (F_{ST} ; Weir and Cockerham 1984) between the URG and the WA source population using the *diveRsity* package in R, with 95% confidence intervals obtained via 1,000 bootstrap iterations. Biologically significant F_{ST} estimates have confidence interval lower bounds >0 and point estimates ≥ 0.05 (Hartl and Clark 1997).

Spatial capture-recapture analysis

We estimated river otter population density (D) and population size (N) using spatial capture-recapture models implemented via maximum likelihood in the R package *secr* (Efford

2004, Efford and Fewster 2013, Efford 2019b). We modeled latrines as ‘count’ detectors for which the detection process followed a Poisson distribution; we did this because the latrines were spatially fixed, multiple river otters could have been detected at a single latrine during a given survey occasion, an individual river otter could have been detected more than once at the same latrine during a given survey occasion, and an individual river otter could have been detected at multiple latrines during a given survey occasion (Royle et al. 2014). We initially considered three separate detection functions (half-normal, exponential, and hazard half-normal) in exploratory analysis, and found that the hazard half-normal detection function best fit our data. Thus, we fit all models with a hazard half-normal detection function that had 2 parameters that were estimated, the baseline detection rate at an otter’s activity (home range) center (λ_0) and the spatial scale parameter (σ). The spatial scale parameter describes animal movement distances over which detection probability varies relative to the locations of their home range centers and detectors; contingent on assumptions, σ can be converted to an estimated 95% home range radius (Royle et al. 2014). We did not survey all latrines during each occasion, so we appropriately accounted for varying survey effort among latrines by employing the hazard-based adjustment developed by Efford et al. (2013). This adjustment mitigated potential confounding between varying survey effort and temporal or spatial variation in density.

The perennial waterways that we surveyed constituted a dendritic network to which available river otter habitat was restricted (Campbell Grant et al. 2007). Although river otters can traverse favorable non-riparian environments to ‘shortcut’ movement among proximal waterways (Spinola et al. 2008), the Rio Grande Gorge was up to 240 m deep and bounded by steep cliffs, and most terrestrial habitats in the intervening areas between waterways were arid and sparsely vegetated. It is unlikely that river otters commonly cross expanses of these arid

lands to move among perennial waterways, instead preferentially moving along the river and stream corridors that collectively comprise the dendritic network (Sauer et al. 1999). As a result, the array of latrines that we surveyed were spatially oriented along the primarily linear directionality of river otter movements and, thus, the likely direction of home range elongation. Spatial capture-recapture models assume that animal home ranges are approximately circular; failure to account for home range elongation or linearity can severely bias D estimates, particularly if the detector array aligns with the direction of home range elongation (Royle et al. 2013, Murphy et al. 2016, Efford 2019a). Therefore, we used the R package `seclinear` to estimate a dendritic state space mask (i.e., area of integration) that was restricted to the network of perennial waterways within a recommended $\sim 4 \times$ estimated σ from latrines and had a point-spacing resolution of 100 m (Royle et al. 2014; Efford 2017, 2019a). Additionally, instead of using the default spatial capture-recapture 2-dimensional Euclidean distance model, which is predicated on the aforementioned assumption of home range circularity, we created a 1-dimensional non-Euclidean specification of the distance model that represented the actual sinuous distances (river and stream km) along the dendritic network of perennial waterways (Efford 2017). This network distance effectively removes the default circular home range assumption to appropriately accommodate river otter movements and home ranges being predominantly restricted to riparian areas in the dendritic network, thereby mitigating bias in σ and D estimates (Efford 2019a).

Spatial capture-recapture models account for heterogeneity in detection that results from the spatial arrangement of detectors relative to the locations of animal activity centers, whereas traditional non-spatial capture-recapture models do not (Royle et al. 2014). However, non-spatial sources of detection heterogeneity can induce bias in spatial capture-recapture density estimates

if those sources are not appropriately included as effects on detection function parameters. Therefore, we fit models with and without potential sources of non-spatial heterogeneity modeled on detection function parameters: a) a latrine-specific behavioral response, bk , because we sampled during the breeding season when otters may repeatedly revisit the same latrine to seek mates, and also because romps often maintain smaller home ranges than lone individuals and therefore visit latrines in close proximity (Gallant et al. 2007); and b) the sex of river otters, because male and female otters may have different detection rates as a result of males often being the wider-ranging sex (Gallant et al. 2007, Spinola et al. 2008, Mowry et al. 2011). We modeled sex as 2-class finite mixtures (Pledger 2000, Gardner et al. 2010), modeled all possible additive and interactive effects on λ_0 , and modeled only sex on σ .

Because of the approximately linear north-south orientation of the dendritic network that we sampled and the location of the reintroduction release site relative to sampled latrines, river otter density may have spatially varied with latitude or distance from the release site. For example, Murphy et al. (2016) found that density of a reintroduced American black bear (*Ursus americanus*) population significantly decreased with increasing distance from the release site. Thus, in addition to fitting spatial capture-recapture models in which the spatial distribution of individual otter activity centers followed a homogeneous Poisson point process (i.e., locations of river otter activity centers were spatially random within the state space), we also fit inhomogeneous Poisson point process models that allowed the spatial distribution of otter activity centers to vary with latitude or distance from the reintroduction release site (Royle et al. 2014). Specifically, we modeled river otter density as a log-linear function of latitude or distance from the release site. The latitude covariate was comprised of UTM northings at 100-m intervals,

and the distance from release site covariate was comprised of sinuous distances along all waterways within the extent of the dendritic state space.

We used Akaike's Information Criterion (AIC_c) corrected for small sample size for model selection when analyzing the river otter capture histories. We considered all models $\leq 2 \Delta AIC_c$ of the top ranked model competing (Burnham and Anderson 2002). If >1 model met this threshold and log-likelihood values among competing models were similar when the number of model parameters differed, then we model-averaged competing models to produce parameter estimates. To estimate the average annual river otter population growth rate (r) since the founder event, we used the exponential growth model developed by Gotelli (2008); this model assumes that density-dependent population regulation is absent and, thus, that the population had not reached carrying capacity by the time that our study occurred (Murphy et al. 2015, 2016). None of the 33 founder river otters were radio-monitored, so the number of founders that survived post-release is unknown; we optimistically assumed that all founders survived the founder event, specifying $N = 33$ otters as the initial population size. We expected river otters in the URG would have elliptical or linear home ranges (Sauer et al. 1999). Therefore, to derive an approximate estimate of elliptical river otter home range size (HR_E), assuming river otter activity/movement was normally distributed and directly related to detection probability, we adapted the equation for calculating the size of an elliptical area to our σ estimates: $HR_E = \pi \times (2 \times \sigma) \times (0.5 \times \omega)$, where ω represents the maximum width of riparian areas (including the river/stream channel) along the waterways that we sampled. Similarly, to derive an approximate estimate of linear river otter home range size (HR_L), assuming river otter activity/movement was normally distributed and directly related to detection probability: $HR_L = 4 \times \sigma$ (M.G. Efford, University of Otago, unpublished data). These approaches relaxed the circularity assumption that is typical of most

area equations to estimate home range sizes, thereby reflecting preferential movements along generally linear waterways with home range widths restricted to available riparian habitats (Sauer et al. 1999).

