Assessing Population Demographics and Genetics Following Reintroduction of Amphibians

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Abstract

To resist the modern global decline of biodiversity, proactive conservation efforts are crucial to bolster species persistence before local extinctions become inevitable. Such extirpations of species have become increasingly common in fragmented landscapes resulting from land-use change. Amphibians are especially threatened by this biodiversity crisis as they are particularly sensitive to environmental change. Reintroduction by translocation is one method for re-establishing populations, although there has been limited research of such conservation efforts. To address this knowledge gap, we assessed two reintroduced populations of wood frogs (*Lithobates sylvaticus*) within isolated habitat patches that have been monitored annually for over a decade. The populations followed vastly different trajectories, with one (Sharon Woods Metro Park) increasing dramatically in population size and the other (Blacklick Woods Metro Park) declining to a consistently small breeding population. Our results indicate that the reintroduced populations have higher embryo non-viability rates, are larger on average as adults, and tend to produce larger egg masses when compared to their source population (Clear Creek Metro Park). These trends were especially prominent at Blacklick Woods, the site of the lowest breeding population size. Yet despite the population bottlenecks resulting from reintroduction, neither of the reintroduced populations shows a significant reduction in genetic diversity or high levels of differentiation. Results from this study suggest that demographic histories of reintroduced populations can directly impact important life history traits, potentially affecting their persistence. However, concerns of loss of genetic diversity may not be as pertinent

at the scale of this study. Future translocation attempts may benefit from additional supplementation over time to support the newly established population, but lack of severe genetic consequences is promising for the future of reintroduced populations.

Introduction

The most significant driver of current global extinction events is anthropogenic land-use conversion resulting in habitat loss and fragmentation (Pimm & Reven, 2000; Pimm et al., 2014; Foley et al., 2005). Fragmentation can create isolated habitat patches surrounded by a matrix of low-suitability landscape that serves as a barrier to dispersal – this essentially transforms habitat patches into islands for the species inhabiting them (Haila, 2002). Increased isolation and decreased patch area escalate the likelihood of local extinction (Fahrig, 2002). Moreover, increased isolation reduces the ability of species with low vagility to successfully disperse to other patches, reducing the possibility for potential recolonization or rescue (Brown & Kodric-Brown, 1977), and decreasing habitat area results in a lower population size (Bender et al., 1998). Small population size and barriers to dispersal increase the threat of descent into an extinction vortex in which the reinforcement among biotic and abiotic factors drives populations in a downward spiral towards extinction (Reed, 2004; Blomqvist et al., 2010; Gilpin & Soulé, 1986). As landscapes become increasingly fragmented, conservationists are faced with the challenge of preserving populations within patches to ensure longevity (Wilcove et al., 1986).

Fragmentation can also impact population health by disrupting gene flow between adjacent populations and sub-populations. Gene flow may increase a population's genetic diversity by contributing novel alleles to a population's gene pool as well as preventing genetic drift and high levels of inbreeding (Schlaepfer et al., 2018). Thus, disruption to gene flow and the

45 associated negative effects on population genetics is of concern for many taxa of conservation
46 concern (Spielman et al., 2004).

47 An associated conservation issue is inbreeding in small, isolated populations, as it
48 increases homozygosity in the offspring. This may be problematic when heterozygotes are more
49 fit than both homozygous types, and/or when deleterious recessive alleles are exposed in
50 homozygous recessive individuals (Edmands, 2007). The fitness consequences of high
51 homozygosity under either of these hypotheses suggests that highly inbred populations may face
52 a higher risk of extinction.

53 Reduced genetic diversity due to restricted gene flow can furthermore decrease a
54 population's ability to adapt to environmental change or resist novel diseases, as adaptation is
55 dependent on standing genetic variation (Reed & Frankham, 2003). Adaptive variation may be
56 crucial for populations facing habitat fragmentation as they may be forced into new or altered
57 habitats with altered species assemblages (Jump et al., 2009). Thus, preserving genetic diversity
58 within and between populations is a highly relevant goal for species conservation (Engelhardt et
59 al., 2014).

60 The trajectory of small wild populations may be especially influenced by the stochastic
61 effects of both inbreeding and genetic drift (Keller & Waller, 2002). Small population size
62 necessitates a higher degree of relatedness between interbreeding individuals, and genetic drift
63 has a more extreme effect on small populations (Kimura, 1995). Therefore, small populations
64 may be especially at risk of extinction as the demographic stochasticity associated with small
65 population size is compounded by greater threats of reduced fitness from genetic factors
66 (Spielman et al., 2004).

Amphibians are the most at-risk taxa in the global biodiversity crisis due to a plethora of reasons, including life history and dispersal traits that make them less tolerant to habitat degradation (Stuart et al., 2004). Amphibians tend to be poor dispersers and/or move short distances, fluctuate drastically in population size, and exhibit site fidelity, all of which contribute to their sensitivity to habitat loss and fragmentation (Blaustein et al., 1994). Conservation efforts to reestablish extirpated populations are likely to be critical in slowing or reversing their declines. In North America, local amphibian populations are experiencing an alarming average rate of decline of 3.79% from metapopulations per year (Grant et al., 2016). Furthermore, amphibian species of Least Conservation Concern have experienced declines of 2.7% each year, suggesting even the most abundant species are at risk (Adams et al., 2013).

Species translocation may be essential to reintroduce amphibian populations and promote local population persistence (Marsh & Trenham, 2001). In fragmented habitats, amphibians are unlikely to be able to disperse sufficiently to recolonize a habitat patch without human intervention (Blaustein et al., 1994). However, there is a deficiency of literature investigating amphibian translocation (Seigal & Dodd, 2002; Dodd & Seigal, 1991; Germano & Bishop, 2009), and many questions remain regarding best practices. For example, how often should translocations occur to successfully establish a robust, self-sustaining population, and with how many individuals? What geographic considerations are required for source populations? Attention to genetic and demographic considerations are scarce, effectiveness and success rates are unclear, and protocols are generally absent (Marsh & Trenham, 2001; Dodd & Seigal, 1991). This lack of understanding generates concern for wildlife managers given that translocation success rates for vertebrates across all taxa appear quite low, especially in amphibians (Fischer & Lindmayer, 2000). For reintroduction by translocation to be a viable conservation and

management tool, it is imperative to more critically evaluate the science behind the translocation process.

The wood frog (*Lithobates sylvaticus*) is the most widely distributed native anuran species in North America (Dodd, 2013). In Ohio, their range covers over two-thirds of the state, and they are not considered a species of concern (IUCN, 2021). Nonetheless, they have been extirpated from numerous areas in the central Ohio region due to habitat loss and alteration, primarily because of urban development. Throughout the latter half of the 20th century, central Ohio (specifically Franklin County, which includes Columbus, the state capitol) experienced a massive increase in human population growth and, subsequently, urban development. From 1950 to 1975, the Franklin County population grew from ~500,000 to ~800,000 people, and then to over one million by the turn of the century (U.S. Census Bureau, 1950; U.S. Census Bureau, 1975; U.S. Census Bureau, 2000). Blacklick Woods Metro Park and Sharon Woods Metro Park, both part of the Columbus and Franklin County Metro Parks, historically contained populations of wood frogs until the early 1970's (TEH, personal observation), after which no individuals have been documented. These areas have been naturally reforested since their conversion to parks, but functionally both are islands surrounded by urban development (Fig. 1). To reintroduce the species, wood frog egg masses were collected from the robust population at Clear Creek Metro Park and translocated among Sharon Woods and Blacklick Woods in the spring of 2006 and 2007 (TEH). Following the translocations, all potential breeding pools have been surveyed annually at both sites. While they both had very similar initial founding populations, the total number of egg masses each year at Blacklick Woods has never exceeded 63 (mean=39) and has dropped as low as 9 individual egg masses. On the other hand, Sharon Woods has

averaged 337 egg masses per year, with a range of 31 to 763, indicating that Blacklick Woods and Sharon Woods have substantially different carrying capacities (Fig 2).

This repatriation and monitoring effort provides the basis for the current study, where we seek to understand the demographic and/or genetic differences that emerge between source and reintroduced populations. Specifically, we compare adult body mass, egg mass size, and embryo non-viability between the source and repatriated populations.

We additionally use 3RAD sequencing and population genomics to investigate potential impacts of translocation on genetic diversity, heterozygosity, inbreeding, and drift. Analysis of allelic variation at a diversity of loci can provide insight into genomic consequences of population founders and decrease in population size (Andrews et al. 2016). By assessing these parameters, in conjunction with demographic data, we can evaluate and compare the potential fitness of each population, which is critical for maximizing the success of future translocations of at-risk species.

