12-2014

# Genetic Health and Population Viability of Reintroduced American Marten in Michigan

Tamara L. Hillman
*Grand Valley State University*

# GENETIC HEALTH AND POPULATION VIABILITY OF REINTRODUCED AMERICAN MARTEN IN MICHIGAN

Tamara L. Hillman

A Thesis Submitted to the Graduate Faculty of

GRAND VALLEY STATE UNIVERSITY

In

Partial Fulfillment of the Requirements

For the Degree of

Master of Science

Biology

December, 2014

## Dedication

I would like to dedicate this to my family, with special thanks to my parents, Randy and Linda Hillman, and my sisters, Maegan Weighman and Katie Hillman for their unending love and support during my pursuit of higher education, without you this would not have been possible.

**Acknowledgements**

I wish to thank the members of my thesis committee: Dr. Paul Keenlance, Dr. Joseph Jacquot, Dr. Michael Henshaw, and Dr. Jennifer Moore for their support and guidance throughout the course of my degree. I would like to thank Dr. Jill Witt, Maria Spriggs DVM, Bob Sanders, Chris Schumacher, Dr. Ari Cornman, Dr. David Unger, and Dr. Bradley Swanson for their efforts in developing and expanding the American marten project, as well as for their invaluable support and advice. I also wish to thank all of the past, present, and future graduate and undergraduate students that have assisted in this project: Sara Bicker, Melissa Cannan, Michael Rossi, Danielle Bradke, Rachel Hughart, Aubri Spenski, Julie Watkins, David Haskins, Angela Kujawa, Patrick Laarman, and hopefully many more to come. I am indebted to the many institutions and agencies that have supported and contributed to the marten project: Grand Valley State University, Little River Band of Ottawa Indians, Mesker Park Zoo and Botanic Garden, Sault Ste. Marie Tribe of Chippewa Indians, Maryville College, Detroit Zoo, Central Michigan University, USDA Forest Service Huron-Manistee National Forests, and Michigan Department of Natural Resources. Finally, I want to give a special thank you to Melissa Cannan, Justin Wegner, Jeff Pashnick, Alynn Martin, Nichole Kupisz, Danielle Bradke, and Bre Gould for their unwavering support of me through my frustrations and triumphs whilst getting this degree.

## Abstract

American marten (*Martes americana*) were extirpated from Michigan's Lower Peninsula (LP) in 1911, and subsequently from the Upper Peninsula (UP) in 1939 due to habitat loss and unregulated trapping. The species was later reintroduced in the UP in the mid-1950s, and to the LP in the mid-1980s. Previous research has determined the small founding sizes used in the LP reintroductions have resulted in losses of genetic diversity, while research in the UP has produced discordant results concerning the effects of the reintroduction methods on genetic health and population structure. Since past research of marten in the LP, no additional reintroductions have occurred to mitigate further diversity loss, and little is known of the current status of marten genetic health or long-term population viability. The objectives of this research were to reevaluate the current genetic health of marten in Michigan, and determine the long-term population viability of marten in the Manistee National Forest (MNF). Microsatellite markers were used to calculate estimates of genetic health, and population viability analysis (PVA) was performed to determine viability over the next 100 years. Results of this research indicate the reintroduced marten populations in Michigan's LP display evidence of genetic diversity loss due to small founding size and isolation in a fragmented habitat. Marten exhibited low levels of allelic richness, effective breeding size, and showed increases in inbreeding levels when compared to previous marten research. Marten in Michigan's eastern UP also displayed signs of reduced genetic diversity, which was congruent with previous findings indicating population structuring reflective of multiple source locations. PVA indicated the MNF population was likely to maintain demographic viability, but would lose genetic viability within the next 100 years, although the use of non-population specific

reproduction and survival rates may have overestimated viability results. It is recommended further research take place to identify factors that may be limiting population growth in marten populations of the LP. A translocation of marten to the LP populations is also recommended to mitigate further genetic diversity loss and preserve long-term viability of marten in Michigan.

## Table of Contents

## List of Tables

**Table** **Page**

## List of Figures

**Figure** **Page**

# Chapter I

## Introduction

A reintroduction is defined by the International Union for Conservation of Nature and Natural Resources (IUCN) as an attempt to reestablish a species in an area that was once a part of its historical range, from which it has become extirpated or extinct (IUCN 1998). Reintroductions have become an important conservation technique to reestablish or augment a species in its native range, and at times are the only remaining method for conserving a species in the wild (Banks *et al.* 2002, Ebenhard 1995, Griffith *et al.* 1989, Sarrazin & Barbault 1996, Wolf *et al.* 1996). Reintroduced populations are often affected by a suite of interrelated demographic and genetic problems due to small founding size, isolation in fragmented habitats, and reduced genetic variation compared to source populations (Banks *et al.* 2002, Lacy 1987, Nei *et al.* 1975, Stockwell *et al.* 1996). Small founding size and reduced genetic variation can contribute to strengthened genetic drift and further loss of diversity, which in turn can lead to increased levels of homozygosity, and inbreeding (Nei *et al.* 1975, Stockwell *et al.* 1996). Such declines in diversity have been seen in the Scandinavian wolf, which saw a 30% reduction in heterozygosity after a bottleneck reduced the population to only 2 individuals (Vilá *et al.* 2002), and similarly in the Greater Prairie Chicken, in which a bottleneck caused a 39% reduction in allelic richness, and 10% reduction in heterozygosity compared to historic levels (Bouzat *et al.* 2009). In the long-term, losses of diversity can cause a population to enter an extinction vortex, in which inbreeding depression leads to reduced adaptive potential, increasing disease susceptibility and reduced ability to survive stochastic events (Lacy 1997).

Perhaps the best example of such genetic erosion is in the Florida panther, which after a severe bottleneck and years at small population size, displayed several signs of inbreeding depression, and increased susceptibility to disease, including cryptorchidism, reduced semen quality, cardiac defects, and a high accumulation of diverse pathological agents (Roelke *et al.* 1993). Similar declines in genetic diversity of reintroduced or bottlenecked populations have been observed in bighorn sheep (Berger 1990), Mexican wolves (Fredrickson *et al.* 2007), alpine ibex (Maudet *et al.* 2002), and wolves on Isle Royale (Wayne *et al.* 1991).

Close monitoring of short- and long-term genetic diversity, and viability of reintroduced populations, can allow managers to assess reintroduction success, detect losses of diversity, and take management action long before severe inbreeding, or population declines begin to occur. Demographic monitoring of populations to document reproduction, survival, and growth trends can be expensive and time consuming, which often results in the fate of reintroductions remaining unknown (Griffith *et al.* 1989, Sarrazin & Barbault 1996). In contrast, genetic monitoring is capable of measuring current and long-term changes in a suite of important population metrics at low cost and effort (Schwartz *et al.* 2007). Animals can be assigned individual genetic tags noninvasively through the collection of hair, feces, feathers, or other tissue types, which saves managers time and expenses, and also greatly reduces or even eliminates behavioral trap response by captured individuals. Once tagged, individuals and populations may be monitored for changes in abundance, genetic variation, geographic range shifts, migration, population structure, and dispersal, as well as survival, and reproductive output through parentage analysis (Schwartz *et al.* 2007). Genetic

monitoring is especially appealing for reintroductions, because animals are capable of being sampled prior to release, and the fates of reintroduced individuals can be tracked through the recapture or absence of that animal's genetic tag in future noninvasive sampling (Schwartz *et al.* 2007). In the long-term, genetic monitoring of reintroduced populations can detect changes in genetic variation, and provide an indication of genetic health decline long before a population is at risk of extinction.

Molecular markers and population viability analysis (PVA) are two methods that can be used to perform genetic monitoring of reintroduced populations to answer questions regarding genetic and demographic health. Reduced costs for analyzing molecular markers, and the prevalence of free PVA software have allowed for more extensive application of these methods by the scientific and management community. Neutral molecular markers, such as microsatellites, allow for the calculation of genetic health measures including allelic diversity, levels of heterozygosity, effective population size, and inbreeding. Molecular markers can also be used to detect population structure, estimate levels of dispersal, and measure fluctuations in population size (i.e. bottleck). In addition to molecular markers, PVA can be used to model long-term extinction risk of a population by incorporating demographic, genetic, and environmental stochasticity (Lacy 1993). The capability of PVA to simulate fluctuations in population connectivity and landscape structure, model occurrence of natural catastrophes, and investigate impacts in modification of harvest or logging levels makes PVA a powerful management tool (Bach *et al.* 2010, Lacy 1993, Shaffer 1990). However, the power and accuracy of PVA is directly related to the accuracy of input parameters, which must be gathered from previous studies for the species of interest, or ideally, obtained from the population being

modeled (Beissinger & Westphal 1998, Boyce 1992). Due to the large number of input parameters required for PVA modeling, it is important to investigate the sensitivity of simulations to uncertain parameters by testing a range of values. Through interpretation of model results and parameter sensitivity, PVA can be an effective tool for determining population viability, or at minimum, can serve as an aid to managers by identifying sensitive model parameters, and areas of research that require further study for improved accuracy. Together molecular markers and PVA can aid in estimation of short- and long-term stability of a population, and can serve as a guide for management to increase probability of reintroduction success.