RESULTS

Genetic sampling

We located and surveyed 20 individual latrines along the Rio Grande, Red River, Rio Hondo, and Rio Pueblo de Taos. Across the 8 capture-recapture survey occasions, we collected 1,184 fecal DNA samples from 622 individual scats. An average of 31 scats were sampled at each latrine during the survey period (95% CI: 23–39). We also received 10 tissue samples from river otters in the WA source population that were collected during the 2017–2018 trapping season; additional tissue samples were collected by WDFW biologists during the 2018–2019 season to increase our sample size, but genotyping of these recent samples has not yet been completed by the laboratory (expected completion by August/September 2019).

Laboratory genotyping

The subsampling protocol resulted in the selection of 543 fecal DNA samples from the URG population for genotyping. Based on genotypes obtained for the 10 tissue samples from the WA source population, PI_{sibs} indicated that a total of 7 loci ($PI_{\text{sibs}} = 0.006$) would be necessary to attain the threshold of 0.01 for reliably distinguishing among unique genotypes. However, while running the matching analysis, laboratory personnel noticed that several genotypes differed at only 1 locus, suggesting that the PI_{sibs} value differed between the source and reintroduced populations, likely due to a founder effect in the latter. Thus, PI_{sibs} was recalculated using the individual genotypes that were detected only once in the matching, which resulted in a total of

≥ 8 loci ($PI_{\text{sibs}} = 0.01$) being necessary to distinguish among unique genotypes in the reintroduced population.

Consensus genotypes at 8–10 loci were obtained for 77 total samples from the URG population, representing a 14% genotyping success rate; partial genotypes at < 8 loci were obtained for an additional 15 samples. Under the strict matching rules, any genotypes that differed at up to 2 loci, with the difference being due to allelic dropout, would be compressed into the same individual. However, laboratory personnel found several instances in which groups of genotypes differed at only 1 locus. This suggested that, a) 8 loci may not have been sufficiently conservative to distinguish among unique genotypes, and b) genotyping results should be presented for both conservative and lenient matching rules. The matching rules for conservative genotypes were outlined above; the matching rules for lenient genotypes entailed splitting genotypes that differed at only 1 locus into separate individuals. Application of said matching rules resulted in a conservative capture history comprised of 30 river otters (12 M:16 F:2 unconfirmed M) that were detected a total of 77 times, and a lenient capture history comprised of 37 river otters (17 M:18 F: 2 unconfirmed M) that were detected a total of 77 times.

The uncertainty surrounding sex identification for 2 unconfirmed male otters is the direct consequence of the SRY method that is commonly used for otters (Dallas et al. 2000). This method attempts to amplify the male Y-chromosome, resulting in a positive male amplification and no PCR products for females; however, the method suffers from the fact that a negative amplification could be either a male with allelic dropout or a female (Statham et al. 2007, Mowry et al. 2011). These are difficult to differentiate between, but all sex identification was completed in 3–6 replicates per sample. Thus, laboratory personnel chose to label an individual as male if

any of the samples in a group were identified as male via the SRY method in that set of matching genotypes.

Population genetics analysis

We detected violation of Hardy-Weinberg equilibrium at loci RIO12 and RIO13 in the lenient genotypes from the URG population, following application of a Bonferroni correction for multiple comparisons ($\alpha < 0.005$); no violations were found in the WA source population genotypes or the conservative genotypes from the URG population. Following Bonferroni correction ($\alpha < 0.001$), we detected linkage disequilibrium in 4% and 8% of 45 loci comparisons in the conservative and lenient genotypes from the URG population, respectively; no evidence of linkage disequilibrium was found in the WA source population genotypes. Estimates of allelic richness were similar among the WA source population and both sets of the URG population genotypes (A_R range: 3.35–3.84; Table 1). However, estimates of observed and expected heterozygosity were 20–26% higher in the WA source population ($H_O = 0.72$; $H_E = 0.67$) compared with the URG population ($H_O = 0.57$ – 0.60 ; $H_E = 0.53$ – 0.54). All inbreeding coefficient estimates were negative (F_{IS} range: -0.10 – 0.07), suggesting that river otters in both the source and reintroduced populations were less related than expected under a random mating model. Estimates of effective number of breeders and genetic effective population size were 106–170% larger for the WA source population compared with the URG population (N_B range: 14–35; N_E range: 7–19). We found support for a genetic bottleneck in the reintroduced URG population, based on both the conservative ($P = 0.006$) and lenient genotypes ($P = 0.01$). A moderate level of genetic differentiation has developed between the WA source population and the URG population ($F_{ST(\text{cons})} = 0.09$ [95% CI: 0.05–0.14]; $F_{ST(\text{len})} = 0.10$ [95% CI: 0.06–0.15]),

indicating that genetic drift has occurred at a rapid rate in the URG since the founder event (average F_{ST} increase of $\sim 0.01/\text{year}$, or $0.06/\text{generation}$).

Table 1. Parameter estimates for measures of population genetic diversity, genetic fitness, and non-random mating/inbreeding of river otters in the Puget Sound, WA, source population and the reintroduced Upper Rio Grande Basin (URG) population. Two estimates are provided for the URG population based on unique genotypes according to conservative and lenient matching rules. 95% confidence intervals are presented in parenthesis; infinity is denoted by ∞ .

Population	A_R^a	H_O^b	H_E^c	N_B^d	N_E^e	F_{IS}^f
WA Source	3.84 (3.49–4.19)	0.72 (0.62–0.82)	0.67 (0.62–0.72)	35 (15– ∞)	19 (8– ∞)	-0.08 (-0.20–0.04)
URG Conservative	3.37 (2.66–4.08)	0.60 (0.48–0.72)	0.54 (0.46–0.61)	17 (11–25)	9 (6–13)	-0.10 (-0.23–0.03)
URG Lenient	3.35 (2.67–4.03)	0.57 (0.46–0.68)	0.53 (0.44–0.60)	14 (8–20)	7 (4–11)	-0.07 (-0.19–0.05)

^a Allelic richness.

^b Observed heterozygosity.

^c Expected heterozygosity.

^d Genetic effective number of breeders.

^e Genetic effective population size.

^f Inbreeding coefficient (non-random mating).