Methods

The three study sites are part of the Columbus and Franklin County Metro Parks system (Fig. 1a). The source site, Clear Creek Metro Park, is located in Hocking County, Ohio. The park contains 2,145 hectares comprised of a wide diversity of landscape forms including ravines, prairie, wetlands, and forested habitat, surrounded by continuous woodland (Fig. 1d). Forested areas are characterized by lowland and ravine hemlock forests, with a mix of xeric oak-hickory and mesic mixed hardwood forests in the uplands. The amount of suitable habitat, which we broadly characterized by forested area, amounted to 2,084 hectares, or 97% of the park's total area. Blacklick Woods Metro Park and Sharon Woods Metro Park are the reintroduced sites and

are both located in Franklin County, Ohio. Sharon Woods Metro Park encompasses 308 hectares of grassland and upland woodlands dominated by oak, hickory, and beech, along with numerous vernal pools. It is, in essence, an isolated habitat island due to being surrounded by urban development (Fig. 1b). The amount of suitable habitat amounted to 220 hectares, or 71% of the park's total area. Similarly, Blacklick Woods Metro Park is 260 hectares, of which 95 hectares is a golf course, and is completely encompassed by urban development (Fig. 1c). It contains a vast swamp forest with expansive buttonbush wetlands and limited upland habitat, dominated by beech-maple and oak. The amount of suitable habitat amounted to 111 hectares, or 43% of the park's total area.

During the spring of 2006, 180 wood frog egg masses were translocated from Clear Creek and distributed among Blacklick Woods and Sharon Woods (90 egg masses placed evenly among two vernal pools within each park). The following year, the same process was repeated with 525 egg masses, 270 of which were placed among five pools in Sharon Woods and 255 among three pools in Blacklick Woods. Since these initial translocations, yearly surveys of egg masses in potential breeding pools have been conducted each spring, beginning in 2008 when the first signs of breeding were observed (Fig. 2). These surveys solely assessed the number of egg masses within each park until 2019 when we began this study.

We assessed three parameters that have potential to influence population demographics at Clear Creek, Blacklick Woods, and Sharon Woods: adult body size, egg mass size, and egg viability. Female wood frogs lay 300-1500 eggs that cluster and fuse together into a single egg mass (Meeks & Nagel, 1973; Berven, 1982; Corn & Livo, 1989). A single mass provides an estimate for an individual female's reproductive output, making egg mass surveys effective for

estimating population size (Crouch & Paton, 2000). Larger females tend to produce more eggs per mass, which positively correlates with the number of offspring that successfully metamorphose (Berven, 1982; Gibbons & McCarthy, 1986; Berven & Grudzien, 1990). Similarly, low embryo viability decreases the proportion of eggs that hatch and potentially metamorphose. More offspring successfully metamorphosing increases potential surviving individuals that contribute to the breeding population. We characterized embryos that failed to develop by a discolored and cloudy appearance, likely due to mold (Karracker & Ruthig, 2009), as well as lack of developmental when compared to other embryos within the mass.

In the spring of 2019, we captured adult wood frogs during the onset of breeding from each site using collapsible minnow traps and weighed each frog with a digital balance to the nearest 0.1 g. Due to a limited capture of females, we used 25 males from each site for subsequent analysis. After females deposited their eggs, we evaluated egg mass sizes by placing each mass (25 from Clear Creek and Sharon Woods, 22 from Blacklick Woods due to camera error) into white dissecting trays, and gently flattening them with clear plexiglass. We took an image of each and, using ImageJ (Schneider et al., 2012), digitally counted the number of eggs in each mass. We determined egg viability by repeating the flattening process and photographing 25 different egg masses from each site roughly 10 days after oviposition to establish the proportion of embryos that were failing to develop. We compared demographic data between reintroduced sites and to the source site using linear (body mass) and generalized linear models fit with the brms R package (Bürkner, 2018). Differences in egg mass size between sites were assessed using a generalized linear model with a negative binomial distribution, while embryo non-viability within egg masses was assessed using a model with a binomial distribution.

In 2019, wood frog toe clips were collected from adults at each study site. The sample set consisted of 18 individuals from Blacklick Woods, 19 individuals from Clear Creek, and 21 individuals from Sharon Woods. DNA was extracted from tissue samples following manufacturer protocol for the DNeasy blood and tissue kit (Qiagen, Hilden, Germany). 3RAD library preparation and sequencing was performed by Tangled Bank Conservation (Ashville, North Carolina). 3RAD, a refinement of RAD (Restriction site Associated DNA) sequencing is a reduced representation sequencing approach that allows for consistent and efficient population-scale genotyping of shared markers (Davey et al., 2013; Bayona-Vásquez et al., 2019). The cut site enzymes used were BamHI and MspI, as well as the adapter dimer cutting enzyme ClaI. Fragments were barcoded and sequenced on an Illumina platform.

Demultiplexing and de novo sequence assembly with minimal filtering was performed by Tangled Bank Conservation with ipyrad (Eaton & Overcast, 2020). We then imported VCF-format data into R using the package pegas (Paradis, 2010). We converted the data from a VCF format to a genlight format with the package vcfR (Knaus & Grünwald, 2017). Post-assembly iterative filtering of loci (focusing on loci present in the Clear Creek source population) and individuals was performed in R with adagenet (Jombart et al., 2008). As the reintroductions occurred within 6-12 generations (Dodd), genetic variation present in the relocated populations is likely to be a subset of the variation in the source population. Therefore, variation observed in the reintroduced populations is likely to also exist in the source population, but may not necessarily appear in our dataset due to random absence from the individuals we sampled or low sequencing coverage. Loci sampled in the source population are thus more informative for our comparison with the reintroduced populations.

We calculated basic population genetics statistics for the filtered dataset with the dartR package (Gruber et al., 2018). With adegenet's Hs.test function, we used a Monte-Carlo test with 999 replicates to individually assess whether gene diversity in either reintroduced population was significantly lower than gene diversity in Clear Creek. We calculated pairwise Fst in the package StAMPP (Pembelton et al., 2013) with 999 bootstraps. We obtained bootstrapped 95% confidence intervals for Fis with the boot.ppfis function in hierfstat (Goudet & Jombart, 2020). DAPC cross-validation and analysis was performed with the adagenet package following the publisher's tutorial accessible with the R command adegenetTutorial("dapc").

Results

The average mass of adult male frogs was 13.7 g (Standard Deviation = 1.31) at Blacklick Woods, 13.0 g (SD = 1.95) at Sharon Woods, and 12.6 g (SD = 1.21) at Clear Creek (Fig. 3). The Blacklick Woods adult male frogs were unequivocally larger than Clear Creek male frogs (Mean difference = 1.1g, 95% CRI = 0.11 to 2.05). There was less difference between male frogs from Sharon Woods and Clear Creek (Mean difference = 0.43g, 95% CRI = -1.37 to 0.51), but 82% of Sharon Woods' frogs were predicted to be larger. Male frogs from Blacklick Woods were also predominantly larger (92% probability) than males from Sharon Woods mean mass was 0.68g greater than Sharon Woods' (Mean difference = 0.68g, 95% CRI = -0.27 to 1.61g).

At Blacklick Woods, we first sampled all 17 egg masses within one vernal pool, all of which contained less than 500 eggs per mass. We then sampled 8 egg masses from out of over 50 from another vernal pool, all of which contained over 900 eggs per mass. Because all egg masses sampled from that one vernal pool contained over 900 eggs, we determine them to be representative of the other egg masses in that pool. Additionally, we did not observe any egg

masses containing less than 500 eggs within the other sites (Fig. 4 and 5). The coefficient of variance calculated for Blacklick Woods was 0.78, while it was 0.16 for Sharon Woods and 0.16 for Clear Creek. The observations of these small clutches may be due to females splitting egg masses and depositing eggs into two or more separate, smaller masses (Herreid & Kinney, 1967; Davis & Folkertz, 1986). We considered the egg masses from Blacklick Woods that contained less than 500 eggs to be outliers and not representative of a single female's total reproductive output (Fig. 5). We instead make the assumption that the average of the larger egg masses is representative of Blacklick Woods' female's total reproductive output. Thus, we provide analyses with only the large Blacklick Woods egg masses (those containing over 900 eggs).

Excluding masses containing less than 500 eggs, the mean Blacklick Woods egg mass contained 1,034 eggs (Range = 912–1148, SD = 88.3, Fig. 6). The mean Sharon Woods egg mass contained 796 eggs (Range = 526–1123, SD = 123) and the mean Clear Creek egg mass contained 757 eggs (Range = 525–982, SD = 118, Fig. 6). Blacklick Woods egg masses were larger than the Clear Creek egg masses by an average of 269 eggs (95% CRI = 137 to 114), and larger than Sharon Woods egg masses by an average of 242 eggs (95% CRI = 109 to 394). The Sharon Woods egg masses contained similar numbers of eggs as masses from Clear Creek (mean difference = 36 eggs, 95% CRI = -92 to 40), but are predicted to be larger 79% of the time.

The mean embryo non-viability rate was 24% at Blacklick Woods (Range = 1 – 64%; SD = 19%), 20% at Sharon Woods (Range = 2 – 43%; SD = 14%), and 11% at Clear Creek (Range = 2 – 50%; SD = 12%, Fig. 7). Our fitted model indicates that the embryo non-viability rate at Blacklick Woods was significantly higher than either Clear Creek (2.1x higher; CRI = 1.96 to 2.14x higher) and Sharon Woods (1.2x higher; 1.13 to 1.26x higher). The estimated non-viability

rate at Sharon Woods was also significantly greater than the rate estimated for Clear Creek (1.8x higher; CRI = 1.32 to 1.43x higher).