The American marten (*Martes americana*) in Michigan have been historically extirpated and reintroduced, and are an example of populations that would benefit from genetic monitoring and PVA to aid managers in determining if the reintroduced populations require further management. Marten are carnivorous mammals native to the northern reaches of North America. The species is often considered a habitat specialist requiring late successional coniferous forests, total canopy closure greater than 50%, and course woody debris coverage ranging from 20-50% of ground surface cover (Allen 1982, Buskirk & Powell 1994, Poole *et al.* 2004, Thompson & Colgan 1994, Slauson *et al.* 2004). Large trees provide dens and resting sites (Hargis & McCullough 1984), while adequate canopy closure protects marten from aerial predators (Hargis & McCullough 1984), and coarse woody debris provides den habitat and easier access to prey during winter (Allen 1982, Bull & Heater 2000). The loss of these key habitat characteristics due to logging, fire, settlement, and unregulated trapping led to the extirpation of marten in

many parts of their native range, including the state of Michigan (Berg 1982, Hodgeman *et al*. 1994, Thompson 1994).

Marten once colonized Michigan as far south as Allegan County, but combinations of habitat loss and overharvest resulted in drastic reductions in their historical range (Williams *et al.* 2007). The species was believed to be extirpated from the state by the 1940s based on the diminution of sightings and incidental trappings (Manville 1948, Wood & Dice 1924). The last confirmed sighting of marten in the more populous Lower Peninsula (LP) occurred in 1911 near Lewiston in Montmorency County (Wood & Dice 1924). The less fragmented Upper Peninsula (UP) provided some refuge for the species until 1939, when the last confirmed sighting occurred in the Huron Mountains of Marquette County (Manville 1948). Years of implementing more conservative logging and land use practices resulted in regeneration of more continuous secondary forest and allowed reintroductions of marten to take place (Shands 1991, Williams *et al.* 2007). The marten was considered a desirable part of the ecosystem, and it was thought the reintroduction of the species would fill an ecological role as a mid-sized predator, that was lost with its extirpation (Berg 1982, Williams *et al.* 2007).

Marten were first released back into Michigan in the UP in 1955-57 in the Porcupine Mountains. Two marten (1 M, 1 F) were trapped in White River Country, Algoma District, Ontario, Canada in February of 1955 and released to Porcupine Mountains Wilderness State Park in Ontonagon County. Also released at this time were an additional two marten (1 M, 1 F) originally from British Columbia, Canada that were acquired from a local fur farm in Perkins, Michigan. Shortly after, the Porcupine Mountains received two marten (1 M, 1 F) from White River Country in March of 1955,

and 23 marten (15 M, 8 F) from Crown Chapleau Game Preserve, Ontario, from July, 1955 to April, 1957. The UP then received subsequent reintroductions including 99 marten (62 M, 37 F) from Port Arthur District, Ontario released to the western unit of the Hiawatha National Forest (HNF) in 1969-70, and 148 marten (77 M, 71 F) from Algonquin Provincial Park, Ontario released in Marquette, Baraga, and Iron Counties in 1979-81 (Williams *et al.* 2007). By the end of the 26-year period, the UP had received 276 marten (157 M, 119 F) from three regions of Ontario.

Following the reintroductions, the Michigan Department of Natural Resources (MDNR) and United States Forest Service (USFS) conducted subsequent translocations of marten to aid in long-distance dispersal of the species across its historical range in the region (Williams *et al.* 2007). In 1989, 20 marten were translocated by the USFS from the HNF west unit to the HNF east unit in the Tahquamenon Bay area (Williams *et al.* 2007). The HNF east unit received an additional 27 marten from Iron County during the winter of 1989-90 by the MDNR (Williams *et al.* 2007). The sex ratio of these two translocations to the eastern UP was unclear in available records, so it is difficult to determine if uneven sex ratio may have impacted the founding population's growth in this area. The final translocation in the UP took place in 1992, when the MDNR moved 19 marten (14 M, 5 F) from southern Houghton County to southeastern Keewenaw County.

The LP did not receive its first reintroduction of marten until 1985, after habitat in the LP succeeded to mixed coniferous and northern hardwood forest (Earle 1996, Williams *et al.* 2007). This forest composition was different than the conifer dominated habitat in the UP and traditional old-growth coniferous marten habitat, but was

considered acceptable for a reintroduction because it was thought the mixed habitat types would contribute to the understanding of marten preference and avoidance (Earle 1996; Williams *et al.* 2007). In 1985-86, 49 marten (25 M, 24 F) were reintroduced into the Pigeon River Country State Forest (PGR), and 36 marten (20 M, 16 F) were released into the Manistee National Forest (MNF) and Pere Marquette State Forest from Crown Chapleau Game Preserve (Williams *et al.* 2007). The MDNR originally planned to introduce 220 to 240 marten in the LP over the course of 2 to 3 years, with a goal of five to six releases spaced 32 to 64 km apart, in order to maintain genetic diversity through natural dispersal (Williams *et al.* 2007). However, when the MDNR sought to continue the reintroductions the Ontario Ministry of Natural Resources was concerned that removing 200 marten from any one area of the province could have negative effects on the remaining marten population in Ontario (Williams *et al.* 2007). As a result of this concern, there were no further reintroductions of marten to the LP, which left only the original 85 marten (45 M, 40 F) in two small pockets spaced over 160 km apart (Williams *et al.* 2007).

In 1999, marten were removed from Michigan's list of threatened species after 21 years of listing (Earle *et al.* 2001). This delisting, along with an increase in incidental captures and field sign allowed for the opening of a limited trapping season on marten in the UP in 2000 (Frawley 2002). Currently, state registered trappers may bag one marten/person/year from December 1-15. At the time of this study, members of Michigan Native American tribes included in the 2007 Inland Consent Decree (Bay Mills Indian Community, Grand Traverse Band of Ottawa and Chippewa Indians, Little River Band of Ottawa Indians, Little Traverse Bay Band of Odawa Indians, and Sault Ste. Marie Tribe

of Chippewa Indians) could bag three marten/member/year from October 1 to March 15. This limit was reduced in 2014 to two marten/member/year, with the same season length. Trapping of marten is currently banned in the LP.

Previous research investigating marten in the UP concluded that the methods used to reintroduce the species to the peninsula were successful in establishing populations, and in preventing bottlenecks across the peninsula (Swanson *et al.* 2006, Williams & Scribner 2010). Although past studies are in agreement that the reintroductions successfully established marten in the UP, there is disagreement regarding the number of source populations, and the level of genetic structure across the peninsula (Swanson & Kyle 2007, Williams & Scribner 2007). Swanson *et al.* (2006) found marten in the UP to function as a single genetically panmictic population distinct from one source population in Chapleau, Ontario, and from 24 additional Canadian populations. Williams and Scribner (2010) have disagreed with this finding, based on their research showing three significantly distinct genetic clusters (Porcupine Mountains, Huron Mountains, and Hiawatha populations), each of which was reflective of a different respective source population (Crown Chapleau Game Preserve, ON, Algonquin Provincial Park, ON, and undetermined, but potentially Colorado). Additionally, Swanson *et al.* (2006) found the UP marten population to have significantly higher allelic diversity than the source ($A$ = 7.4 in UP, $A$ = 5.8 in Chapleau, ON), whereas Williams and Scribner (2010) found the three genetic clusters to have equal to or significantly lower levels of diversity compared to multiple sources ($A$ = 4.2 to 5.0 in UP, $A$ = 5.0 to 5.4 in source populations). The confounding results of these studies highlight the importance of obtaining accurate

release histories for the study of reintroduced populations, and also promote further study in the UP to determine population genetic structuring across the peninsula.