Spatial capture-recapture analysis

We fit the same set of *a priori* models to both the conservative and lenient capture histories to produce a range of parameter estimates. Models that allowed river otter density to spatially vary as a function of distance from the release site failed to converge (variance-covariance matrices were comprised of all zeros); therefore, we excluded this density-covariate specification from our final set of candidate models. Four models were competing ($\leq 2 \Delta AIC_c$) for both capture histories, with population density (D) as a homogeneous Poisson point process, baseline detection rate (λ_0) varying between sexes, and spatial scale of detection (σ) shared between sexes as commonalities among most competing models (Tables 2 and 3). The top, most parsimonious model for both capture histories was identical. One competing model for the conservative capture history included a positive density-latitude relationship that indicated river otter density increased northward ($\beta_{Lat} = 0.33$); however, the confidence interval reflected much uncertainty about this relationship (95% CI: -0.18 – 0.83) and suggested that no relationship or even a negative relationship also may have been compatible with the data. A latrine-specific behavioral response (bk) that varied between sexes was strongly supported; two competing models for the conservative capture history each included these as additive or interaction effects, and one competing model for the lenient capture history included these as an interaction. Sex-specific σ was present in only one competing model for the lenient capture history and none of the competing models for the conservative capture history, thereby strongly supporting similar movements and home range sizes between male and female river otters during our survey.

Table 2. Spatial capture-recapture model selection from analysis of conservative river otter capture histories. Estimated model parameters were population density (D), baseline detection rate (λ_0), and the spatial scale of detection (σ). Models were fit that fixed those parameters as constant (1); allowed D to spatially vary as a function of latitude (Lat); accommodated sex-specific variation in both λ_0 and σ ; a trap-specific behavioral response (bk) on λ_0 ; and additive (+) and interaction (\times) effects between Sex and bk on λ_0 .

Model	K^a	AIC ^b	AIC _c ^c	ΔAIC_c^d	logLik ^e	Deviance ^f	Wt ^g
$D \sim 1 \lambda_0 \sim \text{Sex} \sigma \sim 1$	5	527.03	529.53	0.00	-258.17	516.34	0.31
$D \sim 1 \lambda_0 \sim bk + \text{Sex} \sigma \sim 1$	6	526.92	530.56	1.04	-257.14	514.28	0.18
$D \sim \text{Lat} \lambda_0 \sim \text{Sex} \sigma \sim 1$	6	527.48	531.13	1.60	-257.48	514.96	0.14
$D \sim 1 \lambda_0 \sim bk \times \text{Sex} \sigma \sim 1$	7	527.57	531.23	1.69	-257.79	515.58	0.10
$D \sim 1 \lambda_0 \sim \text{Sex} \sigma \sim \text{Sex}$	6	528.60	532.25	2.72	-258.30	516.60	0.08
$D \sim \text{Lat} \lambda_0 \sim bk + \text{Sex} \sigma \sim 1$	7	527.28	532.37	2.84	-256.64	513.28	0.07
$D \sim 1 \lambda_0 \sim bk + \text{Sex} \sigma \sim \text{Sex}$	7	528.55	533.65	4.11	-257.28	514.56	0.04
$D \sim \text{Lat} \lambda_0 \sim bk \times \text{Sex} \sigma \sim 1$	8	527.07	533.92	4.39	-255.53	511.06	0.03
$D \sim \text{Lat} \lambda_0 \sim \text{Sex} \sigma \sim \text{Sex}$	7	529.14	534.23	4.70	-257.57	515.14	0.03
$D \sim 1 \lambda_0 \sim bk \times \text{Sex} \sigma \sim \text{Sex}$	8	528.73	535.59	6.05	-256.36	512.72	0.01
$D \sim \text{Lat} \lambda_0 \sim bk + \text{Sex} \sigma \sim \text{Sex}$	8	529.01	535.87	6.33	-256.50	513.00	0.01
$D \sim \text{Lat} \lambda_0 \sim bk \times \text{Sex} \sigma \sim \text{Sex}$	9	529.07	538.07	8.53	-255.53	511.06	0.00
$D \sim 1 \lambda_0 \sim 1 \sigma \sim \text{Sex}$	5	536.12	538.62	9.09	-263.06	526.12	0.00
$D \sim 1 \lambda_0 \sim bk \sigma \sim \text{Sex}$	6	536.08	539.73	10.20	-262.04	524.08	0.00
$D \sim \text{Lat} \lambda_0 \sim 1 \sigma \sim \text{Sex}$	6	536.79	540.45	10.91	-262.40	524.8	0.00
$D \sim \text{Lat} \lambda_0 \sim bk \sigma \sim \text{Sex}$	7	536.65	541.74	12.21	-261.33	522.66	0.00
$D \sim 1 \lambda_0 \sim bk \sigma \sim 1$	5	540.61	543.11	13.58	-265.30	530.60	0.00
$D \sim 1 \lambda_0 \sim 1 \sigma \sim 1$	4	541.58	543.18	13.64	-266.79	533.60	0.00
$D \sim \text{Lat} \lambda_0 \sim 1 \sigma \sim 1$	5	541.88	544.38	14.85	-265.94	531.88	0.00
$D \sim \text{Lat} \lambda_0 \sim bk \sigma \sim 1$	6	540.77	544.42	14.89	-264.38	528.76	0.00

^a Number of model parameters.

^b Akaike's Information Criterion.

^c AIC corrected for small sample size.

^d Difference between AIC_c of model and AIC_c of top ranked model.

^e log-likelihood.

^f Model deviance ($-2 \times \log$ -likelihood).

^g Model weight.

Table 3. Spatial capture-recapture model selection from analysis of lenient river otter capture histories. Estimated model parameters were population density (D), baseline detection rate (λ_0), and the spatial scale of detection (σ). Models were fit that fixed those parameters as constant (1); allowed D to spatially vary as a function of latitude (Lat); accommodated sex-specific variation in both λ_0 and σ ; a trap-specific behavioral response (bk) on λ_0 ; and additive (+) and interaction (\times) effects between Sex and bk on λ_0 .