The full assembled dataset consisted of 250,909 SNPs in 58 individuals: 18 from Blacklick Woods, 19 from Clear Creek, and 21 from Sharon Woods. Due to low representation of loci across individuals, iterative filtering produced a final dataset of 1,300 loci retained and 16 individuals from Blacklick Woods, 10 from Clear Creek, and 17 from Sharon Wood. This dataset represents a tradeoff between missingness in individuals and retention of a sufficiently large sample size of loci and individuals.

The gene diversity in Blacklick Woods is not significantly less than in Clear Creek (p-value = 0.344). The gene diversity in Sharon Woods is also not significantly less than in Clear Creek (p-value = 0.948). Observed gene diversities in all populations are roughly similar (0.110, 0.117 and 0.134 for BW, CC, and SW, respectively, Table 1).

Observed heterozygosity is consistently lower than expected heterozygosity for all populations, and observed heterozygosity is similar across all populations (Table 1). There is a lower percentage of loci out of Hardy-Weinberg equilibrium in the Clear Creek population than either of the reintroduced populations (0.54% at Clear Creek, 3.85% at Blacklick Woods, and 4.54% at Sharon Woods, Table 1). The inbreeding coefficient F_{is} is similar in all three populations with bootstrapped 95% confidence intervals overlapping for all three populations (C.I. = (0.2391, 0.3377) for BW, (0.2385, 0.3371) for CC and (0.2165, 0.2877) for SW)).

Blacklick Woods was significantly genetically differentiated from both Clear Creek and Sharon Woods (Pairwise Fst for BW-CC = 0.0425, p-value < 0.001; Pairwise Fst for BW-SS =

0.04, p-value < 0.001) but Sharon Woods and Clear Creek were not significantly differentiated (Pairwise Fst = 0.0074, p-value = 0.143).

DAPC cross-validation suggested that retaining 14 PCs optimizes prediction success of individual assignments to populations. The DAPC plot with 14 PCs shows some clustering of individuals by population but with notable overlap in geometric space between populations (Fig. 8). There is a higher degree of geometric spatial overlap between Clear Creek and Sharon Woods than between Blacklick Woods and either of the other populations in the DAPC plot.

Discussion

Our results indicate that wood frogs at the reintroduced sites (Sharon Woods Metro Park and Blacklick Woods Metro Park) are heavier in mass, produce larger egg masses, and have higher embryo non-viability rates than their source population (Clear Creek Metro Park). This provides strong evidence that life history traits may experience consequences of translocation efforts. The deviation in life history traits from the source population is especially notable within Blacklick Woods, the reintroduced population of lower resulting size, as all three parameters were distinct from the source population. In contrast, the only parameter that differed significantly between Sharon Woods and Clear Creek was embryo viability.

Neither the bottleneck event associated with reintroduction nor subsequent stochastic reductions in population size in the reintroduced populations have had a significant effect on gene diversity. For all populations, observed heterozygosity is lower than expected heterozygosity, which may be attributable to inbreeding (Keller & Waller, 2002). However, observed heterozygosity and the inbreeding coefficient F_{is} is roughly similar across populations,

again suggesting that bottleneck events and differences in effective population size have not had a substantial effect on population genetics.

Discriminant Analysis of Principal Components (DAPC) clusters individuals somewhat within their populations, but with notable overlap. Pairwise Fst suggests that Clear Creek and Sharon Woods are not significantly genetically differentiated from each other, while Blacklick Woods is differentiated from both. The higher degree of genetic similarity between Clear Creek and Sharon Woods may reflect their greater similarity in demographic trends and larger population size. Blacklick Woods is somewhat more differentiated, likely owing to its divergent demographic trends, but the population still clusters in close proximity to the other two populations. Combined with the conclusions of little differentiation from the above genetic statistics, the overall trend is one of broad similarity between populations, with some fine-scale differentiation in association with demography.

Evidence for population differences appears stronger for life history trait variation than for population genetics. The variation among these life history parameters may be explained largely by site differences, including, but not limited to, location, size, degree of isolation, succession, disturbance history, forest type, and hydraulics. Any of these differences in site characteristics may have an effect on demographic traits, survival, or carrying capacity. The amount of available habitat area is likely the biggest factor contributing to limits on population size. However, the dynamics among the reintroduced vs. source populations are only one factor that may contribute to differences between populations, and it is difficult to differentiate effects of reintroduction from effects of site differences without further experimentation. We provide additional explanations below for observed differences.

Life history trait and genetic variation may also be partially resultant from the population bottleneck associated with a translocation; as a small sample of the original population becomes isolated, the genetic variation is reduced, and the effects of genetic drift are amplified. Reintroduction attempts can impose both genetic and demographic bottlenecks on newly established populations (Keller & Waller, 2002; Jamieson et al., 2007). Dramatically reducing standing genetic variation may reduce the long-term fitness of a population by decreasing adaptive potential and disease resistance while increasing the prevalence of physiological issues, thus threatening the viability of newly established populations (Spielman et al., 2004; Allentoft & O'Brien, 2010). Inbreeding can cause harmful mutations that are typically segregated to recombine and manifest as a recessive disease or deleterious mutation (Wang et al., 1999; Keller & Waller, 2002). As a result, low genetic variation can contribute to an extinction vortex when population size is minimal (Reed & Frankham, 2003; Blomqvist et al., 2010), especially if the population is established in an isolated region and thus unable to be "rescued" by recruitment from other populations (Reed, 2004).

The bottleneck impacting wood frogs in this study is extreme, as the Blacklick Woods population was reduced to as few as nine breeding pairs in 2014 and remained at a low population, while the population in Sharon Woods dropped dramatically several times. Despite the known founder event and subsequent population fluctuations, the genetic diversity, as measured through our SNP data, does not indicate loss of genetic diversity or an increase in inbreeding in the reintroduced populations. This is promising for the persistence of the reintroduced study populations, as it suggests the aforementioned issues sometimes associated with bottlenecks and isolation are not identifiable in the populations at this time.

Pairwise Fst suggests Blacklick Woods is significantly genetically differentiated from the other two populations, which are not differentiated from each other. DAPC shows a finer-scale clustering of individuals within their respective populations, but with Clear Creek and Sharon Woods sharing more overlap than Blacklick Woods does with either of them. This follows the logic that the more drastic population bottleneck following reintroduction and sustained smaller population size in Blacklick Woods has led to a greater degree of genetic differentiation. The observation of fewer loci out of Hardy-Weinberg equilibrium at Clear Creek is unsurprising as well, given its much larger population size than either reintroduced population and the association of Hardy-Weinberg equilibrium with large population size (Mayo, 2008). These findings in the absence of significant differences in genetic diversity or inbreeding suggest that genetic consequences from the reintroductions did occur, but were not severe enough to result in changes to the parameters associated with concern for population persistence.

Embryo non-viability showed the starkest contrast between source and reintroduced populations. Typical non-viability rates in wood frog eggs are reported to be <10%, although it is not widely assessed and may be variable (Herreid & Kinney, 1967; Porter, 1969; Seigel, 1983; Corn & Livo, 1989; Gomez-Mestre et al., 2006). High embryo mortality in anurans has been linked to several abiotic factors. Low pH of vernal pools, temperature, and high UV-B exposure have all been documented to increase embryo mortality (Herreid & Kinney, 1967; Freda, 1987; Kiesecker et al., 2001). The presence of pathogens like fungus can also decrease survival of embryos (Blaustein et al., 1994; Gomez-Mestre et al., 2006; Haislip et al., 2011; Hall et al., 2020). Amphibians may be more susceptible to pathogens when under stress, such as poor environmental conditions, and stress on a population may result in outbreaks (Blaustein et al., 1994). Additionally, the effects of inbreeding could cause higher susceptibility of embryos to

abiotic factors such as poor water quality or disease (Armbruster & Reed, 2005; Smallbone et al., 2016). Ficetola et al. (2007) and Okamiya and Kusano (2018) documented a significant correlation between inbreeding and embryo mortality in isolated amphibian populations, and correlations between inbreeding and hatching success has been documented in several studies of inbred bird populations (e.g., Daniels & Walters, 2000; Briskie & Macintosh, 2004; Jamieson et al., 2006). Other studies have also noted high embryo mortality accompanying inbreeding (e.g., Richter & Nunziata, 2014; Michaelides et al., 2016). A more likely possibility is that these embryos were never fertilized. This could have been the result of reproductive incompatibility or poor sperm quality (Hinkson & Poo, 2020). Infertility issues could be linked to and manifested from inbreeding or genetic drift (Ruiz-Lopez et al., 2010; Hinkson & Poo, 2020). Additionally, low water temperatures can reduce fertilization success by decreasing sperm motility (Herreid & Kinney, 1967).

