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ALL IN DUE TIME: MULTI-TRAIT ASSESSMENT OF ELK ACCLIMATION TO
TRANSLOCATION

By

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Dissertation

presented in partial fulfillment of the requirements
for the degree of

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in Fish and Wildlife Biology

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All in due time: Multi-trait assessment of elk acclimation to translocation

Chairperson: Dr. Joshua Millspaugh

ABSTRACT

Wildlife translocation – the intentional movement of animals – is a crucial conservation tool for restoring species and halting global biodiversity decline. However, this practice is challenging for wildlife, and animals must adjust to their release landscapes for restoration to be successful. The period following release is a vulnerable time for translocated wildlife and determining when and how animals eventually acclimate following releases allows researchers to efficiently tailor post-release management to each species' needs, thus maximizing the success of translocations while minimizing costs of an already expensive conservation practice. In this dissertation, I investigate changes in the physiological, behavioral, and social dynamics of 106 elk (*Cervus canadensis*) during the 6-8 years following their release to Missouri, U.S.A. in 2011-2013. I define the acclimation period throughout this work as the duration of time prior to stabilization in each investigated response relative to time from release. In Chapter 1, I analyzed changes in glucocorticoid metabolites (fGCMs) as an indicator of physiological acclimation. Fecal GCM levels declined following translocation and subsequently stabilized relative to days from release at approximately 42 days. The fast physiological acclimation by Missouri elk relative to other species suggests relatively low sensitivity by elk to translocation and effective use of temporary post-release management efforts. In Chapter 2, I investigated changes in elk spatial behavior (movements and resource selection patterns) using location data from GPS-collars deployed on all translocated elk. Changes in resource selection and monthly individual range sizes and overlap relative to time from release stabilized within the first year of translocation. Sexes varied in their post-release movement dynamics, with females showing faster and stronger evidence of acclimation following translocations that occurred during the parturition season. Significant temporal dynamics in selection for multiple resource covariates indicated that elk did not demonstrate a simple forage-refuge tradeoff while acclimating to their release landscape. In Chapter 3, I investigated dynamics in elk mating structure using paternity analysis on DNA extracted from tissue samples of all translocated elk and subsequently captured adults and calves. Following a translocation tactic favoring releases of young-aged males, initial polygyny in the restored Missouri population was low; however, polygyny levels increased and stabilized to expected values within four years of the last translocation event. Importantly, initial dampened polygyny may facilitate retention of genetic variation by maximizing the genetic contribution of more founding individuals. In Chapter 4, I investigated retention of genetic diversity over initial generations following release and projected future losses over a management-relevant time period. The Missouri elk population retained relatively high levels of genetic diversity as evidenced by minimal losses in allelic richness and expected heterozygosity (H_e), and we projected similarly stable H_e levels for the next 130 years (loss < 10%). Together, these results suggest translocated wildlife acclimate to their release landscapes in a continuum of response, with behavior lagging physiological responses, and larger-scaled population processes, such as mating structure, sitting at the ultimate end of this spectrum. Investigating the manifold changes of translocated animals as they acclimate to their release landscape represents an opportunity to improve post-release monitoring and assessment while directly informing dynamic management needs of restored populations.

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A final bid of gratitude to the wild life and places that surround us. They are why I am here today. “As we work to heal the earth, the earth heals us.” – Robin Wall Kimmerer

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Chapter 1: Physiological acclimation of elk during population restoration in the Missouri Ozarks, U.S.A.¹

ABSTRACT

Conservation translocations -- the intentional movement of animals to restore populations -- have increased over the past 30 years to halt and reverse species declines and losses. However, there are many challenges translocated animals face which should be considered for restoration programs to be successful. Understanding how long it takes for translocated animals to acclimate to these challenges and their new landscape is a critical component of post-release population management. Physiological measures such as hormone responses are increasingly used to assess animal responses and acclimation to disturbances including translocation. We determined the physiological acclimation period of elk (*Cervus canadensis*) translocated to the Missouri Ozarks, USA as part of a restoration effort. From 2011 to 2013, we translocated 108 GPS-radio-collared elk from Kentucky, USA to Missouri, USA, and collected fecal samples for glucocorticoid metabolite extraction to use as an indicator of physiological acclimation. We modeled the response of population-wide fGCMs across the initial nine years of the restoration in response to days following release and additional site-specific covariates. Presence of white-tailed deer (*Odocoileus virginianus*) hunts and monthly precipitation levels were positively and negatively associated with fGCM levels, respectively. Concurrent with influences from site-specific conditions on the release landscape, fGCM levels declined following release. We identified a breakpoint in fGCM decline at approximately 42 days following translocation releases

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suggesting elk acclimated physiologically relatively quickly compared to other species. The fast physiological acclimation by Missouri elk suggests effective use of temporary post-release management efforts. Determining how quickly animals acclimate following translocations allows researchers to tailor post-release management plans to each species' needs, thus maximizing the success of future translocation efforts while minimizing costs.

INTRODUCTION

Biological communities are experiencing declines worldwide in what has been called the 'sixth great extinction' (Ceballos et al. 2017). Terrestrial communities have lost over 20% of their original biodiversity globally and three quarters of large land mammals have been extirpated from their original ranges (Diaz et al. 2019). Conservation translocation -- the intentional movement of animals to restore populations (IUCN 2013) -- has emerged over the past thirty years as an important conservation tool to halt and reverse species declines. Nearly 700 reintroduction-based translocation efforts occurred in the United States alone by 1989 (Griffith et al. 1989) and the number has subsequently increased (Seddon and Armstrong 2016). Despite increases in the practice, translocation projects have been plagued by failures often attributed to unavoidable challenges and disruptions to translocated individuals (Griffith et al. 1989; Teixeira et al. 2007).

Wildlife experience challenges associated with the translocation process during their acclimation to the new landscape (Teixeira et al. 2007; Dickens et al. 2010). For example, during translocation, animals often experience multiple captures, periods of captivity and/or quarantine, disease testing and intervention, containment and transfer, and release into foreign systems with novel pressures (Dickens et al. 2010). This series of successive translocation challenges represents a prolonged exposure to stress and is one of the biggest threats to restoration success

(Teixeira et al. 2007; Dickens et al. 2010; Armstrong et al. 2017). If translocated wildlife are unable to adequately respond to prolonged challenges through behavioral and physiological modifications, animals risk physiological disruption (Romero et al. 2009). Physiological disruptions in turn make animals more susceptible to increased mortality and reproductive failure when acclimating to their new landscape, and these post-release effects can determine whether a translocation is successful (Armstrong and Reynolds 2012).

Post-release effects can be mitigated through management interventions (Harrington et al. 2013). In particular, managers can provide supplemental food (Castro et al. 2003) or protection from predators (Villemey et al. 2013) during the acclimation period. Managers may also choose to limit the amount of human viewing or recreation opportunities available to the public while a population acclimates to minimize additional challenges to translocated populations. For example, managers closed trapping seasons within a 625-km² area to protect a recently translocated fisher population (*Martes pennanti*) in southwestern Oregon (Aubry and Lewis 2003). However, such management actions are expensive and sometime controversial (Coz and Young 2020). Understanding how long provisions or protections need to be applied following a translocation effort can maximize time- and cost-efficiency (Moehrensclager and Lloyd 2016). For this reason, knowing the length of time necessary for a population to acclimate to its new landscape can inform post-release management and is important to translocation success.

With recent attention on population acclimation, it is thought that duration of time required to reach acclimation following translocation varies among species (Armstrong et al. 2017); however, species-specific data on acclimation duration is limited. Understanding the time to acclimation and variation across species could help determine species-specific sensitivities to post-release effects and how reactive species are to translocation challenges. Base knowledge of

species-specific sensitivities to translocation challenges may ultimately assist biologists in planning future translocation efforts. Understanding the spectrum of translocation sensitivities across species is also necessary to inform species- or taxa-specific translocation guidelines recommended by the IUCN (IUCN 2013).

Previous investigations on acclimation have focused on estimating duration through changes in survival (Armstrong et al. 2017), but the demographic data required is resource intensive (e.g., mark-recapture studies) and does not reflect finer scale impacts. Moreover, because mortality is ostensibly the coarsest metric to gather, managers may benefit from finer scale bioindicators of acclimation that may be useful in forecasting ultimate demographic trends. Measuring the behavioral or physiological acclimation of wildlife may provide a more sensitive response metric to translocation. Glucocorticoid hormones (GCs) are highly conserved steroid hormones that regulate and, in turn, reflect physiological and behavioral responses to environmental challenges (McEwen et al. 2003). GCs secreted into the blood are metabolized and present in multiple non-plasma materials that can be collected frequently and noninvasively to reflect integrated GC levels over tissue- and species-specific excretion intervals (Dantzer et al. 2014). Fecal GC metabolites (fGCMs) are one non-plasma material commonly used when sampling plasma is not preferred or possible (Palme et al. 2019). Researchers increasingly use GCs as sensitive physiological markers of individual and population response to translocation (Teixeira et al. 2007; Dickens et al. 2010) and commonly observe elevations in GCs following release (Franceschini et al. 2008; Jachowski et al. 2013). As such, the return of GC levels to baseline may be used to indicate physiological acclimation following translocation.

Although GCs and their metabolites are commonly used to indicate responses to translocation challenges, they are less commonly used to understand the duration of acclimation

and, in turn, inform the sensitivity of species to translocation-related conservation actions. To bridge this information gap, we use fGCMs as an indicator of acclimation status in a translocated elk (*Cervus canadensis*) population in Missouri, U.S.A. Evidence suggests elk acclimate well to different forms of disturbance (Van Dyke et al. 2012) to the point that concern exists for high levels of elk habituation in un hunted populations (Thompson and Henderson 1998). Further, increasing evidence associates underlying GC physiology with animal movement behavior (Jachowski et al. 2013, 2018; Jachowski and Singh 2015), and initial investigation into the movements made by elk translocated to Missouri suggested little behavioral disruption following release (Bleisch et al. 2017). We hypothesized that the recently translocated Missouri elk population would similarly show little physiological sensitivity to translocation by demonstrating a relatively fast period of fGCM acclimation. In addition to estimating the physiological acclimation period for Missouri elk, we compared our results to durations for other species to consider a broader species-specific spectrum of translocation sensitivity. A better understanding of species-specific sensitivities to translocation will ultimately inform species-specific translocation protocols as advocated by the IUCN to improve conservation efforts (IUCN 2013).

METHODS

Animal translocations — We translocated 108 elk from Kentucky, USA to the southeastern Missouri Ozarks, USA (91°24' to 90°58'W and 37°0' to 37°19'N: Bleisch et al. 2017) in three successive cohorts from 2011 to 2013. The nearest neighboring restored elk population was in Arkansas and separated from the Missouri elk range by approximately 250 mi (Dent et al. 2012). We captured elk from the source population in January of each year (2011–2013) and held them in quarantine corral facilities at the capture site for 102–129 days before overnight trailer transport to Missouri. Upon arrival in Missouri, and prior to release, we held elk for an additional

quarantine period of 19–34 days in outdoor holding corrals at Peck Ranch Conservation Area, which is managed by the Missouri Department of Conservation (MDC).

We released elk in Missouri in June of each year. The demographic composition of release cohorts differed in each year: 2011 (n = 34) – 15 adult females (2+ years), 5 yearling females, 6 two-year-old males, 8 yearling males; 2012 (n = 33) – 22 adult females, 3 yearling females, 4 two-year-old males, 4 yearling males; 2013 (n = 39) – 20 adult females, 16 yearling females, 3 yearling males. Prior to release, we fit all elk with GPS-VHF collars (RASSL custom 3D cell collar, North Star Science and Technology, King George, VA, or G2110E Iridium-GPS series model, Advanced Telemetry Systems, Isanti, Minnesota, USA) and affixed PIT- and ear-tags.

Sample collection — We collected fresh fecal samples with semi-regular frequency (from September 2011 to December 2014 and from January 2018 to November 2019) without observation or knowledge of individual elk identity. We randomized collection of elk fecal samples across the landscape by randomly selecting GPS-collared elk IDs and collecting a fresh fecal sample from the area of their most recent location within the previous 6 hours. Previous studies found little difference in fGCM estimates between anonymous and individual based collection approaches in ungulate species (Huber et al. 2003; Corlatti 2018).

Upon sample collection, we randomly subsampled 5-10 fecal pellets from pellet groups that appeared fresh. We avoided collecting samples after rain events to preserve the integrity of the fGMs within fecal samples (Washburn and Millspaugh 2002) and facilitate confidence around recency of pellet deposition. We homogenized pellets with a mallet prior to storage within a -20°C freezer until assay preparation (Millspaugh and Washburn 2003).

Sample preparation and assay — We followed established protocols for fGM extraction, dilution, and assay outlined by Wasser et al. (2000) and physiologically validated for elk (Millspaugh et al. 2001). Briefly, we freeze-dried samples then ground and sifted them through stainless steel mesh for thorough mixing. We subsampled dried and sifted feces to a standardized weight of ~0.2 g for each sample. We extracted metabolites by washing dried feces in 2.0 mL 90% methanol, vortexing for 30 min, and centrifuging for 20 min at 4°C. We stored the resulting supernatant in a -20°C freezer until assayed. We used corticosterone I125 radioimmunoassay kits (MP Biomedicals, Solon, OH) and followed MP Biomedical assay protocol except for halving reagent volumes (Millspaugh et al. 2001).

We assayed a first batch of samples collected in 2011–2014 ($n = 935$) in a randomized order in 2014 over 12 assays. Average inter-assay variation for 2011-14 assays was 2.92% and intra-assay variation was 1.51%. We assayed a second batch of samples collected in 2018 and 2019 ($n = 236$) together in a randomized order in 2020 over 6 assays. Average inter-assay variation for 2018-19 assays was 6.99% and intra-assay variation was 1.63%. We duplicated the assay of 50 freeze-dried fecal samples collected in 2011–2014 at the time of assay for the 2018 and 2019 samples to test for bias between batches. We stratified selection of the duplicated samples across low [$n = 18$; 0 – 20 ng/g], medium [$n = 14$; 21 – 50 ng/g], and high [$n = 18$; 51 – 200 ng/g] fGCM values. Samples were highly correlated (Pearson's $r = 0.95$), and we did not detect any difference in fGCM values between batches that was beyond a consistent, marginal decline expected with extended storage (6–9 y) of lyophilized samples in a -20°C freezer (paired-samples t-test: $t = -6$, $p < .05$, mean difference [95% CI] = -7.27 [-9.78—4.77]).

Statistical analyses — We modelled the dynamics of elk fGCM responses to translocation with a two-step process. First, we built a generalized linear model to draw inferences on fGCM

responses relative to the effect of translocation along with other covariates hypothesized to influence elk fGCMs. We then performed a breakpoint analysis (Muggeo 2003) on the model to identify when physiological acclimation occurred as evidenced by a significant change in the slope of fGCM response in the days following translocation releases. Because we were unable to collect pre-translocation fecal samples to determine within-population baseline fGCM values, we relied on comparison to reference values from established elk populations that were determined using the same laboratory methodology and reported elsewhere in the literature (Washington: Jachowski et al. 2015; South Dakota: Millspaugh et al. 2001).

To build the generalized linear model for the first step in our analysis, we considered covariates in three categories hypothesized to challenge elk: translocation factors, climate, and human disturbance (Table 1-1). Translocation covariates included days from most recent translocation release, year of restoration, and the proportion of animals released within the year (Table 1-1). Climate covariates included temperature and precipitation covariates averaged over the month and previous day to reflect potential thermoregulatory and/or drought challenges (Romero 2002; Table 1-1). We used measures from the previous day for daily averages of climatic variables to align with the GCM excretion profile of elk (Wasser et al. 2000). Human disturbance covariates included factors related to the occurrence and type (gun, bow, or muzzleloader) of managed white-tailed deer (*Odocoileus virginianus*) hunts that took place sporadically October – December within Peck Ranch Conservation Area. Hunting is a major challenge to target animals (Santos et al. 2018). Although elk were not hunted, we included these human disturbance covariates to reflect potential challenges associated with human activity and use of firearms on the landscape. As we were unsure the duration of potential challenge

following the end of the managed deer hunts, we compared models reflecting a 3-day, 5-day, and 10-day period wherein fecal samples were considered to be within the hunting window.

Within each category of covariates, we fit models with each covariate separately and added two additional variables reflecting day-of-year terms (Eq. 1-1 and 1-2: Jammalamadaka and Lund 2006) (Table 1-2).

$$\text{Sine day of year} = \text{sine} \left(\frac{2\pi[\text{day of year}]}{365} \right) \quad [\text{Eq. 1-1}]$$

$$\text{Cosine day of year} = \text{cosine} \left(\frac{2\pi[\text{day of year}]}{365} \right) \quad [\text{Eq. 1-2}]$$

We included these day-of-year terms across all models to control for the strong seasonal rhythms of fGCMs (Romero 2002). We compared support for each model within these three categories using Akaike Information Criterion for small sample sizes (AICc; Burnham and Anderson 2002) in program R using the ‘performance’ package (Lüdecke et al. 2021).

We based inference on a model which combined the most supported model within each covariate category. To address model uncertainty, we retained all covariates from models that were within 2 AICc units of the most supported model within each of the three categories to the combined model. If supported covariates showed multicollinearity (defined as VIFs > 5: Thompson et al. 2017), we selected covariates from only the most supported model in that category for the combined model. We examined normality assumptions and model fit using the R package “performance” (Lüdecke et al. 2021).

For the second step of our analysis, we assessed fGCM acclimation using piecewise linear regression to test for the occurrence of a breakpoint at which the regression curve from the combined model characterizing fGCMs changed its slope relative to the explanatory variable of ‘days from release’ (package “segmented”; Muggeo 2008). Convergence of the algorithm from

the function “segmented” demonstrates the existence of a breakpoint and a change in the linear relationship within the regression model (Muggeo 2003).

RESULTS

We collected and assayed 1,171 elk fecal samples from 2011 to 2019. Days from release, average monthly precipitation and average daily temperature, and occurrence and/or type of deer hunt within 10-day or 5-day intervals were most supported within translocation, climate, and disturbance categories, respectively (Table 1-3). Within the disturbance category, the three top models reflecting occurrence of a deer hunt within 10-day and 5-day intervals and the model reflecting both occurrence and type of hunt within a 10-day interval (gun, bow, muzzleloader, or no hunt) were within 2 AICc units of each other (Table 1-3). Because these three hunting covariates were highly correlated, we only included the covariates from the lowest AIC model reflecting occurrence of a hunt within a 10-day interval into the global model (Eq. 1-3).

$$fGCM = \sin.day + \cos.day + m.precip + d.temp + hunt.10d + df.release \quad [\text{Eq. 1-3}]$$

Results from the final model indicated that fecal GCs decreased with number of days following release ($\beta = -0.0024$, $SE = 0.0005$, $p < 0.001$). Higher average monthly precipitation was marginally associated with lower fGCMs ($\beta = -0.1372$, $SE = 0.079$, $p < 0.1$; Figure 1-1), while average daily temperature had no significant relationship with fGCMs ($\beta = 0.0676$, $SE = 0.076$, $p > 0.1$). Higher fGCMs were associated with the occurrence of a deer hunt within a 10-day interval ($\beta = 2.3082$; $SE = 0.8678$, $p < 0.01$; Figure 1-2). Circular day of year covariates were strongly associated with fGCMs ($\sin.day$: $\beta = -2.2413$, $SE = 0.645$, $p < 0.001$; $\cos.day$: $\beta = -7.1297$, $SE = 1.131$, $p = 0.001$).

Segmented analysis detected a breakpoint in fGCM values at 41.99 days following release (Figure 1-3) suggesting physiological acclimation occurred rather quickly. The effect of

days from release continued to be negative after 42 days, suggesting elk showed continued adjustment to their landscape following the initial indication of acclimation at 42 days. However, the size of negative effect was marginal relative to before the breakpoint (days from release before breakpoint: $\beta = -0.2657$, SE = 0.203; days from release after breakpoint: $\beta = -0.0065$, SE = 0.002), indicating minimal continued acclimation of fGCMs.

DISCUSSION

Glucocorticoid hormones regulate and reflect physiological responses to environmental challenges (McEwen et al. 2003), and animals typically respond to the challenge of translocation with elevated levels of GCs (Dickens et al. 2010). The duration of elevated GC levels is not well described across species but has implications for post-release management and ultimate success of translocation. We observed a breakpoint in the decline of fGCMs after 42 days post-release, reflecting a relatively fast population-level acclimation period by elk to the Missouri Ozark landscape. Because we took a population-level approach, the effect of days from release was likely diluted across years as proportionally less of the population was actively released during the second and third translocation years. Thus, lower fGCMs from animals translocated in previous years would dilute the observed response, making the decrease in fGCMs after release more gradual for years two and three after translocation. However, we still observed a significant decline in fGCMs and a breakpoint at the first 42 days following release of animals across all years, which suggests a strong effect.

While we observed an approximate 42-day physiological acclimation period in Missouri elk, comparisons among species with available data suggest there is considerable variation in acclimation duration (Dickens et al. 2010; Jachowski et al. 2013). Such variability in acclimation periods indicates there is likely a wide spectrum of variation in species sensitivity to

translocation. For example, researchers detected elevated fGCM levels 20 years after translocation in African elephants (*Loxodonta africana*) (Jachowski et al. 2013). The greater sensitivity to translocation observed in elephants suggested by the long-term physiological acclimation may be expected for a species with strong and complex social systems (Wittemeyer et al. 2005), long memories, and advanced cognitive capacities (Byrne et al. 2009). Conversely, captive bred Przewalski's horses (*Equus ferus przewalskii*) appear to be relatively insensitive to translocation challenges, indicated by physiological acclimation within 72 hours of release (Ji et al. 2013). The fast acclimation observed for Przewalski's horses may be attributed to generations of captive breeding (Ji et al. 2013); however, which species-specific traits contribute to variation in sensitivities to translocation remains an open area of investigation. Together with white rhinoceroses (*Ceratotherium simum*: 32 days; Yang et al. 2019) and Grevy's zebras (*Equus grevyi*: 11-18 weeks; Franceschini et al. 2008), the physiological acclimation period of elk falls between the long-term duration of African elephants and the near immediate response by Przewalski's horses. There are myriad additional factors that may influence a population's response to translocation, including number, intensity, and duration of challenges associated with translocation and the release landscapes (Dickens et al. 2010; Romero and Wingfield 2015). Species-specific sensitivity may thus be most appropriately used to form baseline expectations for anticipating species-specific population response to translocation and informing post-release management plans.