Model	K^a	AIC ^b	AIC _c ^c	Δ AIC _c ^d	logLik ^e	Deviance ^f	Wt ^g
$D\sim 1 \lambda_0\sim \text{Sex} \sigma\sim 1$	7	545.16	549.02	0.00	-265.58	531.16	0.19
$D\sim 1 \lambda_0\sim bk \times \text{Sex} \sigma\sim 1$	5	547.56	549.49	0.47	-268.78	537.56	0.15
$D\sim 1 \lambda_0\sim 1 \sigma\sim \text{Sex}$	5	548.18	550.11	1.09	-269.09	538.18	0.11
$D\sim 1 \lambda_0\sim 1 \sigma\sim 1$	4	549.47	550.72	1.70	-270.73	541.46	0.08
$D\sim \text{Lat} \lambda_0\sim bk \times \text{Sex} \sigma\sim 1$	8	546.01	551.15	2.13	-265.00	530.00	0.07
$D\sim \text{Lat} \lambda_0\sim \text{Sex} \sigma\sim 1$	6	548.41	551.21	2.19	-268.20	536.80	0.06
$D\sim 1 \lambda_0\sim \text{Sex} \sigma\sim \text{Sex}$	6	548.79	551.59	2.57	-268.39	536.78	0.05
$D\sim \text{Lat} \lambda_0\sim 1 \sigma\sim \text{Sex}$	6	549.12	551.92	2.91	-268.56	537.12	0.05
$D\sim \text{Lat} \lambda_0\sim 1 \sigma\sim 1$	5	550.25	552.18	3.16	-270.12	540.24	0.04
$D\sim 1 \lambda_0\sim bk \times \text{Sex} \sigma\sim \text{Sex}$	8	547.14	552.28	3.26	-265.57	531.14	0.04
$D\sim 1 \lambda_0\sim bk + \text{Sex} \sigma\sim 1$	6	549.54	552.34	3.32	-268.77	537.54	0.04
$D\sim 1 \lambda_0\sim bk \sigma\sim \text{Sex}$	6	550.10	552.90	3.88	-269.05	538.10	0.03
$D\sim 1 \lambda_0\sim bk \sigma\sim 1$	5	551.44	553.37	4.35	-270.72	541.44	0.02
$D\sim \text{Lat} \lambda_0\sim \text{Sex} \sigma\sim \text{Sex}$	7	549.72	553.58	4.57	-267.86	535.72	0.02
$D\sim \text{Lat} \lambda_0\sim bk + \text{Sex} \sigma\sim 1$	7	550.40	554.26	5.24	-268.20	536.40	0.01
$D\sim 1 \lambda_0\sim bk + \text{Sex} \sigma\sim \text{Sex}$	7	550.75	554.62	5.60	-268.38	536.76	0.01
$D\sim \text{Lat} \lambda_0\sim bk \times \text{Sex} \sigma\sim \text{Sex}$	9	548.00	554.67	5.65	-265.00	530.00	0.01
$D\sim \text{Lat} \lambda_0\sim bk \sigma\sim \text{Sex}$	6	551.07	554.93	5.91	-268.53	537.06	0.01
$D\sim \text{Lat} \lambda_0\sim bk \sigma\sim 1$	8	552.23	555.03	6.01	-270.11	540.22	0.01
$D\sim \text{Lat} \lambda_0\sim bk + \text{Sex} \sigma\sim \text{Sex}$	8	551.70	556.84	7.83	-267.85	535.70	0.00

^a Number of model parameters.

^b Akaike's Information Criterion.

^c AIC corrected for small sample size.

^d Difference between AIC_c of model and AIC_c of top ranked model.

^e log-likelihood.

^f Model deviance ($-2 \times$ log-likelihood).

^g Model weight.

We model-averaged competing models to produce final parameter estimates. Estimates of σ were similar between the two capture histories (range: 11.03–12.11 km) and resulted in an optimal sinuous buffer of 50 km upstream and downstream of latrines (Fig. 2). Thus, our models indicated that parameter estimates applied to a 359-km dendritic state space (area of integration) that was comprised of perennial waterways in the URG Basin, ranging from the southern tip of the Alamosa National Wildlife Refuge in Colorado to the Rio Grande and Chama rivers at La Mesilla and Chili, New Mexico, respectively (Fig. 3).

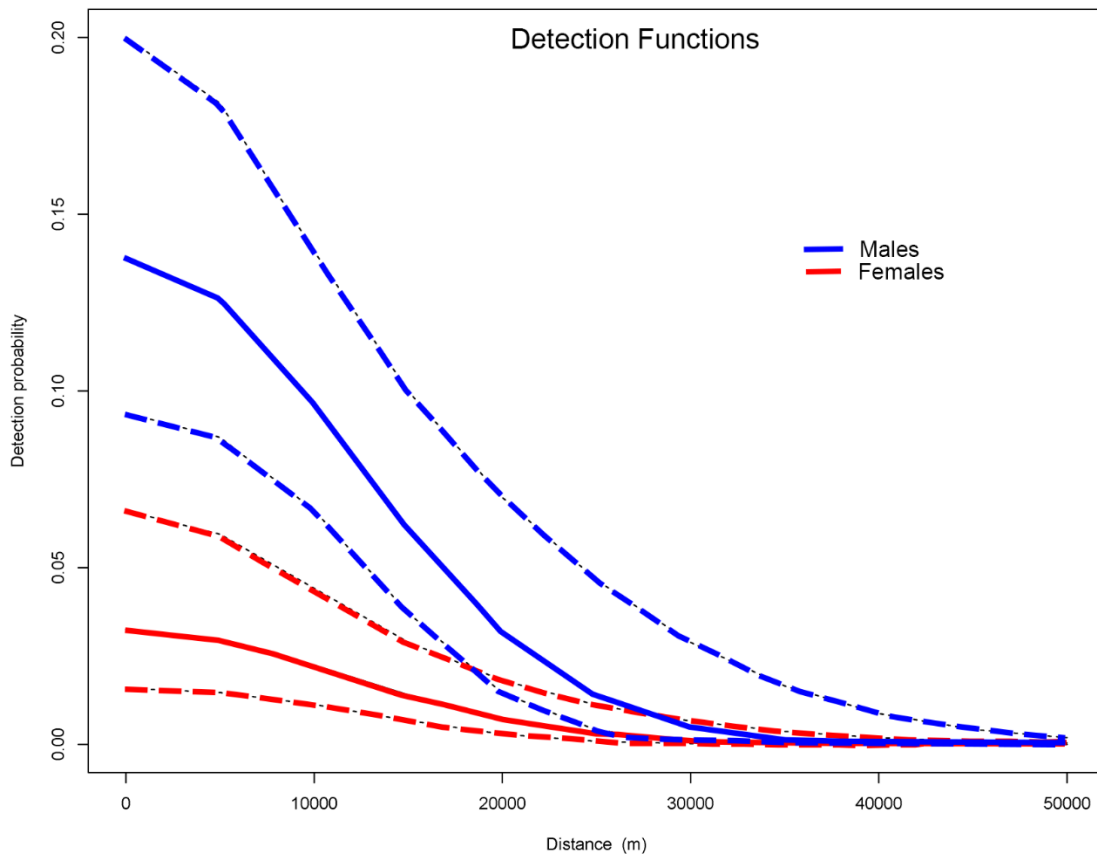


Figure 2. Plot of hazard half-normal detection functions for male and female river otters from the most parsimonious spatial capture-recapture models. Depicts the rate of detection probability decline as a function of increasing distance between a river otter activity center and a latrine. Solid lines represent point estimates and dashed lines represent 95% confidence intervals.