The observation of small egg masses at Blacklick Woods could have been due to females splitting masses; that is, a female not depositing all of her eggs at once into a single mass, but distributing them across multiple, smaller masses. This has been observed to occur in wood frogs by Herreid and Kinney (1967) and Davis and Folkertz (1986), but to our knowledge has not otherwise been documented, nor do we know the mechanism behind it. If this is the case and Blacklick's wood frogs are splitting egg masses, their breeding population may be much smaller than previously estimated. This could raise some speculation as to whether egg mass surveys are an accurate estimate of breeding population size. This was only observed in one of the vernal pools within Blacklick, but considering that wood frogs return to the same vernal pool they were born in to breed, it wouldn't be unusual to find that frogs in one vernal pool could display

different behavior from frogs in another. We make the assumption that the larger egg masses are representative of Blacklick Woods' female wood frogs' total reproductive output.

An explanation for the variation in body size and egg mass sizes is phenotypic plasticity in response to density-dependent resource availability. Lower population density results in reduced intraspecific competition, which could allow individuals to have greater mass as adults, and larger females tend to produce bigger egg masses (Berven, 1982; Berven, 1990; Berven, 2009). Harper and Semlitsch (2007) observed the average mass of wood frogs after one year to be nearly threefold higher when raised in lower densities. A Berven (2009) study on density dependence in wood frogs revealed that at a lower juvenile population size, females were larger at first reproduction and produced larger egg masses. Blacklick Woods contained the lowest population density, which may have produced larger frogs yielding larger egg masses. The Sharon Woods population had a much higher density, which would explain its similarity to Clear Creek in those parameters.

Additionally, larger adult mass and egg mass sizes at Blacklick Woods and Sharon Woods may demonstrate microevolution. As female body size and egg mass size are positively correlated for some anurans, as are yearly average male and female body size, (Green, 2015), a genetic component to variation in adult size may be selected for. These larger frogs would contribute disproportionately to the gene pool, with directional selection resulting in a larger average adult size. In smaller populations, selection may act more rapidly because each individual constitutes a larger portion of the population, so their genetic contribution through offspring has a larger proportional effect on the overall makeup of the population. This may

explain why Blacklick Woods had a greater increase in adult mass and egg mass sizes in comparison to the source population, as Blacklick Woods had a lower population size.

These reintroduced populations are of very few documented amphibian translocations to have succeeded and persisted long term. Not only is it important to re-establish a lost species to an ecosystem, but the information these reintroduction efforts provide can be learned from to improve the success of future translocation attempts (Sarrazin & Barbault, 1996; Runge, 2013). While we expected to see decreased genetic variation in the reintroduced populations, our results do not suggest that this is the case, which is promising for future reintroductions of amphibians. Our results do suggest that reintroduction by translocation can directly impact life history traits. A way to combat this dilemma as a consideration for future translocations is to continuously supplement the population over time with additional translocations as the population stabilizes (Johnson & Dunn, 2006), although care should be taken to prevent outbreeding depression (Sagvik et al., 2005). Reintroductions may require continuous management and monitoring in order to see success in the long term (Ewen & Armstrong, 2007; Heezik et al., 2009; Buk et al., 2018). Lack of long-term monitoring has been an issue with past translocation efforts, but it is a necessity to prevent any adverse outcomes, in addition to promoting and assessing the success of a translocation (Griffith et al., 1989; Seddon, 1999; Dodd & Seigal, 1991; Seigal & Dodd, 2002; Heezik et al., 2009; Germano & Bishop, 2009).

Literature Cited

Adams, M. J., Miller, D. A. W., Muths, E., Corn, P. S., Grant, E. H. C., Bailey, L. L., Fellers, G. M., Fisher, R. N., Sadinski, W. J., Waddle, H., & Walls, S. C. (2013). Trends in Amphibian Occupancy in the United States. *PLOS ONE, 8*(5), e64347.

418 Allentoft, M. E., & O'Brien, J. (2010). Global Amphibian Declines, Loss of Genetic Diversity
419 and Fitness: A Review. *Diversity*, *2*(1), 47–71. https://doi.org/10.3390/d2010047

420 Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P. A. (2016). Harnessing
421 the power of radseq for ecological and evolutionary genomics. *Nature Reviews. Genetics*,
422 *17*(2), 81–92.

423 Armbruster, P., & Reed, D. H. (2005). Inbreeding depression in benign and stressful
424 environments. *Heredity*, *95*(3), 235–242.

425 Bayona-Vásquez, N. J., Glenn, T. C., Kieran, T. J., Pierson, T. W., Hoffberg, S. L., Scott, P. A.,
426 Bentley, K. E., Finger, J. W., Louha, S., Troendle, N., Diaz-Jaimes, P., Mauricio, R., &
427 Faircloth, B. C. (2019). Adapterama III: Quadruple-indexed, double/triple-enzyme RADseq
428 libraries (2RAD/3RAD). *PeerJ*, *7*, e7724.

429 Bender, D. J., Contreras, T. A., & Fahrig, L. (1998). Habitat Loss and Population Decline: A
430 Meta-Analysis of the Patch Size Effect. *Ecology*, *79*(2), 517–533.

431 Berven, K. A. (1982). The Genetic Basis of Altitudinal Variation in the Wood Frog Rana
432 sylvatica. I. An Experimental Analysis of Life History Traits. *Evolution*, *36*(5), 962–983.

433 Berven, K. A. (1990). Factors Affecting Population Fluctuations in Larval and Adult Stages of
434 the Wood Frog (Rana Sylvatica). *Ecology*, *71*(4), 1599–1608.

435 Berven, K. A., & Grudzien, T. A. (1990). Dispersal in the Wood Frog (rana Sylvatica):
436 Implications for Genetic Population Structure. *Evolution*, *44*(8), 2047–2056.

437 Berven, K. A. (2009). Density Dependence in the Terrestrial Stage of Wood Frogs: Evidence
438 from a 21-Year Population Study. *Copeia*, *2009*(2), 328–338.

Blaustein, A. R., Grant Hokit, D., O'Hara, R. K., & Holt, R. A. (1994). Pathogenic fungus contributes to amphibian losses in the pacific northwest. *Biological Conservation*, *67*(3), 251–254.

Blaustein, A., Wake, D., & Sousa, W. (1994). Amphibian Declines: Judging Stability, Persistence, and Susceptibility of Populations to Local and Global Extinctions. *Conservation Biology - CONSERV BIOL*, *8*, 60–71.

Blomqvist, D., Pauliny, A., Larsson, M., & Flodin, L.-Å. (2010). Trapped in the extinction vortex? Strong genetic effects in a declining vertebrate population. *BMC Evolutionary Biology*, *10*(1), 33.

Briskie, J. V., & Mackintosh, M. (2004). Hatching failure increases with severity of population bottlenecks in birds. *Proceedings of the National Academy of Sciences*, *101*(2), 558–561.

Brown, J. H., & Kodric-Brown, A. (1977). Turnover Rates in Insular Biogeography: Effect of Immigration on Extinction. *Ecology*, *58*(2), 445–449.

Buk, K. G., van der Merwe, V. C., Marnewick, K., & Funston, P. J. (2018). Conservation of severely fragmented populations: Lessons from the transformation of uncoordinated reintroductions of cheetahs (Acinonyx jubatus) into a managed metapopulation with self-sustained growth. *Biodiversity and Conservation*, *27*(13), 3393–3423.

Bürkner, P. C. (2018). *Advanced bayesian multilevel modeling with the R package brms. R J. 10, 395–411. doi: 10.32614*. RJ-2018-017.

Corn, P. S., & Livo, L. J. (1989). Leopard Frog and Wood Frog Reproduction in Colorado and Wyoming. *Northwestern Naturalist*, *70*(1), 1–9.

Crouch, W. B., & Paton, P. W. C. (2000). Using Egg-Mass Counts to Monitor Wood Frog Populations. *Wildlife Society Bulletin (1973-2006)*, *28*(4), 895–901.

Daniels, S. J., & Walters, J. R. (2000). Inbreeding Depression and its Effects on Natal Dispersal in Red-Cockaded Woodpeckers. *The Condor*, *102*(3), 482–491.

Davey, J. W., Cezard Timothée, Fuentes-Utrilla, P., Eland, C., Gharbi, K., & Blaxter, M. L. (2013). Special features of rad sequencing data: implications for genotyping. *Molecular Ecology*, *22*(11), 3151–3164.

Davis, M. S., & Folkerts, G. W. (1986). LIFE-HISTORY OF THE WOOD FROG, RANA-SYLVATICA LECONTE (AMPHIBIA, RANIDAE), IN ALABAMA. *Brimleyana*, (12), 29-50.

Dodd, C. K. (2013). *Frogs of the United States and Canada, 2-vol. set* (Vol. 1). JHU Press.

Dodd, C. K., & Seigel, R. A. (1991). Relocation, Repatriation, and Translocation of Amphibians and Reptiles: Are They Conservation Strategies That Work? *Herpetologica*, *47*(3), 336–350.

Eaton, D. A. R., Overcast, I., & Schwartz, R. (2020). Ipyrad: interactive assembly and analysis of radseq datasets. *Bioinformatics*, *36*(8), 2592–2594.

Edmands, Suzanne. (2007). Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology*, *16*(3), 463–475.

Engelhardt, K. A. M., Lloyd, M. W., & Neel, M. C. (2014). Effects of genetic diversity on conservation and restoration potential at individual, population, and regional scales. *Biological Conservation*, *179*, 6–16.