Additional context-specific factors should be considered as potentially influencing a population's acclimation period. For example, the relatively fast acclimation of the restored Missouri elk population could have been affected by lactation status of females as calves moved from nursing to foraging; however, calving dates in Missouri were wide-ranging over the

restoration (Keller et al. 2015), making it unlikely that lactation status could drive the response we saw in the breakpoint analysis. Likewise, there are documented seasonal patterns of declining fGCMs from summer to fall (Millspaugh et al. 2001), but such a seasonal pattern does not align with the distinct breakpoint we detected. Given our attempt to control for such potential effects via day of year terms, it seems more likely that in addition to underlying species-specific sensitivity, fast acclimation may have been facilitated by post-release management intended to assist acclimation. The MDC bolstered forage resources through planting of high-quality food plots, limited human disturbance by restricting public elk-viewing opportunities during calving, and prohibited elk hunting on the recently restored population (Dent et al. 2012). While durations of physiological acclimation are unknown for other translocated elk populations, comparisons of movement patterns between the restored Missouri population and a restored Ontario population receiving less post-release intervention may suggest indication of faster behavioral acclimation in the Missouri population (Ontario: 1-3 yrs, Fryxell et al. 2008; Missouri: < 6 months, Bleisch et al. 2017).

The relatively fast physiological acclimation in the Missouri population was discernable despite subsequent climatic and human disturbance stressors occurring on the release landscape. For example, human disturbance is known to be a primary challenge influencing fGCM response in established elk populations (Millspaugh et al. 2001, Jachowski et al. 2015), and we did observe increased fGCMs associated with hunting activity associated with managed deer hunts. However, the timing of a breakpoint in fGCM decline prior to hunts suggests a fast physiological acclimation to the challenge of translocation that was earlier and more influential than the subsequent effect of deer hunting.

Understanding the duration of acclimation can inform the length of time that post-release management activities intended to facilitate acclimation are necessary. For example, the MDC maintained restrictions on public elk-viewing opportunities annually within the core elk range during the calving season until 2017 (3 years after final release of elk). The rapid acclimation in fGCMs we observed following translocation supports the benefits of public-viewing restrictions in the initial months following releases but suggests such restrictions may not be necessary over subsequent years. Conversely, our finding of a persisting decline, though minimal, in fGCMs after the signal of acclimation suggests elk may continue to adjust to their landscape beyond the primary period of initial physiological acclimation. Thus, our results indicate that maintaining a longer period of protections against larger-scale human disturbances may benefit elk. For example, MDC waited 7 years to initiate the first hunting season on the restored elk population. Though this was due primarily to meeting minimum population size and robust population growth thresholds, this extended period without elk hunting likely also provided more time for elk to acclimatize to their new environment. While we detected relatively fast initial physiological acclimation by elk in spite of deer hunting activity across elk range, hunting directed toward elk themselves likely represents an even greater disturbance to the elk population.

Glucocorticoid physiology is complicated, and the interpretation of data relative to population health can be nuanced. For example, low GC or fGCM levels on their own do not necessarily signify healthy functioning (Romero and Beattie 2021). However, our results indicate that with sufficient long-term monitoring and access to adequate baseline or reference levels, fGCMs serve as a useful noninvasive bioindicator for assessing physiological acclimation. Adequate long-term monitoring and project reporting remain an issue for animal translocation

projects (Berger-Tal et al. 2020; Resende et al. 2020). As wildlife restoration is a costly conservation practice (Weise et al. 2014), being able to use resources most efficiently is crucial to continued successful implementation. Our study supports the use of fGCMs as an innovative and efficient monitoring method called for by translocation specialists (Berger-Tal et al. 2020).

Management Implications

We used fecal glucocorticoid metabolites as a noninvasive bioindicator of physiological acclimation in the restored Missouri elk population. We identified a relatively fast physiological acclimation period for Missouri elk compared to other large mammals for which physiological acclimation data are available. As such, post-release management at the release site relative to resource availability and disturbance reduction may facilitate acclimation and reduce the period of time recently translocated populations are at risk of post-release effects. Species-specific differences in translocation sensitivity likely contributes to the duration of the acclimation period and the period of time post-release management actions may be necessary. Increased resolution of number of species with known acclimation durations may thus contribute to improving the efficacy and efficiency of species-specific translocation guidelines and post-release management protocols.

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TABLES

Table 1-1. Covariate table including covariate name, description, and possible values for three categories of variables hypothesized to explain fGCM variation in the restored Missouri elk (*Cervus canadensis*) population.

Category	Covariate	Description	Values
Translocation	Proportion translocated	Proportion of population translocated in year	1, 0.5, 0.33, 0
	Restoration year	Year of restoration effort	1-9
	Days from release	Number of days following most recent translocation release	1-2357 days
Climate	Daily precipitation	Average precipitation from previous day in alignment with fGCM passage time for elk (Wasser et al., 2000)	0-5.99 (cm.)
	Daily temperature	Average temperature from previous day in alignment with fGCM passage time for elk (Wasser et al., 2000)	-12.31-29.44 (°C)
	Monthly precipitation	Average precipitation across month	1.68-23.87 (cm.)
	Monthly temperature	Average temperature across month	-2.76-27.80 (°C)
Disturbance	3-day hunt window	Occurrence of deer (<i>Odocoileus virginianus</i>) hunt in area within 3-d window	Yes/No
	5-day hunt window	Occurrence of deer hunt in area within 5-d window	Yes/No
	10-day hunt window	Occurrence of deer hunt in area within 10-d window	Yes/No
	3-day hunt type window	Occurrence and type of deer hunt in area within 3-d window	None, archery, rifle, muzzleloader
	5-day hunt type window	Occurrence and type of deer hunt in area within 5-d window	None, archery, rifle, muzzleloader
	10-day hunt type window	Occurrence and type of deer hunt in area within 10-d window	None, archery, rifle, muzzleloader

Table 1-2. Model table including model descriptions and model structures for each of three categories of variables hypothesized to explain fGCM variation in the restored Missouri elk (*Cervus canadensis*) population.

Category	Model description	Model structure
Translocation	Null	fGCM ~ 1
	Day of year	fGCM ~ sin.day + cos.day
	Day of year + proportion translocated in year	fGCM ~ sin.day + cos.day + prop.trans
	Day of year + days from most recent release	fGCM ~ sin.day + cos.day + df.release
	Day of year + year of restoration	fGCM ~ sin.day + cos.day + restor.yr
Climate	Null	fGCM ~ 1
	Day of year	fGCM ~ sin.day + cos.day
	Day of year + avg daily precipitation	fGCM ~ sin.day + cos.day + d.prcp
	Day of year + avg daily temperature	fGCM ~ sin.day + cos.day + d.temp
	Day of year + avg monthly precipitation	fGCM ~ sin.day + cos.day + m.prcp
	Day of year + avg monthly temperature	fGCM ~ sin.day + cos.day + m.temp
Disturbance	Null	fGCM ~ 1
	Day of year	fGCM ~ sin.day + cos.day
	Day of year + deer hunt in 3-day window	fGCM ~ sin.day + cos.day + hunt.3d
	Day of year + deer hunt in 5-day window	fGCM ~ sin.day + cos.day + hunt.5d
	Day of year + deer hunt in 10-day window	fGCM ~ sin.day + cos.day + hunt.10d
	Day of year + deer hunt type in 3-day window	fGCM ~ sin.day + cos.day + hunttyp.3d
	Day of year + deer hunt type in 5-day window	fGCM ~ sin.day + cos.day + hunttyp.5d
	Day of year + deer hunt type in 10-day window	fGCM ~ sin.day + cos.day + hunttyp.10d

Table 1-3. Model selection results for three categories of variables hypothesized to explain fGCM variation in the restored Missouri elk (*Cervus canadensis*) population. We report number of parameters (K), difference Akaike Information Criterion (AIC) corrected for small sample sizes from the most supported model (ΔAICc), log likelihood (LL), and the Akaike weights (w_i) for each model.

Category	Model	K	ΔAICc	LL	w_i
Translocation	fGCM ~ sin.day + cos.day + df.release	5	0	-4544.97	0.91
	fGCM ~ sin.day + cos.day + restor.yr	5	4.53	-4547.23	0.09
	fGCM ~ sin.day + cos.day + prop.trans	5	17.71	-4553.82	0.00
	fGCM ~ sin.day + cos.day	4	30.59	-4561.27	0.00
	fGCM ~ 1 (intercept only)	2	168.82	-4632.40	0.00
Climate	fGCM ~ sin.day + cos.day + m.prcp	5	0	-4557.92	0.58
	fGCM ~ sin.day + cos.day + d.temp	5	1.84	-4558.84	0.23
	fGCM ~ sin.day + cos.day + d.prcp	5	3.71	-4559.77	0.09
	fGCM ~ sin.day + cos.day	4	4.68	-4561.27	0.06
	fGCM ~ sin.day + cos.day + m.temp	5	5.23	-4560.53	0.04
	fGCM ~ 1 (intercept only)	2	142.92	-4632.40	0.00
Disturbance	fGCM ~ sin.day + cos.day + hunt.10d	5	0	-4556.47	0.34
	fGCM ~ sin.day + cos.day + hunt.5d	5	0.48	-4556.71	0.27
	fGCM ~ sin.day + cos.day + huntyp.10d	7	1.28	-4555.09	0.18
	fGCM ~ sin.day + cos.day + hunt.3d	5	2.63	-4557.79	0.09
	fGCM ~ sin.day + cos.day + huntyp.5d	7	3.46	-4556.18	0.06
	fGCM ~ sin.day + cos.day + huntyp.3d	7	4.28	-4556.59	0.04
	fGCM ~ sin.day + cos.day	4	7.57	-4561.27	0.01
	fGCM ~ 1 (intercept only)	2	145.80	-4632.4	0.00

FIGURES

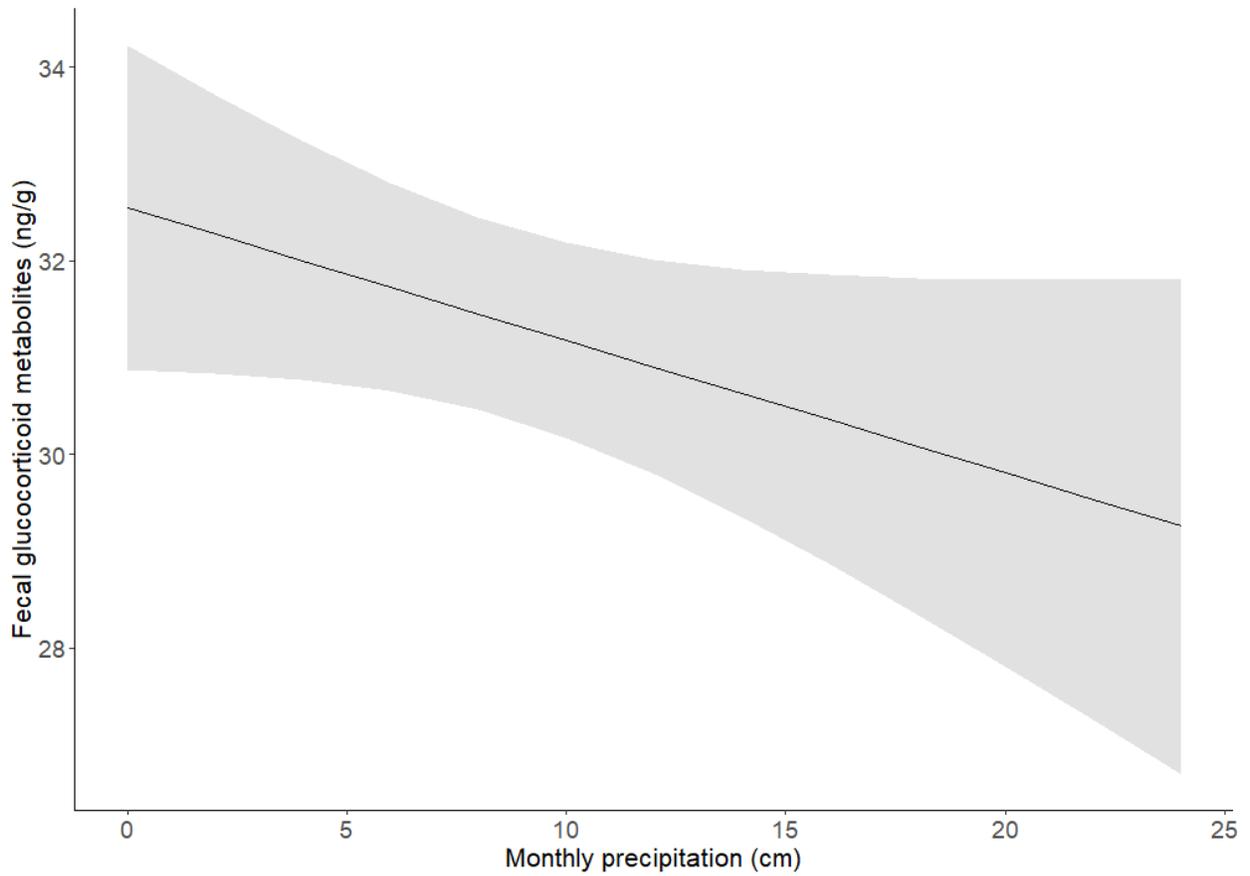


Figure 1-1. Predicted effect of average monthly precipitation on fecal glucocorticoid metabolite (fGCM) response in the restored Missouri elk (*Cervus canadensis*) population in the initial 9 years of restoration (2011-2019).

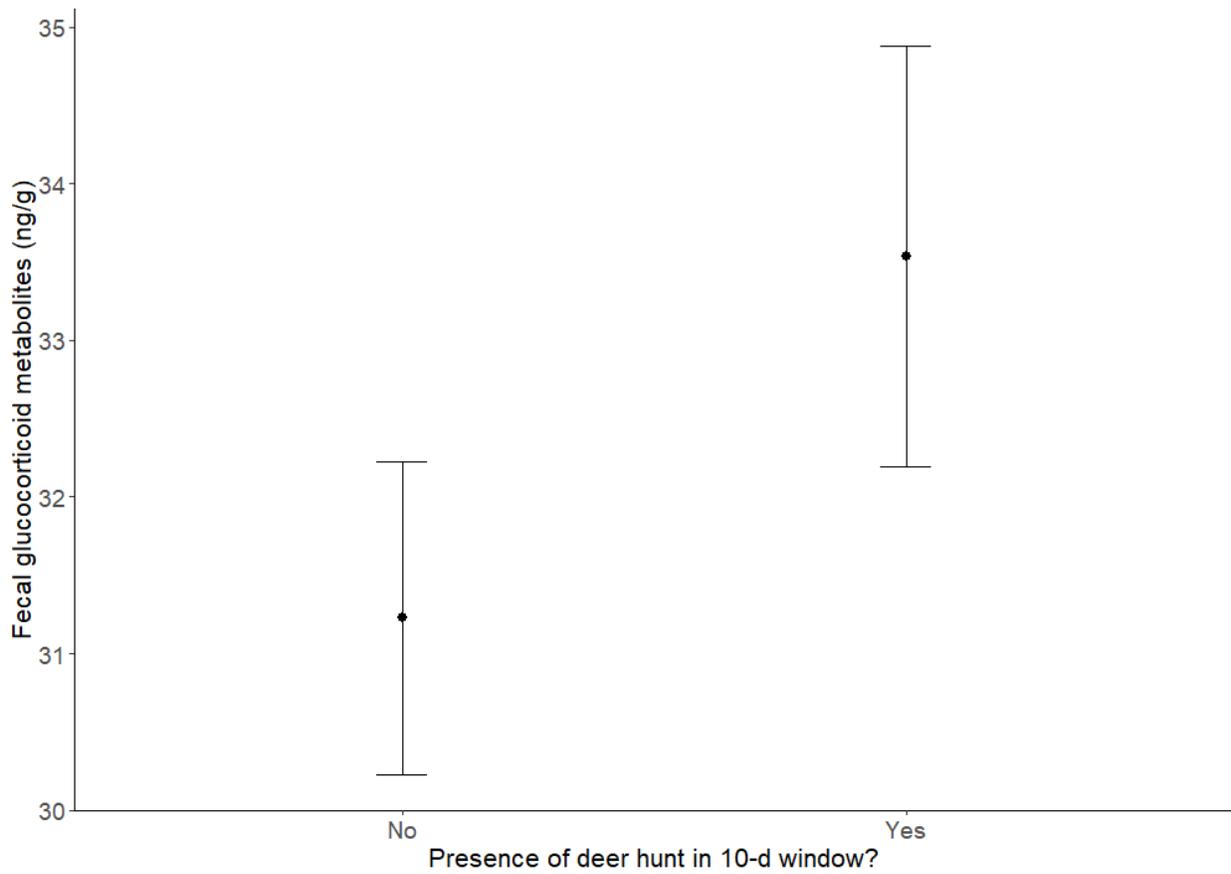


Figure 1-2. Predicted effect of the presence (no/yes) of a white-tailed deer (*Odocoileus virginianus*) hunt within a 10-day window on fecal glucocorticoid metabolite (fGCM) response in the restored Missouri elk (*Cervus canadensis*) population in the initial 9 years of restoration (2011-2019).

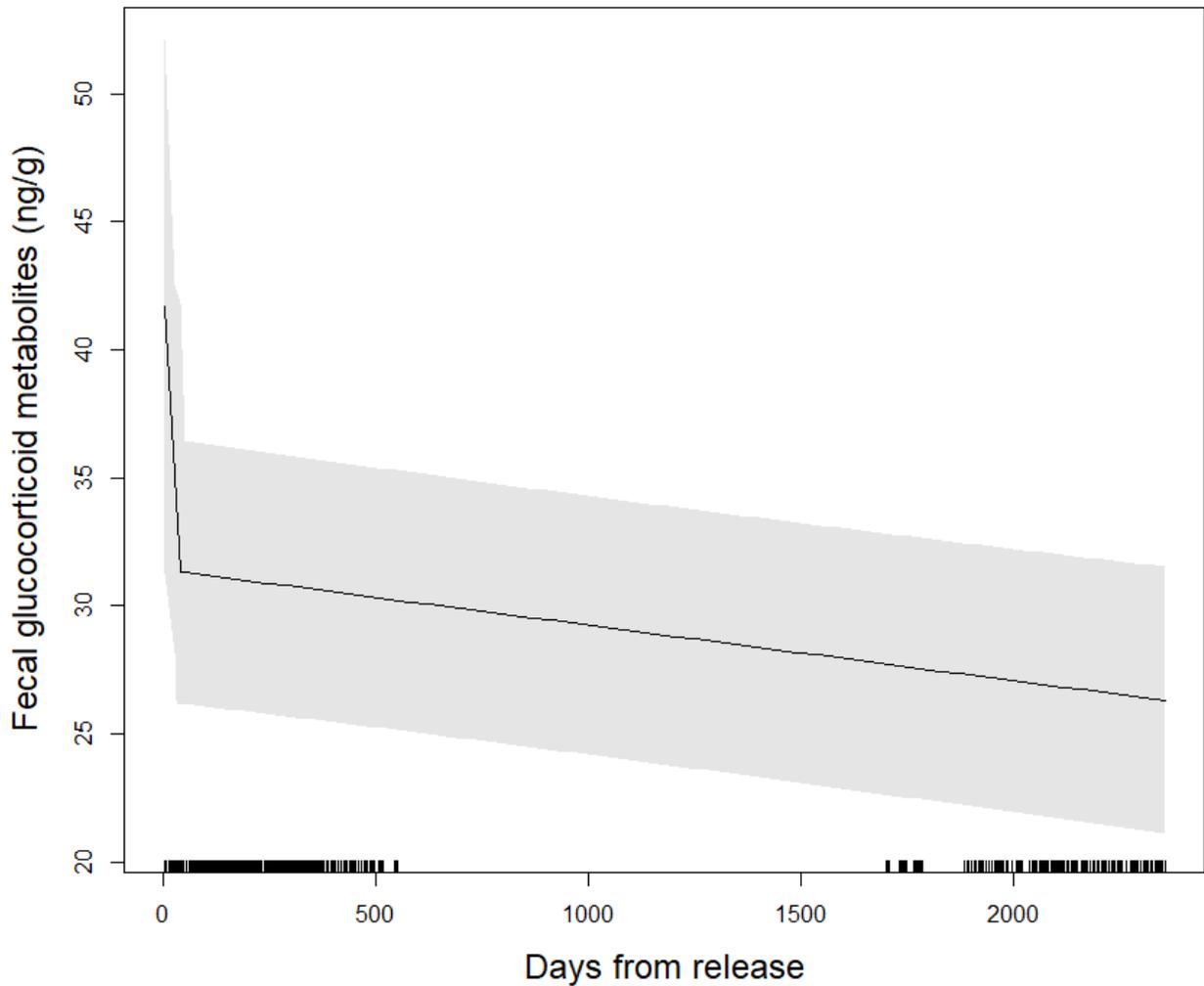


Figure 1-3. Predicted effect of days from most recent translocation release on fecal glucocorticoid metabolite (fGCM) response in the restored Missouri elk (*Cervus canadensis*) population in the initial 9 years of restoration (2011-2019), with estimated breakpoint and indication of physiological acclimation occurring at 41.99 days. Rugs indicate sampling occurrences.

Chapter 2: Spatial acclimation of elk following translocation to the Missouri Ozarks, U.S.A. ²

ABSTRACT

Wildlife translocation is an important conservation tool but challenging for animals. For translocations to be successful, animals must adjust to their release landscape. Investigating how animals acclimate to their release landscape improves post-release monitoring and informs needs of translocated populations. We investigated movements and resource selection dynamics of 106 elk (*Cervus canadensis*) during the 6-8 years following release to Missouri, U.S.A. in 2011-2013. We observed spatial acclimation by elk within their first year of translocation as determined by cessation of time from release effects in resource selection and monthly individual range sizes and overlap. Females showed faster and stronger evidence of acclimation following release. While range overlap for both sexes stabilized within approximately 6 months, female range size stabilized within approximately 3 months and males within approximately 11 months. Elk selection for multiple resources also generally stabilized within a year. A simple refuge-forage tradeoff alone did not explain acclimation in resource selection dynamics as elk selected high quality forage resources across the temporal extent of restoration, while summer selection for cover resources increased after elk acclimated. Together, spatial acclimation generally lagged behind post-release physiological responses, adding to the increasing evidence that translocated animals display acclimation patterns across trait-specific time periods. Our approach demonstrates the utility of estimating acclimation duration across multiple spatial response

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metrics for improving post-release monitoring, evaluation, and management of restored wildlife populations.

INTRODUCTION

Translocation of animals to restore wildlife populations is an important technique in conservation biology. The initial period following release is often most critical in determining success of a translocation program. While acclimating, animals are most prone to post-release effects including increased mortality and desertion (Armstrong and Reynolds 2012; Le Gouar et al. 2012). Project failures typically occur during initial stages of release, even when conditions at the release site are deemed sufficient to support population persistence (Armstrong and Seddon 2008). In response, release designs may reduce the time to acclimation to limit the period of time animals are vulnerable to post-release effects (Batson et al. 2015a). For example, managers may choose to bias release cohorts towards particular age classes or sexes that are most likely to acclimate (Hayward et al. 2012; Batson et al. 2015b; Moehrensclager and Lloyd 2016). Beyond decisions about the design of translocation releases, post-release effects can also be mitigated through post-release management of the release landscape. For example, managers may increase food availability or offer supplementary protections to reduce challenges during the vulnerable acclimation period to facilitate animal adjustment to their new landscape (Aubry and Lewis 2003; Hayward and Slotow 2016). However, these management actions are costly and sometimes controversial (Coz and Young 2020), so knowledge of how long it takes for a released population to acclimate to its new landscape can inform how long provisions or protections are required. Accurate estimation of acclimation duration may thus benefit the post-release management of restored populations and improve assessment of decisions made regarding the translocation process.