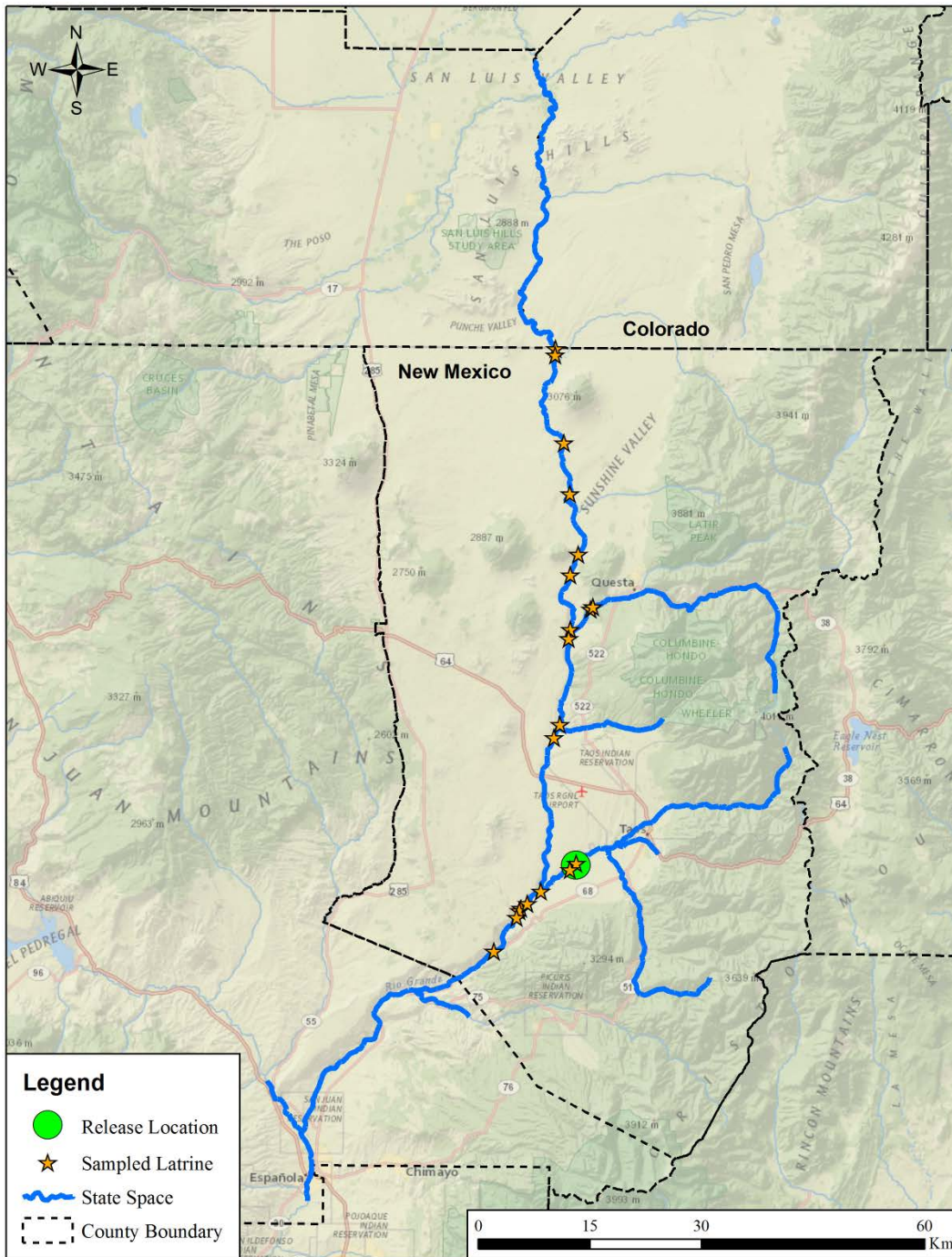


Figure 3. Extent of the 359-km dendritic state space that was comprised of perennial waterways to which spatially explicit estimates of river otter population size and density applied.

The lenient capture history resulted in 50–69% lower estimates of male λ_0 and 21.7% larger estimates of D compared with the conservative capture history, whereas estimates of female λ_0 were similar between capture histories (Tables 4 and 5). The conservative estimate of D was 0.23 otter/km (95% CI: 0.13–0.40), or 1 otter/4.35 km (95% CI: 2.50–7.69) of waterway, whereas the lenient estimate of D was 0.28 otter/km (95% CI: 0.17–0.49), or 1 otter/3.57 km (95% CI: 2.04–5.88) of waterway. Models estimated that the population sex ratio was female-biased, though the conservative estimate had a 24% larger female component (0.28M:0.72F) than the lenient estimate (0.42M:0.58F). The D estimates corresponded to conservative and lenient N estimates of 83 (95% CI: 47–144) and 100 (95% CI: 61–176) total river otters, respectively, in the 359 km of perennial waterways within the dendritic state space. Conservative and lenient average annual exponential population growth rates (r) from 2010 to 2018 were 1.12/year (95% CI: 1.05 – 1.20) and 1.15/year (95% CI: 1.08–1.23), respectively. Based on a maximum riparian area width of ~200 m, estimated elliptical river otter home range (HR_E) sizes were 7.16 km² (95% CI: 5.47–9.36) from the conservative σ estimate, and 6.93 km² (95% CI: 4.62–10.39) for females and 7.61 km² (95% CI: 5.58–10.37) for males from the respective lenient σ estimates, or an average lenient population HR_E of 7.27 km² (*Note*: sex-varying σ was not supported by any competing models for the conservative capture history, so conservative sex-specific σ and HR estimates were not produced). Ellipticity, or degree of deviation from circularity, ranged from 0.004 to 0.005 for all 3 HR_E estimates. If home ranges were instead approximately linear, then estimated linear home range (HR_L) sizes were 45 km (95% CI: 35–60) from the conservative σ estimate, and 44 km (95% CI: 29–66) for females and 48 km (95% CI: 36–66) for males from the respective lenient σ estimates, or an average lenient population HR_L of 46 km.

Table 4. Conservative parameter estimates from model-averaging of 4 competing ($\leq 2 \Delta AIC_c$) spatial capture-recapture models that analyzed conservative capture histories.