Skalski *et al.* (2011) used harvest data and statistical population reconstruction to estimate marten abundance in the UP, and found a downward trend in abundance estimates from 2000 to 2007 (N = 1733.3 $\pm$ 861.3 SE in 2000, N = 1163.9 $\pm$ 520.1 SE in 2007). Skalski *et al.* (2011) concluded the level of harvest in the UP may be unsustainable, and changes to harvest strategies should be considered in order to maintain a harvest with a stable or increasing population. The studies of reintroduced marten in the UP emphasize the importance of obtaining accurate reintroduction histories, and the need for continued monitoring of reintroduced and harvested populations over the long-term. Additionally, if the UP were to serve as a source population for supplementing the LP marten populations, it must first be determined that it is genetically and demographically sound to do so.

Previous research on marten in Michigan's LP found the populations had expanded in range from reintroduction sites (Harden 1998), utilizing upland areas of secondary forest dominated by conifers or of a coniferous-deciduous mix (Buchanan 2008, Harden 1998, McFadden 2007), and with high levels of coarse woody debris (60 coarse woody debris poles ≥15 cm per hectare, Buchanan 2008). Genetically, marten in the LP have reduced allelic richness, but have maintained heterozygosity compared to the source population (Bicker 2007). Bicker (2007) found evidence of a bottleneck in both the PGR and MNF, but did not detect inbreeding in either area. Although inbreeding was not present at the time of study, Bicker (2007) emphasized inbreeding could be a threat to future population viability if diversity loss continued. Additionally, Nelson (2006) found

evidence of genetic divergence between the PGR, MNF, and the source population due to low levels of dispersal (2.6% per generation) between the LP populations. Nelson (2006) also cited small founding sizes, and low recruitment as potential contributing factors to genetic divergence in the LP. Together the studies on marten in the LP examined important habitat characteristics of marten, and highlighted early signs of genetic isolation and decline in the LP populations. Since these studies, no additional reintroductions or management have been implemented to mitigate further loss of genetic diversity, and it is unknown if genetic variation has continued to decline. Additional research on genetic health and long-term population viability may aid managers in determining if management actions are necessary to conserve marten in the LP.

In this study, I estimate the current genetic health, and long-term population viability of the marten populations in Michigan's LP and eastern UP. To do so, I used microsatellite markers to calculate a suite of genetic health parameters, and PVA to determine population viability over the next 100 years. The overall goal of my research is to determine if the reintroduced populations of marten in Michigan's LP are demographically and genetically viable or if further augmentation is necessary to ensure long-term population survival. I analyze the eastern UP marten population to determine if this area would be an adequate source population if a translocation to the LP were needed. Finally, I use my research to provide management recommendations for maintenance or increase of genetic diversity of marten populations in Michigan.

# Chapter II

## Methods

### Study area

My study area encompassed two locations in Michigan's LP (the MNF and the PGR), and one location in the eastern UP (Figure 1). The MNF and PGR are characterized by secondary forest up to 120 years old consisting of a mixture of coniferous species and northern hardwoods. Dominant tree species include red pine (*Pinus resinosa*), white pine (*P. strobus*), jack pine (*P. banksiana*), American beech (*Fagus grandifolia*), red maple (*Acer rubrum*), sugar maple (*A. saccharum*), red oak (*Quercus rubra*), white oak (*Q. alba*), and black oak (*Q. veluntina*). Elevation in the LP study area ranges from approximately 259 to 305 meters above sea level with some areas of rolling hills. Some fragmentation is present in the form of road networks varying in traffic volume from USFS service roads to highly traveled paved highways, and moderate levels of private inholdings. The eastern UP study area is also characterized by a mixture of coniferous and northern hardwood species, but is more heavily dominated by conifers. Dominant tree species in the UP include northern white cedar (*Thuja occidentalis*), white pine, red pine, tamarack (*Larix laricina*), balsam fir (*Abies balsamea*), sugar maple, red maple, and American beech. The UP study area is generally lower in elevation from approximately 207 to 274 meters above sea level and contains many wetland areas. Fragmentation is less severe in the UP, primarily present as USFS two-tracks, unpaved roads that are often limited to seasonal use due to snowfall, and few private inholdings.

## Sample collection

Sample collection began in 2004-06 by Dr. Bradley Swanson and graduate student Sara Bicker of Central Michigan University (CMU) in the PGR and MNF (Bicker 2007). Samples were collected from live captured individuals in the form of an ear tissue punch, and one sample of hair was collected non-invasively using hair snares (Bicker 2007). I received stock DNA samples from CMU in winter of 2012 for re-analysis using the same methodology I used for samples collected from the field in 2011-13. Detailed methodology on DNA extraction methods for the CMU samples can be reviewed in Bicker (2007).

Samples collected from 2011-2013 in the LP were primarily blood samples from marten that were live-trapped as part of a larger collaborative study on habitat characteristics, reproduction, dispersal, and health assessment of the MNF population. I performed trapping sessions in 2-3 week intervals beginning in May and July, 2011, and continued the following year in January, May, July, and December, 2012. In 2013, trapping sessions took place in May and opportunistically throughout the summer in an effort to capture juveniles and individuals needing radio collar replacement or removal. I also collected two additional tissue samples in the MNF, one taken from a road-killed marten in the study area, and a second from a juvenile mortality discovered while tracking a collared female.

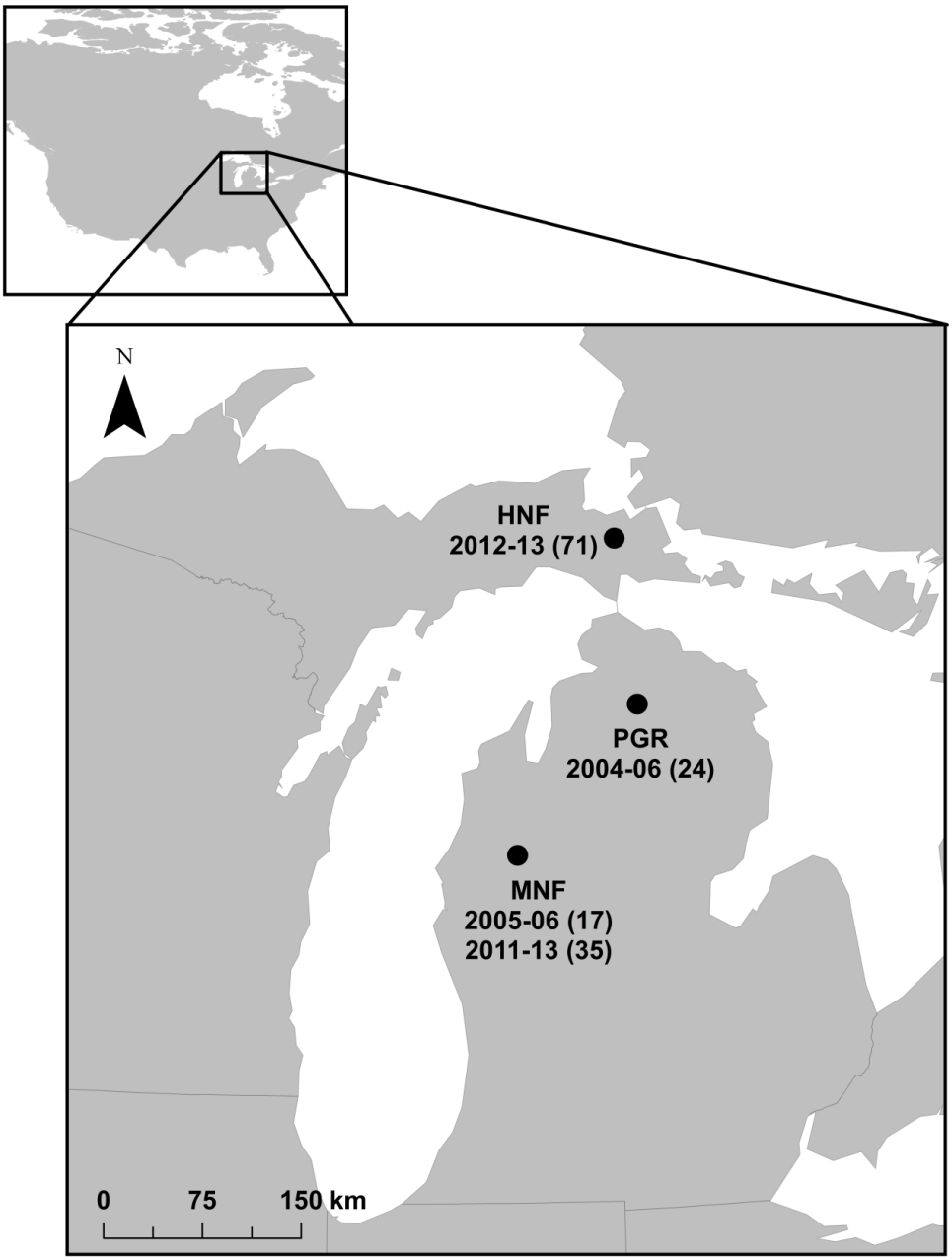


Figure 1. Map of Michigan displaying the reintroduction and sampling sites of American marten in this study. Sampling sites (black dots) are accompanied by the years of sample collection and sample size (in parentheses). HNF = Hiawatha National Forest in the eastern Upper Peninsula, PGR = Pigeon River Country State Forest in the northern Lower Peninsula, and MNF = Manistee National Forest in the west-central portion of the Lower Peninsula.