Previous investigations into acclimation have focused primarily on changes in animal survival (Armstrong et al. 2017). For example, Hamilton et al. (2010) defined the acclimation duration by the period of time in which survival rates were temporarily depressed after release of Riparian brush rabbits (*Sylvilagus bachmani riparius*) during a reintroduction event in California. However, recent studies demonstrate similar transitory dynamics following release across additional metrics besides survival. For example, physiological (Chapter 1, *this dissertation*), social (Poirier and Festa-Bianchet 2018; Chapter 3, *this dissertation*), and behavioral (Shmitz et al. 2015; Flanagan et al. 2016) attributes all show a similar pattern of acclimation wherein response attributes show temporarily high rates of change immediately following release of animals that stabilize after some period of time. The similarity of patterns across a suite of ecological metrics suggests a broader definition of acclimation (e.g., the period of time before responses stabilize) would be useful for extending acclimation estimation beyond survival. Finer-scaled bioindicators may provide more sensitive estimates of acclimation that facilitate assessment of and mechanistic explanations for animal response to translocation (Tarszisz et al. 2014; Poirier and Festa-Bianchet 2018).

Spatial behaviors are emerging as promising indicators of translocation acclimation. Translocation projects often fail if animals flee from the selected release site (e.g., Viljoen et al. 2008; Le Gouar et al. 2012), and dispersal after initial release is linked to increased energy expenditures and mortality events (Haydon et al. 2008). Failure of translocated animals to establish stable home ranges may indicate that mitigative actions are required within the release area (Griffith et al. 1989; Armstrong and Seddon 2008), while the individual nature of location data that comes from VHF- or GPS-collars allows researchers to draw inference on if and how some animals might vary in their acclimation patterns. For example, if a certain sex or age class

acclimates more quickly, we can relate these results back to translocation decisions pertaining to cohort composition to inform successful translocation strategies. While researchers increasingly use movement metrics such as home range size and overlap to indicate acclimation (Flanagan et al. 2016; Mertes et al 2019; Werdel et al. 2021), few researchers seek to investigate acclimation dynamics of resource selection patterns within translocated wildlife.

Wildlife may vary their resource selection patterns in predictable ways relative to acclimation. Researchers increasingly link animal physiology to animal space use (Schick et al. 2008; Jachowski and Singh 2015; Jachowski et al. 2018). For example, in African elephants (*Loxodonta africana*), elevated concentrations of glucocorticoid metabolites are associated with greater use of resources associated with refugia (Jachowski et al. 2012). Translocated animals typically experience elevations in glucocorticoid hormones while acclimating to their release landscapes (Teixeira et al. 2007; Dickens et al. 2010; Chapter 1) and thus may be expected to demonstrate increased selection for resources associated with safety (e.g., cover or increased distance to human disturbance). Results from recent investigations lend support to a trade-off between post-release resource selection for resources associated with safety versus forage after translocation (Maor-Cohen et al. 2021; Picardi et al. 2021). If translocated animals predictably vary their selection patterns relative to acclimation status, managers may use this information to plan habitat interventions (e.g., manage for abundant cover resources during acclimation) that support the changing needs of translocated wildlife as they adjust to their new landscapes. Conversely, if researchers fail to account for temporally dynamic resource selection patterns of translocated wildlife, they may draw incorrect inference on the changing resource needs of restored populations (Picardi et al. 2021). By integrating acclimation effects into resource selection dynamics we might better understand the dynamic resource needs for restored

populations as well as elucidate potential mechanisms driving spatial acclimation patterns in restored populations.

In this study, we evaluated post-release spatial acclimation relative to movement and resource selection of translocated elk restored to the Missouri Ozark landscape in the central United States. Elk (*Cervus canadensis*) are frequently translocated (Popp et al., 2014). While movement dynamics have been studied for several restored elk populations (Larkin et al. 2004; Fryxell et al. 2005; Haydon et al. 2008; Bleisch et al. 2017), release effects have not been investigated in studies of resource selection by restored elk populations (e.g., Andersen et al. 2005; Popp et al. 2013; Trent et al. 2019: this system). In response to the need for a broader definition of acclimation that extends beyond survival (e.g., Hamilton et al. 2010; Armstrong et al. 2017), we define acclimation in this study as the period of time prior to stabilization of response metrics. Given demonstrated links between spatial behavior and underlying physiological state in large mammals (e.g., Jachowski et al. 2012, 2013), we hypothesized that elk would show similar rates of acclimation in their spatial behavior as observed for their physiological response within this system (< 3 mos; Chapter 1). Specifically, we expected to observe high rates of change across time in both movement and resource selection metrics, which would stabilize within the initial weeks on the Missouri landscape. Given recent evidence suggesting a forage-refuge trade-off between selection for forage and safety resources by recently translocated wildlife (Maor-Cohen et al. 2021; Picardi et al. 2021), we further hypothesized that a refugia-seeking process would drive elk spatial acclimation in Missouri. Elk typically respond to the challenge of human disturbance (a dominant disturbance-type associated with translocation) by selecting areas associated with cover and low road density (Skovlin et al. 2002; Proffitt et al. 2010). We therefore predicted that elk would seek resources associated with

cover and low-road densities close to the time of release, but would demonstrate avoidance for such forage-poor resources once they acclimated to the landscape. Forage-rich landcover types are typically highly selected by elk in established populations, however, as open lands they are associated with limited cover and increased risk (DeVoe et al. 2019). As such, we predicted elk would increase selection for forage resources in open areas after acclimating to their new landscape. We evaluated the existence and duration of post-release spatial acclimation period across sex and age groups to enhance assessment and implementation of population restoration efforts.

METHODS

Study area

We translocated elk into an Elk Restoration Zone (ERZ) that spans three counties of southeastern Missouri Ozarks (Carter, Reynolds, and Shannon). The ERZ was comprised predominantly of land owned by state, federal, and non-governmental land management entities. The Missouri Department of Conservation (MDC), National Park Service, and United States Forest Service managed 49% of the ERZ, while the Nature Conservancy owned 3% of the ERZ, and an additional 27% was held by a sustainable forest products initiative (L-A-D: Missouri Department of Conservation 2010). The ERZ was dominated by forest and woodland ecotypes (93%) with the sparse open lands (5%) comprised mostly of managed food plots and natural forage pasturelands (Missouri Department of Conservation 2010).

Animal translocations and sample collection

We translocated and released 106 elk to the ERZ in Missouri in three cohorts over the years 2011-2013. In January of each year, elk were captured in Kentucky, USA, held in quarantine corrals for 102 – 129 days, then transported overnight on trailers to Missouri. Elk were

quarantined for an additional 19 – 34 days in holding pens before release on the Missouri landscape. The demographic composition of release cohorts varied slightly by year: 2011 (n = 34) – 15 adult females (2+ yrs), 5 yearling females, 6 two-year-old males, 8 yearling males; 2012 (n = 33) – 22 adult females, 3 yearling females, 4 two-year-old males, 4 yearling males; 2013 (n = 39) – 20 adult females, 16 yearling females, 3 yearling males.

We fitted all translocated elk with GPS-VHF collars prior to release (RASSL custom 3D cell collar, North Star Science and Technology, King George, VA, or G2110E Iridium-GPS series model, Advanced Telemetry Systems, Isanti, Minnesota, USA) and affixed PIT-tags for permanent identification. We performed subsequent captures of individuals on the Missouri landscape between 2015 and 2018 via darting to maintain functional collars. Collars were programmed with 1- and 5-hr fix rates; however for each elk we filtered the location interval to 5-hrs to maintain a constant sampling rate across individuals for movement and resource selection analyses.

Data analysis

Movement dynamics – We used probabilistic path reconstruction (Fleming et al. 2016) to estimate individual occurrence distributions (OD) for each monthly period following release for each translocated elk. We selected the Ornstein-Uhlenbeck motion model (“OU”: Uhlenbeck and Ornstein 1930; Fleming et al. 2014) and fit ODs with the “hr_od” function from the “amt” (Signer et al. 2019; Signer and Fieberg 2021) package in the R software environment to fit animal ODs. A probabilistic, continuous-time movement framework allowed us to account for serial autocorrelation issues with movement data and appropriately estimate confidence intervals (Fleming et al. 2015). We estimated home range areas as the 95% isopleth of the estimated ODs for every individual for each monthly period containing more than 30 locations following release

(Seaman et al. 1999). To evaluate site fidelity, we calculated the volume of intersection (Seidel 1992; Millsaugh et al. 2004) between ODs for all successive monthly periods following release for each animal with the “hr_overlap” function in the “amt” package (Signer et al. 2019).

Next, we built LMEs to evaluate the effect of month from release, individual sex and capture age, and cohort release number on both response metrics (home range size and fidelity). We controlled for seasonality in movement patterns with a circular transformation on day of year resulting in two day of year terms (Eq. 1 and 2: Jammalamadaka and Lund 2006; Table 2-1).

$$\text{Sine day of year} = \text{sine} \left(\frac{2\pi[\text{day of year}]}{365} \right) \quad [\text{Eq. 2-1}]$$

$$\text{Cosine day of year} = \text{cosine} \left(\frac{2\pi[\text{day of year}]}{365} \right) \quad [\text{Eq. 2-2}]$$

We included interactions between month from release and the categorical translocation covariates to evaluate whether acclimation duration varied by capture age, sex, or release cohort (Table 2-1). Our primary goal was to determine which combination of covariates best predicted changes in home range size and fidelity across time from release. Therefore, we fit all combinations of covariates and selected the simplest model from the top models within 2 AICc (Table 2-1), which was used to predict acclimation period during breakpoint analysis (below). We used ANOVA analyses on top performing LME models to determine the significance of predictors. We assessed normality and model diagnostics with the “check_model” function in the “performance” package, and we evaluated model fit with typical metrics (R^2 and RMSE) using the “check_performance” function.

We assessed acclimation duration for movement responses with piecewise linear regression. We tested for the occurrence of a breakpoint in both the range size and fidelity models wherein the regression curve for each modelled response metric changed slope relative to the explanatory variable of ‘month from release’ (package “segmented”; Muggeo 2008).

Convergence of the algorithm from the function “segmented” demonstrates the existence of a breakpoint and a change in the linear relationship within the regression model (Muggeo 2003).

We used separate range size breakpoint analyses by sex to allow the location of the breakpoint to change between sexes given the significant interaction between sex and month from release in the range size model (Table 2-1).

Resource selection dynamics – We evaluated acclimation dynamics in post-release resource selection behavior using step-selection functions (SSF; Thurfjell et al. 2014) with generalized linear mixed models (GLMMs). Within GLMMs, we investigated the effect of time from release on the relative probability of selection for resources by interacting a “days from release” term with resource covariates.

Resource covariates: We modeled elk post-release selection dynamics for resource covariates important to elk resource use that informed the refuge-forage tradeoff hypothesized for translocated animals during acclimation to the release landscape. We evaluated elk selection for the following variables derived at 30 m resolutions: 1) road density (km of paved and public gravel road: from Smith et al. 2019), 2) percent canopy cover (from 2011, 2013, 2016, and 2019 United States Forest Service National Land Cover Database [NLCD]; Homer et al. 2015), 3) landcover type (cover, food plot, forage categories: reclassified from the 2011, 2013, 2016, and 2019 NLCD and merged with a food plot management layer from the Missouri Department of Conservation; Supplementary Information Table. S2-1), 4) Euclidean distance to cover (from the landcover category map and the ‘Calculate Distance’ tool in ArcGIS v. 10.8), and 5) slope (from a digital elevation model [DEM] obtained from the USDA Geospatial Data Gateway). We included both food plot and natural forage categories as landcover types given the management relevance of food plots and because we expected elk selection acclimation patterns to be more

pronounced for food plots that are intensively managed to provide high-quality forage for elk than for naturally occurring but less intensively managed forage cover types. If resource variables had $|r| < 0.7$, we used AICc to select the top performing variable.

We investigated the effect of time from release on the relative probability of selection for resources by interacting a continuous “days from release” [DFT] term with resource covariates. We considered multiple functional forms of DFT in interactions with resource covariates. We evaluated the best functional form of DFT interactions among each resource covariate independently using AICc (Supplementary Information Table S2-2). Within each model set, we included null models that only had the resource covariate and no interaction with a DFT term to allow the possibility that selection for that resource was not affected by time from release. We considered the selection of models with DFT interactions to indicate an effect of time on elk resource selection for that resource covariate, and we considered the selection models with logarithmic, square root, and exponential decay DFT functional forms to indicate some degree of acclimation behavior for selection of that resource (i.e., functional forms that demonstrated a period of large rates of change followed by stabilization; Supplementary Information Figure S2-1a). The equation for exponential decay (Eq. 2-3) includes a multiplier, α , that corresponds to the rate of decay (Nielsen et al. 2009; Fortin et al. 2021):

$$-\exp(-\alpha\text{DFT}) \quad [\text{Eq. 2-3}]$$

We considered 6 multipliers that allowed us to test the best-performing acclimation duration (i.e., the number of days until stabilization occurred; Supplementary Information Figure S2-1b) for each resource covariate where the top performing DFT interaction was exponential decay.

Resource selection models: We selected a movement-based SSF analysis (Avgar et al. 2016).

SSFs follow a used-available design to estimate relative probabilities of selection for resources

that are proportional to true selection probabilities (Manly et al. 2002; Millspaugh et al. 2020). Availability was determined through random draws from empirical distributions fit to used step lengths and turning angles (Forester et al. 2009; Thurfjell et al. 2014). Resources associated with each used step (i.e., location along an animal's movement path) were compared to resources associated with a matched set of unused but available steps at that same time point to create a stratum of matched used and available locations (Signer et al. 2019).

We created strata containing one used step with 5 matched available steps within the R package “amt” (Signer et al. 2019). We then estimated relative intensities of selection by comparing resources associated with used and unused steps within strata using mixed conditional Poisson GLMM regression with stratum-specific intercepts that we modeled as random effects with a fixed large variance to prevent their regression towards the mean (Muff et al. 2019). We fit models using the R package “glmmTMB” (Brooks et al. 2017). We accounted for variable responses and sample sizes across individuals to reduce bias in population-level fixed effects by including individual-level random coefficients (Gillies et al. 2006; Duchesne et al. 2010; Muff et al. 2019).

To control for seasonal variation in selection patterns, we restricted data to the summer when all release events occurred (May 1 – Aug 31); however, we observed similar patterns of acclimation, with few exceptions, across seasons (rut: Sept 1- Dec 31 and gestation: Jan 1 – April 31; Supplementary Information Figure S2-2). Because we detected a significant interaction between sex and month from release in analysis of elk home range size, we fit separate models by sex. We interpreted positive selection coefficients whose 95% confidence interval (CI) did not overlap zero as relative selection for that resource and negative coefficients whose 95% CIs did not overlap zero as relative avoidance for that resource. We plotted relative selection as a

function of days from release for all significant interactions between resource covariates and DFT terms, using $p < 0.05$ as significant, to assess acclimation duration and patterns; we considered acclimation complete when the interaction slope approximated zero. Across models, we only included data from individuals marked for >1 month following release.

We validated both sex-specific models using k-fold cross-validation with 10 folds by iteratively fit 10 models, each with 90% of individual elk, and we used model fixed-term coefficients to predict RSF scores for the remaining 10% of animals (Roberts et al. 2017). We ranked predictions into 6 bins for used locations against predictions for associated available locations within strata (5 available locations and 1 used location), and we calculated the Spearman rank correlations (r_s) to test whether higher ranking bins included more used locations (Fortin et al. 2009).

RESULTS

We used approximately 335,000 locations from 103 individuals, and the average number of locations collected per individual was 3,350 (range = 55 – 11,209; SD = 3,112).

Movement dynamics -- We created 2,774 monthly ODs from 103 individuals. The average number of ODs per individual was 26.9 (range = 1-79; SD = 21), and the average number of locations per OD was 119 (range = 31-146; SD = 27.3). Time from release was associated with elk OD overlap ($F_{1, 2491} = 99.19, p < 0.001$); however, no dependencies relative to animal sex, translocation age, or release cohort were retained in the top performing range fidelity model (i.e., no interactions with month from release; Table 2-1b). We estimated a population-wide breakpoint and subsequent stabilization in fidelity at approximately 6 months (breakpoint: 6.79 months [95% CI: 5.36 – 8.22]; Figure 2-1a).

Month from release was associated with elk range size, and this effect depended on sex ($F_{1, 2705.71} = 46.35, p < 0.001$), with males using larger home ranges in initial months following release, but not later (Figure 2-1b). Neither translocation age nor release cohort were retained in the top performing range size model (Table 2-1b). A breakpoint and relative stabilization in female range size occurred at approximately 3 months (female breakpoint = 2.82 months [95% CI: 2.27-3.37]; Figure 2-1b). The male breakpoint in range size occurred approximately 8 months later (11.27 months [95% CI: 8.14-14.41]) but indicated a greater lasting effect of time from release after the breakpoint (i.e., greater absolute slope after breakpoint; Figure 2-1b).

Resource selection dynamics – Models of summer resource selection dynamics included 101,011 used GPS locations for 74 female elk and 17,360 used GPS locations for 25 males. Univariate model selection identified the exponential decay functional form of DFT with different decay rates depending on the resource as top performing for interactions with all variables except distance to cover (Supplementary Information Table S2-2). Top performing decay rates showed the faster acclimation for landcover, slope, and canopy variables (decay rates of 0.02 and 0.01 associated with acclimation durations within ~ 4 and 8 mos, respectively; both before individuals' second summers) relative to road density (decay rate = 0.002 associated with an acclimation duration of ~ 3 years). Distance to cover was removed from final models because it was highly correlated with the cover category of landcover and performed poorly in model selection (Supplementary Information Table S2-3).

Males and females demonstrated strong selection for food plot landcover types that was uninfluenced by time from release (95% CI overlapping zero for food plot and DFT interaction terms, Figure 2-2). Selection for forage landcover types, while lower than food plots, was also positive in both sexes and selection showed marginal increases following acclimation by females

only (Figure 2-2; Figure 2-3). Males and females showed neutral or marginally positive selection for cover landcover types that was greater after acclimation for males only (Figure 2-2, Figure 2-3); however, selection for cover decreased after acclimation for females in non-summer seasons (Supplementary Information Figure S2-2).

Male and female elk demonstrated opposite temporal dynamics relative to road density. As predicted, females selected areas associated with low road densities during initial post-release years but showed only marginal avoidance for these areas upon acclimation (Figure 2-4a). Conversely, males showed neutral selection for areas of low road density upon initial release, but selected sites with low road density after acclimation (Figure 2-4a). Contrary to predictions, elk of both sexes selected for lower canopy cover which was uninfluenced by acclimation (Figure 2-2). Males and females avoided resources associated with steep slopes in the initial months post-release, and avoidance for these resources increased after acclimation (Figure 2-4b).

Both male and female models validated well with high predictive performance. Mean (\pm SD) r_s across 10 withheld folds was 1.0 (\pm) 0.0 for females and 0.99 (\pm) 0.02 for males (Supplementary Information Figure S2-3).

DISCUSSION

We evaluated temporal dynamics of range size, range fidelity and resource selection of elk translocated to Missouri and identified patterns indicative of spatial acclimation within the first year of their release. We identified a faster and clearer acclimation response in the movements of female elk released during the parturition season, suggesting a translocation tactic biased towards pregnant females may promote faster spatial acclimation. A simple refuge-forage tradeoff alone did not explain acclimation in resource selection dynamics, as elk selected high quality forage resources across the temporal extent of restoration, while summer selection for cover resources increased after elk acclimated. Together, these results add increasing evidence that translocated

animals display acclimation patterns across trait-specific time periods, and we extend methods for directly estimating acclimation duration of translocated animals to their new landscape.

We observed stabilization in elk home range size and fidelity that varied with sex. Increasing and stabilizing fidelity in elk home ranges suggests elk were initially exploring the landscape before establishing a home range area (Maor-Cohen et al. 2021). The longer acclimation duration we observed for male home range size relative to females, together with their stronger continued decline in home range size after initial indication of acclimation, suggests males had a stronger exploration response than females (Mertes et al. 2019). The faster and stronger acclimation response by females may be attributed to releases occurring during the parturition season. Initial investigation into elk movements during their first 6 months on the landscape in this system indicated maternal females had smaller home ranges than non-maternal females (Bleisch et al. 2017). This maternal effect likely influenced sex-specific durations in home range size acclimation; however, the temporal dynamics of resource selection patterns offered additional insight into the drivers of spatial acclimation.

Temporal dynamics in summer resource selection patterns varied by resource covariate. As predicted, females initially showed avoidance of areas with high road densities following release that dissipated after acclimation. This result was consistent with our predictions and provides some support for temporary selection away from areas of greater human disturbance after initial release and that females adopted the forage-refuge trade-off (Maor-Cohen et al. 2021; Picardi et al. 2021). However, over the same period males showed the opposite trend wherein they neither selected nor avoided areas of high road density initially, yet they avoided these areas after acclimating to the landscape. While not apparent in earlier resource selection research in this system that did not incorporate acclimation effects (Smith et al. 2019), the sex-specific

differences in the effect of roads on elk summer selection patterns that we observed following acclimation, are consistent with the literature within established elk populations (Skovlin et al. 2002; McCorquodale, 2003). Given the tendency for males to demonstrate avoidance for roads, it is surprising their avoidance was not greater upon initial release. The combination of greater range size, together with evidence that males did not initially demonstrate avoidance of areas associated with higher human disturbance may indicate that their spatial acclimation was driven more by an exploration and learning (Schmitz et al 2015) process rather than a refugia-seeking process (Maor-Cohen et al. 2021).

The mixed support we found for the hypothesis that elk resource selection patterns would be governed by a refugia-seeking process was further corroborated by the temporal selection patterns we observed among landcover types and canopy cover. Contrary to predictions, neither male or female elk showed decreased use of open, high-quality food plot areas while acclimating, and only females showed decreased use of lower-quality forage while acclimating. Similarly, neither sex showed predicted increased selection for cover resources upon initial release. Elk selection for cover resources during acclimation may have been obscured by the high degree to which cover dominated the ERZ landscape in Missouri. However, any such influence of a “functional response” (Myerud and Ims 1998), wherein selection for a given landcover type decreases with its relative availability on the landscape, does not explain the relative increase we observed in male elk selection for cover landcover types across days following release. The lack of support for increased use of cover resources while acclimating by females was only apparent in the summer season (Supplementary Information Figure S2-2) and may also be explained in part by maternal behavior females. Near to and following parturition, female ungulates typically show increased selection for forested resources to promote hiding

cover and protection of their calves (Long et al. 2009; Wright et al. 2021). As such, the lack of acclimation effect in female elk selection for cover resources during the parturition season may reflect their needs relative to calving.