Parameter	Estimate	SE	95% CI	CV
<i>D</i> (otter/km)	0.23	0.07	0.13–0.40	0.30
<i>N</i>	83	25	47–144	0.30
Sex Ratio				
Females (%)	0.72	0.09	0.51–0.87	0.12
Males (%)	0.28	0.09	0.13–0.48	0.32
λ_0				
Females, bk = 0	0.03	0.01	0.01–0.07	0.33
Males, bk = 0	0.14	0.03	0.09–0.22	0.21
Females, bk = 1	0.04	0.02	0.02–0.12	0.50
Males, bk = 1	0.16	0.05	0.09–0.28	0.31
σ (km)	11.39	1.57	8.71–14.90	0.14

Table 5. Lenient parameter estimates from model-averaging of 4 competing ($\leq 2 \Delta AIC_c$) spatial capture-recapture models that analyzed lenient capture histories.

Parameter	Estimate	SE	95% CI	CV
<i>D</i> (otter/km)	0.28	0.08	0.17–0.49	0.29
<i>N</i>	100	29	61–176	0.29
Sex Ratio				
Females (%)	0.58	0.12	0.34–0.79	0.21
Males (%)	0.42	0.12	0.21–0.66	0.28
λ_0				
Females, bk = 0	0.03	0.02	0.01–0.10	0.67
Males, bk = 0	0.07	0.02	0.04–0.11	0.29
Females, bk = 1	0.06	0.03	0.02–0.17	0.50
Males, bk = 1	0.05	0.03	0.01–0.15	0.60
σ (km)				
Females	11.03	2.30	7.36–16.53	0.21
Males	12.11	1.93	8.88–16.51	0.16

DISCUSSION

Our estimates of spatially explicit river otter density (0.23–0.28 otter/km) for the URG population are within the range of reported densities for the species, but are towards the lower bound. River otter densities tend to vary between inland and coastal systems, with densities of 0.07–0.51 otter/km and 0.28–0.93 otter/km, respectively (Melquist and Hornocker 1983, Testa et al. 1994, Bowyer et al. 2003, Mowry et al. 2011, Brzeski et al. 2013). This discrepancy between systems is primarily the result of coastal bays generally providing higher quantities of suitable habitats and food resources than inland rivers and streams (Kruuk 1995, Blundell et al. 2000, Brzeski et al. 2013). Nevertheless, comparisons among our estimates of river otter density for the URG population and reported density estimates for other river otter populations must be interpreted with caution. Previous estimates of river otter densities were primarily derived from population sizes that were estimated using traditional non-spatial capture-recapture models. Non-spatial estimates of population density are typically inaccurate, because the effective sampling area to which population size estimates apply is unknown and must be approximated with *ad hoc* methods (Borchers and Efford 2008, Obbard et al. 2010, Sutherland et al. 2016). In contrast, spatial capture-recapture models explicitly define the geographical area to which density estimates apply and can produce unbiased estimates of population density. To our knowledge, our study is the first to estimate river otter population density using spatial capture-recapture models with an ecologically realistic non-Euclidean distance submodel and a state space (parameter estimation area) restricted to the dendritic network of waterways.

We are aware of only one previous study that estimated river otter density using spatial capture-recapture models. Forman (2015) applied spatially explicit models to detection data obtained from river otter fecal DNA in Pennsylvania, USA. However, he used the default

Euclidean distance submodel that is predicated on home ranges being approximately circular in shape, and also used a convex hull state space that included all non-riparian habitats in the intervening areas among waterways. Although river otters are capable of traversing non-riparian habitats to move among waterways, they do so very infrequently relative to the amount of time spent moving within riparian corridors along waterways (Spinola et al. 2008); thus, river otters typically have home ranges that are elliptical, sinuous, or linear in shape (Sauer et al. 1999). For populations or species that have such non-circular home ranges, using the default Euclidean distance submodel will result in biased estimates of population density (Efford 2019a). Additionally, using a convex hull state space that includes large areas of non-riparian habitats for a riparian-obligate species, without incorporating a non-Euclidean distance submodel, will negatively bias density estimates (e.g., Royle et al. 2013, Efford 2019a). In contrast, our use of a non-Euclidean distance submodel that reflected the sinuous distances along the dendritic network, as well as a state space that was comprised of only the dendritic network, mitigated bias and likely improved the accuracy of our density estimates (Efford 2019a).

Nonetheless, our estimates of river otter density for the URG population may not be completely free from bias. First, noninvasive fecal DNA sampling of scats results in the detection of all age classes of a population that defecate at sampling sites (Mowry et al. 2011, Brzeski et al. 2013, Davidson et al. 2014, Morin et al. 2016). Although the level of variation in defecation rates among adult, subadult, and juvenile otters at latrines is unclear, differentiating among scats or genotypes by age class is not possible. We likely sampled at least some scats deposited by juveniles; therefore, our density estimates are not restricted solely to the reproductively active cohort of the population and, as a result, should be considered total population estimates that include all age classes (Mowry et al. 2011, Brzeski et al. 2013).

Second, genotyping error in the form of allelic dropout, and possibly false alleles, was present in our detection data. Genotyping error is common in all types of noninvasively collected genetic material, but it may be more prevalent in scat, because DNA is generally of lower quantity and poorer quality compared with hair and tissue (Stenglein et al. 2010). This problem is often exacerbated in scat from carnivores, primarily because of the high lipid and low fiber content of consumed meats, which can reduce intestinal cell slough rates (Murphy et al. 2003). Genotyping error or failure may be further elevated in scat from otters, as well as other carnivores that also have diets primarily comprised of aquatic fauna, because the byproducts from consumed fish and other aquatic species may interfere with the chemistry of the DNA extraction protocol (Dallas et al. 2000, Murphy et al. 2003, Aristizábal Duque et al. 2018). For example, Brzeski et al. (2013) encountered 28.6% and 1.5% rates of allelic dropout and false alleles, respectively, from genotyping of fecal DNA in river otter scats that were collected in California.

Analytical methods that incorporate genotyping error have been implemented in traditional non-spatial capture-recapture models to mitigate bias in population size estimates (Lukacs and Burnham 2005, Knapp et al. 2009, Wright et al. 2009). However, no such methods have been developed yet for implementation in spatial capture-recapture models; though extending the recently developed spatial partial-identity class of spatial capture-recapture models has considerable potential (Augustine et al. 2019). Given the current lack of methods for incorporating genotyping error in spatial capture-recapture models, we chose to provide 2 separate sets of parameter estimates based on analysis of both conservative and lenient capture histories. Although it is unclear which capture history and corresponding estimates are most accurate, we suspect that the conservative estimates are more reliable; genotyping error typically inflates the number of unique genotypes and leads to overestimates of population size and

density (Lukacs and Burnham 2005, Knapp et al. 2009, Wright et al. 2009). The difference in river otter density estimates from the two sets of capture histories was 21.7%, but this corresponded to a population size discrepancy of only 17 river otters. This is relatively nominal realized difference, and confidence intervals overlapped considerably, which increases confidence that the true population size is 83 to 100 river otters.