I collected samples from the eastern UP in the form of muscle tissue from harvested marten, or blood samples from live captured individuals. Muscle tissue (tongue, deltoid or abdominal) samples from the eastern UP were collected from marten carcasses that had been harvested by tribal trappers during the 2012-13 season. I collected blood samples from marten live captured in 2013 as part of a collaborative study by the Sault Ste. Marie Tribe of Chippewa Indians and GVSU to study marten ecology and estimate population size in the eastern UP.

I captured marten in the LP and UP from 2011-13 using collapsible and rigid, single-door live traps (Models 103-105 and 201, Tomahawk Live Trap, Hazelhurst, WI) baited with raw meat or smoked pork, and a long-distance commercial call lure (Caven's Gusto Lure, F&T Fur Harvester's Trading Post, Alpena, MI). I placed traps on the ground near coarse woody debris and covered traps in natural materials (leaves, bark, moss) to both disguise traps and to protect captured individuals from excessive heat or precipitation. During winter, I surrounded traps with straw, and sheltered traps with a plastic half-barrel to provide added insulation. I utilized the network of forest service roads to opportunistically place traps in a roughly uniform distribution throughout the study areas in locations I deemed suitable marten habitat. These areas were usually characterized by adequate mixed forest cover and often the presence of coarse woody debris. I checked traps daily between 0700 and 1200. Undisturbed traps were checked for bait and scent supply, and left open for the following morning. I released non-target captures immediately, and processed marten captures after the completion of trap checking in order to be aware of number and location of captured marten.

I processed marten captures in a mobile laboratory with the assistance of a certified wildlife veterinarian, Maria Spriggs DVM of Mesker Park Zoo and Botanic Garden (Evansville, IN). A denim cone attached to the door end of the trap allowed marten to voluntarily exit the trap, and funneled captured individuals towards a portable anesthesia machine (Model M3000, Parkland Scientific Inc., Coral Springs, FL) administering isofluorane inhalant anesthetic (Desmarchelier *et al.* 2007, IsoFlo®, Abbott Laboratories, Abbott Park, IL) delivered in oxygen. Upon anesthetization, I examined captured marten for overall physical health, performed sex, body length, and weight measurements, and inspected individuals for external parasites. I approximated age based on dental wear patterns, and collected blood (≤ 1 % body mass), feces, hair, urine, and ear tissue punches for use in genetic, disease, and overall health assessments. All marten captures were implanted with a passive integrated transponder tag (PIT, AVID, Norco, CA), and marten of sufficient size (≥ 500 g) were fitted with radio collars (either ≤ 25 g VHF or ≤ 50 g GPS collar; Holohil Systems Ltd., Carp, Ontario, Canada or Advanced Telemetry Systems, Isanti, MN). Upon completion of data collection I placed marten in a wood, towel lined box until fully recovered (5-10 minutes). Processing lasted 30 minutes or less from anesthetization to release, during which I monitored marten closely for body temperature, heart and respiration rate. I stored blood and tissue samples at -20$^{o}$ to -80$^{o}$ C until time of DNA extraction. All methods followed guidelines set by the American Society of Mammalogists (Sikes & Gannon 2011), and were approved by the GVSU Institutional Animal Care and Use Committee (protocol 12-05-A).

## Laboratory methods

I extracted DNA from 80 to 100 μL of blood, and 15 to 25 mg of tissue using QIAGEN DNeasy Blood and Tissue Kits (QIAGEN Sciences, MD) following the bench protocol for animal blood or tissues. I performed final elution twice for each sample as described in the bench protocol to increase final DNA concentrations. Following final elution, I ran samples on a 1% agarose gel to test for extraction success. I diluted stock DNA samples to 10% working solutions assuming clear band illumination on the extraction gel. Samples appearing clear but faint were diluted to 50%, and very faint samples were left undiluted.

I amplified DNA at eleven microsatellite loci: Ma-1, Ma-2, Ma-3, Ma-5, Ma-7, Ma-10, Ma-11, Ma-15, Gg-3, Gg-7 (Davis and Strobeck 1998), and Mvis072 (Fleming *et al.* 1999) using polymerase chain reaction (PCR) in 20-μL reactions consisting of 10x Colorless Go Taq reaction buffer (1.5 mM $MgCl_2$), 0.25 mM dNTP mix, 0.1 μM forward and reverse primers, 0.1 μM M13 primer labeled with FAM, VIC, NED, or PET dyes, 0.3 units of Go Taq DNA polymerase, and 3 μL of genomic DNA. I used a touchdown thermal protocol for PCR to increase sensitivity and specificity of primer amplification (Korbie & Mattick 2008). My thermal protocol consisted of 95 $^{o}$C for 4 min, followed by 20 cycles of 95 $^{o}$C for 30 s, 60 $^{o}$C for 30 s (decreasing 0.5 $^{o}$C each cycle), and 72 $^{o}$C for 30 s, then followed by 36 cycles of 95 $^{o}$C for 30 s, 50 $^{o}$C for 30 s, and 72 $^{o}$C for 30 s, ending with an extension of 72 $^{o}$C for 7 min. I visualized PCR products on a 2% agarose gel to test for amplification success before sending samples for fragment analysis on a 3130 xl Genetic Analyzer (Applied Biosystems Inc.) by the Annis Water Resources Institute (Muskegon, MI). I scored alleles using the program Peak Scanner (v1.0,

Applied Biosystems, Inc.) to assign genotypes to individuals at all loci. I randomly selected ten percent (10%) of all samples for re-scoring to calculate scoring error. Any errors discovered during scoring error calculation were corrected before final genetic diversity analyses.

## Genetic diversity analysis

I analyzed each of the sampling locations (MNF, PGR, eastern UP) and periods (MNF in 2005-08 and MNF in 2011-13) separately to observe presence or absence of population structure or potential loss or gain of genetic variation. I was particularly interested in comparing the genetic diversity of the two sampling periods in the MNF for signs of diversity loss or gain. I was also interested in measuring levels of population structure to examine how presence of fragmentation between sampling locations may be affecting dispersal rates.

I tested for deviations from Hardy-Weinberg equilibrium (HWE) using the program GenAlEx 6.5 (Peakall & Smouse 2006). I assessed genetic variation using observed heterozygosity ($H_O$) and expected heterozygosity ($H_E$), calculated in GenAlEx, as well Wright's inbreeding coefficient ($F_{IS}$) and allelic richness (A), calculated in FSTAT 2.9.3 (Goudet 1995). I chose allelic richness for comparison of genetic diversity over raw number of alleles for its ability to correct for sample size differences between sampling areas (El Mousadik & Petit 1996). I tested for differences in genetic diversity between sampling areas and periods using Friedman tests conducted in Program R (v.3.0.0, R Development Core Team 2005). I examined significant Friedman tests using Wilcoxon signed-rank tests for pairwise comparisons. Wilcoxon signed-rank tests were

also used for comparisons of observed and expected heterozygosity to determine if sampling areas expressed significant reduction in heterozygosity. I evaluated genetic structure of populations using $F_{ST}$ calculated with an Analysis of Molecular Variance (AMOVA), and I also measured the number of private alleles in each population using GenAlEx. All test statistics were assessed at an alpha level of 0.05.

I sought to estimate effective population size in order to determine if population size may be playing a role in genetic diversity loss or gain between sampling periods or areas. Effective population size represents the ideal population size that would experience the same level of genetic drift that is observed in the population of interest (Allendorf *et al.* 2013). For species with overlapping generations, such as marten, effective population size estimates functionally represent the effective number of breeders ($N_b$, Allendorf *et al.* 2013), and I chose to estimate $N_b$ to give an indication of the number of individuals contributing genes to the population. I also used my $N_b$ estimates to calculate a range of possible census population size estimates based on the finding of Frankham (1995), who found effective population size estimates are typically 10% to 50% of the adult census population size.