Ewen, J. G., & Armstrong, D. P. (2007). Strategic monitoring of reintroductions in ecological restoration programmes. *Ecoscience*, *14*(4), 401–409.

Fahrig, L. (2002). Effect of Habitat Fragmentation on the Extinction Threshold: A Synthesis*. *Ecological Applications*, *12*(2), 346–353.

Ficetola, G. F., Garner, T. W. J., & Bernardi, F. D. (2007). Genetic diversity, but not hatching success, is jointly affected by postglacial colonization and isolation in the threatened frog, Rana latastei. *Molecular Ecology*, *16*(9), 1787–1797.

Fischer, J., & Lindenmayer, D. B. (2000). An assessment of the published results of animal relocations. *Biological Conservation*, *96*(1), 1–11.

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., Chapin, F. S., Coe, M. T., Daily, G. C., Gibbs, H. K., Helkowski, J. H., Holloway, T., Howard, E. A., Kucharik, C. J., Monfreda, C., Patz, J. A., Prentice, I. C., Ramankutty, N., & Snyder, P. K. (2005). Global Consequences of Land Use. *Science*, *309*(5734), 570–574.

Freda, J. (1987). The Influence of Acidic Pond Water on Amphibians: A Review. In H. C. Martin (Ed.), *Acidic Precipitation* (pp. 439–450). Springer Netherlands.

Germano, J. M., & Bishop, P. J. (2009). Suitability of Amphibians and Reptiles for Translocation. *Conservation Biology*, *23*(1), 7–15.

Gibbons, M. M., & McCarthy, T. K. (1986). The reproductive output of frogs Rana temporaria (L.) with particular reference to body size and age. *Journal of Zoology*, *209*(4), 579–593.

Gilpin, M. E. & Soulé, M.E. (1986). Minimal viable populations: processes of species extinction. *Conservation biology: the science of scarcity and diversity*.

Gomez-Mestre, I., Touchon, J. C., & Warkentin, K. M. (2006). Amphibian Embryo and Parental Defenses and a Larval Predator Reduce Egg Mortality from Water Mold. *Ecology*, *87*(10), 2570–2581.

Goudet, J. & Jombart, T. (2020). hierfstat: Estimation and Tests of Hierarchical F-Statistics. R package version 0.5-7. https://CRAN.R-project.org/package=hierfstat

Grant, E. H. C., Miller, D. A. W., Schmidt, B. R., Adams, M. J., Amburgey, S. M., Chambert, T., Cruickshank, S. S., Fisher, R. N., Green, D. M., Hossack, B. R., Johnson, P. T. J., Joseph, M. B., Rittenhouse, T. A. G., Ryan, M. E., Waddle, J. H., Walls, S. C., Bailey, L. L., Fellers, G. M., Gorman, T. A., … Muths, E. (2016). Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. *Scientific Reports*, *6*(1), 25625.

Green, D., M. (2015). Implications of female body-size variation for the reproductive ecology of an anuran amphibian. *Ethology Ecology & Evolution*, *27*(2), 173–184.

Gruber, B., Unmack, P.J., Berry, O.F., & Georges, A. (2018). dartR: An R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources*, **18**, 691–699.

Griffith, B., Scott, J. M., Carpenter, J. W., & Reed, C. (1989). Translocation as a Species Conservation Tool: Status and Strategy. *Science*, *245*(4917), 477–480.

Haila, Y. (2002). A Conceptual Genealogy of Fragmentation Research: From Island Biogeography to Landscape Ecology*. *Ecological Applications*, *12*(2), 321–334.

Haislip, N. A., Gray, M. J., Hoverman, J. T., & Miller, D. L. (2011). Development and Disease: How Susceptibility to an Emerging Pathogen Changes through Anuran Development. *PLOS ONE*, *6*(7), e22307.

Hall, E. M., Brunner, J. L., Hutzenbiler, B., & Crespi, E. J. (2020). Salinity stress increases the severity of ranavirus epidemics in amphibian populations. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1926), 20200062.

Harper, E. B., & Semlitsch, R. D. (2007). Density dependence in the terrestrial life history stage of two anurans. *Oecologia*, *153*(4), 879–889.

Heezik, Y. van, Maloney, R. F., & Seddon, P. J. (2009). Movements of translocated captive-bred and released Critically Endangered kaki (black stilts) Himantopus novaezelandiae and the value of long-term post-release monitoring. *Oryx*, *43*(4), 639–647.

Hein, E. W. (1997). Improving translocation programs. *Conservation Biology*, *11*(6), 1270-1271.

Herreid, C. F., & Kinney, S. (1967). Temperature and Development of the Wood Frog, Rana Sylvatica, in Alaska. *Ecology*, *48*(4), 579–590.

Hinkson, K. M., & Poo, S. (2020). Inbreeding depression in sperm quality in a critically endangered amphibian. *Zoo Biology*, *39*(3), 197–204.

Jamieson, I. G., Wallis, G. P., & Briskie, J. V. (2006). Inbreeding and Endangered Species Management: Is New Zealand Out of Step with the Rest of the World? *Conservation Biology*, *20*(1), 38–47.

Jamieson, I. G., Tracy, L. N., Fletcher, D., & Armstrong, D. P. (2007). Moderate inbreeding depression in a reintroduced population of North Island robins. *Animal Conservation, 10*(1), 95–102.

Johnson, J. A., & Dunn, P. O. (2006). Low Genetic Variation in the Heath Hen Prior to Extinction and Implications for the Conservation of Prairie-Chicken Populations. *Conservation Genetics*, *7*(1), 37–48.

Jombart T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**: 1403-1405.

Jump, A. S., Marchant, R., & Peñuelas J. (2009). Environmental change and the option value of genetic diversity. *Trends in Plant Science*, *14*(1), 51–8.

Karraker, N. E., & Ruthig, G. R. (2009). Effect of road deicing salt on the susceptibility of amphibian embryos to infection by water molds. *Environmental Research*, *109*(1), 40–45.

Keller, L. F. & Waller, D.M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, *17*(5), 230–241.

Kiesecker, J. M., Blaustein, A. R., & Belden, L. K. (2001). Complex causes of amphibian population declines. *Nature*, *410*(6829), 681–684.

Kimura, M. (1955). Random genetic drift in multi-allelic locus. *Evolution*, *9*(4), 419–435.

Knaus, B.J. & Grünwald, N.J. (2017). "VCFR: a package to manipulate and visualize variant call format data in R." *Molecular Ecology Resources*, **17**(1), 44–53.

559 Marsh, D. M., & Trenham, P. C. (2001). Metapopulation Dynamics and Amphibian
560 Conservation. *Conservation Biology*, *15*(1), 40–49.

561 Mayo, O. (2008). A century of hardy-weinberg equilibrium. *Twin Research and Human*
562 *Genetics*, *11*(3), 249–256.

563 Meeks, D. E., & Nagel, J. W. (1973). Reproduction and Development of the Wood Frog, Rana
564 sylvatica, in Eastern Tennessee. *Herpetologica*, *29*(2), 188–191.

565 Michaelides, S. N., While, G. M., Zajac, N., Aubret, F., Calsbeek, B., Sacchi, R., Zuffi, M. A. L.,
566 & Uller, T. (2016). Loss of genetic diversity and increased embryonic mortality in non-
567 native lizard populations. *Molecular Ecology*, *25*(17), 4113–4125.

568 Okamiya, H., & Kusano, T. (2018). Lower genetic diversity and hatchability in amphibian
569 populations isolated by urbanization. *Population Ecology*, *60*(4), 347–360.

570 Paradis E (2010). "pegas: an R package for population genetics with an integrated–modular
571 approach." *Bioinformatics*, **26**, 419-420.

572 Pembleton L, Cogan N, Forster J (2013). "StAMPP: an R package for calculation of genetic
573 differentiation and structure of mixed-ploidy level populations." *Molecular Ecology*
574 *Resources*, **13**, 946-952.

575 Pimm, S. L., Jenkins, C. N., Abell, R., Brooks, T. M., Gittleman, J. L., Joppa, L. N., Raven, P.
576 H., Roberts, C. M., & Sexton, J. O. (2014). The biodiversity of species and their rates of
577 extinction, distribution, and protection. *Science*, *344*(6187).

578 Pimm, Stuart L., & Raven, P. (2000). Extinction by numbers. *Nature*, *403*(6772), 843–845.

Porter, K. R. (1969). Evolutionary Status of the Rocky Mountain Population of Wood Frogs. *Evolution*, *23*(1), 163–170.

Reed, D. H. (2004). Extinction risk in fragmented habitats. *Animal Conservation*, *7*(2), 181–191.

Reed, D. H., & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conservation biology*, *17*(1), 230-237.

Richter, S. C., & Nunziata, S. O. (2014). Survival to metamorphosis is positively related to genetic variability in a critically endangered amphibian species. *Animal Conservation*, *17*(3), 265–274.

Ruiz-Lopez, M. J., Evenson, D., Espeso, G., Gomendio, M., & Roldan, E. (2010). High Levels of DNA Fragmentation in Spermatozoa Are Associated with Inbreeding and Poor Sperm Quality in Endangered Ungulates. *Biology of Reproduction*, *83*, 332–338.