By integrating acclimation effects into our resource selection investigation, we identified shifting patterns of post-release elk resource selection and provided insight into the duration and drivers of spatial acclimation. Previous investigations of post-release resource selection behavior largely have failed to account for transient acclimation effects (e.g., Trent et al. 2019), or accounted for them by discarding data from an arbitrary time period (e.g., Mondal et al. 2013). We used a similar method as Picardi et al. (2021) to directly incorporate dependence on time from release into our modelling approach for elk resource selection. We determined the exponential decay functional form performed best for all resources whose selection patterns were time dependent, indicating complete cessation of acclimation effects. Our results indicated that patterns of selection pertaining to variables associated with road density took the longest period to stabilize (e.g., landcover, canopy, slope: < 1 year vs road density: ~3 years). Such results may indicate that elk require longer periods of time to adjust to the unpredictability associated with variable human disturbance levels associated with multiple road types (Montgomery et al. 2013).

Our results add to increasing evidence suggesting translocated animals acclimate to their release landscape on different temporal scales depending on the ecological metric under investigation (e.g., this study; Chapter 1; Chapter 3). Given the influence of underlying physiological processes to animal space use (Nathan et al. 2008; Jachowski & Singh 2015), we expected elk would demonstrate similar temporal dynamics in acclimation effects between the spatial metrics we investigated in this study and the physiological stress response observed in this system (< 3 months; Chapter 1). Although we observed variation relative to sex and specific

movement response or resource covariate under investigation, acclimation durations for both movement responses and most resource variables all occurred between approximately 3 mos. and 1 year. Thus, contrary to predictions, spatial acclimation lagged behind physiological acclimation, suggesting that additional underlying processes contribute the post-release spatial acclimation patterns that we observed. For example, multiple external (e.g., landscape composition [Fryxell et al. 2005], weather events and climate [Myserud et al. 2007], inter- and intraspecific interactions [Creel et al. 2005; van Beest et al. 2014] and internal factors (e.g., reproductive state [Long et al. 2009], navigational capacity [Tsoar et al. 2011], memory [Ranc et al. 2020]) contribute to animal space use and thus may interact with animal response to translocation to mediate spatial acclimation. Together, acclimation in spatial behavior appears to fall on a response continuum lagging behind physiological acclimation (Chapter 1), but before acclimation in larger-scaled population processes (e.g., population mating system [Chapter 3]). While not yet investigated, we would predict changes in survival, as the product of finer-scaled responses, to sit at the final and ultimate end of this acclimation spectrum.

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TABLES

Table 2-1. Covariate tables demonstrating variables hypothesized to influence changes in monthly elk (*Cervus canadensis*) 95% occurrence distribution (OD) size and overlap across 6-8 years following translocation to the Missouri landscape from Kentucky in 2011-2013. **2-1a)** demonstrates variables considered in linear mixed models (in addition to a random individual effect), while **2-1b)** demonstrates covariates retained in final model for each movement response (range size and overlap) following dredged model selection with AICc.

a) Covariate consideration

Covariate category	Covariate
Extrinsic	Sin(day of year)
	Cosine(day of year)
Intrinsic	Sex
	Age class at capture
Translocation	Month from release
	Cohort release group
	Month from release: sex
	Month from release: age class at release
	Month from release: cohort release group

b) Covariate selection

	<i>Range Overlap</i>	<i>Range Size</i>
Covariates retained by model selection	Sin(day of year)	Sin(day of year)
	Cosine(day of year)	Cosine(day of year)
	Sex	Sex
	Month from release	Month from release
	Cohort release group	Month from release*sex

FIGURES

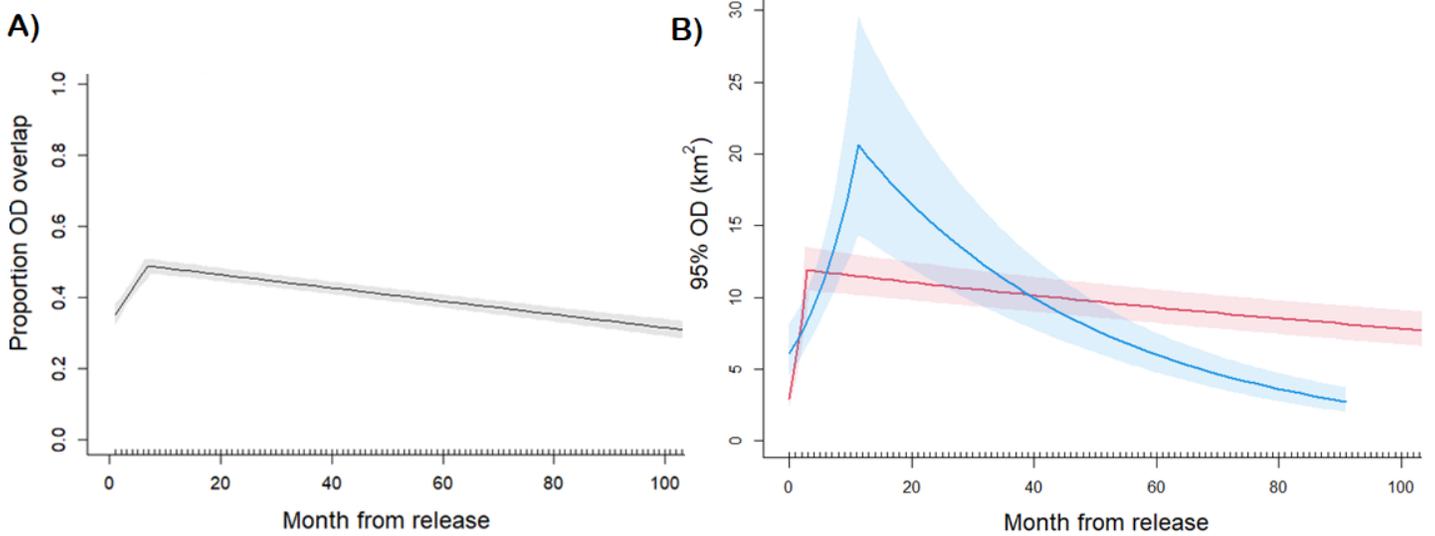


Figure 2-1a. Predicted effect of month from release on volume of intersection between successive monthly 95% occurrence distributions for individual elk (*Cervus canadensis*) translocated to the Missouri landscape in 2011-2013, including estimated breakpoint and indication of spatial acclimation occurring at 6.79 months following translocation. Rugs indicate sampling occurrences. **2-1b)** Predicted effect of month from release on 95% occurrence distribution area (km²) for individual male (blue) and female (red) elk translocated to the Missouri landscape in 2011-2013, including estimated breakpoint and indication of spatial acclimation occurring at 2.82 and 11.27 months following translocation for females and males, respectively. Rugs indicate sampling occurrences.

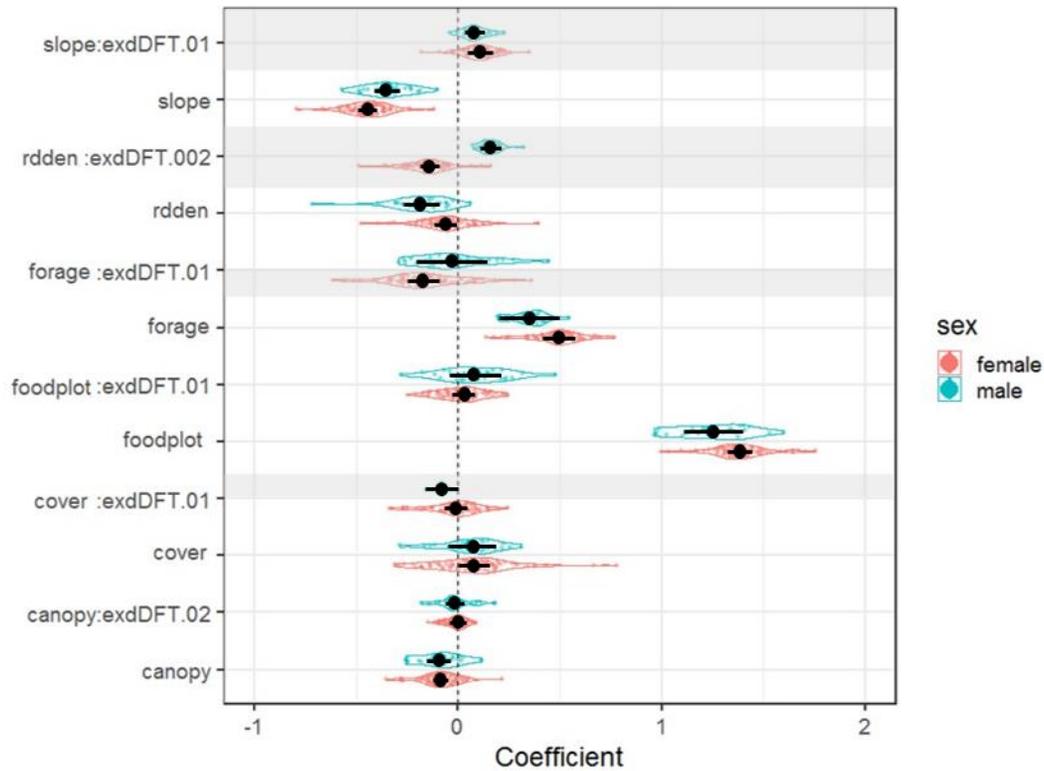


Figure 2-2. Individual- and population-level selection coefficients and 95% confidence intervals for female (red) and male (teal) elk (*Cervus canadensis*) across the 6-8 years following translocation to Missouri from Kentucky in 2011-2013. Population-level effects are symbolized by the larger black dots and error bars. Distributions of individual-level variation are captured by violin plots and selection coefficients for each individual are symbolized by small colored dots within violins. Significant acclimation effects are demonstrated by gray shading. Interaction terms include top-performing functional forms DFT; exdecayDFT.02 represents an exponential decay transformation with a decay rate of 0.02, exdecayDFT.01 represents an exponential decay transformation with a decay rate of 0.01, and exdecayDFT.002 represents an exponential decay transformation with a decay rate of 0.002.

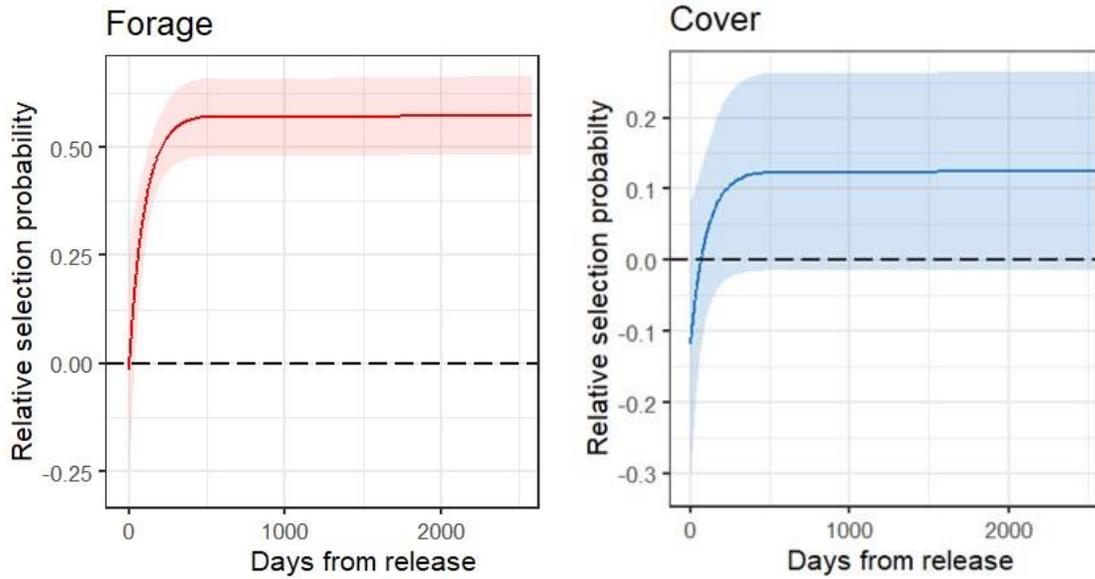


Figure 2-3. Predicted effects of days from release on relative selection for landcover types for female (pink) and male (blue) elk (*Cervus canadensis*) over the 6-8 years following translocation to Missouri from Kentucky in 2011-2013. Absence of change over time (i.e., a flat slope) indicates cessation of translocation effect and complete spatial acclimation. Only significant ($p < 0.05$) interactions are plotted.

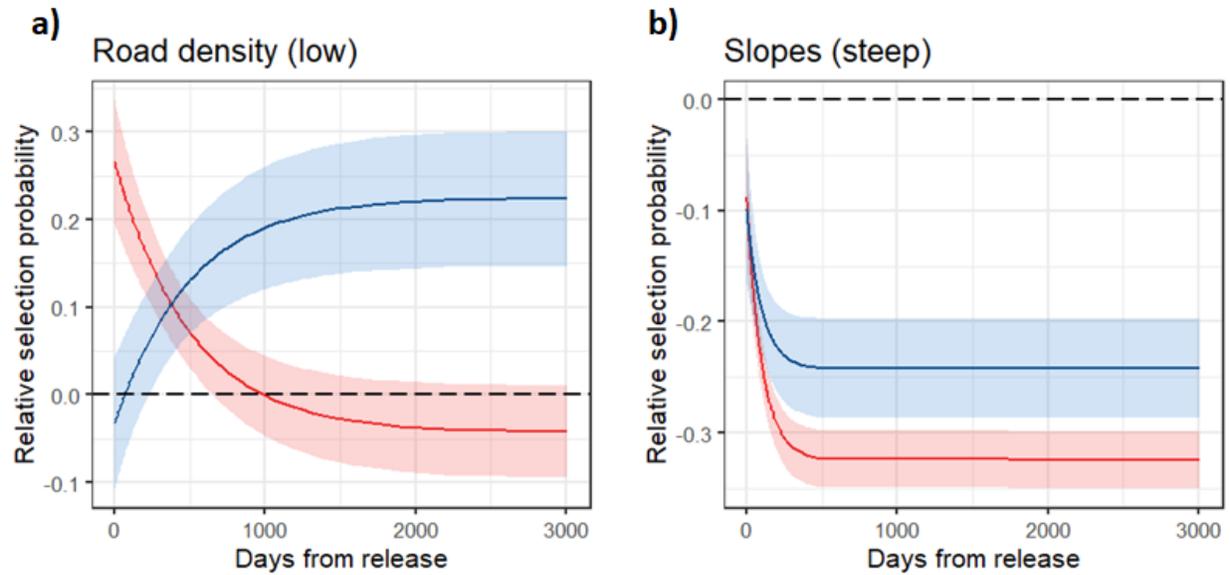


Figure 2-4. Predicted effects of days from release on the relative selection for a) areas of low (1st quartile) road density and b) steep (3rd quartile) slope grades for female (pink) and male (blue) elk (*Cervus canadensis*) over the 6-8 years following translocation to Missouri from Kentucky in 2011-2013. Absence of change over time indicates cessation of translocation effect and complete spatial acclimation.

SUPPLEMENTARY INFORMATION

Table S2-1) Original U.S. Forest Service National Land Cover Database classification legend from 2011-2019 alongside our reclassification of cover types for investigation of post-release resource selection temporal dynamics of elk (*Cervus canadensis*) following translocation to Missouri from Kentucky in 2011-2013. We include the original NLCD categories in italicized dark gray text beneath the landcover categories we reclassified. The Missouri Department of Conservation (MDC) food plot layers were merged with the NLCD raster and thus do not have an original NLCD coding. Reclassification was driven by the focus on evaluating potential trade-offs between elk selection for resources associated with safety (i.e., cover) and forage as they acclimate from translocation to the post-release landscape.

NLCD Land Cover Classification	NLCD Reclassification for Missouri elk resource selection
Open Water	Open (elk food plot):
Perennial Ice/Snow [^]	<i>MDC-managed food plot</i>
Developed, Open Space	Open (forage):
Developed, Low Intensity	<i>Grassland/Herbaceous</i>
Developed, Medium Intensity	<i>Pasture/Hay</i>
Developed, High Intensity	<i>Cultivated Crops</i>
Barren Land	<i>Emergent Herbaceous Wetlands</i>
Deciduous Forest	Cover (wooded):
Evergreen Forest	<i>Deciduous Forest</i>
Mixed Forest	<i>Evergreen Forest</i>
Dwarf Scrub*	<i>Mixed Forest</i>
Shrub/Scrub	<i>Shrub/Scrub</i>
Grassland/Herbaceous	<i>Woody Wetlands</i>
Sedge/Herbaceous*	Other:
Lichens*	<i>Open Water</i>
Moss*	<i>Developed, Open Space</i>
Pasture/Hay	<i>Developed, Low Intensity</i>
Cultivated Crops	<i>Developed, Medium Intensity</i>
Woody Wetlands	<i>Developed, High Intensity</i>
Emergent Herbaceous Wetlands	

*Alaska only; [^] Not present in elk study area

Table S2-2) Model selection criteria competing functional forms of days from release (DFT) terms to be carried forward in interactions with resource covariates in models of temporally dynamic elk (*Cervus canadensis*) resource selection in the 6-8 years following translocation to Missouri from Kentucky in 2011-2013. Each model set included a “Null” model containing only the resource covariate and no interaction with DFT. Models were fitted without random coefficients due to model convergence issues and were competed for each resource separately. $\Delta AICc$ indicates delta AICc units with the top model assigned a value 0, K denotes the number of parameters in the model, and w_t indicates model weight.

Road density			
Functional Form	K	$\Delta AICc$	w_t
Exd002	3	0.00	0.95
Exd001	3	5.88	0.05
Log	3	28.54	0
Sqrt	3	28.63	0
Exd005	3	46.50	0
Linear	3	72.81	0
Exd01	3	82.46	0
Exd02	3	114.34	0
Sq	4	156.20	0
Exd05	3	184.20	0
Null	2	268.31	0
Canopy			
Functional Form	K	$\Delta AICc$	w_t
Exd02	3	0.00	0.72
Exd05	3	1.92	0.28
Exd01	3	11.48	0
Sq	4	21.24	0
Exd005	3	24.10	0
Linear	3	32.18	0
Log	3	34.23	0

Exd002	3	35.88	0
Null	2	36.27	0
Sqrt	3	37.84	0
Exd001	3	38.88	0

Landcover			
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Functional Form	K	$\Delta AICc$	w_t
Exd01	3	0.00	0.59
Exd02	3	2.05	0.21
Exd005	3	2.23	0.19
Exd002	3	10.33	0
Log	3	10.67	0
Exd05	3	10.76	0
Exd001	3	16.95	0
Null	2	18.34	0
Sqrt	3	19.23	0
Linear	3	22.71	0
Sq	4	23.34	0

Slope			
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Functional Form	K	$\Delta AICc$	w_t
Exd01	3	0.00	0.88
Exd02	3	3.94	0.12
Exd005	3	14.46	0
Log	3	57.78	0
Exd002	3	79.74	0
Exd001	3	189.55	0
Sqrt	3	222.36	0
Exd05	3	243.80	0
Linear	3	399.39	0
Sq	4	593.09	0

Null	2	788.56	0
Dist to cover			
Functional Form	K	ΔAICc	w_t
Sq	4	0.00	1
Linear	3	66.19	0
Exd01	3	90.05	0
Exd005	3	91.35	0
Exd02	3	93.35	0
Sqrt	3	96.51	0
Exd002	3	103.37	0
Null	2	103.56	0
Exd05	3	103.68	0
Exd001	3	104.84	0
Log	3	105.54	0

Table S2-3) Model selection results from comparing the performance of full SSF models with highly correlated $|r| > 0.7$ variables distance to cover (“d2cover”) and the landcover (“landcover”) for explaining female and male elk (*Cervus canadensis*) resource selection in the 6-8 years following translocation to Missouri from Kentucky in 2011-2013. ΔAICc indicates delta AICc units with the top model assigned a value 0, K denotes the number of parameters in the model, and w_t indicates model weight. For females and males, models with landcover outperformed models with distance to cover.

Model	Females			Males	
	K	ΔAICc	w_t	ΔAICc	w_t
Full_landcover	25	0.00	1	0.00	1
Full_d2cover	18	7735.69	0	1118.74	0

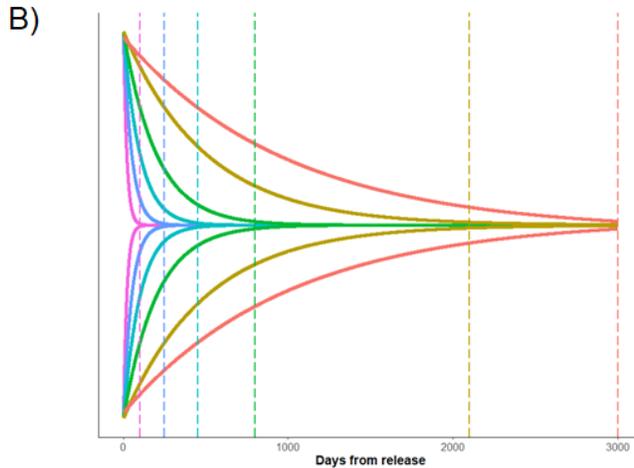
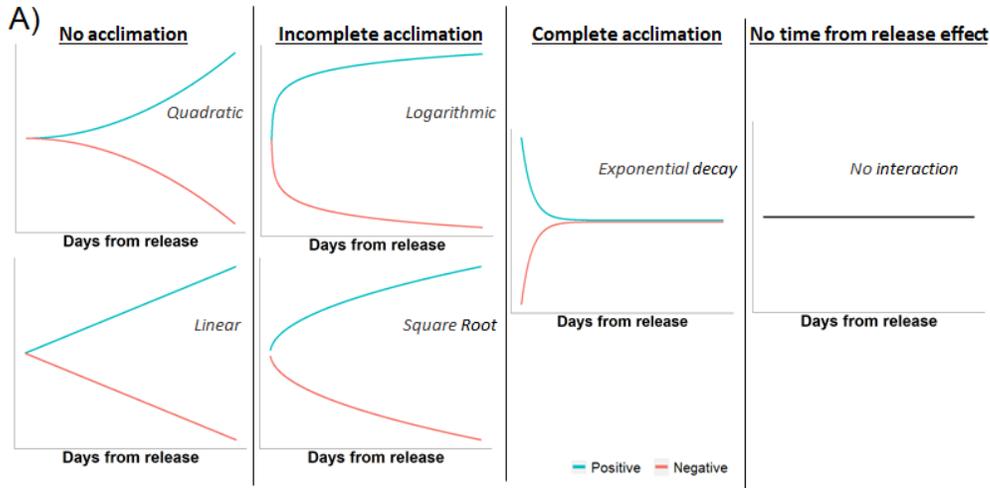


Figure S2-1a) Conceptual figure demonstrating effects (positive in teal, and negative in pink) of interactions with various functional forms of a days from release term (DFT) on resource selection patterns for resource covariates. Absence of change over time (i.e., a flat slope) indicates cessation of translocation effect and complete spatial acclimation (exponential decay DFT form). Quadratic and linear DFT forms show no decrease in the effect of DFT over time, and thus no indication of acclimation. Logarithmic and square root DFT forms show decreased effect of DFT with time, and thus indication of partial acclimation. Absence of interaction also indicates no effect of time from release and no acclimation effect. **S2-1b)** Conceptual figure demonstrating the effect of varying constants in the exponential decay DFT term on the time it takes to reach acclimation as indicated by absence of change over (i.e., a flat slope) time following release. Constants range from 0.05 (pink), 0.02 (violet), 0.01 (blue), 0.005 (green), 0.002 (gold), 0.001 (red), and the color-match vertical dotted line approximates when the slope reaches zero indicating absence of time from release effect and complete acclimation.