I estimated the effective number of breeders for each sampling area and period using Bayesian and linkage disequilibrium methods. I also used the temporal method for the MNF marten population, for which two sets of samples separated by greater than one generation (4-6 years) were available. Marten generation time was estimated as the average age of breeding individuals based on marten life history characteristics (Clark *et al.* 1989). I chose the approximate Bayesian computation estimator ONeSAMP (Tallmon *et al.* 2008) based on its ability to be robust even with small sample size and low levels of

polymorphic data (Beebee 2009), which I expected due to the elusive behavior of marten, and small founding sizes in the LP. I also calculated estimates of effective breeding size using the bias-corrected version based on linkage disequilibrium (Hill 1981, Waples 2006, Waples & Do 2010), and the temporal method (Pollak 1983, Waples 1989) using the program NeEstimator V2.01 (Do *et al.* 2014). For the temporal method I set the MNF (2005-06) samples as generation zero, and the MNF (2011-13) samples as generation 1.3, based on the length of time between these sampling periods (5-8 years), and marten generation time (4-6 years). I calculated multiple estimates for effective population size in order to be cautious of estimator tendencies to produce large or infinite confidence intervals when tested using small sample sizes (Beebee 2009). I rounded all $N_b$ estimates up to the nearest whole number to represent the number of complete individuals as fractions of individuals would be inappropriate for this parameter.

## Population viability analysis

I used VORTEX v. 10.0 (Lacy 1993) to model genetic and demographic viability of marten in the MNF over the next 100 years. I chose to model viability of only the most recent MNF population data (2011-13) after tests of genetic differentiation ($F_{ST}$) displayed significant differences between the older MNF samples (2005-06), and the most recent MNF samples (2011-13, see Results). I obtained general life history characteristics from past studies of marten ecology, and only modified characteristics if I directly observed different values during the course of this study (i.e. litter sizes, Table 1). I maintained general life history characteristics for all model simulations, for which I only modified the parameter of interest (see below sections). I ran each model for 1000

iterations, and I defined extinction as only one sex remaining. I considered populations to be demographically viable if fewer than 10% of simulated populations went extinct within the 100 years modeled (probability of survival > 0.90), and I considered populations genetically viable if less than 10% of genetic diversity was lost over the 100 year period (Soulé *et al.* 1986). Genetic diversity in VORTEX was measured using the mean proportion of expected heterozygosity remaining from year 0 to year 100 of the simulations.

I set the breeding system of marten to be polygynous based on past observations of breeding male home ranges encompassing on average three female home ranges (Clark *et al.* 1989). I followed the assumption of Lacy (1993) that males will on average successfully breed with three females, and thus males would sire approximately three litters each year. In order for males to sire, on average, three litters per year without requiring a highly female-biased sex ratio, I excluded a portion of males from the breeding population, as would happen naturally through male territorial interactions (Hawley & Newby 1957). To do this, I used a Poisson distribution of reproductive success calculated by the VORTEX program, to estimate that approximately 63% of males should be included in the breeding population (37% male exclusion, Lacy 1993). It should also be noted that marten may be capable of multiple paternity through female superfetation, as has been observed in free-ranging mink (Yamaguchi *et al.* 2004), and the stoat (Holland & Gleeson 2005). Because female marten have been observed to have two or more periods of estrus separated by 1 to 2 weeks (Ashbrook & Hanson 1930), and have been noted to mate with multiple males (Markley & Bassett 1942), it is possible ≥2 males may fertilize ova produced in different ovulations in a single female

(superfetation). If this does occur in marten, males would be capable of siring three litters through either mating with three females that subsequently do not mate with any other males, or through the siring of partial litters of >3 females.

In order to determine how the percentage of males breeding may affect population viability, I increased the percentage of males in the breeding pool to 100%, and also decreased this parameter to 50% of males breeding. I found that percentage of males breeding had little effect on genetic or demographic population viability, so I retained the original value of 63% for all further analyses.

Inbreeding & genetic parameters

I used the default value for inbreeding depression of 6.29 lethal equivalents based on O'Grady *et al.* (2006). To accurately model the Michigan populations from their current state, I included starting allele frequencies, and set the starting inbreeding level equal to the inbreeding coefficient ($F_{IS}$) calculated using FSTAT.

Initial population size & carrying capacity

Lacking accurate estimates of current population size and carrying capacity of the surrounding suitable marten habitat, I modeled the sensitivity of population viability to initial population size and carrying capacity. I tested initial population size values of N = 50 and N = 100, based loosely on capture data obtained over the course of this study. At the time of population viability modeling, I did not have an estimate of population size calculated from mark-recapture data, and thus based initial population sizes on the minimum number of marten I knew to be alive based on live capture data. I tested a wide

range of carrying capacity values (K = 50, K = 100, K = 250, K = 500, K = 1000) because although the national forests within which the marten populations of Michigan reside potentially encompass hundreds of thousands of acres of habitat, it was unknown at the time of this study how the level of patch connectivity or how moderate levels of fragmentation such as roads may affect marten movement or habitat selection.

Table 1. Input parameters used in VORTEX for American marten in Michigan. Life history characteristics were the same in all models.

| Parameter | Input value | Reference |
|---|---|---|
| Female age of first reproduction | 2 yrs | Lacy 1993, Strickland *et al.* 1982 |
| Male age of first reproduction | 3 yrs* | Lacy 1993 |
| Maximum age of reproduction | 12 yrs | Strickland & Douglas 1987 |
| Frequency distribution of litter sizes† | 1 kit: 10%<br>2 kits: 30%<br>3 kits: 55%<br>4 kits: 5% | Lacy 1993, Ongoing study of Michigan populations |
| Sex ratio at birth | 1:1 | Clark *et al.* 1987 |
| Female mortality | 1st year: 50%<br>1+ annual: 10% | Lacy 1993, Strickland *et al.* 1982 |
| Male mortality | 1st year: 50%<br>2nd year: 25%<br>2+ annual: 10% | Lacy 1993, Strickland *et al.* 1982 |

*Although both sexes mature as yearlings, it was assumed that most males do not successfully breed until their second year, resulting in first offspring born at about 3 years (Lacy 1993).
† Research on marten reproduction was being performed concurrently as this study, but sample sizes were not yet adequate for modeling strictly Michigan data. Values from Lacy (1993) included litter sizes up to 3 kits, but during the course of the reproduction study two cases of 4 kits were observed. As a result, values from Lacy (1993) were modified to include a litter size of 4 kits

Density dependence

I modeled three levels of density dependent population growth. Under mild density dependence, 90% of females bred at low density (P(0) = 90), 70% of females bred at carrying capacity (P(K) = 70), and Allee effect was minor (A = 1). Under moderate density dependence, 85% of females bred at low density (P(0) = 85), 50% of females bred at carrying capacity (P(K) = 50), and Allee effect was moderate (A = 2). Under strong density dependence, 80% of females bred at low density (P(0) = 80), only 30% of females bred at carrying capacity (P(K) = 30), and Allee effect was high (A = 4).

Percentage of females reproducing

To determine how the percentage of females successfully producing offspring would affect population viability, I modified the brood distribution parameter in

VORTEX. Because female marten are capable of producing only one litter per year, I modified the brood distribution parameter by varying the percentage of breeding age females producing one litter per year, and the percentage of breeding age females producing no litters. I varied the percentage of females successfully producing one litter per year from 50% to 100% in 10% increments. To account for the remaining females of breeding age, I varied the percentage of females producing no litters from 0% to 50% in 10% increments (i.e. models with 80% of females producing one litter had 20% of females producing no litters to account for 100% of breeding age females in the population).

Catastrophes

Reintroductions may result in large losses of genetic variation if founding sizes are small and populations do not rapidly expand following release (Lacy 1997, Stockwell *et al.* 1996). In the long-term, low diversity may lead to reduced adaptive potential, making populations susceptible to stochastic events. In an effort to gauge how robust the Michigan marten populations would be to such stochastic events, and to estimate long-term adaptive potential, I modeled two types of catastrophes—disease and fire.