Runge, M. C. (2013). Active Adaptive Management for Reintroduction of an Animal Population. *The Journal of Wildlife Management*, *77*(6), 1135–1144.

Sagvik, J., Uller, T., & Olsson, M. (2005). Outbreeding depression in the common frog, Rana temporaria. *Conservation Genetics*, *6*(2), 205–211.

Sarrazin, F., & Barbault, R. (1996). Reintroduction: Challenges and lessons for basic ecology. *Cambridge University Press*, 5.sarra

Schlaepfer, D. R., Braschler, B., Rusterholz, H.-P., & Baur, B. (2018). Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: a meta-analysis. *Ecosphere*, *9*(10).

599 Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of
600 image analysis. *Nature Methods*, *9*(7), 671–675.

601 Seddon, P. J. (1999). Persistence without intervention: Assessing success in wildlife
602 reintroductions. *Trends in Ecology & Evolution*, *14*(12), 503.

603 Seigel, R. A. (1983). Natural Survival of Eggs and Tadpoles of the Wood Frog, Rana sylvatica.
604 *Copeia*, *1983*(4), 1096–1098.

605 Seigel, R. A., & Dodd, C. K. (2002). Translocations of Amphibians: Proven Management
606 Method or Experimental Technique? *Conservation Biology*, *16*(2), 552–554.

607 Smallbone, W., van Oosterhout, C., & Cable, J. (2016). The effects of inbreeding on disease
608 susceptibility: Gyrodactylus turnbulli infection of guppies, Poecilia reticulata. *Experimental*
609 *Parasitology*, *167*, 32–37.

610 Spielman, D., Brook, B. W., Briscoe, D. A., & Frankham, R. (2004). Does Inbreeding and Loss
611 of Genetic Diversity Decrease Disease Resistance? *Conservation Genetics*, *5*(4), 439–448.

612 Spielman, D., Brook, B. W., Frankham, R., & Schaal, B. A. (2004). Most species are not driven
613 to extinction before genetic factors impact them. *Proceedings of the National Academy of*
614 *Sciences of the United States of America*, *101*(42), 15261–15264.

615 Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L., &
616 Waller, R. W. (2004). Status and Trends of Amphibian Declines and Extinctions
617 Worldwide. *Science*, *306*(5702), 1783–1786.

618 *The IUCN Red List of Threatened Species*. (2021). IUCN Red List of Threatened Species.
619 Retrieved April 12, 2021, from https://www.iucnredlist.org/en

U.S. Census Bureau. (1950). United States Census 2000. Available online: http://www.census.gov

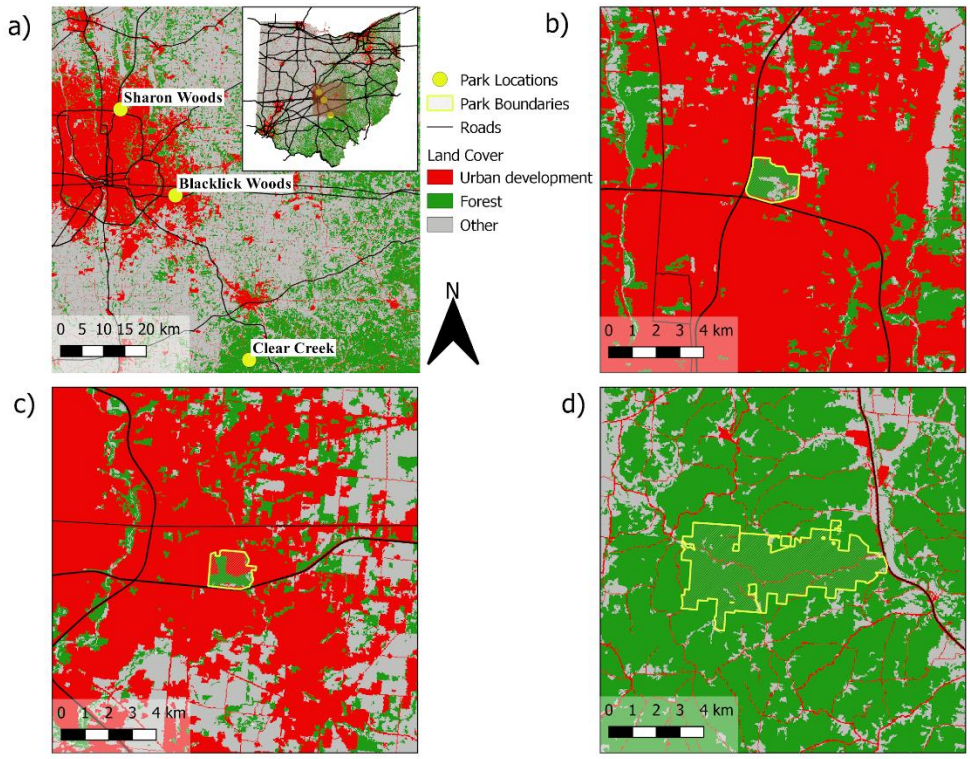
U.S. Census Bureau. (1975). United States Census 2000. Available online: http://www.census.gov

U.S. Census Bureau. (2000). United States Census 2000. Available online: http://www.census.gov

Wang, J., Hill, W. G., Charlesworth, D., & Charlesworth, B. (1999). Dynamics of inbreeding depression due to deleterious mutations in small populations: Mutation parameters and inbreeding rate. *Genetics Research*, *74*(2), 165–178.

Wilcove, D. S., McLellan, C. H., & Dobson, A. P. (1986). Habitat fragmentation in the temperate zone. *Conservation biology*, *6*, 237-256.