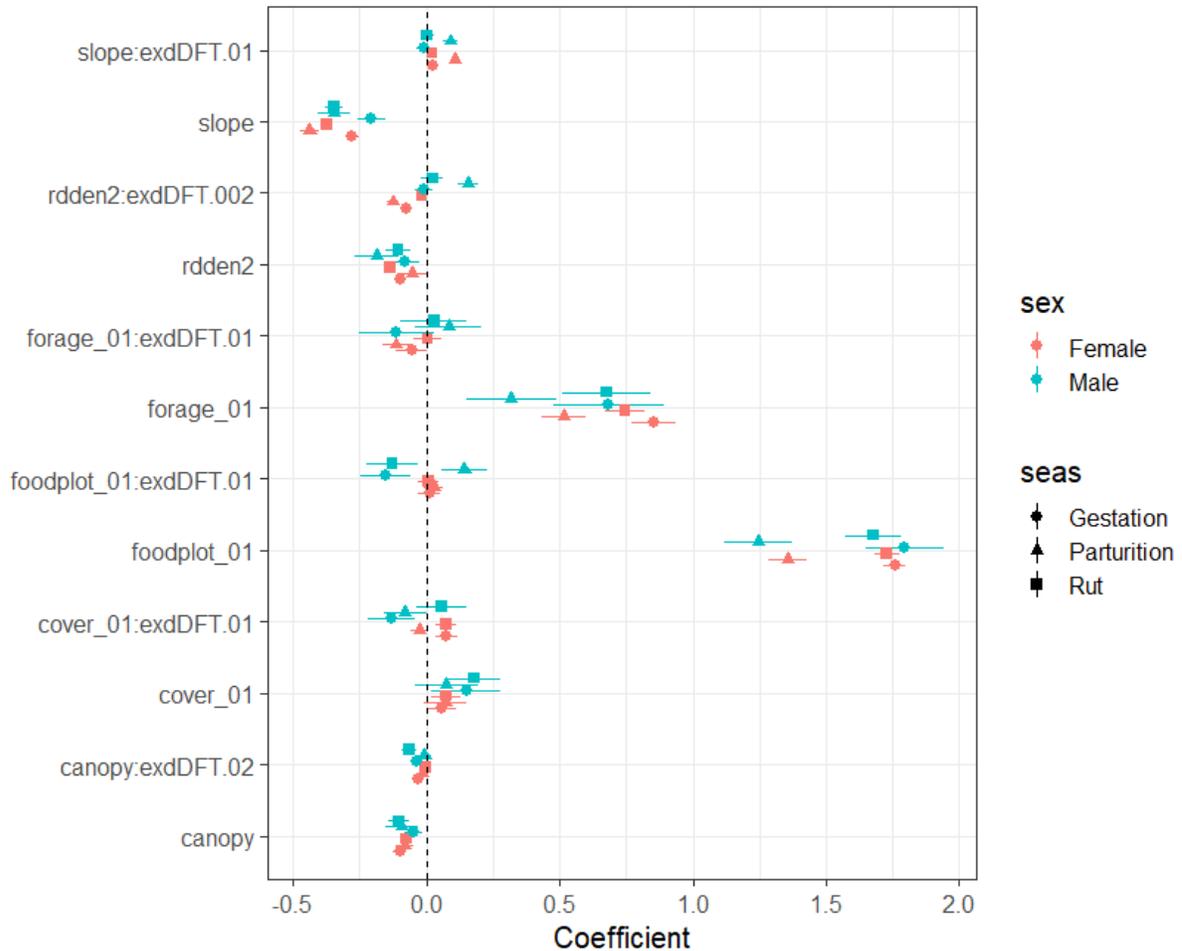


Figure S2-2) Population-level selection coefficients and 95% confidence intervals for female (in red) and male (in teal) elk (*Cervus canadensis*) for all seasons across the 6-8 years following translocation to Missouri from Kentucky in 2011-2013. Parturition season (May 1 – Aug 31) is represented by circular symbols, rutting season (Sept 1 – Dec 31) is symbolized by squares, and the gestation season (Jan 1 – April 31) is symbolized by triangles. Interaction terms include top-performing functional forms DFT; exdecayDFT.02 represents a exponential decay transformation with a decay rate of 0.02, exdecayDFT.01 represents a exponential decay transformation with a decay rate of 0.01, and exdecayDFT.002 represents a exponential decay transformation with a decay rate of 0.002). Positive coefficients for interactions with exdecayDFT signify a decrease in relative selection with acclimation while negative coefficients signify an increase in relative selection with acclimation for the interacted resource.

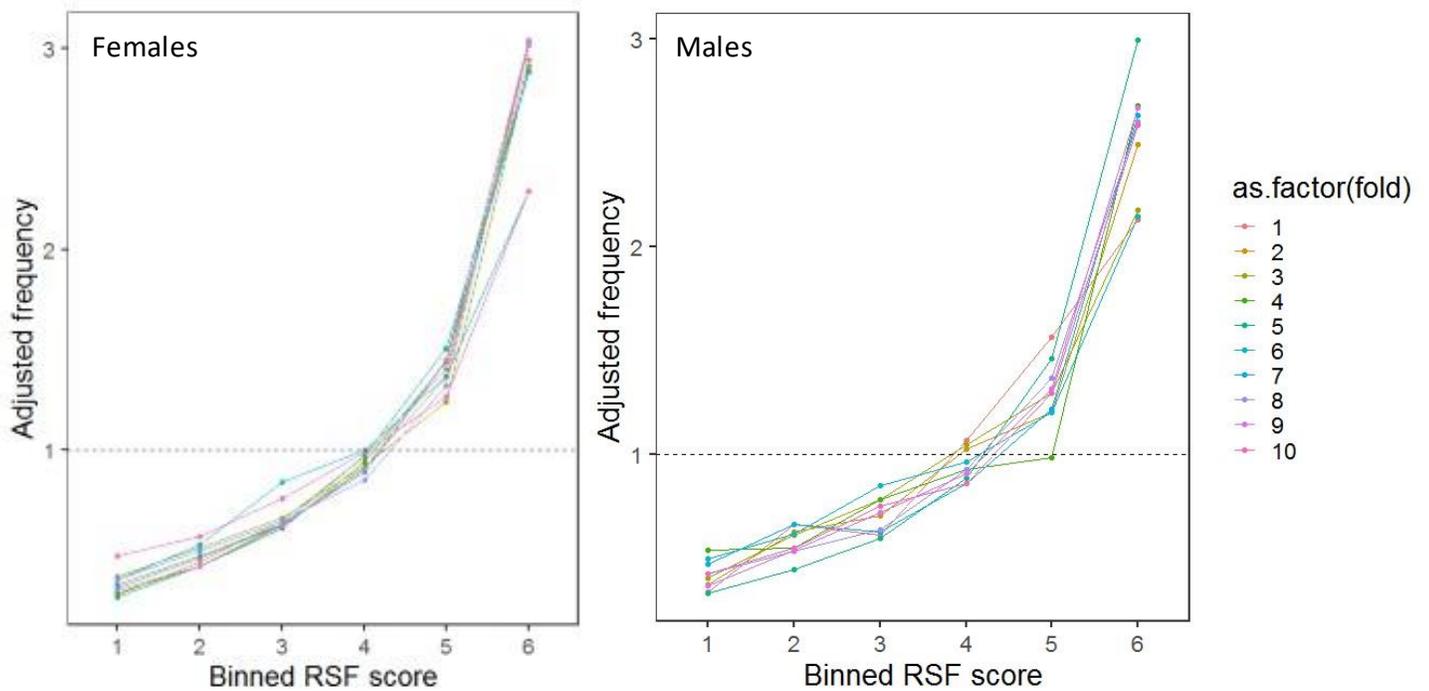


Figure S2-3) Out-of-sample cross validation results for resource selection models of male and female elk (*Cervus canadensis*) over the 6-8 years following translocation to Missouri from Kentucky in 2011-2013. Adjusted frequencies for each fold represents the cumulative frequency of predicted RSF scores for used locations that fall into each of 6 equal-interval bins representing 5 available location and 1 used location per stratum. Values above 1 indicate that cross-validated used locations occur at rates higher than expected by chance. For each sex, models were fit to 90% of individual elk, and model coefficients were used to predict RSF scores for the remaining 10%.

Chapter 3: Acclimation of elk mating system following restoration to the Missouri Ozarks, U.S.A.³

ABSTRACT

Biologists increasingly use translocation to restore animals to areas where they have been extirpated. However, we know little about how translocation decisions influence translocation-mediated social dynamics. Breeding in polygynous ungulate mating systems is typically dominated by prime age males, but founding males within translocated ungulate populations often are comprised of only young individuals. We investigated the influence of releasing exclusively young-aged males on mating system and male reproductive success during an elk (*Cervus canadensis*) restoration program in Missouri. From 2011 to 2013, we translocated and released 106 elk from Kentucky to Missouri, USA. We collected tissue samples for DNA from all translocated elk and subsequently captured adults and calves in Missouri during 2014 – 2018 for paternity analysis. Initial levels of polygyny were low but increased and acclimated over the initial years following translocation, commensurate with advancing sire age structure and increasing population density. Sire age was positively associated with individual male reproductive success initially, but the effect of age decreased as polygyny acclimated and sire age structure became older and more variable. Polygyny levels in the reintroduced population were restored to expected values within four years of the last translocation event, demonstrating the acclimation of mating structure despite a translocation tactic favoring young-aged males. Importantly, initial dampened polygyny may facilitate retention of genetic variation by

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maximizing the genetic contribution of more founding individuals; however, benefits to genetic variation retention should be considered against potential demographic consequences to calves sired by young males.

INTRODUCTION

Following release into a new environment, translocated animals must adjust to novel biotic and abiotic environments (i.e., the acclimation period). During acclimation, animals experience increased mortality and dispersal (Le Gouar et al. 2012), termed ‘post-release effects’ (Armstrong and Reynolds 2012). Reintroduction failures most often occur during the acclimation period, even when conditions at the release site are deemed sufficient to support population persistence (Armstrong and Seddon 2008). Managers must make numerous decisions when planning and implementing translocations that may influence acclimation of the founding population to its new landscape and ultimately affect project success. Particular attention should thus be placed on understanding how various decisions influence recently translocated animals during their initial period in their new landscape (IUCN 2013).

Aspects of social behavior have garnered recent attention as proximate factors mediating higher-level population processes (i.e., survival, reproduction, and dispersal) that ultimately influence restoration success (Berger-Tal et al. 2016; Greggor et al. 2016). Translocations may disrupt social behavior in newly founded populations stemming from changes to group sizes, membership, and underlying environmental drivers of aggregation (e.g., landscape composition and distribution of resources (He et al. 2019)). These social disruptions may have important consequences on translocated individuals and populations. For example, translocated bighorn sheep (*Ovis canadensis*) suffered from increased conspecific aggression and subsequently decreased body condition and delayed reproduction during supplemental translocations in

Alberta, Canada (Poirier and Festa-Bianchet 2018). Similarly, translocation disrupted social structure and increased mortality in hihi (*Notiomystis cincta*) that lost cohort members during a reintroduction in New Zealand (Franks et al. 2020). Despite growing evidence that social behavior can affect translocated populations and individuals, limited research has investigated the extent and dynamics of translocation-mediated social disruptions. A better understanding of how translocation decisions influence behavioral interactions may thus aid population establishment and persistence.

Individual reproductive behavior and mating system dynamics are aspects of social behavior that can affect demographic and genetic aspects of wildlife restoration given their direct contributions to reproduction and population growth (Sigg et al. 2005). An immediate goal of most wildlife restoration events is to promote population growth by maximizing survival and reproduction of founders. As such, mating system structure is an important aspect of the reproductive potential and population growth rate of a recently established population (Lee et al. 2011; Schindler et al. 2013). Further, retention of genetic variation improves long-term restoration success by buffering against the harmful effects of inbreeding and genetic drift and maximizing adaptive potential (Biebach et al. 2016). Effective population size and rate of genetic variation loss are also dependent on population mating system (Nunney 1993). Inequality in reproductive success decreases effective population size, facilitating observed loss of genetic variation in small, restored populations during the crucial acclimation period (Wright 1938; Crow and Kimura 1970). Polygynous mating systems may thus be particularly sensitive to decreased effective population size and losses in genetic variation because they are characterized by high variability in reproductive success. Simulation studies show polygynous mating systems lead to shorter times to extinction for small populations (Conard et al. 2010; Lee et al. 2011), and

restored populations undergo at least an initial period of low abundance during the vulnerable acclimation phase (IUCN 2013). Thus, the effect that a population's mating system has on facilitating or inhibiting acclimation to a new environment, and ultimately on restoration success, warrants further investigation. Because mating systems are dynamic and influence retention of genetic variability, reintroduction planning would benefit from a greater understanding of translocation effects on mating systems.

Reintroductions cause large-scale changes to underlying demographic factors that may influence a population's mating system in predictable ways. Mating systems are dynamic in response to a population's demography and environment (Emlen and Oring 1977; Clutton-Brock 1989; Lott 1991), and density is a primary demographic factor hypothesized to influence variation in reproductive success (Apollonio 1989). As density increases in polygynous systems, sexual selection theory predicts increased opportunities for male-male encounter and competition, resulting in an increased level of polygyny (i.e., inequality in reproductive success; Emlen and Oring 1977; Eshel 1979; Clutton-Brock 1989; Kokko and Rankin 2006). Beyond population density, the distribution of male quality and competitive ability are also pivotal components influencing a population's mating system (Shuster and Wade 2003; Klug et al. 2010) and may be influenced by translocation decisions. In species where age is associated with competitive ability, decisions regarding the age structure of release cohorts and subsequent demographic age-shifts may directly influence mating system dynamics in the years following reintroduction events. Population mating system and individual reproductive success in restored populations may thus be influenced by the composition of age-classes within translocation cohorts and by subsequent changes in the age structure and density of founding populations.

Ungulate restorations are ideal for understanding translocation-mediated changes in reproductive success and mating systems. In polygynous ungulates, older males tend to be larger than young males and have higher reproductive success (Coltman et al. 2002; Nussey et al. 2009; Festa-Bianchet 2012). Thus, translocation decisions about founding age structure may influence the degree and dynamics of polygyny in restored ungulate populations. Further, disruptions to polygyny may be particularly pronounced in ungulate restorations given the tendency for male release cohorts to be composed predominately of young individuals (calves to 2-year-olds; Larkin et al. 2002). The strong bias towards young-aged male founders results in populations with a uniformly young male age structure early in reintroduction, but with increasing variation in male age structure later in the reintroduction following subsequent reproduction and supplementation events.

We capitalized on the unique opportunity presented by translocation-mediated demographic shifts that occurred during an elk (*Cervus canadensis*) restoration program in Missouri, U.S.A. to investigate changes in the population mating system in the years following translocations. To our knowledge, no translocation event has tested predictions about factors influencing a population's mating system dynamics. We hypothesized that increasing elk density and variation in relative male competitive ability (proxied by male age) would result in increasing inequality in male reproductive success following translocation. Accordingly, we predicted that levels of polygyny would increase with time following translocation as male density and variation in male age structure increased. Further, we hypothesized that male age would become an increasingly important factor associated with individual male reproductive success as male age structure became older and more variable. Understanding how translocation alters mating structure may allow managers to maximize contributions of translocated

individuals to the founding gene pool to enhance retention of genetic variation and increase restoration success (Haig et al. 1990).

METHODS

Animal translocations and sample collection – From 2011 to 2013, we translocated and released 106 elk to the southeastern Missouri Ozarks (91°24' to 90°58'W and 37°0' to 37°19'N: Bleisch et al. 2017). Elk were captured in Kentucky, USA, in January of each year and quarantined in corral facilities for 102 – 129 days before overnight trailer transport to Missouri. Upon arrival in Missouri, elk were quarantined for an additional 19 – 34 days in holding pens before release. The Missouri elk range is separated from the nearest neighboring restored elk population in Arkansas by approximately 250 mi and managed in isolation (Dent et al. 2012).

To assess location and survival, we fit all elk with GPS-VHF collars prior to release (RASSL custom 3D cell collar, North Star Science and Technology, King George, VA, or G2110E Iridium-GPS series model, Advanced Telemetry Systems, Isanti, Minnesota, USA) and affixed PIT-tags for permanent identification. We released 106 translocated elk from 2011-2013, and the demographic composition of release cohorts was: 2011 (n = 34) – 15 adult females (2+ years), 5 yearling females, 6 two-year-old males, 8 yearling males; 2012 (n = 33) – 22 adult females, 3 yearling females, 4 two-year-old males, 4 yearling males; 2013 (n = 39) – 20 adult females, 16 yearling females, 3 yearling males.

We performed subsequent captures of yearling and adult individuals on the Missouri landscape between 2015 and 2018 via darting. We variously employed vaginal-implant transmitters (Johnson et al. 2006), GPS-based behavioral monitoring (Cartensen et al. 2003), and opportunistic searches (Seward et al. 2005) to locate and capture neonates during summers from 2011 to 2018 (except 2015). We ascribed mother-calf relationships when possible (64.8% of

sampled calves). We took ear notches for microsatellite-based paternity analyses from all translocated and subsequently captured individuals. Tissue samples were placed in 100% ethanol and frozen at -20° C until extraction.

Microsatellite genotyping – We isolated DNA from tissue samples using the Qiagen Dneasy Blood and Tissue Kit (Qiagen). We selected microsatellite loci for paternity analysis based on potential for high degrees of polymorphism and previous use in the literature for elk and red deer (*Cervus elaphus*) diversity and parentage investigation (Polziehn et al. 2000; Williams et al. 2002; Hicks et al. 2007; Conard et al. 2010). We used polymerase chain reaction to amplify extracted DNA at 16 microsatellite loci in 3 multiplex reactions (1) BM888, BL42, BM5004, BM1009, ETH152; (2) BM4107, BM4208, BM1225, BM203, BM4513; and (3) C01, C229, T193, T510, T26, T156 (Kossarek et al. 1993; Bishop et al. 1994; Jones et al. 2002; Meredith et al. 2005). Reactions consisted of 8 uL total volume with 4 uL Platinum PCR Mastermix (Applied Biosystems), 0.6 uL BSA, 0.6 uL GC Enhancer (Applied Biosystems), 0.275 uM of each primer and 1 uL of genomic DNA extract. All multiplex reactions were run at the same cycling conditions consisting of 94° for 15 min then 30 cycles of 94° for 30 sec, 55° for 90 sec, 72° for 60 sec followed by 60° for 30 min. Fragments were analyzed at the University of Missouri DNA Core on an ABI 3730xL with LIZ 600 size standard and scored using GeneMarker (SoftGenetics). We used CERVUS 3.0 (Kalinowski et al. 2007) to estimate annual allele frequencies and null alleles, and to employ χ^2 analyses to estimate departures from Hardy-Weinberg equilibrium with Bonferroni corrections (Rice 1989). We used the package “GENEPOP” (Raymond and Rousset 1995) in program R (R Core Team 2020) to carry out disequilibrium tests on only translocated individuals to avoid multigenerational effects. We also used the Bonferroni method to adjust table-wide significance levels for genotypic disequilibria.

Parentage assessment – For each calf cohort sired in Missouri from 2012 through 2018, we used CERVUS to assess paternity for 133 individuals captured as calves and yearlings and for 14 individuals aged 2.5 years old at the time of capture (because they could be confidently aged [Quimby and Gaab 1957] and retroactively assigned to their appropriate year’s calf cohort). To estimate paternity, CERVUS employs a maximum likelihood approach to estimate the probability that a candidate is the most likely parent for each parent-offspring pairing by calculating a logarithm of odds (LOD) score, which reflects the likelihood of paternity of a given candidate sire relative to an arbitrary individual (Marshall et al. 1998). The program uses simulations based on the population’s allele frequencies from the supplied genotypes and user-defined input parameters (including total male candidate population size, proportion of known candidates that have been sampled [see below], and genotyping error rate). The program then assigns a sire at user-defined confidence levels (often 80% and 95%) after estimating a population-wide critical threshold value (δ) representing the difference in LOD scores between the two most likely candidate sires for a given calf (Marshall et al. 1998). We conducted simulations for each cohort using the genotypes of calves and candidate parents of that year, using 100,000 cycles, a default 1% error rate, and estimated 95% and 99% confidence levels.

We considered all males aged one year or older and not known to have died before the start of the rut (defined as September 1) to be paternal candidates for a calf in a given year. We estimated total annual candidate population sizes and proportions of sampled candidates for 2014-2018 by comparing the number of genetically sampled individuals to the Missouri Department of Conservation (MDC) sex-specific minimum population estimates. Given complete census counts of translocated individuals (2011-2013) and subsequent intensive monitoring and capture efforts, we considered the population count in 2013 to be a complete

census. MDC uses a sex- and stage-structured Lefkovitch population model dependent on observation-based inputs for minimum population counts, survival, and recruitment to estimate minimum population sizes (Table S3-1). Minimum counts were informed by intensive daily monitoring and annual aerial surveys. Survival and recruitment were estimated by daily monitoring of VHF and satellite collars. While MDC estimates were treated as minimum estimates across years, underestimation was likely greater in later years when population growth and expansion increased relative uncertainty (A. Hildreth, 2020, Missouri Department of Conservation, Jefferson City, MO, personal communication).

Relative to MDC's annual male population estimates, we achieved an average sire sampling rate of 90% across the years 2013 – 2018. However, because MDC minimum estimates were assumed to be biased low, particularly in later years, we conservatively fixed the proportion of sampled sires at 75% for all years to consider additional potentially unknown candidate sires in the population (Vanpé et al. 2007). The total number of candidate sires for paternity estimation was then calculated for each year by dividing the total number of sampled males in the population by 0.75. Given our conservatively high sampling rate, we expected high success in paternity assignments even at high thresholds of confidence (95% and 99%). We also performed paternity assignments with the proportion of sampled candidates fixed at 85% and 95% to explore the sensitivity of assignments and subsequent analyses to uncertainty in total male population size (Table S3-1). We include the results of these analyses in the Supplementary Information but do not discuss them further because we observed little sensitivity from the estimated sampled proportion of candidate males and no change in interpretation of paternity (Tables S3-2—S3-4) or polygyny dynamics (Figure S3-1).

Paternity and level of polygyny – We determined annual individual male annual reproductive success (ARS) by summing the number of calves successfully assigned to each candidate male at the 95% confidence level. We then characterized population-wide level of polygyny with Shuster and Wade’s (2003) opportunity for selection, I_m (the mean standardized variance in male ARS = variance in ARS/mean ARS²). I_m is a widely used metric for characterizing the population-wide level of polygyny (Willisch et al. 2012), where higher values signify greater variation in male ARS and thus higher levels of polygyny.