Precision of our population density and size estimates ($CV = 0.29\text{--}0.30$) was nevertheless poorer than desirable (i.e., optimal $CV \leq 0.20$; Laufenberg et al. 2013). The less than optimal precision has a generally minimal influence on point estimates, as these are the most probable values given the data (i.e., values that maximized the likelihood function); rather, poor precision is reflected in the wide confidence intervals around the point estimates. These intervals indicate that a URG population size of as few as 47 river otters and as many as 176 river otters may also be compatible with our data, albeit much less likely than the point estimates (Amrhein et al. 2019, Wasserstein et al. 2019). This poor estimate precision is largely a direct consequence of the low genotyping success rate for our samples. The effect of genotyping failure on spatial capture-recapture model parameter estimates is functionally similar to randomized subsampling, which point estimates from spatial capture-recapture models are nominally affected by (Murphy et al. 2016, 2018). However, both genotyping failure and randomized subsampling result in the loss of spatial recaptures, which can directly degrade estimate precision (i.e., wider confidence intervals; Murphy et al. 2016, Augustine et al. 2019).

The 14% genotyping success rate for our samples was similar to other published otter fecal DNA studies, though towards the lower bound of the range ($\bar{x} = 26\%$, range: 8–60%; Dallas et al. 2003, Ben-David et al. 2004, Kalz et al. 2006, Hájková et al. 2009, Guertin et al. 2010, Mowry et al. 2011, Brzeski et al. 2013). Genotyping success of fecal samples can be influenced

to varying degrees by the environment, including climatic conditions; sources of fecal DNA; age of fecal material; method of sample storage; and PCR-inhibiting substances in scat (Dallas et al. 2003, Fike et al. 2004, Hájková et al. 2009, Stenglein et al. 2010, Mowry et al. 2011). We collected samples during the coldest winter months, stored samples according to established effective protocols, and employed DNA extraction and PCR amplification techniques and used microsatellite loci that were optimized for otters, all of which enhance genotyping success rates (Stenglein et al. 2010, Mowry et al. 2011, Brzeski et al. 2013). Fecal samples collected from otter anal jellies tend to have higher genotyping success rates than samples from scats, but fewer jellies are deposited by river otters compared with scats (Mowry et al. 2011, Brzeski et al. 2013). We attempted to locate anal jellies at each latrine but found only 4 total jellies that were intact; numerous stains on rocks, logs, and soils at multiple sites were indicative of the prior presence of anal jellies that had already decomposed.

We suspect that the high ultraviolet radiation and arid climatic conditions of the study area likely caused rapid drying and decomposition of anal jellies and elevated DNA degradation in scats (Murphy et al. 2007, Lonsinger et al. 2015). Mean ultraviolet index and relative humidity in the study area during sampling were 8.5 and 1.0%, respectively, which are classified as ‘very high’ and ‘arid’, respectively (National Oceanic and Atmospheric Administration 2019). Poor genotyping success rates for noninvasively collected scat and hair samples from other carnivore populations in New Mexico and elsewhere in the southwestern USA were attributed to the same causes (Naidu et al. 2011, Gould et al. 2018). Survey occasions that are <7 days in duration may be necessary to combat the DNA-degrading environmental and climatic conditions of the URG Basin. However, scat accumulation rates at latrines tend to be slow for otters (Gallant et al. 2007,

Rivera et al. 2019), and surveying latrines more frequently than 7-day intervals would be logistically infeasible without doubling manpower and increasing survey costs.

Assuming our spatial capture-recapture models accurately estimated population density and size, that all 33 founders survived the founder event, and that exponential growth was possible, the URG population has exhibited moderate positive population growth to date (1.12–1.15/year). Just 8 years had elapsed between the founder event and our study, which represents ~1.25 generations (average river otter generation time = 6.4 years; Boyle 2006, Mowry et al. 2015). Additionally, limited competition for resources existed, because no resident population of river otters occurred in the URG at the time of reintroduction (NMDGF 2006). Thus, the conditions for exponential growth likely did exist. However, the optimistic population growth rates that we estimated for the URG population are >40% lower than growth rates estimated for most other reintroduced river otter populations (Breitenmoser et al. 2001, Barding and Lacki 2014, Ellington et al. 2018). Although a feasibility study that was conducted prior to the reintroduction indicated that the URG Basin was the most suitable for river otters, relative to other riverine systems in New Mexico (NMDGF 2006), perennial waterways in this arid region are sparsely distributed and more frequently below the minimum flow levels required to support river otter populations, compared with systems in Missouri, Kentucky, and Ohio, where population growth was much higher. Furthermore, otter reintroduction projects in those states released founder groups that were substantially larger than the number of URG founders, ranging from 123 founders in Ohio to 845 founders in Missouri (Hamilton 1998, Barding and Lacki 2014, Ellington et al 2018). The small size of the URG founder group corresponds to much lower breeding opportunity among founders compared with the large founder groups in other states,

which likely mitigated the potential for more rapid population growth to occur (Breitenmoser et al. 2001, Groombridge et al. 2012).

An unfortunate consequence of using small founder groups in reintroductions is the severity of a founder effect, which immediately reduces population genetic diversity, genetic effective population size, and thus, population fitness (Lande et al. 2003, Allendorf and Luikart 2007, Jaimeson 2010). We found considerable evidence that the URG river otter population has experienced a severe bottlenecking founder effect, and that the compounding effect of subsequent genetic drift and isolation has led to rapid genetic divergence of the URG from the WA source population. Estimates of genetic diversity and genetic effective sizes were 20–26% and 106–170% lower, respectively, in the reintroduced URG population compared with the WA source population. Furthermore, estimates of genetic effective population size (N_E) for the URG population are far below the minimum that is necessary for both short- and long-term population viability and to prevent inbreeding depression ($N_E > 50$; Frankham et al. 2014). Although point estimates of inbreeding coefficients did not support non-random mating, confidence intervals overlapped zero with positive upper bounds, suggesting that non-random mating was compatible with our data. Additionally, inbreeding would be difficult to detect so few generations after the founder event and likely would necessitate pedigree (parentage) reconstruction and larger sample sizes of individuals from both the URG and the WA source population to confirm (Biebach and Keller 2010). Nevertheless, the rapid rate of diversity loss and decline in effective sizes in the URG river otter population are alarming. Given that the URG population is likely isolated and the potential for natural immigration from populations in other riparian networks is low, human-assisted genetic restoration via translocations may be required to prevent further genetic degradation.