To model disease I sought to mimic sustained or increased prevalence of two diseases known to already be present in the Michigan marten populations. At the time of this study, approximately 10% of Michigan marten sampled displayed exposure to canine distemper virus (CDV), and approximately 50% exposure to toxoplasmosis (Maria Spriggs DVM, personal communication). CDV has been documented in a large number of mustelid species and is often responsible for causing drastic declines, as has been seen

in captive and free-ranging populations of the highly endangered black-footed ferret (*Mustela nigripes*, Deem *et al.* 2000, Williams *et al.* 1988). Drastic declines in mustelid populations exposed to CDV result from the family's high susceptibility to the virus, with fatality rates close to 100% (Davidson 1986, Deem *et al.* 2000). CDV survival rates of the American marten species specifically are not well known, but it is likely the species is highly susceptible to infection, and it is probable that the current 10% prevalence of CDV seen in my samples underestimates the level of infection in the population, as many individuals may die from infection (Maria Spriggs DVM, person. comm.). To be conservative, I tested CDV in VORTEX at three levels of survival reduction: 50%, 70%, and 90% reduction in survival, with no effect on reproduction, and a 3% probability of occurrence per year. I based probability of occurrence on the review by Reed *et al.* (2003) indicating the frequency of catastrophe occurrence in vertebrates averages 14% per generation, which for marten (4-6 year generation time) equated to approximately 3% probability of occurrence per year.

I modeled toxoplasmosis because of its relatively common prevalence in our populations and due to its potentially negative impact on female reproductive success. Young female marten previously unexposed to toxoplasmosis may abort or have reduced litter sizes if exposed to toxoplasmosis during pregnancy (Maria Spriggs DVM, person. comm.). To model sustained toxoplasmosis exposure I reduced reproduction by 10%, with no effect on survival, and 3% probability of occurrence. To model increased toxoplasmosis exposure I reduced reproduction by 10%, with no effect on survival, and increased probability of occurrence to 6%.

I modeled fire as a second type of environmental stochasticity that could impact survival and reproduction through both direct mortality and sustained impacts from habitat and resource loss. Although fire occurrence may be rare in Michigan, I sought to model a catastrophe type that would impact both reproduction and survival. Furthermore, the simulation of fire in VORTEX may be likened to the effects experienced by wild populations undergoing range constriction due to habitat loss from settlement. I modeled fire as a reduction in survival by 30%, and a reduction in reproduction by 10%, with a 3% probability of occurrence.

Lastly, I modeled canine distemper, toxoplasmosis, and fire together to represent a naturally existing population in which exposure to multiple environmental risks could occur. For this model I reduced survival due to distemper by 50%, with no impact on reproduction and a 3% probability of occurrence, I modeled toxoplasmosis by reducing reproduction by 10%, with no impact on survival, and a 3% probability of occurrence, and maintained the fire parameters of 30% reduction in survival, 10% reduction in reproduction, and 3% probability of occurrence.

Supplementation

I modeled a translocation of marten into the MNF to determine how an influx of new individuals could impact long-term demographic and genetic viability of the reintroduced population. VORTEX assumes translocated individuals are unrelated to one another, and unrelated to individuals in the release population of interest, so a translocation modeled with VORTEX is likely to estimate the highest possible increase in genetic viability. In an effort to represent a translocation I believed possible by managers

within the next ten years, I modeled introductions using 20 to 40 marten released over two consecutive years or every other year. Specifically, I modeled an introduction of 20 marten: releasing 10 marten per year at years 5 and 6 of the simulation, a release of 40 marten total: 20 released per year at years 5 and 6 of the simulation, an introduction of 20 marten: releasing 10 marten per year at years 5 and 7 of the simulation, and finally a release of 40 marten: 20 released per year at years 5 and 7 of the simulation. I modeled translocated individuals as adults only with a sex ratio of one male to 1.5 females. I chose to model translocations with a female biased sex ratio because male marten typically mate with multiple females (Clark *et al.* 1989), and a reintroduction of equal sex ratio could result in unsuccessful male breeders failing to provide an influx of genetic material to the population. I also considered an introduction of more females to be advantageous, for the possibility of increasing release sizes indirectly through the introduction of pregnant females (Slough 1994). Overall, I thought that by using a female biased sex ratio, managers would be more likely to release individuals that would successfully contribute new genetic information to the receiving population, while also reducing the number of individuals (likely males) that would fail to breed, or who could contribute territorial conflict with resident marten.

# Chapter III

## Results

In total, I analyzed 147 (51 M, 36 F, 60 unk) marten samples in this study (Appendix A). I collected 35 (20 M, 14 F, 1 unk) marten samples from the MNF in 2011-13, and 71 (6 M, 8 F, 57 unk) samples from the eastern UP in 2012-13. I re-analyzed 17 (10 M, 6 F, 1 unk) samples from the MNF collected by CMU in 2005-06, and 24 (15M, 8 F, 1 unk) samples from the PGR collected by CMU in 2004-06. Scoring error rate for all samples over the 11 loci was 2.4%.

### Genetic diversity & effective breeding size

I detected three of 11 loci in the earlier MNF sample (2005-06) to be out of HWE (Ma-10, Ma-11, Gg-3), I detected seven loci out of equilibrium in the MNF in 2011-13 (Ma-1, Ma-2, Ma-5, Ma-7, Ma-11, Ma-15, Mvis-072), two loci out of equilibrium in the PGR (Ma-1, Ma-10), and five loci out of equilibrium in the eastern UP (Ma-1, Ma-5, Ma-10, Ma-15, Mvis-072). I retained all loci in subsequent analyses with the exception of Ma-3 in the MNF (2005-06) and eastern UP due to being monomorphic. Deviations from HWE suggest the populations are experiencing non-random mating, increased levels of inbreeding, or contain underlying genetic structure (Wahlund effect). This finding is not surprising considering the reintroductions for all areas were under the recommended size of 50 individuals for combating negative genetic effects in reintroduced populations (Slough 1994). It is most likely that small founding size, and the isolation of the LP

populations, has resulted in increased genetic drift and inbreeding, which over the long-term has caused the populations to drift out of HWE.

I did not observe any significant differences in genetic diversity measures (A, $H_O$, and $F_{IS}$) between sampling areas or periods, indicating that all Michigan marten populations are similar in genetic diversity levels, and that the MNF has not experienced significant changes in genetic diversity between sampling periods (Friedman, d.f. = 3, $\chi^2$ = 7.514, $P$ = 0.057 for A; $\chi^2$ = 3.165, $P$ = 0.367 for $H_O$; and $\chi^2$ = 7.200, $P$ = 0.066 for $F_{IS}$). Mean allelic richness, adjusted to a minimum sample size of 17 individuals, ranged from 3.585 (0.440 SE) in the early MNF sample (2005-06) to 4.344 (0.474 SE) in the most recent MNF sample (2011-13), observed heterozygosity ranged from 0.494 (0.068 SE) in the most recent MNF sample (2011-13) to 0.576 (0.074) in the early MNF sample (2005-06), and $F_{IS}$ ranged from 0.016 in the early MNF sample (2005-06) to 0.238 in the most recent MNF sample (2011-13, Table 2). The PGR and eastern UP samples fell in between the early and most recent MNF samples for allelic richness, observed heterozygosity, and $F_{IS}$. Although no significant differences were detected between the MNF sampling periods, I did observe the greatest differences in diversity measures between these samples. I observed a trend of allelic richness increase, which indicates an increase in genetic diversity. However, this result was contrasted with a decreasing trend in observed heterozygosity, and an increase in inbreeding coefficient, and loci out of HWE. I also observed significant population structure between the MNF sampling periods ($F_{ST}$ = 0.068, $P$ = 0.001). These trends indicate the MNF population is undergoing changes in genetic diversity, which may be a result of genetic drift on a small population in a fragmented habitat.

I observed significant differences between observed and expected heterozygosity in the MNF (2011-13) and the eastern UP, indicating significant heterozygote deficiency (homozygote excess) in these sampling areas (Wilcoxon signed-rank, $P = 0.014$ for MNF 2011-13, and $P = 0.020$ for the eastern UP). The MNF (2005-06) and PGR sampling areas did not display significant differences between observed and expected heterozygosity, indicating these sampling areas do not express heterozygote deficiency (Wilcoxon signed-rank, $P > 0.05$). I observed the highest number of private alleles in the eastern UP (10 private alleles over 6 loci), followed by the most recent MNF samples (2011-13, 9 private alleles over 6 loci), and the PGR (4 private alleles over 3 loci). The samples collected from the MNF in 2005-06 did not contain any private alleles. The large discrepancy in the number of private alleles between the MNF sampling periods is most likely an effect of unequal sample size, and less likely an indication of very high mutation or dispersal rates. I collected and analyzed two-times the number of samples in 2011-13 than in 2005-06, which most likely resulted in a failure to detect alleles in the small sample, that were later detected with the larger sample size, and it is less likely the MNF population is experiencing high mutation or dispersal rates. The private alleles I observed in the eastern UP are likely a result of different original source populations, and very low to zero admixture with marten residing in the LP, due to fragmentation and peninsular geography blocking dispersal routes.