Figures

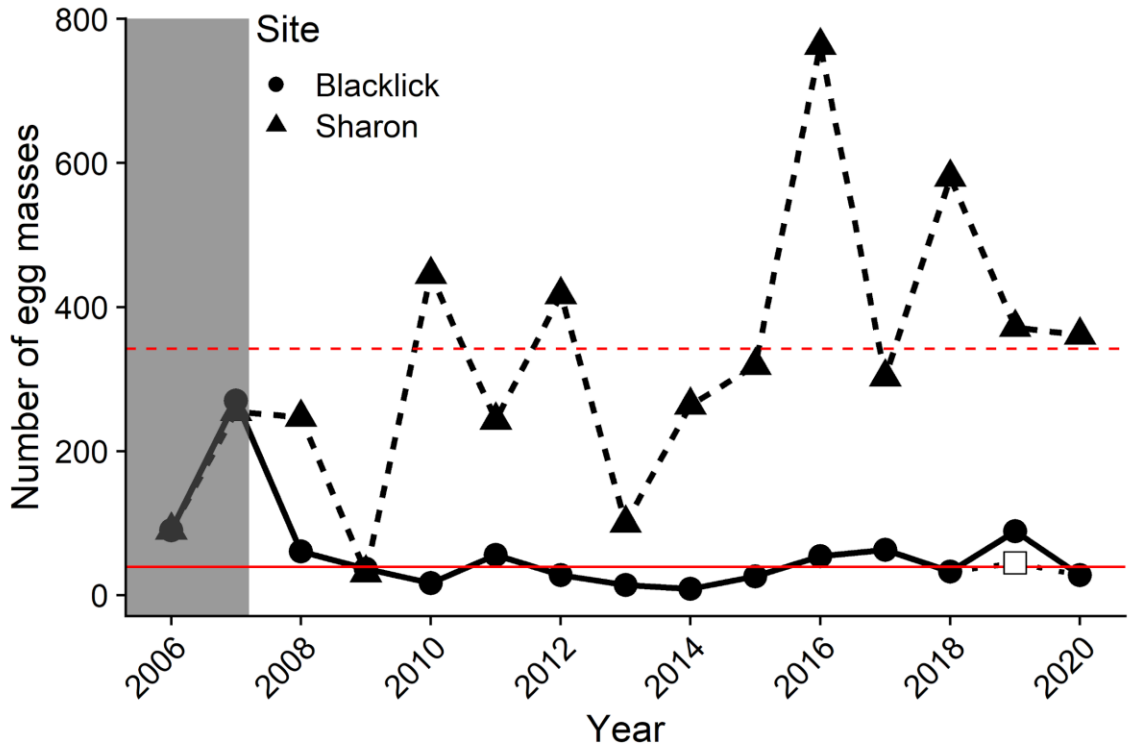


632

633 Figure 1. Maps depicting the land cover type and locations of all three sites. a) Location of all

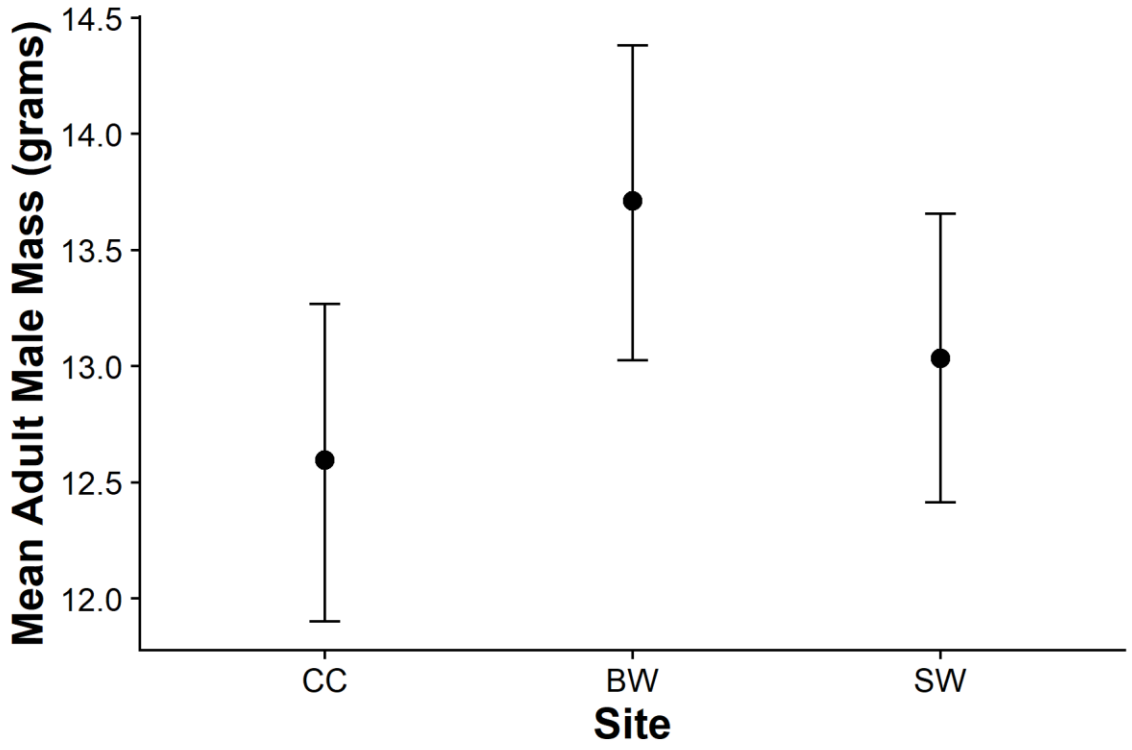
634 three sites relative to Columbus, Ohio, b) Sharon Woods Metro Park, c) Blacklick Woods Metro

635 Park, d) Clear Creek Metro Park.



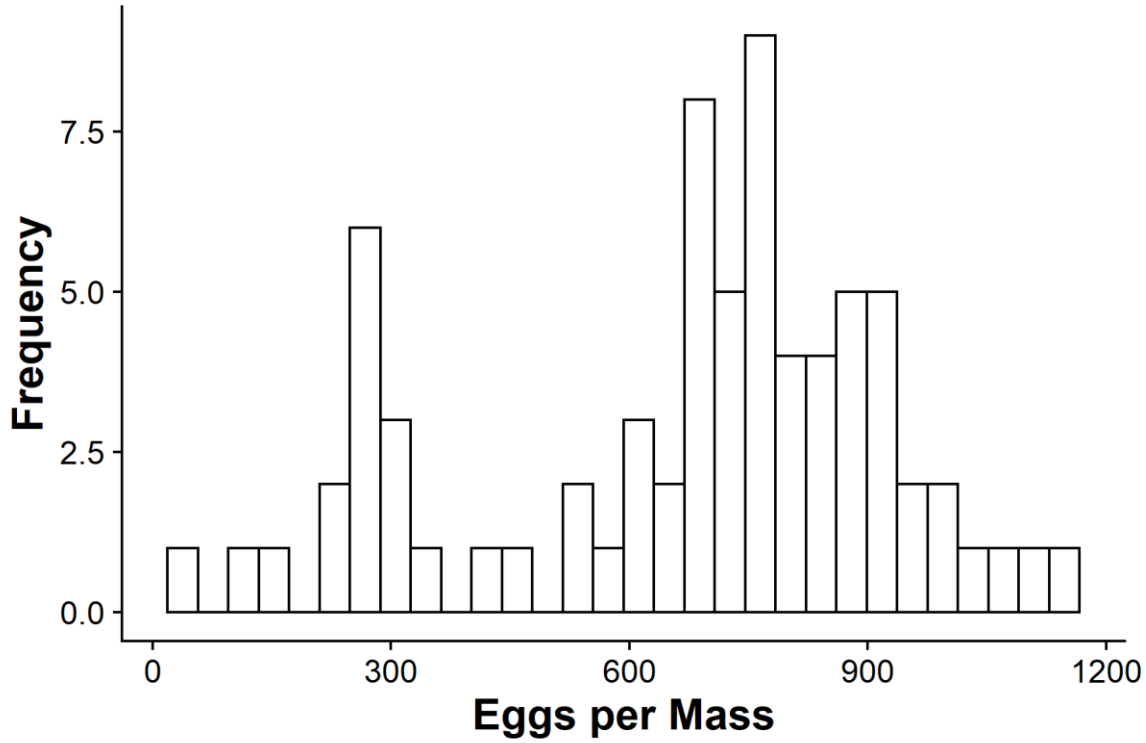
636

637 Figure 2. Yearly egg mass surveys from 2006 to 2020 within Blacklick Woods Metro Parks and
638 Sharon Woods Metro Park. The area shaded in grey are the initial translocated egg masses
639 during 2006 and 2007. The years following are egg masses from natural breeding within the
640 site. The white box in 2018 shows the estimated number of egg masses had they not
641 presumably been split. The red lines indicate the average number of egg masses within each
642 site per year (dashed line= Sharon Woods, solid line = Blacklick Woods).



643

644 Figure 3. The mean adult male mass of wood frogs within each site



645

Figure 4. The amount of egg masses containing varying amounts of eggs, among all sites, including the masses containing less than 500 eggs at Blacklick Woods.

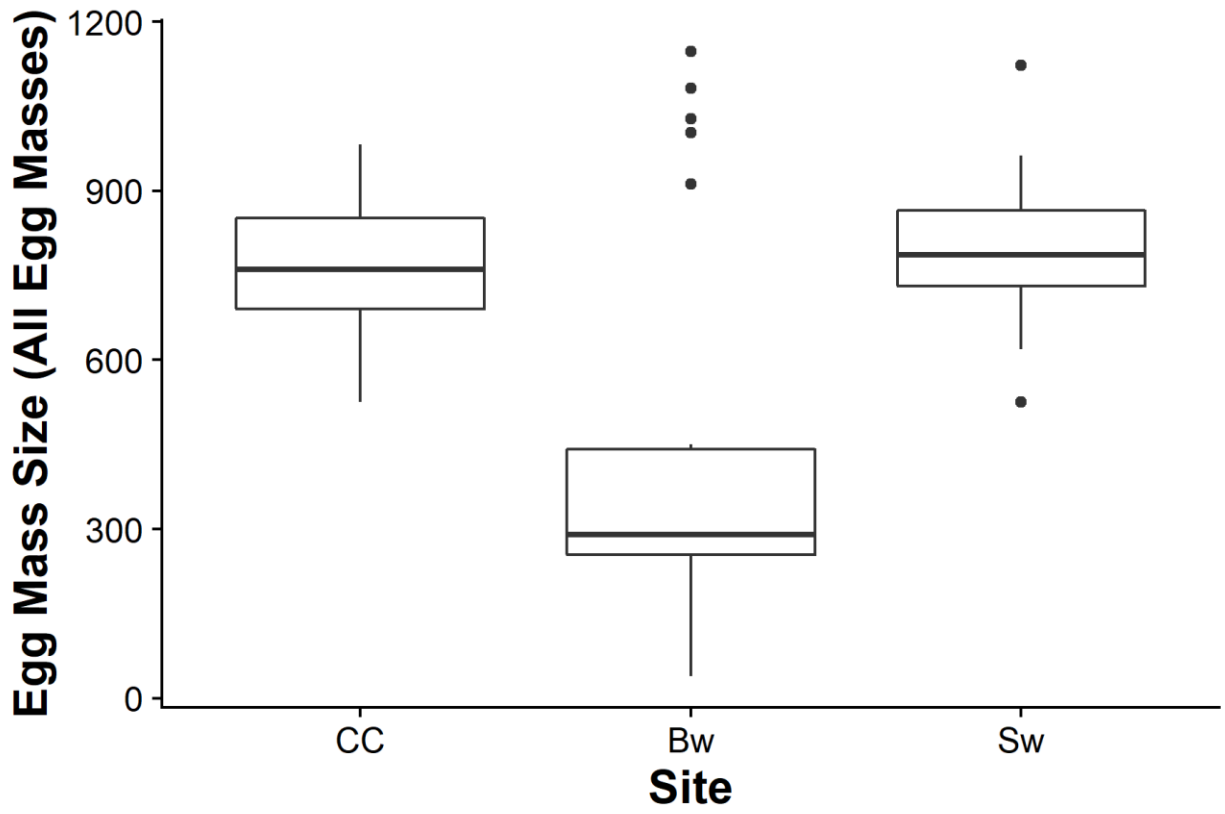
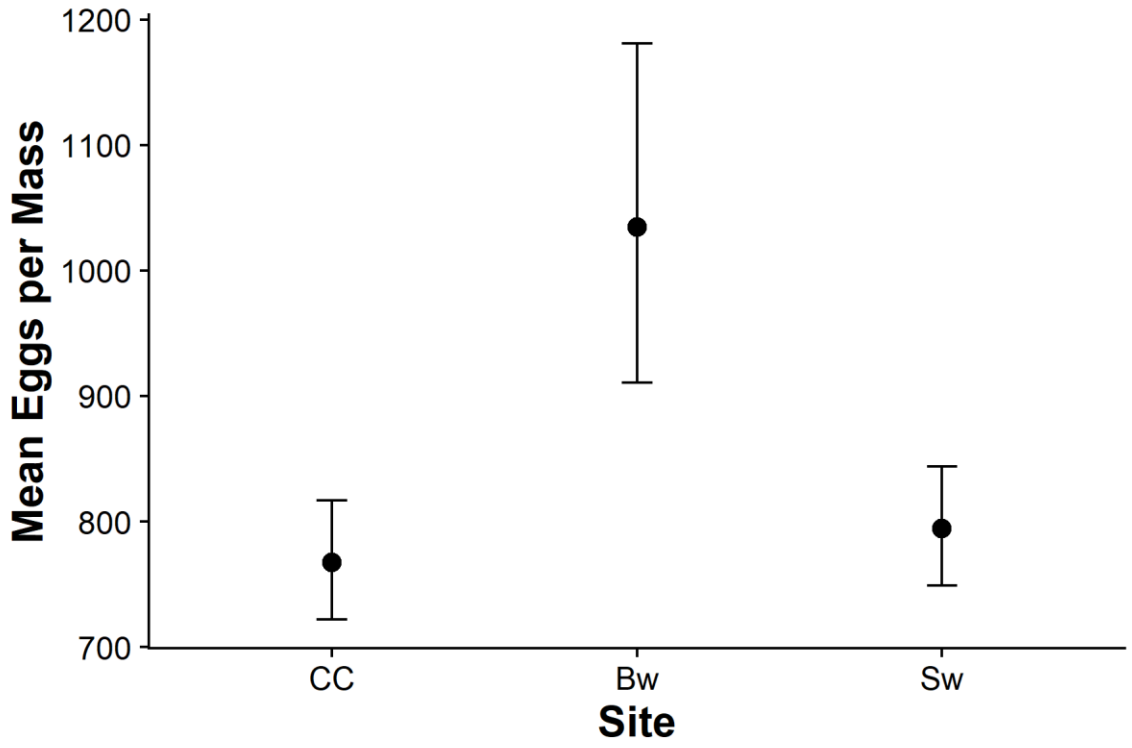