ARS model – To further investigate the influence of age on reproductive dynamics within the restored herd, we used a Bayesian zero-inflated negative binomial model to predict individual annual male reproductive success as a function of male age and other relevant biological covariates. We built a global model to understand factors influencing male reproductive success with package ‘brms’ (Bürkner 2018) in the Bayesian environment STAN (Stan Development Team 2020) within program R (R Core Team 2020). We incorporated additional covariates reflecting whether or not candidates had successfully sired a calf in the previous year, whether or not candidates were known to harbor brain worm (*Parelaphostrongylus tenuis*) parasites (an important source of mortality for Missouri elk [Chitwood et al. 2018]), a fixed year term, and a random intercept for a candidate ID and age interaction (to account for repeated measures of ARS on the same individual candidate sire across years and to allow for individual variation in the effect of age). We allowed covariates to interact with year to investigate whether any factors associated with ARS changed across years following translocation commensurate with shifting population age structure and density. We calculated leave-one-out (LOO) model validation metrics (package ‘loo’; Vehtari et al. 2020) and conducted post-predictive checks (package

‘brms’) on our global model to evaluate ARS model fit. We considered covariates whose 95% credible intervals did not overlap zero to be significant.

RESULTS

Tests of microsatellite markers – We genotyped 306 individuals (188 females; 118 males) and estimated paternity for 145 Missouri-sired calves. We observed a 91.9% typing rate across loci. All loci were polymorphic with an average value of 9.53 alleles per locus (SE = 3.94). We did not find evidence of significant linkage disequilibrium among loci (all pairwise comparisons after Bonferroni correction $p > 0.05$ [Rice 1989]). Following sequential Bonferroni correction, we identified one locus (T193) that significantly deviated from Hardy-Weinberg proportions and removed it from paternity analyses. Total exclusionary power of the 15 retained microsatellites was 0.9997 when one parent was known and 0.9887 when neither parent was known. The probability of identity over all loci was 2.12×10^{-12} among all individuals and 1.08×10^{-5} among siblings.

Paternity and level of polygyny – We successfully assigned paternity at the 95% confidence level for 97 of the 145 Missouri-sired calves. Total number of candidate sires increased across years together with an increasing and more variable candidate sire age structure (Figure 3-1a). Age structure of successful sires likewise increased and became more variable across years (Figure 3-1b). We observed an increasing level of polygyny across years from a minimum value of I_m of 3.55 in 2013 to 5.99 in 2018. The level of polygyny peaked at 7.57 in 2017 (Figure 3-2).

ARS model – Age and the age-by-year interaction were the only covariates with credible intervals not overlapping zero (Figure 3-3). Age was positively associated with annual male reproductive success (Figure 3-3), but the age-by-year interaction had a negative association suggesting that the effect of age decreased across time (Figure 3-3). Thus, older individuals benefitted from a

higher probability of reproductive success within a smaller and more homogenously young male population early in the restoration compared to a relatively larger, more variably aged population later in the restoration (Figure 3-4).

DISCUSSION

As with other elk translocations, the Missouri translocation was biased toward release of females, to maximize immediate population growth, and young males to facilitate transport. Unsurprisingly, we detected a homogenous, young male-age structure and dampened levels of polygyny during initial years of the restoration. Low polygyny observed in the first year following final translocation was similar to values observed in populations of ungulate species that are territorial or more typically adopt tending bonds as opposed to harem strategies (e.g., roe deer *Capreolus capreolus* [I = 2.5; Vanpé et al. 2007], Soay sheep *Ovis aries* [I = 4.0; Coltman et al. 1999], white-tailed deer *Odocoileus virginianus* [I = 1.9; Sorin 2004]). Four years after the final translocation cohort was released and commensurate with increases in population density and variation in male age structure, the Missouri elk population reached polygyny values associated with high levels of reproductive skew characteristic of typical red deer populations (I = 7.2; Pemberton et al. 1992). Thus, the increase in population-wide polygyny to expected levels occurred alongside an advancing and more variable sire-age structure, increasing population density, and temporally dynamic individual male reproductive success. The observed adjustment in polygyny suggests that successful acclimation in mating structure can be attained within the initial years of elk restoration despite a translocation tactic favoring young-aged males.

Intraspecific variation in mating structure is common among ungulates (Rubenstein 1986; Apollonio et al. 1992; Carranza 2000). The degree of mating structure variation we observed in Missouri elk appears less common but does occur in other populations experiencing large-scale

demographic shifts (Isvaran 2005). For example, the mating structure of a pronghorn (*Antilocapra americana*) population on the National Bison Range in western Montana also shifted across years in the face of large-scale changes in male age structure (Byers and Kitchen 1988). Within seasons, populations of moose (*Alces alces*) and reindeer (*Rangifer tarandus*) shift from a harem to tending bond system as the density of available females decreases (Bowyer et al. 2011; Weladji et al. 2017). Such mating system shifts provide evidence for the impact of large-scale changes in demographic factors like density and age structure on mating system dynamics. While shifts in mating structure associated with demographic changes are thus not without precedent across ungulate species, mating system disruptions associated with translocation-mediated demographic changes may have important implications for restoration success relative to retention of genetic variation.

The reduced degree of polygyny in early years following translocation may facilitate success within reintroductions of animals with polygynous mating structures. Theoretical (Wright 1938; Crow and Kimura 1970; Nunney 1993) and empirical investigations (Lee et al. 2020) demonstrate that low levels of reproductive skew increase effective population size and retention of genetic variation by increasing genetic contributions of more individuals. Attenuated polygyny in the Missouri elk population may thus have facilitated retention of genetic variation in the relatively small, restored population. While our observation of normalized polygyny levels within four years following final translocation suggests that translocation-mediated reduction in polygyny is temporary, translocated populations may benefit most from increased effective population size during the initial post-release acclimation period. During acclimation, population size is lowest and thus most vulnerable to adverse effects of genetic bottleneck (Biebach et al. 2016). Thus, it is precisely during this initial period when genetic variation retention is most

crucial and when any boost to retention of genetic variation is most needed. Encouragingly, the potential benefit of initial dampened polygyny to effective population size and genetic diversity retention is supported by evidence indicating high retention of genetic diversity across recent elk restoration efforts (Youngmann et al. 2020).

Opportunity for selection (I_m) is a convenient metric for describing a population's mating system and relative level of polygyny (Krakauer et al. 2011); however, like all characterizations of mating structure, appropriate use is dependent on a high known and sampled proportion of the population. When ungulate populations are not intensively monitored, it is difficult to obtain a sufficiently high sample of the population (Corlatti et al. 2015). Thus, no I_m estimates have been reported for elk populations before this study. We were able to characterize the mating system of the Missouri elk population because we had a small, recently translocated population that was intensively monitored (via capture and collaring efforts) across the restoration. Even with near-perfect knowledge of the population from complete genetic sampling of translocation cohorts and from subsequent years of intensive capture efforts, we were unable to confidently assign paternities for 33% of sampled calves over the 6 years of our investigation. While our proportion of assigned calves is higher than that observed in other investigations of mating structure in wild populations (e.g., Willis et al. 2012), it likely means that we underestimated maximum individual male annual reproductive success and overestimated the number of sires producing no calves. It is unclear however whether overestimating non-breeding males and underestimating maximum annual paternities increases or decreases relative polygyny, as these effects likely have opposite consequences on overall variance in reproductive success (Vanpé et al. 2007). Regardless, we experienced similar proportions of both sampled candidate males and successfully assigned calves across years, suggesting the relative increase and acclimation in

polygyny levels across years was likely unaffected by the imperfect candidate sampling and paternity assignment observed in our study.

We were unable to tease apart influences of increasing density and an increasingly variable age structure on relative polygyny level; however, we identified age as a strong predictor of individual male reproductive success. In male ungulates, sexually selected characteristics and reproductive success are typically functions of male size, which in turn are correlated with male age (Festa-Bianchet 2012). For example, Coltman et al. (2002) saw a non-linear increase in mating success with age in male bighorn sheep, while Nussey et al. (2009) saw a similar relationship between age and male breeding success in red deer. Contrary to our predictions, however, we found that the influence of age on individual male reproductive success decreased with time following translocations, even as male age structure became more variable and population-wide polygyny increased. While contrary to our prediction, this finding corroborates Martin et al.'s (2016) observation that the effect of age on individual reproductive success is independent of the influence of demographic age structure on population-wide variation in reproductive success in male bighorn sheep.

The significant interaction between time from translocation and male age on individual annual reproductive success in the Missouri elk population suggests that male age had the greatest effect on likelihood of siring calves during early years when male age structure was homogeneously young, compared to later years when male age structure was older and more variable. Thus, while it appears advantageous to be older, it appears to be most advantageous to be older when the age structure is compressed (e.g., being 3 when among males that are 1 and 2 is better than being 8 when among males that are from 1-7). In later years, when the age structure is more advanced and variable, factors in addition to age that contribute to male quality and

established dominance hierarchies appear to gain importance. For example, inter- and intra-age differences in antler size, body mass, and fighting ability may all emerge as more important predictors of reproductive success as the young population ages. The lower effect of male age in later years could also suggest females demonstrate avoidance for breeding with the youngest males (i.e., yearlings and two-year-olds) rather than selection for the oldest-males in the relatively young population. While mating systems are typically characterized by male mating patterns, female behavior plays a role in population mating structure across species (Bowyer et al. 2020). In small populations, such as those that have been recently reintroduced, females may be better able to exert some degree of female choice (Morina et al. 2018) and resist undeveloped mating tactics of the most sexually inexperienced yearling males (Clutton-Brock & McAuliffe 2009). However, more data are necessary to partition the influence of male behavior and female choice on observed mating structure (DuVal and Kempenaers 2008).

Regardless of the influence of female behaviors, successful matings achieved by yearling and young-aged males are observed and predictable in restored elk populations where managers only translocate yearling and two-year-old male individuals (Larkin et al. 2001). However, there is no consensus as to whether there are demographic or phenological consequences to calves sired by young-aged males. While life history theory predicts some trade-off between growth and reproduction (Stearns 1989), there is limited evidence for negative associations between sire age and offspring survival in cervids (Kie et al. 2013). Beyond calf survival, there is some evidence that inefficient breeding of young age sires may adversely influence calf survival by delaying conception dates and leading to extended parturition seasons (Mysterud et al. 2002; Noyes et al. 2002; Nussey et al. 2006; Keller et al. 2015). Managers may thus need to consider potential costs to calf survival from young age sires against any likely benefits of the bias toward young males

during translocation, including the maximization of founder genetic contribution via dampened polygyny.

Overall, our results show how factors related to translocation practice may influence the mating system of a restored population following translocation to a new landscape. We demonstrated an initial dampening of polygyny within a reintroduced elk population that was restored using only young-aged males. High levels of polygyny were restored in the population four years following final translocation, suggesting successful mating system acclimation using a young-male translocation tactic. It remains unclear if translocating a higher proportion of males from older age classes might facilitate faster acclimation of polygyny as we were unable to elucidate the influences of increasing density and an increasingly variable age structure on population-wide variation in reproductive success. Further, the initial dampened polygyny may facilitate retention of genetic variation by maximizing the genetic contribution of more founding individuals; however, any benefits to genetic variation retention should be considered against any potential consequences to calves sired by young-aged males. Our work demonstrates the acclimation of high levels of polygyny and potential benefits to genetic variation retention in translocation scenarios favoring young-aged males.

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FIGURES

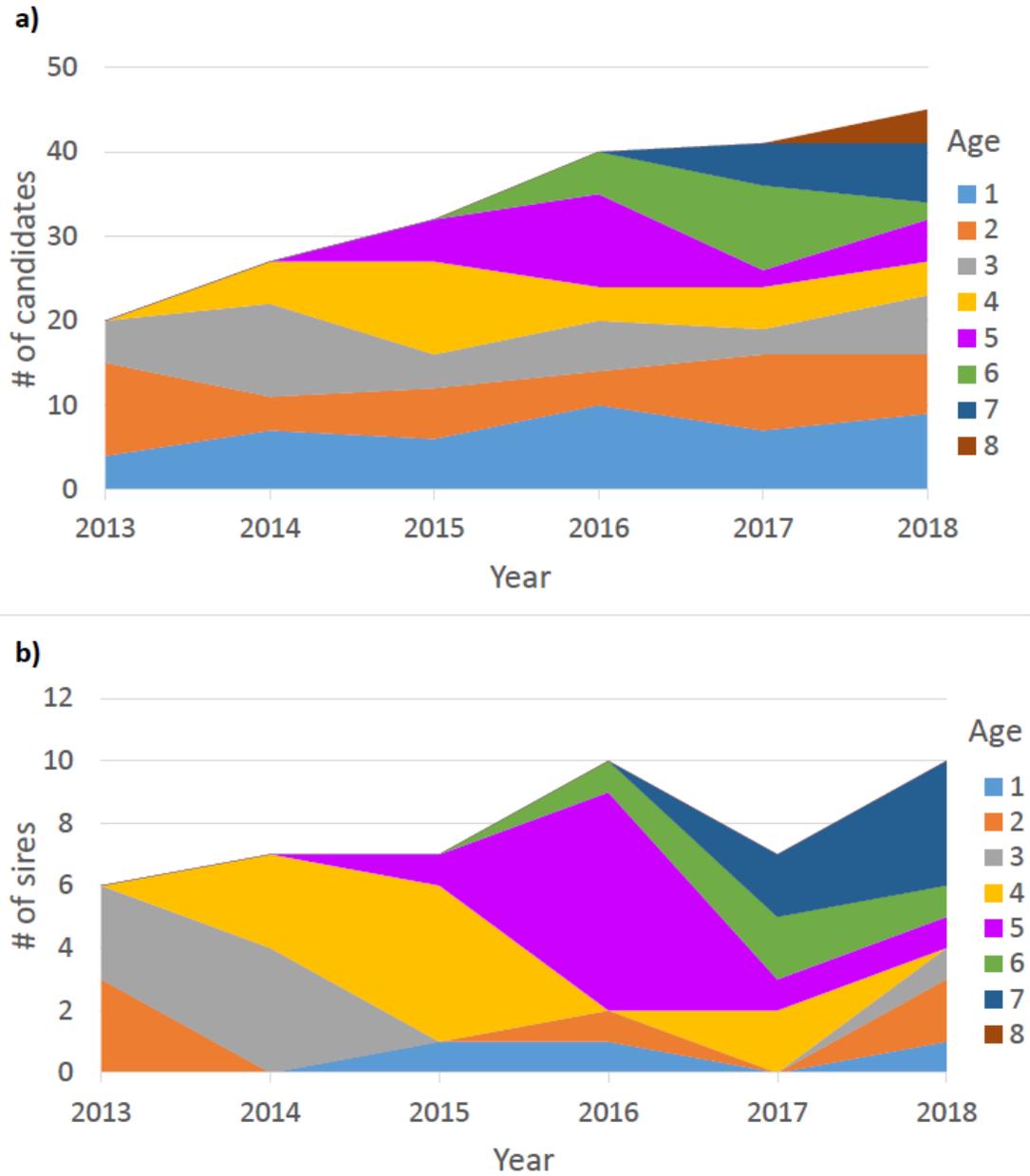


Figure 3-1) Plots representing the **3-1a)** total number of sampled candidate sires, and **3-1b)** counts of successful sires according to age class within the Missouri elk (*Cervus canadensis*) population over the initial years following restoration (2013-2018).

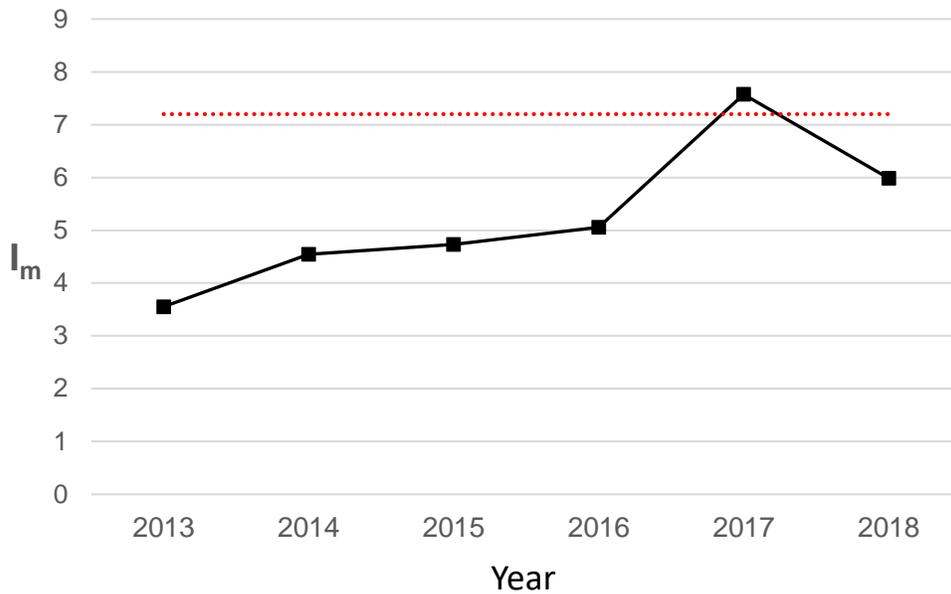


Figure 3-2) Plot of the annual mean standardized variance in male annual reproductive success (I_m) for the Missouri elk (*Cervus canadensis*) population over the initial years following restoration (2013-2018). I_m represents relative level of polygyny. The red dotted line represents the I_m value published for a typical, established red deer (*Cervus elaphus*) population ($I = 7.2$; Pemberton et al. 1992), included for comparison as the taxa most closely related to elk.

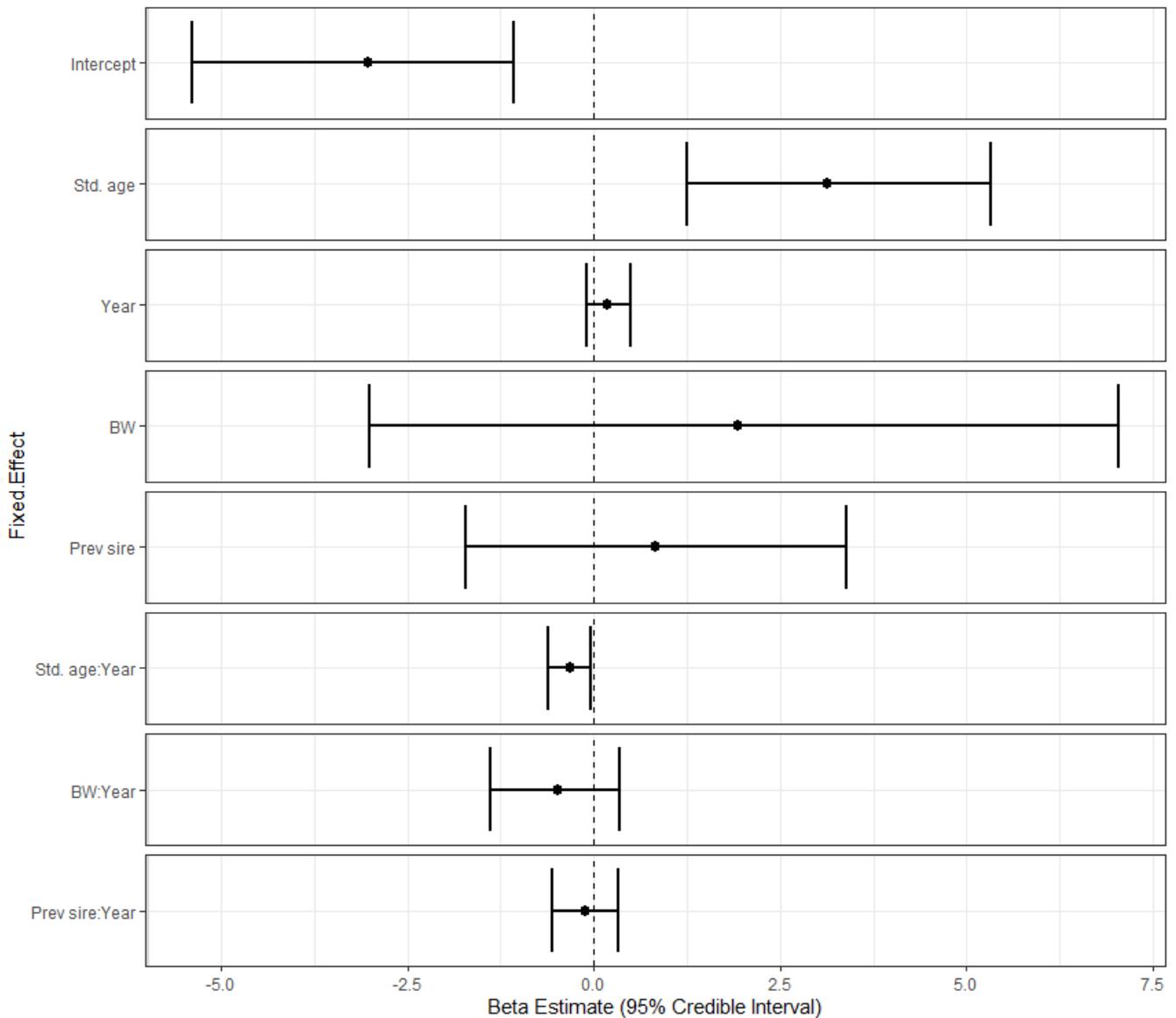


Figure 3-3 Caterpillar plot of β coefficient estimates for Bayesian hierarchical mixed effects model for male elk (*Cervus canadensis*) annual reproductive success (ARS). Model covariates include standardized age (std. age), known diagnosis with brain worm (BW), history of successful sireship (prev. sire), and interactions of each variable with standardized age. Variation around β estimates is represented as 95% credible intervals. Estimates for all fixed and population-level random effects can be found in Table S3-2.