The effective breeding size estimates that I calculated using the Bayesian estimator ONeSAMP ranged from 17 (95% CI: 14-22) in the earlier MNF sampling period (2005-06) to 91 (95% CI: 60-206) in the eastern UP (Table 2). I observed lower $N_b$ estimates using the linkage disequilibrium method, ranging from 10 (95% CI: 4-24) in

the MNF (2005-06) to 17 (95% CI: 10-34) in the PGR sampling area. The $N_b$ estimate for the eastern UP using the linkage disequilibrium method was much lower at only 13 (95% CI: 11-16) individuals, compared to the Bayesian estimate of 91 (95% CI: 60-205). Finally, I utilized the two sampling periods in the MNF to calculate an additional $N_b$ estimate using the temporal method, which resulted in the lowest $N_b$ estimate at only 6 (95% CI: 4-11) individuals (Table 2). Overall, I observed low $N_b$ estimates for marten populations in Michigan, with all LP estimates falling below 50 and the UP estimates falling below 100 individuals.

I utilized the $N_b$ estimates calculated with the Bayesian estimator ONeSAMP to estimate census population sizes ($N_c$), based on the finding of Frankham (1995), who found effective population size estimates are typically 10% to 50% of the adult census population size. Using this method, $N_c$ estimates were 34-170 (95% CI: 28-220) for the MNF (2005-06), 54-270 (95% CI: 44-420) for the MNF (2011-13), 46-230 (95% CI: 38-320) for the PGR, and 182-910 (95% CI: 120-2060) for the eastern UP. All of the $N_c$ estimates for the LP fell below 500 individuals, and the UP estimate was below 1000 individuals, indicating the LP populations in particular are likely of small census population size, which may be amplifying the effects of genetic drift and diversity loss.

Table 2. Summary of genetic diversity measures for reintroduced American marten populations in Michigan based on 11 microsatellite loci. Samples were obtained from the Manistee National Forest (MNF), the Pigeon River Country State Forest (PGR), and the eastern Upper Peninsula (UP), with years of sampling indicated in parentheses. Sample size of each sampling area (N), number of alleles (alleles), allelic richness (A) with standard error (SE), number of loci (of 11) in Hardy-Weinberg Equilibrium (HWE), observed heterozygosity ($H_O$) with SE, expected heterozygosity ($H_E$) with SE, Wright's inbreeding coefficient ($F_{IS}$), number of private alleles, and effective breeding size ($N_b$) estimates with 95% confidence intervals. Bolded heterozygosity values indicate significant reduction in $H_O$ compared to $H_E$ at $P < 0.05$. Comparison tests between sampling areas for A, $H_O$, and $F_{IS}$ were non-significant ($P > 0.05$).

| | | | | | | | | | $N_b$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sampling area (Years sampled) | N | Alleles | A (SE) | HWE | $H_O$ (SE) | $H_E$ (SE) | $F_{IS}$ | Private Alleles | Bayesian* | LD† | Temporal‡ |
| MNF (2005-06) | 17 | 3.727 | 3.585 (0.440) | 7 | 0.576 (0.074) | 0.565 (0.066) | 0.016 | 0 | 17 (14-22) | 10 (4-24) | 6 (4-11) |
| MNF (2011-13) | 35 | 5.364 | 4.344 (0.474) | 4 | **0.494 (0.068)** | **0.636 (0.066)** | 0.238 | 9 | 27 (22-42) | 13 (10-18) | 6 (4-11) |
| PGR (2004-06) | 24 | 4.091 | 3.842 (0.368) | 9 | 0.561 (0.070) | 0.585 (0.058) | 0.063 | 4 | 23 (19-32) | 17 (10-34) | - |
| UP (2012-13) | 71 | 5.091 | 4.138 (0.491) | 4 | **0.517 (0.078)** | **0.598 (0.072)** | 0.142 | 10 | 91 (60-206) | 13 (10-16) | - |

*Bayesian estimator ONeSAMP (Tallmon et al. 2008)

†LD = Linkage Disequilibrium method, bias-corrected version (Hill 1981, Waples 2006, Waples & Do 2010), estimated using program NeEstimator V2.01 (Do et al. 2014).

‡Temporal method (Pollak 1983, Waples 1989), generations set to 0 and 1.3, estimated using program NeEstimator V2.01.

Table 3. Pairwise $F_{ST}$ values between sampling areas of reintroduced American marten in Michigan's Lower Peninsula and eastern Upper Peninsula. Lower half of matrix displays $F_{ST}$ values, upper half of matrix displays *P*-values, calculated using an Analysis of Molecular Variance (AMOVA). MNF = Manistee National Forest (sampled in 2005-06 and again in 2011-13), PGR = Pigeon River Country State Forest (sampled in 2004-06), and UP = eastern Upper Peninsula (sampled in 2012-13).

| | MNF (2005-06) | MNF (2011-13) | PGR (2004-06) | UP (2012-13) |
|---|---|---|---|---|
| MNF (2005-06) | - | 0.001 | 0.001 | 0.001 |
| MNF (2011-13) | 0.068 | - | 0.001 | 0.001 |
| PGR (2004-06) | 0.043 | 0.093 | - | 0.001 |
| UP (2012-13) | 0.051 | 0.104 | 0.070 | - |

## Population viability

### Initial population size & carrying capacity

I found all initial population size and carrying capacity model variations to be demographically viable over the next 100 years (Figure 3A). Models that I tested with larger carrying capacities retained a greater percentage of genetic diversity, while populations with carrying capacities of 100 or less were not genetically viable (Figure 3B). Populations with carrying capacities larger than 100 lost only 1.74% to 6.49% of heterozygosity, whereas populations with carrying capacities of 100 or less lost 15.51% to 31.32% of heterozygosity from year 0 to 100 (Figure 3B). In general, I noticed that populations with carrying capacities greater than 100 individuals increased in size quickly from initial size to carrying capacity, which mitigated genetic diversity loss (Figure 3C).

### Density dependence

I found that increasing strength of density dependence had little effect on population viability. Mild and moderate levels of density dependence had nearly identical results both demographically and genetically, each maintaining demographic viability, but losing approximately 16% of heterozygosity over 100 years (Figure 4). Strong density dependence resulted in a greater loss of genetic diversity (19.46% loss), and although 8 of the 1000 simulated populations went extinct with strong density dependence this was not enough to cause populations to dip below the demographic viability threshold (Figure 4).