Figure 5. The variation among egg mass sizes within each site, including the egg masses at Blacklick Woods containing less than 500 eggs.



653

654 Figure 6. The mean number of eggs per mass within each site, excluding the egg masses

655 containing less than 500 eggs at Blacklick Woods.

656

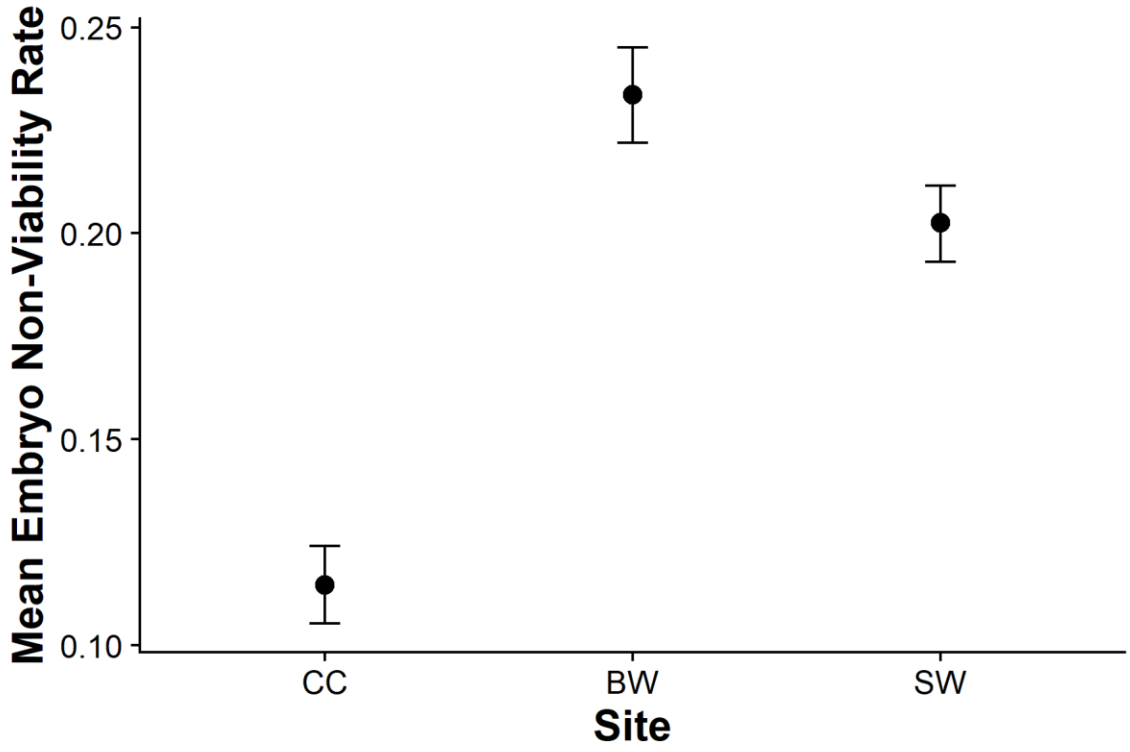


Figure 7. The mean embryo non-viability rate within egg masses among each site.

Population	Observed gene diversity (H_s)	Observed Heterozygosity (H_o)	Expected Heterozygosity (H_e)	% of Loci out of HWE	Inbreeding Coefficient (F_{is})
BW	0.110	0.078	0.100	3.85	0.292
CC	0.117	0.083	0.103	0.54	0.290
SW	0.134	0.100	0.123	4.54	0.253

Table 1. Basic population genetics statistics by population. HWE indicates Hardy-Weinberg Equilibrium.

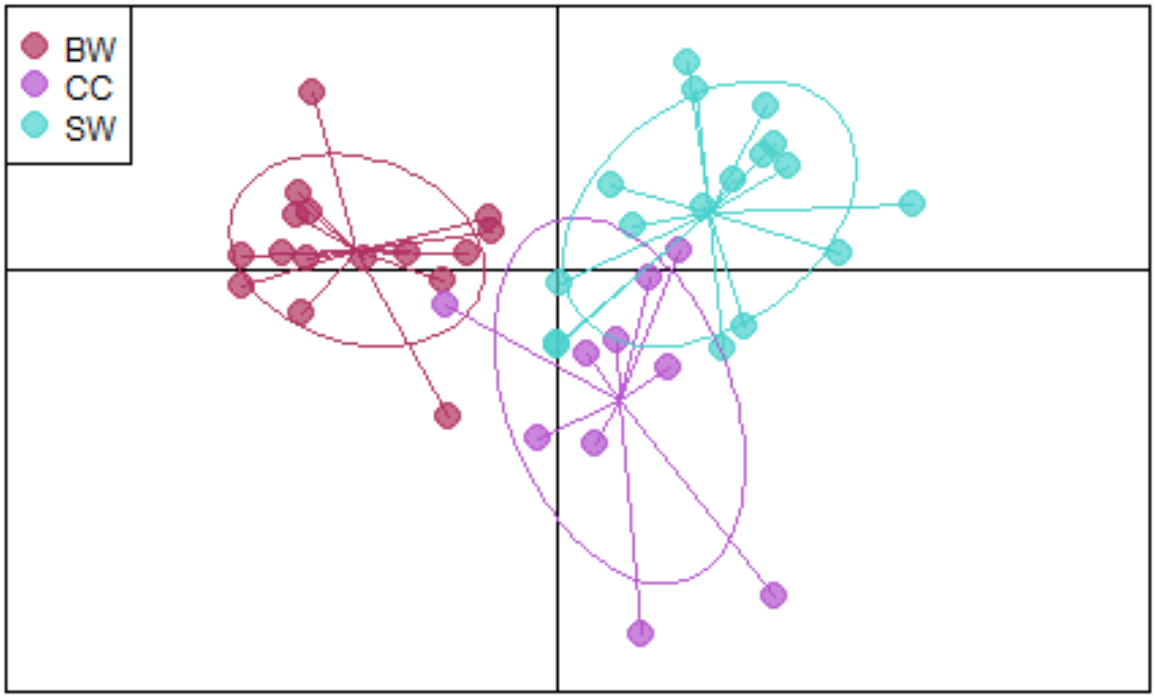


Figure 8. Discriminant Analysis of Principal Components (DAPC) of population genetic data, with 14 PCs retained. Clear Creek and Sharon Woods share more overlap, indicating higher genetic similarity. Blacklick Woods is slightly more differentiated but still shows close genetic similarity to the other populations.