MANAGEMENT IMPLICATIONS

Although our estimates of population density, size, and growth rate support short-term reintroduction success for the URG river otter population, the genetic results do not support short-term success, and the criteria for long-term reintroduction success have not been attained (Robert et al. 2015). The URG river otter population is small, genetically depauperate, likely isolated, and consequently has elevated vulnerability to acute population decline, inbreeding, and loss of fitness (Lande et al. 2003, Allendorf and Luikart 2007, Jaimeson 2010, Frankham et al. 2014). Therefore, we recommend that protections currently afforded to river otters in New Mexico be maintained into the foreseeable future. River otters in the URG dendritic network are presently listed as threatened in Colorado, so if protections in New Mexico continue, substantial opportunity would exist for further growth of this transboundary population. Additionally, we suggest that managers consider augmenting the URG population with river otters from other populations, perhaps those in geographically proximal waterways in neighboring states (e.g., San Juan River or Verde River), to attempt to increase genetic diversity and effective population size, as well as reduce the potential for inbreeding. At minimum, demographic and genetic monitoring of the URG river otter population should be implemented at regular intervals (e.g., once per generation [~6 years]) to afford managers the opportunity to implement timely conservation actions if a population decline or further genetic erosion occur.

Assuming our estimates of river otter density remain spatially and temporally constant throughout the additional 355 km of waterways in the URG dendritic network where otter occurrences have been documented (714 km total, including our 359-km parameter estimation area; Savage and Klingel 2015, Colorado Parks and Wildlife 2018), potentially as many as 164–200 river otters could inhabit the network from the headwaters of the Rio Grande near Creede,

Colorado, south to the Rio Chama and Rio Grande rivers at Chama River Canyon Natural Area and Cochiti Lake, New Mexico, respectively (Fig. 4). Thus, despite having a female-biased sex ratio that is indicative of range expansion (Murphy et al. 2016), potential for the URG population to increase to the recommended Mustelidae minimum viable population size of ≥ 400 individuals in the near future is likely low (Reed et al. 2003). Based on our estimated average annual rates of URG population growth since the founder event, and an optimistic but unverifiable assumption that exponential population growth will continue, the URG river otter population could reach said minimum viable population size by the years 2030–2033 (i.e., 11–14 years from present). However, this assumes that perennial waterways in the URG Basin, from Cochiti Lake, New Mexico to the headwaters of the Rio Grande in Creede, Colorado, collectively have sufficient resources to support an extraordinarily high river otter population density of approximately 0.50 otter/km of waterway, or 1 otter/2.0 km; this would be among the highest river otter densities ever reported. Habitat availability is likely the principal limiting factor to growth of the URG river otter population, because most drainages south of Cochiti Lake were identified as unsuitable or of limited suitability for river otters (NMDGF 2006). Nevertheless, the river otter food habits study that we are conducting should provide at least partial insight into whether sufficient resources exist to support such a high population density.

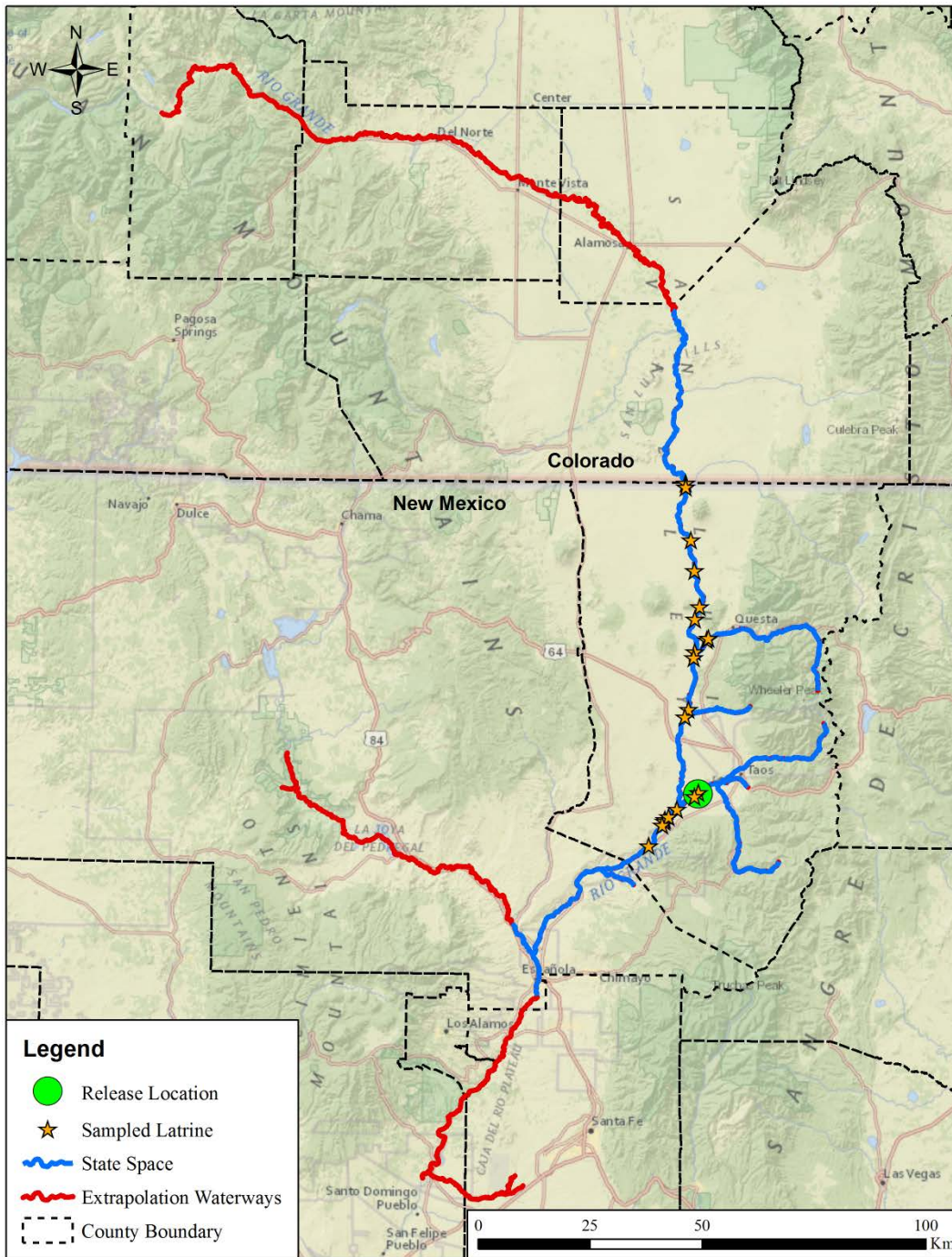


Figure 4. The extent of waterways to which spatially explicit river otter population estimates applied (state space) and additional reaches where river otter occurrence has been documented in the URG Basin since 2008 (extrapolation waterways; Savage and Klingel 2015, Colorado Parks and Wildlife 2018) relative to the URG reintroduction release site and sampled latrines.

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