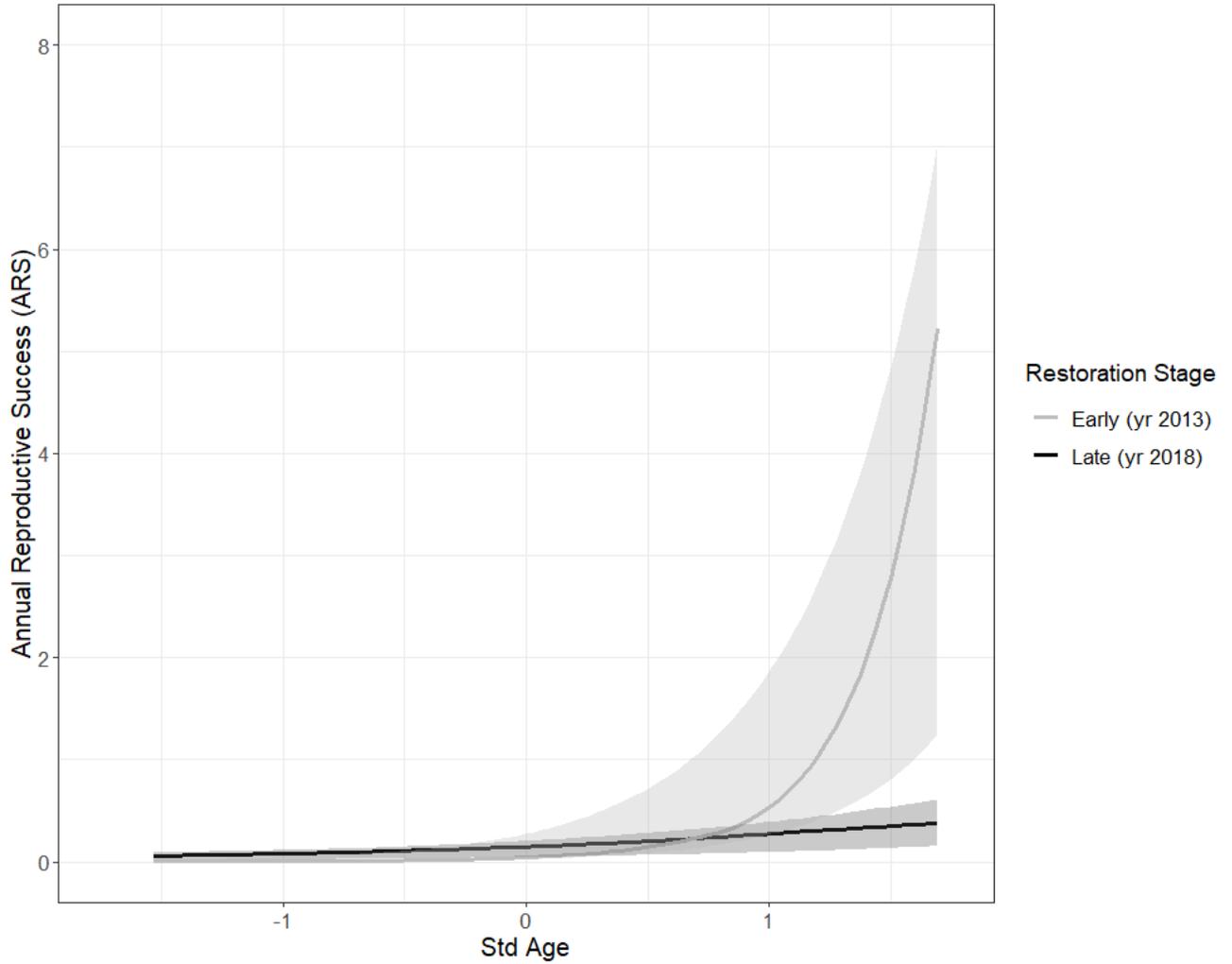


Figure 3-4) Plot representing the significant interaction effect of standardized male age and year of restoration on annual male elk (*Cervus canadensis*) reproductive success (ARS). The light grey line represents the effect of standardized age on ARS early in the restoration (year 2013) while the black line represents the effect of standardized age on ARS late in the restoration (year 2018). Grey shading represents the 95% credible interval.

SUPPLEMENTARY INFORMATION

Table S3-1) Comparison of the number of candidate elk (*Cervus canadensis*) sires genetically sampled across years relative to total minimum male population estimates from the Missouri Department of Conservation (MDC) and total candidate population sizes estimated by assuming sampled individuals account for 75%, 85% and 95% of the total candidate population. The 2013 MDC population count was considered a complete census given the known number of translocated individuals and subsequent, intensive monitoring of survival and births.

Year	Sampled	MDC min est	75% est	85% est	95% est
2013	20	27	27	24	21
2014	27	35	36	32	28
2015	32	44	43	38	34
2016	40	50	53	47	42
2017	41	34	55	48	43
2018	45	40	60	53	47

Table S3-2) Mean posterior estimates and 95% credible intervals for all parameters estimating male elk (*Cervus canadensis*) annual reproductive success (ARS) assuming 75% of the male population was genetically sampled across years. StdAge represents standardized male age, BW represents a binary factor indicating whether a male was known to have brain worm, and PrevSire represent a binary factor indicating whether a male sired a calf in a previous year. Model statement: $ARS \sim \text{Std Age} * \text{Year} + \text{BW} * \text{Year} + \text{PrevSire} * \text{Year} + (\text{Std Age} | \text{ID})$.

Parameter	Estimate	Est. Error	2.50%	97.50%
<i>Population level effects</i>				
Intercept	-3.02	1.10	-5.38	-1.08
StdAge	3.13	1.04	1.26	5.33
Year	0.19	0.15	-0.10	0.50
BW	1.94	2.55	-3.00	7.04
PrevSire	0.83	1.31	-1.72	3.39
StdAge: Year	-0.31	0.14	-0.63	-0.04
BW: Year	-0.48	0.44	-1.38	0.35
PrevSire: Year	-0.12	0.22	-0.56	-0.32
<i>Group level effects</i>				
σ (Intercept)	1.38	0.48	0.55	2.46
σ StdAge (intercept)	1.00	0.56	0.07	2.16
cor Intercept, StdAge	0.17	0.48	-0.76	0.95
<i>Family specific parameters</i>				
ϕ	1.74	0.94	0.60	4.19
z_i	0.12	0.09	0.01	0.33

Table S3-3) Mean posterior estimates and 95% credible intervals for all parameters estimating male elk (*Cervus canadensis*) annual reproductive success (ARS) assuming 85% of the male population was genetically sampled across years. StdAge represents standardized male age, BW represents a binary factor indicating whether a male was known to have brain worm, and PrevSire represent a binary factor indicating whether a male sired a calf in a previous year. Model statement: $ARS \sim \text{Std Age} * \text{Year} + \text{BW} * \text{Year} + \text{PrevSire} * \text{Year} + (\text{Std Age} | \text{ID})$.

Parameter	Estimate	Est. Error	2.50%	97.50%
<i>Population level effects</i>				
Intercept	-2.95	1.12	-5.30	-0.92
StdAge	2.86	1.35	0.39	5.78
Year	0.22	0.16	-0.08	0.54
BW	3.11	2.74	-2.20	8.72
PrevSire	1.35	2.02	-2.71	5.28
StdAge: Year	-0.28	0.19	-0.68	0.08
BW: Year	-0.70	0.48	-1.71	0.19
PrevSire: Year	-0.19	0.32	-0.82	0.44
<i>Group level effects</i>				
σ (Intercept)	1.21	0.46	0.38	2.21
σ StdAge (intercept)	0.89	0.54	0.05	2.05
cor Intercept, StdAge	0.22	0.49	-0.79	0.96
<i>Family specific parameters</i>				
ϕ	1.56	0.89	0.52	3.89
z_i	0.15	0.10	0.01	0.37

Table S3-4) Mean posterior estimates and 95% credible intervals for all parameters estimating male elk (*Cervus canadensis*) annual reproductive success (ARS) assuming 95% of the male population was genetically sampled across years. StdAge represents standardized male age, BW represents a binary factor indicating whether a male was known to have brain worm, and PrevSire represent a binary factor indicating whether a male sired a calf in a previous year. Model statement: $ARS \sim \text{Std Age} * \text{Year} + \text{BW} * \text{Year} + \text{PrevSire} * \text{Year} + (\text{Std Age} | \text{ID})$.

Parameter	Estimate	Est. Error	2.50%	97.50%
<i>Population level effects</i>				
Intercept	-2.60	0.94	-4.53	-0.86
StdAge	2.10	1.08	0.10	4.35
Year	0.23	0.13	-0.03	0.50
BW	0.92	2.32	-3.71	5.42
PrevSire	1.86	1.76	-1.59	5.37
StdAge: Year	-0.19	0.15	-0.50	0.10
BW: Year	-0.26	0.38	-1.01	0.47
PrevSire: Year	-0.29	0.28	-0.85	0.26
<i>Group level effects</i>				
σ (Intercept)	1.00	0.36	0.32	1.75
σ StdAge (intercept)	0.68	0.43	0.03	1.61
cor Intercept, StdAge	0.19	0.49	-0.80	0.96
<i>Family specific parameters</i>				
ϕ	1.55	0.78	0.58	3.54
z_i	0.11	0.09	0.00	0.31

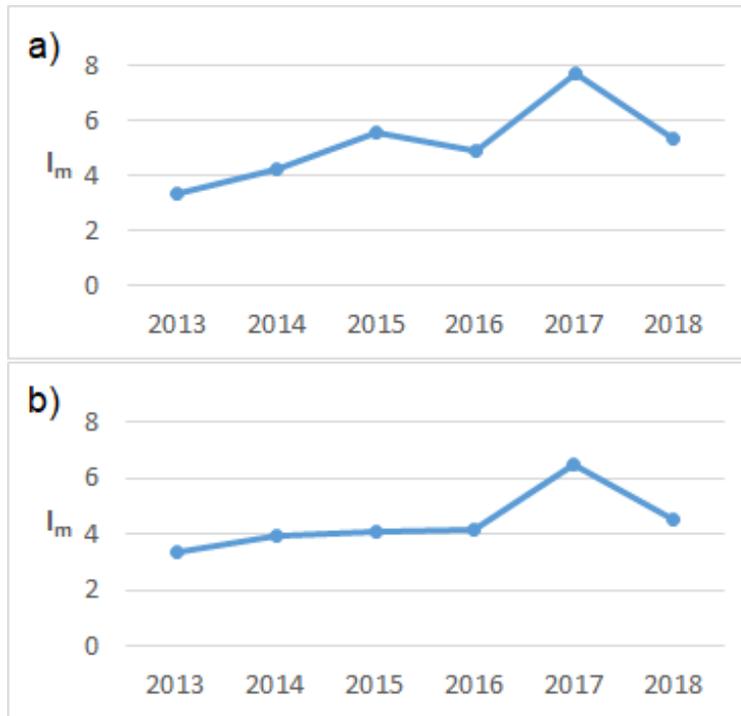


Figure S3-1) Plots of the mean standardized variance in male annual reproductive success (I_m) for the Missouri elk (*Cervus canadensis*) population over the initial years following restoration (2013-2018) assuming **S3-1a)** 85%, and **S3-1b)** 95% of the male population was genetically sampled across years. I_m represents relative level of polygyny

Chapter 4: One size does not fit all: genetic considerations from the Missouri elk restoration⁴

ABSTRACT

Population restoration is an inherently costly conservation practice typically reliant on animal translocations. There are many approaches to translocation and consideration is paid to understanding how various translocation models influence restoration success. Translocation strategies are often designed to meet site-specific objectives, minimize cost, and maximize success. We investigated genetic diversity retention associated with the low-founder, multi-release, single admixed stock translocation model of the Missouri elk (*Cervus canadensis*) restoration in 2011-2013. We further estimated effective population size and projected future losses in genetic diversity if the restored Missouri elk herd is maintained at the population size objective with no immigration from neighboring states. We observed relatively high levels of genetic diversity retention as evidenced by minimal losses in allelic richness and expected heterozygosity. Our projections indicated 90% genetic diversity retention within the Missouri population for roughly 130 years. Where number of progeny or source stocks are limited by resource or disease considerations, use of a relatively low-founder, single admixed source may enable retention of genetic variation, while minimizing costs.

INTRODUCTION

Wildlife translocations are an important conservation tool and are increasingly prevalent in the face of heightened human-induced ecosystem change and biodiversity loss. Estimates of annual restoration-based animal translocation projects in the US alone neared 700 in 1989 (Griffith et al.

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1989), and the number has risen in the 30 years since (Seddon & Armstrong 2016; Resende et al. 2020). While early translocation practice was plagued by failures, the rate of reintroduction success is increasing (Chauvenet et al. 2016). However, translocation remains a costly conservation tool. Total price tags for individual translocation projects may total millions of US dollars across the extent of a project (Weise et al. 2014). Thus, in addition to maximizing project success, cost and resource efficiency is often a stated goal of translocation projects.

Managers must make several decisions when planning and implementing restoration efforts that influence project success and resource needs. Some important considerations for planning translocations include: How many individuals should be translocated? How many recurrent releases are necessary? Should a single source stock or multiple source stocks be used? What should the demographic structure of translocated individuals be? Should wild or captive stocks be used (IUCN 2013; Converse and Armstrong 2016)? These decisions each may be influenced by potential cost or ecological limitations. For example, number of source individuals or stocks may be limited by disease considerations (Germano & Bishop 2009) or by available habitat at the translocation site (Griffith et al. 1989). Each choice ultimately results in a different translocation model with commensurate resource expenditures and ecological implications which may affect project fate. Alongside population demography, translocation decisions that influence population genetics within newly restored, small populations may have important consequences on the success and adaptive potential of a restored population.

Immediately following translocation, a population must have adequate genetic variation to adapt to its new environment, and ultimately achieve an effective population size sufficient to prevent rapid loss of genetic variation and high levels of inbreeding (Hedrick and Miller 1992). Numerous aspects of restoration events (e.g., source population with low genetic diversity, small

founding size, serial population bottlenecks) risk leaving fish and wildlife populations depauperate in genetic diversity (Biebach et al. 2016). Thus, genetic considerations remain of high importance for successful restoration projects. Maintaining sufficient levels of genetic diversity is a primary factor associated with successful restoration efforts, but is often not fully considered. For example, managers have conducted elk (*Cervus canadensis*) translocations for over a century (Popp et al. 2014), but only recently have investigators considered and reported genetic diversity in restored elk populations (though see Polziehn et al. 2000; Williams et al. 2002; Hicks et al. 2007; Conard et al. 2010; Hundertmark and Van Daele 2010).

Elk are an ideal species to consider the genetic response to various translocation models given the variety of strategies employed to restore elk populations across the United States. For example, in eastern North America alone, 25 U.S. states and Canadian provinces together employed many different translocation models. These models range from low founder, single source, single release efforts (e.g., Wisconsin: Anderson et al. 2005) to one of the largest big mammal restorations to date in which over 1500 founding animals were translocated from 6 different sources (Kentucky: Wichrowski et al. 2005). Youngmann et al. (2020) recently reported that the multiple source stocks and high numbers of founders used for this latter large restoration effort contributed to high levels of genetic variation retention, and they encouraged wildlife managers to model future translocations after Kentucky's large-scale elk restoration effort. However, before reaching consensus on an optimal taxa-specific translocation model for restoring elk populations, we seek to evaluate similar genetic metrics among alternative translocation tactics.

The Missouri elk restoration relied on a translocation model of moderate scale. Missouri capitalized on the single highly admixed population that resulted from the Kentucky elk

restoration effort but used 1/15th the number of founding individuals as the Kentucky restoration. While multiple source stocks and high founding numbers may lead to retention of genetic variation and restoration success (Youngmann et al. 2020), we hypothesized that high levels of genetic variation can be retained through use of a single, highly genetically admixed source stock and modest founding numbers (> 60 individuals [Tracy et al. 2011; Groombridge et al. 2012; Biebach et al. 2016]). Further, we assessed whether future genetic management of the restored elk population was warranted. We estimated effective population size and predicted losses in genetic diversity if the Missouri population is managed at an agency-established target population size in isolation from other states, as is common in the eastern U.S.

METHODS

Study Area

From 2011-2013, 108 elk were translocated to the southeastern Missouri Ozarks (91°24' to 90°58'W and 37°0' to 37°19'N; Bleisch et al. 2017). The Missouri elk range is separated from the nearest neighboring restored elk population in Arkansas by approximately 250 mi and managed in isolation (Dent et al. 2012). Elk were captured in Kentucky during January and subsequently held in quarantined corral facilities for 102-129 days before overnight trailer transport to MO. Upon arrival in MO, elk were held for an additional 19-34 days of quarantine in holding pens at Peck Ranch Conservation Area before release.

Animal Translocations and Sample Collection

Prior to releases, all elk were fitted with GPS-VHF collars (RASSL custom 3D cell collar, North Star Science and Technology, King George, VA, or G2110E Iridium-GPS series model, Advanced Telemetry Systems, Isanti, MN, USA), PIT-tagged, and ear-tagged. The demographic composition of cohorts was: 2011 (n = 34) – 15 adult females [2+ years], 5 yearling females, 6

two-year-old males, 8 yearling males; 2012 (n = 33) – 22 adult females, 3 yearling females, 4 two-year-old males, 4 yearling males; 2013 (n = 39) – 20 adult females, 16 yearling females, 3 yearling males.

We acquired ear notches for tissue-based genetic microsatellite analyses from all translocated individuals from 2011-2013 at the time of translocation (total genetically sampled from translocation efforts: n = 105). We also collected tissue samples for genetic analysis from individuals born on the Missouri landscape during all neonate, yearling and adult capture efforts from 2011-2018 (n = 131). We placed tissue samples in 100% ethanol and froze them at -20° C until extraction.

Genotyping and Genetic Analyses

We extracted DNA from tissue samples using DNeasy Blood and Tissue kits (Qiagen Inc., Valencia, CA, U.S.A.) according to manufacturer instructions. We conducted DNA extraction, amplification, and genotyping at 16 microsatellite loci in 3 multiplexes: (1) BM888, BL42, BM5004, ETH152, BMC1009; (2) BM4107, BM203, BM1225, BM4208, BM4513; (3) C01, C229, T193, T510, T26, and T156 [Kossarek et al. 1993; Bishop et al. 1994; Talbot et al. 1996; Jones et al. 2002; Meredith et al. 2005]) chosen for high degrees of polymorphism in elk. Multiplex reactions consisted of 8 µL with 0.48 µL PCR water, 3.9 µL Platinum Multiplex PCR Master Mix (Applied Biosystems), 1.1 µL 2 µM multiplex primer mix, 0.6 µL BSA (Ambion), 0.9 µL GC enhancer (Applied Biosystems), and 1 µL DNA. DNA concentration was not measured for every sample but extraction procedures typically >10 ng/uL of genomic DNA. All multiplexes were run under the following thermocycler conditions: pre-denaturation at 95°C for 15 minutes; 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1.5 minutes, and extension at 72°C for 1 minute; and final extension at 60°C for 30 minutes. PCR products

were then submitted to the University of Missouri DNA Core Facility (Columbia, MO) for fragment analysis on an ABI 3730xl DNA Analyzer with LIZ 600 GeneScan Marker (ThermoFisher). Microsatellite panels were repeated for 104 individuals to amplify single loci that did not amplify in the first PCR attempt. We used genotypes from these repeated attempts to calculate error rates with Gimlet (Valière 2002). To produce an accurate consensus genotype, PCR was repeated at least 1 more time in the case that genotypes from 2 PCRs mismatched.

We tested for deviations from Hardy–Weinberg proportions and linkage disequilibrium using the R package “pegas” (Paradis 2010). We applied a sequential Bonferroni correction for multiple comparisons at an alpha of 0.05 to correct for inflated type I error rates due to multiple testing (Rice 1989). We calculated microsatellite genetic diversity metrics (allelic richness [AR] and expected heterozygosity [H_e]) for two groups: (1) the entire cohort of translocated individuals and (2) the 2018 population. We defined the 2018 population as including any individual alive during any portion of 2018 reproductive year (1 Sept. 2017 – 1 Sept. 2018), which included 2018 calves and translocated elk still on the Missouri landscape.

We used the R package “hierfstat” (Goudet 2005) to calculate AR and the package “strataG” (Archer et al. 2017) to calculate heterozygosity. We regarded the translocated group as a censused population, but to account for uncertainty in our estimates of population level heterozygosity within the 2018 sampled group, we bootstrapped across individuals to create 1000 resampled datasets and report the 95% confidence intervals associated with H_o and H_e estimates. All analyses were conducted in R version 4.0.4.

Predicted Genetic Diversity Loss

We employed the recursive equation (Wright 1969):

$$h_{t+1} = \left(1 - \frac{1}{2N_{e(future)}}\right) h_t \quad [\text{Eq. 4-1}]$$

to project future losses in heterozygosity if the Missouri elk population is managed at or near a future N_c size of 400, which represents the conservative, low end of a 400-500 population target set by the Missouri Department of Conservation (Dent et al. 2012). We used our mean estimate of H_e for the 2018 population to represent starting heterozygosity (h_t) in the first generation of our future projections ($t = 1$), and the equation was reiterated for 200 years (~ 52 generations). We derived future effective population size ($N_{e(future)}$) from an $N_{e(past)}/N_c$ ratio that we calculated over the years 2013-2018. We calculated average $N_{e(past)}$ for the years 2013-2018 using an extension of Eq. 4-1 over multiple generations and solving for N_e (Hedrick 2011):

$$N_{e(past)} = \frac{1}{2(1 - e^{(\ln(H_t/H_0))/t})} \quad [\text{Eq. 4-2}]$$

where H_0 is the H_e within the translocated population, H_t is the H_e in 2018, and t is the number of elapsed generations. We used the average male breeder age in the Missouri elk population in 2013-2018 (3.3 years) as an estimate of generation length, resulting in an estimated 1.51 elapsed generations. We calculated average N_c over the same time period using the harmonic mean of estimated population size in 2013 (i.e., 105, when nearly all elk were marked and monitored) and 2018 (i.e., 170, when ~70% of the population was marked and regularly monitored leading to reasonably high confidence in population estimates [A. Hildreth, personal communication, Missouri Department of Conservation]). We used this average 2013-2018 N_e/N_c ratio to derive future N_e for when the population is held at a N_c of 400 in Eq. 4-1. To incorporate uncertainty in our estimates of future heterozygosity loss, we repeated this analysis using the upper and lower 95% confidence interval of our estimated H_e in 2018.

We explored uncertainty in N_e estimation using multiple alternative N_e estimators; however, we observed no meaningful differences in future N_e that would influence interpretation or implications stemming from projections of future N_e and predicted heterozygosity loss (Table S4-1, available online in Supporting Information). In addition to estimating our N_e/N_c ratio using the N_e estimate we calculated over the years 2013-2018, we also explored the effect of projecting future N_e by using alternative N_e/N_c ratios reported in the literature for red deer (*Cervus elaphus*) and elk (Reed et al. 1986; Glenn 1990; Waples et al. 2013). We likewise observed no meaningful difference in interpretation or management implications relative to the N_e/N_c ratios we employed, estimated future N_e , and predicted heterozygosity loss (Table S4-2, available online in Supporting Information).

We calculated predicted heterozygosity loss per generation, as well as the number of generations and years until 10% of heterozygosity is lost, assuming no migrants from surrounding populations and no mutation. We converted between estimated generations and years to 10% heterozygosity loss by multiplying estimated elk generations by the 2018 elk generation length (2018 average male breeding age = 3.9 years). Reported elk and red deer generation lengths for established populations are typically longer (Reed et al. 1986; Hard et al. 2006; Conard et al. 2010; Hundertmark and Van Daele 2010) and we do expect the restored Missouri elk generation length to continue increasing while the population age structure continues to advance. However, we chose to use the shorter generation length from the 2018 population for a more conservative estimate of future H_e loss.

RESULTS

We successfully genotyped 236 individuals from the restored Missouri elk population at 16 microsatellite loci. We observed a 92.8% typing rate across loci with an average allelic dropout

rate of 0.017 (range: 0-0.046) and average false allele rate of 0.003 (0-0.029). All loci were polymorphic with an average of 6.9 alleles per locus (SE = 0.755). Following sequential Bonferroni correction (Rice 1989), we identified three loci (C01, C229, and T193) that significantly deviated from Hardy-Weinberg proportions and removed them from our analyses. We found no evidence of significant linkage disequilibrium among loci. We genotyped 105 released individuals in the translocated population and 186 individuals in the 2018 population (131 individuals born on the Missouri landscape and 55 of the translocated individuals that were also alive and present in the 2018 calving season).

Allelic richness and expected heterozygosity were similar between the translocated and 2018 population. H_e declined from 0.65 in 2013 to 0.64 (95% CI: 0.63 to 0.65) in 2018, but the confidence interval in 2018 overlapped the 2013 census value (Table 4-1). AR slightly decreased from 6.39 in 2013 to 5.83 in 2018 (Table 4-1).

The average N_e/N_c ratio from 2013-2018 was 0.41 (95% CI: 0.23 to 1.03) with an average N_e of 53 (95% CI: 30 to 133; Table 4-1) and an N_c harmonic mean of 129.82. Using this ratio and the future target N_c size of 400, we estimated future N_e in the Missouri elk population to be 164 (95% CI: 92 to 411; Table 4-1). We estimated a loss of 0.002 h_t per generation (95% CI: 0.001 to 0.003; Table 4-1), and thus estimate it will take 34 generations (95% CI: 19 to 86), or 134 years (95% CI: 75 to 338), to realize a 10% heterozygosity loss in the restored Missouri elk population (Figure 4-1).

DISCUSSION

Translocation models for eastern elk restoration vary substantially in their scale and resource costs consistent with targeted population size and objectives. While past large-scale restoration efforts are associated with large and genetically admixed populations, future feasibility of high

founder and multi-source translocation models, particularly for cervids, may be limited by a variety of factors. We thus investigated genetic variation retention as a factor associated with restoration success within a moderately scaled restoration effort involving modest founder numbers (~100 animals) and a single, genetically diverse source stock. We observed minimal loss in genetic variation within a restored elk population over the initial years following translocation to the Missouri Ozarks, and predicted limited future losses in genetic variation over a management-relevant time period.

Ultimate restored population size objectives are state or region specific and dictated by numerous factors ranging from funding, human tolerance, habitat availability, sufficient stock sources, and more. The largest eastern elk restoration effort to date relied on high propagule pressure and a multi-source model to restore an elk population now managed at an estimated size of 14,000 individuals spanning an area of approximately 4.1 million acres in Kentucky (Kentucky Department of Fish and Wildlife Resources 2019). However, beyond limitations posed by high costs and geospatial constraints for restorations of this scale, disease considerations are of growing concern and may pose constraints to ongoing and future cervid restoration efforts. Diseases like brucellosis, tuberculosis, meningeal worm, and chronic wasting disease (CWD) are particularly problematic for managed cervid translocations in the eastern U.S. For example, limiting translocation of cervids is a primary recommendation to limit the spread of CWD (Gillin and Mawdsley 2018). Additionally, the geographic variation of common disease agents is only beginning to emerge (Eggert et al. 2021) suggesting unknown consequences of mixing sources. Such concerns may thus limit the feasibility of employing a high founder, multi-source translocation model for cervid restoration. Disease concerns limiting the scope and scale of translocation efforts are not unique to cervids (Muths and McCallum 2016). For example,

populations of amphibians are declining worldwide, in part due to the chytrid fungus *Batachochytrium dendrobatidis* [Bd]. However, translocation efforts to restore amphibian species decimated by Bd are both limited and hampered by its persistence on the landscape (Germano and Bishop 2009). In such cases where disease concerns or resource limitations restrict number of source stocks and/or founding individuals, it is especially important that retention of high levels of genetic variation is considered and prioritized.

Relative to translocation models of much greater scale, the Missouri translocation model shares important commonalities for maximizing retention of genetic variation. For example, although the larger-scale Kentucky model used about 15x the number of founders as Missouri, the number of founding individuals in both efforts surpassed generalized effective population size recommendations of founder stock size (e.g., 20-40 individuals [Griffith et al. 1989]; >100 individuals [Fischer and Lindenmayer 2000]; >60 individuals [Biebach et al. 2016]). Notably, Conard et al. (2010) found little support for the effect of founding size on genetic variation across 12 elk restoration events within the United States and instead suggested initial positive population growth might be one of the most important factors facilitating retention of genetic diversity in elk, a conclusion supported by theoretical population genetics (Fisher 1930, Kimura and Ohta 1974). Indeed, modern elk translocation efforts, including Missouri (Gitzen et al. 2016), employ serial annual releases into high-forage areas to bolster population increases in initial years and to facilitate early population growth.

Beyond initial propagule pressure and subsequent population growth, additional aspects of translocation models can facilitate retention of high levels of genetic variation within moderately-scaled restoration efforts. For example, a diverse gene pool within founding individuals is widely recommended (Biebach et al. 2016). The Missouri elk restoration used the

genetically diverse, recently admixed restored population of Kentucky as its sole source stock. While multi-source models are often recommended to facilitate high levels of genetic diversity (Tracy et al. 2011; Keller et al. 2012), the intensive multi-source translocation efforts undertaken by Kentucky made available a large and highly admixed population that may be used by future single-source translocation efforts more limited in scale and circumstance (Youngmann et al. 2020). An additional aspect of translocation that may be conducive to high retention of genetic diversity is the age composition of translocation cohorts. Male demographic compositions of modern elk translocation efforts are biased towards young-aged males with few or no mature bulls comprising the founding populations (Larkin et al. 2001). This lack of mature bulls may be associated with dampened polygyny and reduced variation in initial male reproductive success. More equality in reproductive success can increase effective population size of the founding population and facilitate the observed retention of genetic variation in the small, restored population during the crucial acclimation period (Wright 1938; Crow and Kimura 1970).

While we observed relatively high levels of retained genetic diversity in the Missouri elk population, without future migration into the population, loss of standing genetic diversity within relatively small populations (i.e., $N_e < 500$) is expected (Wright 1969). Because many eastern elk populations are intentionally managed to restrict immigration between states (Larkin et al. 2001), consideration of future loss in genetic diversity is crucial. However, future genetic management remains a little considered aspect of eastern elk restoration and management. Genetic management to facilitate success in ungulate restoration within North America is not without precedent. Following near extinction and decades of persistence on the landscape within restored, isolated populations, the US National Park Service recently released a proposed coordinated metapopulation strategy for North American plains bison (*Bison bison bison*)

management explicitly considering genetic diversity retention (to include targeted translocations of select individuals between herds; Hartway et al. 2020).

Using a derived N_e/N_c ratio from the initial phase of the Missouri elk restoration and absent future migration, we estimated annual long-term loss in heterozygosity to be low in the Missouri elk, with the majority of H_e likely to be maintained over the next two centuries of conservation-relevant management. We strove to produce a conservative estimate of future N_e and projected loss of heterozygosity in the restored Missouri elk population. We used a generation length estimated from average male breeder age in 2018, however we expect the generation length in the Missouri population to increase to levels observed in more established populations as the age-structure in the restored population continues to advance (Reed et al. 1986; Hard et al. 2006; Conard et al. 2010; Hundertmark et al. 2010). For a given generational N_e , a longer generation interval will slow the rate of loss of genetic variation as measured in years, and thus we expect it will take more than 134 years to realize a 10% loss in genetic variation. Conversely, expected increases in variation in male reproductive success would decrease N_e (Wright 1938; Crow and Kimura 1970), and increase the rate of loss of genetic variation. We would thus not expect decreases in our future N_e estimates relative to potential increases in reproductive variance.

Although our estimated future N_e value is well above suggested N_e of 50 for short term persistence, the estimated future N_e is under the suggested 500 N_e necessary for long-term maintenance of genetic variation (“50/500 rule”: Franklin 1980; Soulé 1980; Allendorf et al. 2013; Franklin et al. 2014). Further, because of uncertainty in our projections, periodic genetic monitoring (~10 years) should estimate H_e and N_e to ensure they remain at a healthy level. In cases where populations fail to grow or are maintained at N_e significantly smaller than the long-

term target of ~ 500, additional genetic management actions such as increased connectivity to other population or additional, targeted translocations might be warranted to reduce the effects of inbreeding (Whiteley et al. 2015, Bell et al. 2019) and maintain long-term (i.e., 200+ years) adaptive potential.

In light of numerous recently restored elk populations across North America, and expanding translocation efforts across taxa more generally, applied attention to genetic considerations of restored populations is timely, relevant, and instrumental for future translocation efforts. The additional perspective gained from the Missouri elk restoration alongside findings from alternative translocation models within the same taxa is important: one translocation model does not fit all population-specific circumstances and objectives. Similar to findings from large-scale, multi-release, multi-source translocation models (Youngman et al. 2020), we observed high levels of genetic diversity retention from Missouri's small-scale, multi-release, single-source translocation model. Although we estimated future effective population size as roughly half that of future census population size, we projected the retention of at least 90% expected heterozygosity over the next 130 years in the restored Missouri elk population. Because multiple-source translocation models could be discouraged for cervid restoration efforts in light of disease concerns, it is particularly encouraging that we identified similar levels of genetic diversity retention within the single-source translocation model adopted by the Missouri elk restoration.

We affirm the importance of genetic considerations when translocation is used to restore populations. We recommend pursuing an objective-based translocation model that both facilitates retention of genetic diversity and minimizes cost while meeting demographic targets. Successful restoration efforts with high genetic variation retention over management-relevant

time periods may be achieved without relying on multiple source stocks if available source stock are sufficiently numerous and/or genetically diverse and initial population growth is promoted. Managers may thus be better positioned to choose a genetically-informed cost- and time-efficient translocation model that meets their objectives under a variety of circumstances and practical limitations. In the case of future and on-going cervid restorations where live animal transport and number of source stocks are limited by disease considerations, use of a single-source, highly admixed translocation similar to the Missouri elk restoration would minimize costs and disease implications without sacrificing retention of genetic variation over a management-relevant time scale.

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TABLES

Table 4-1) Summary methods and results for reported genetic metrics using 13 microsatellite loci for the restored Missouri elk (*Cervus canadensis*) population that was translocated from Kentucky over the years 2011-2013. Timepoints include the end of translocations (2013), recent (2018), and future (when the population reaches the minimum end of the Missouri Department of Conservation’s population objective of 400). The translocated population in 2013 was treated as a complete census, while 95% confidence intervals associated with H_e in 2018, past N_e from 2013-2018, future N_e , and H_e loss result from incorporating uncertainty in the 2018 H_e estimate via individual bootstrapping to create 1000 resampled datasets.

Metric	Timepoint	Method	n	Result
H_e	2013	‘heterozygosity’ via "strataG" (Archer et al. 2017)	105 (census)	0.65
H_e	2018	‘heterozygosity’ via “strataG” (Archer et al. 2017) + individual bootstrap	186	0.64 (95% CI: 0.63-0.65)
AR	2013	‘allelic.richness’ via "hierfstat" (Goudet 2005)	105 (census)	6.39
AR	2018	‘allelic.richness’ via "hierfstat" (Goudet 2005)	186	5.83
N_c	Past (2013-2018)	Harmonic mean of 2013 census and 2018 population estimate	N/A	129.8
N_c	Future	Minimum agency population objective	N/A	400
N_e	Past (2013-2018)	Eq. 2 in text	N/A	53 (95% CI: 30-133)
N_e	Future	$N_{e(future)} = \frac{N_{e(past)}}{N_{c(past)}} * N_{c(future)}$	N/A	164 (95% CI: 92-411)
H_e loss /generation	Future	Eq. 1 in text	N/A	0.002 (95% CI: 0.001-0.003)

FIGURES

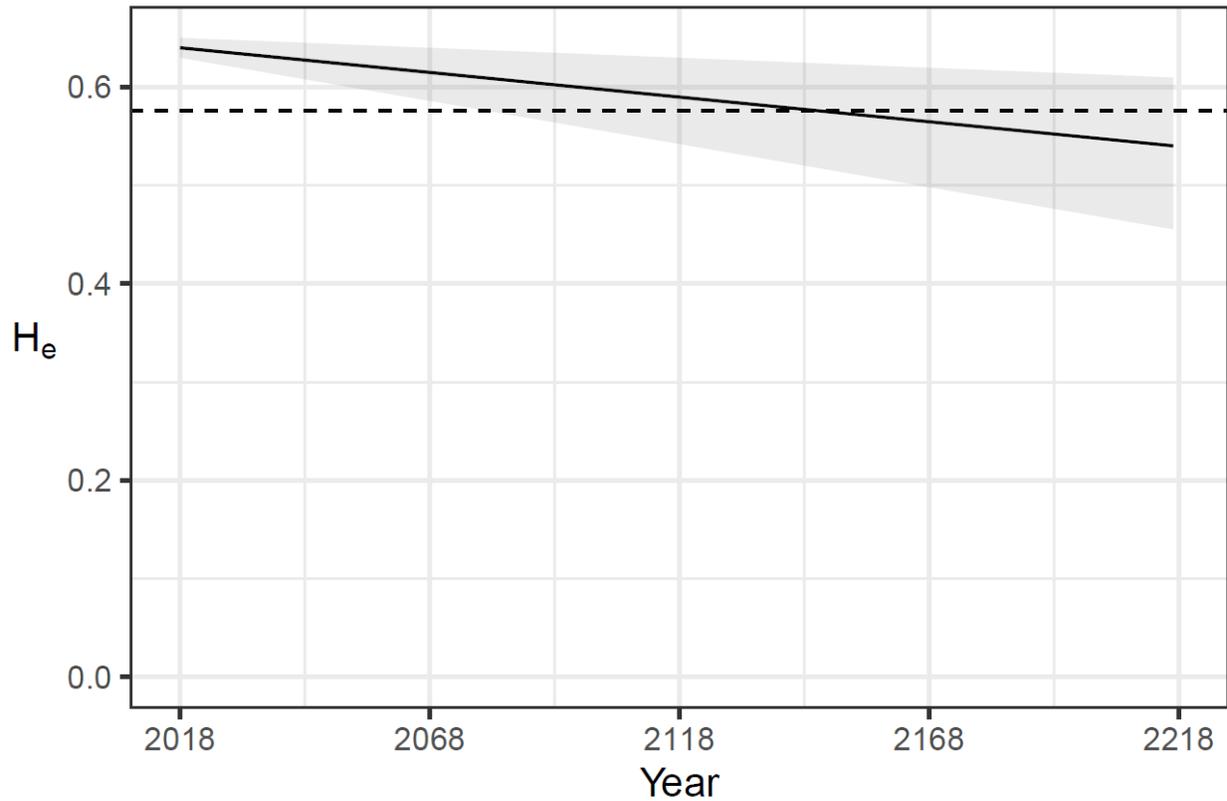


Figure 4-1) Predicted heterozygosity (H_e) and associated 95% confidence interval (represented by gray shading) for the restored Missouri elk (*Cervus canadensis*) population over the next two centuries (2018-2218). The uncertainty depicted by the confidence interval reflects the direct input of the uncertainty from 95% CI of bootstrapped H_e estimates into both our calculation of average N_e over the years 2013-2018 and into uncertainty in starting H_e in the recursive equation for heterozygosity loss (Wright 1969; Eq. 4-1 in text). The horizontal dotted line indicates a threshold of 10% loss in heterozygosity.

SUPPLEMENTARY INFORMATION

Estimating effective population size in the reintroduced Missouri elk population

The translocated Missouri elk population poses significant challenges when estimating effective population size with genetic methods. A consistent and difficult problem with this data set is the admixed nature of this population. For example, we interpret the positive F_{is} that is present in the initial sample of translocated individuals (2013; $F_{is} = 0.013$) as a Wahlund effect that is likely generated by the diverse stocking of the Kentucky source population, spatial substructure within the Kentucky population, or both. In support of the Wahlund effect is the negative F_{is} following *in situ* reproduction in Missouri (2018; $F_{is} = -0.00768$), which is consistent with the mating of genetically divergent individuals generating heterozygote-excess. This intuitively causes problems for the heterozygote-excess method, which relies on the Robertson Effect occurring in a small, randomly mating population (Robertson 1965). Given there was a Wahlund effect occurring in the sample of translocated individuals (2013), there should be upward bias using the heterozygous-excess method estimate. Conversely, the second sample (2018) should be downwardly biased using the heterozygous-excess method. In general, effective use of the heterozygote-excess method requires very small, randomly mating populations (Pudovkin et al. 1996).

The linkage disequilibrium (LD) method is the most widely used and tested single sample estimator of effective population size. Spatial substructure downwardly biases estimates of effective population size when using the LD method (Waples & England 2011). Thus, we expect that estimates from both time periods (2013 and 2018) would be downwardly biased due to linkage disequilibrium generated by admixture and Wahlund effects. In addition to the effects of spatial substructure, both single-sample estimators assume nonoverlapping generations. The effect of iteroparity and overlapping-generations on single, mixed-age samples generally biases estimates low due to mixture linkage disequilibrium occurring between age-classes (Waples et al. 2014). Another important caveat is that the sample of translocated individuals is estimating the effective size in the Kentucky population and does not represent the evolutionary changes that will likely occur due to habitat size and life-history shifts in Missouri. Fortunately, all the sources of bias we have identified should downwardly bias the estimate of effective population size. Both single sample methods, linkage disequilibrium and heterozygote-excess, were estimated in NeEstimator V2.1 (Do et al. 2014) (Table S4-1). For the LD method, we assumed a random mating system and used a critical allele frequency cutoff of 0.02 (Waples & Do 2010).

Estimates of effective size based on genetic changes overtime are also influenced by spatial substructure and admixture (e.g., Araki et al. 2007). For example, the classical estimate of effective size based on heterozygosity loss (Wright 1931) is intuitively negatively affected by the 9 out of 13 loci that increased in observed heterozygosity over the sampling period (i.e. result of admixture; evidenced by negative F_{is}). We therefore used loss in heterozygosity averaged across loci to generate a coarse estimate effective population size, and confidence intervals were generated by bootstrapping across individuals (Table S4-1). We calculated average N_e for the years 2013-2018 using with an extension of Eq. 4-1 (main text) over multiple generations and solving for N_e (Hedrick 2011):

$$N_e = \frac{1}{2(1 - e^{(\ln(H_t/H_0))/t})} \quad \text{Eq. 4-2 (main text)}$$

where H_0 is the H_e in 2013, H_t is the H_e in 2018, and t is the number of elapsed generations. We used the average male breeder age in the Missouri elk population over the years 2013 through 2018 (3.3 years) as an estimate of generation length.

We also employed the temporal method in NeEstimator V2.1 with F -statistics calculated according Jorde and Ryman 1995 (Table S4-1). This method estimates effective size based upon change in allele frequency over 1.51 generations in Missouri. As with all methods discussed, the temporal method also assumes semelparity and non-overlapping generations and violation of this assumption can bias results significantly (Luikart et al. 2010). An important distinction between the heterozygosity-loss method and the temporal method is that they calculate inbreeding and variance effective size, respectively. In a rapidly expanding population, we expect very large, potentially infinite, estimates of temporal effective size, while we expect estimates of heterozygosity loss to be smaller because it is more dependent on the number of contributing parents rather than the number of progeny (Harris & Allendorf 1989). We indeed observe this pattern, where we obtain a negative estimate of effective size with the temporal method (interpreted as infinite; Waples and Do 2010) and an estimate of 53 with the heterozygosity loss method. It is also worth noting that 1.51 generations is an exceptionally short sampling interval for the temporal method, and bias is greatly reduced when sampling spans five or more generations (Waples and Yokota 2007). It is likely that the short sampling interval paired with the admixed and expanding nature of population contributed to an infinite estimate of temporal effective size. As the Missouri elk population stabilizes, variance and inbreeding effective size will begin to converge. Ultimately, the long-term genetic and detailed demographic information that is currently being collected for this population will enable bias to be minimized and a quality estimate of effective size to be obtained.

Although assumptions were violated for all estimation methods, taken together these estimates suggest that the effective size of the Missouri elk population is likely greater than 50. The heterozygote excess method had a single estimate below 50, which we expect to be biased low due to heterozygote-excess generated through admixture (Table S4-1). In the main text, we chose to use the classical loss of heterozygosity estimate for simplicity, its similarity with one sample estimators (biased low), and for it producing finite estimates when using both sampling periods. An effective size of this magnitude ($N_e > 50$) in the infancy of a species reintroduction bodes well for restored Missouri elk population in the conservation-relevant near-term. Genetic monitoring and estimating effective population size after several generations of reproduction in Missouri will permit a higher resolution description of the evolutionary trajectory of Missouri elk.

As a final measure, we compared projections for future N_e and heterozygosity loss stemming from our calculation of average N_e over the years 2013-2018 in the main text to projections reached by employing N_e/N_c ratios reported in the literature for elk and red deer. We compared to two demographic-based N_e/N_c ratios from two elk populations ($N_e/N_c = 0.23$: Reed et al. 1986; $N_e/N_c = 0.41$: Glenn 1990) and one genetic-based ratio from a red deer population ($N_e/N_c = 0.926$: Waples et al. 2013) to capture variation in reported N_e/N_c ratios across estimation methods and populations. We compared future N_e estimates from these three ratios independently, and together as a composite average ± 1 standard error (SE) (Table S4-2). We determined that our projection of future N_e based on our calculation of average N_e over the years 2013-2018 fell well within the bounds of projections based on literature-reported N_e/N_c ratios while still demonstrating the uncertainty inherent to these projections.

Table S4-1) Estimates of effective population size using the linkage disequilibrium, heterozygote-excess, loss of heterozygosity, and temporal methods using 13 microsatellite loci (described in the main text). We denote whether the parameter estimated is variance effective size (N_{ev}) or inbreeding effective size (N_{ei}). We present estimates for both sample periods when using single sample estimators. The negative point estimate in the temporal method indicates that allele frequency variation can be explained by sampling variance, which is generally interpreted as an infinite estimate (Waples & Do 2010).

Estimation Method	Program	Parameter estimated	Year(s)	\hat{N}_e	Confidence Interval
Linkage Disequilibrium	NeEstimator v2.1	N_{ei}	2013	123.7	86.7 – 198.3
			2018	55.6	47.5 – 65.4
Heterozygote Excess	NeEstimator v2.1	N_{ei}	2013	53.0	50.1 – Infinite
			2018	47.0	18.3 – Infinite
Heterozygosity Loss	According to Wright 1930	N_{ei}	2013-2018	53	30 – 133
Temporal Method	NeEstimator v2.1	N_{ev}	2013-2018	-660.3	106.2 – Infinite

Table S4-2) Estimates of future effective population size using calculated (this study) and literature reported N_e/N_c ratios. We report future N_e estimates from independent literature-reported N_e/N_c ratios, as well as from the average (+/- 1 SE) from all 3 studies. The uncertainty in the confidence interval for this study stems from directly inputting the initial variation derived from bootstrapped H_e estimates into our calculation of average N_e 2013-2018.

N_e/N_c ratios	Source	Future \hat{N}_e
0.23	Reed et al. 1986	92
0.41	Glenn 1990	164
0.926	Waples et al. 2013	370.4
0.52 (0.31-0.73)	Average lit. sources: Reed et al.1986, Glenn 1990, Waples et al. 2013	208.8 (125.2 – 292.4)
0.54 (0.32 – 1.23)	This study	163.6 (92 – 411.09)

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