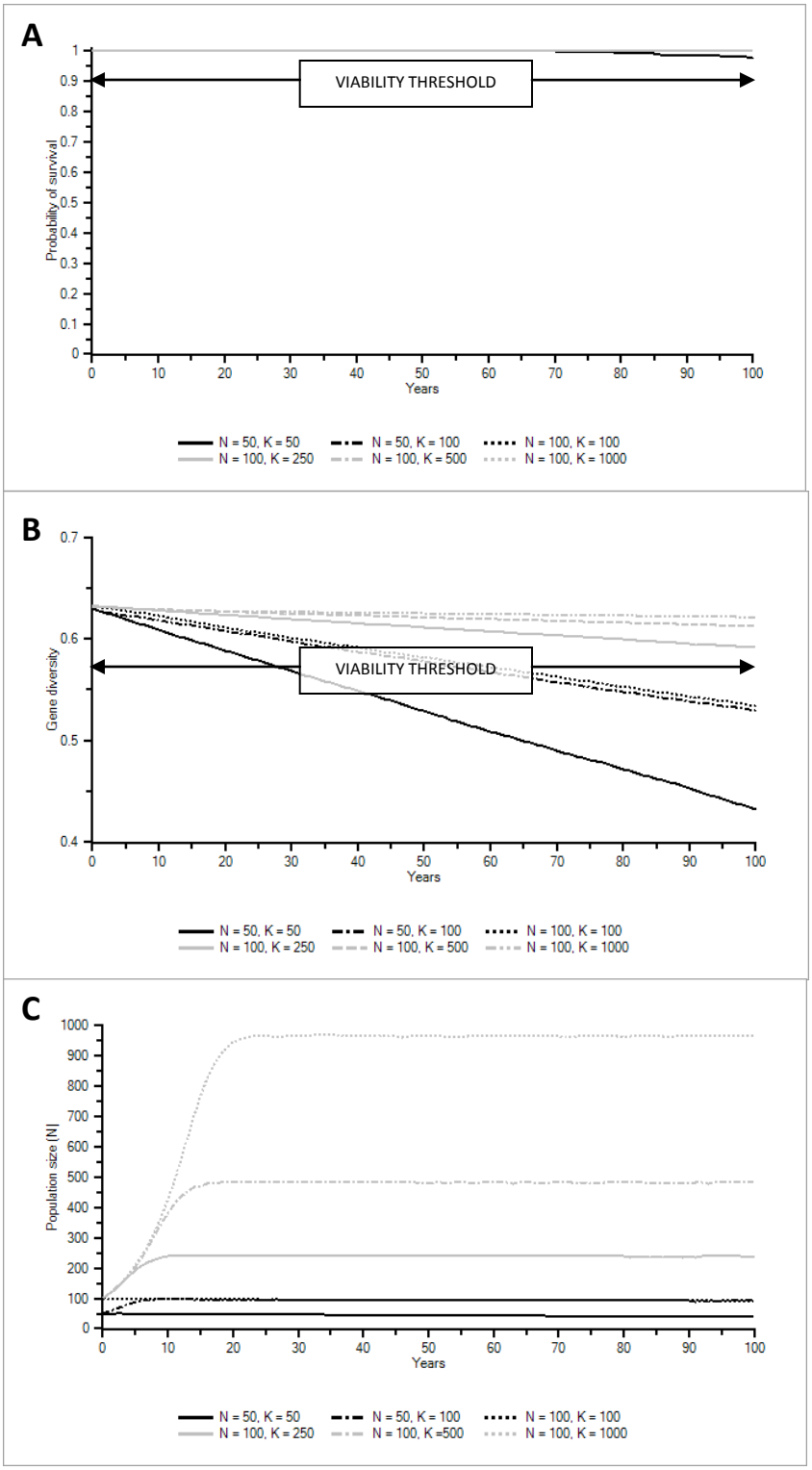


Figure 2. Predicted (A) probability of survival, (B) genetic diversity (heterozygosity), and (C) population size for American marten in Michigan's Manistee National Forest over 100 years with differing levels of initial population size (N) and carrying capacity (K). Simulations were run for 1000 iterations. Viability threshold indicates the critical level below which populations lose long-term viability (demographic viability = fewer than 10% of populations go extinct, genetic viability = less than 10% heterozygosity loss).

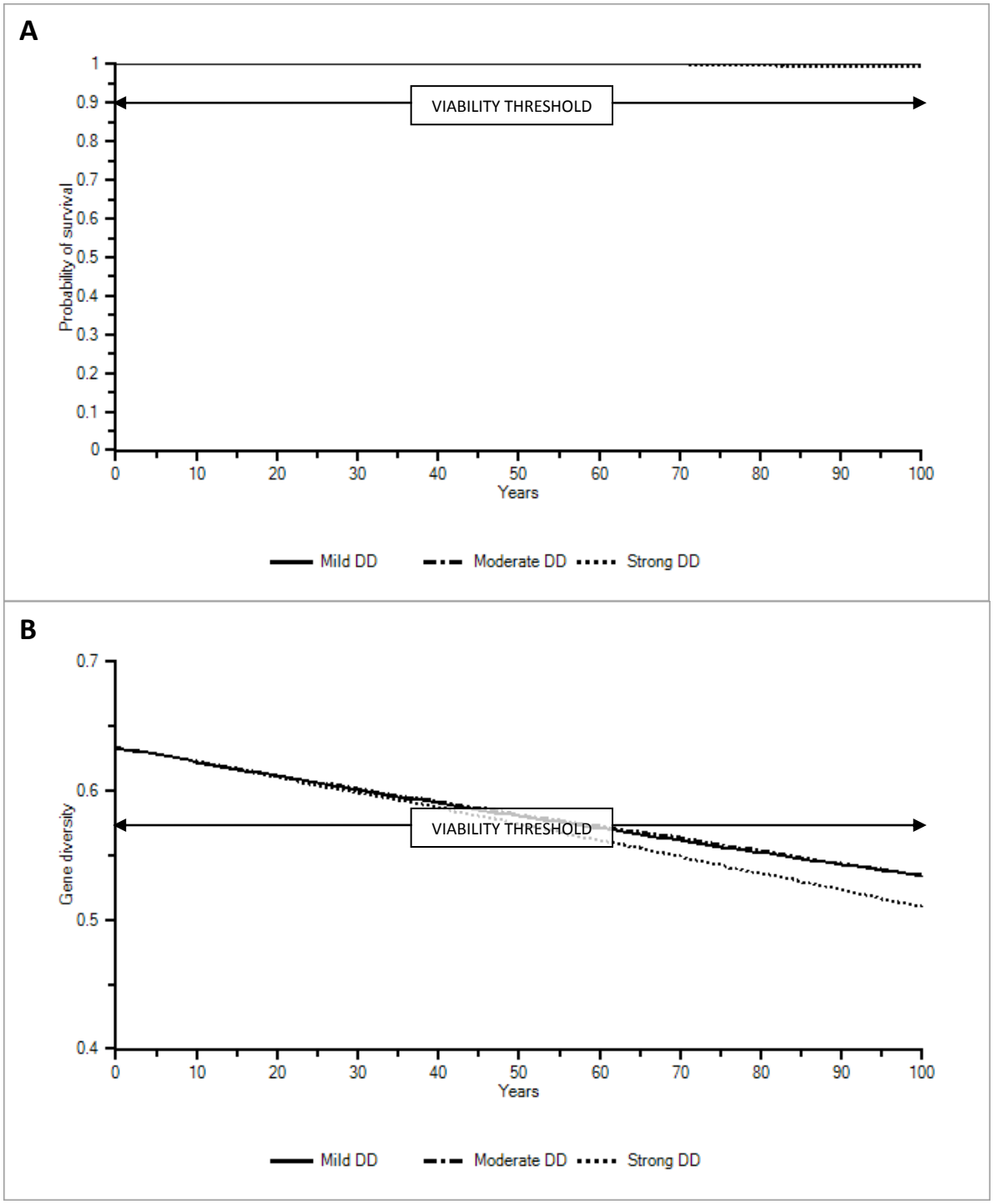


Figure 3. Predicted (A) probability of survival and (B) genetic diversity (heterozygosity) for American marten in Michigan's Manistee National Forest over 100 years with different levels of density dependence (DD). Mild: P(0) = 90, P(K) = 70, A = 1, Moderate: P(0) = 85, P(K) = 50, A =2, Strong: P(0) = 80, P(K) = 30, A =4. Where P(0) indicates the percentage of females breeding at low density, P(K) indicates the percentage of females breeding near carrying capacity, and A indicates the strength of the Allee effect on locating mates. Simulations were run for 1000 iterations. Viability threshold indicates the critical level below which populations lose long-term viability (demographic viability = fewer than 10% of populations go extinct, genetic viability = less than 10% heterozygosity loss).

Percentage of females reproducing

I found that decreasing the percentage of females producing litters had a strong effect on both demographic and genetic viability of marten populations. Populations in which I modeled ≥70% of females producing litters maintained demographic viability (Figure 5A), but even 100% of females reproducing was not sufficient to maintain genetic diversity over 100 years (Figure 5B). Heterozygosity loss ranged from 15.48% to 18.51% in simulations modeling 100%, 80%, and 70% of females reproducing.

Populations that I modeled with fewer than 70% of females producing litters lost demographic viability, and continued to lose heterozygosity over time (Figure 5). As I decreased the percentage of females producing litters from 70% to 60% I saw an increase in probability of extinction from 0.017 (0.004 SE) to 0.190 (0.002 SE). I continued to see probability of extinction rise to 0.806 (0.013 SE) when I decreased the percentage of females breeding to 50%. When fewer than 70% of females successfully produced litters I found populations lost a quarter or more of heterozygosity over the 100 year simulation (Figure 5B). Models with 60% of females reproducing lost 23.85% of heterozygosity and models with 50% of females reproducing lost 34.44% of original heterozygosity. It was clear from simulations in which I modified the brood distribution parameter that the percentage of females successfully reproducing strongly affected long-term population viability, and highlighted the importance of obtaining accurate data on population reproduction rates to improve viability modeling.

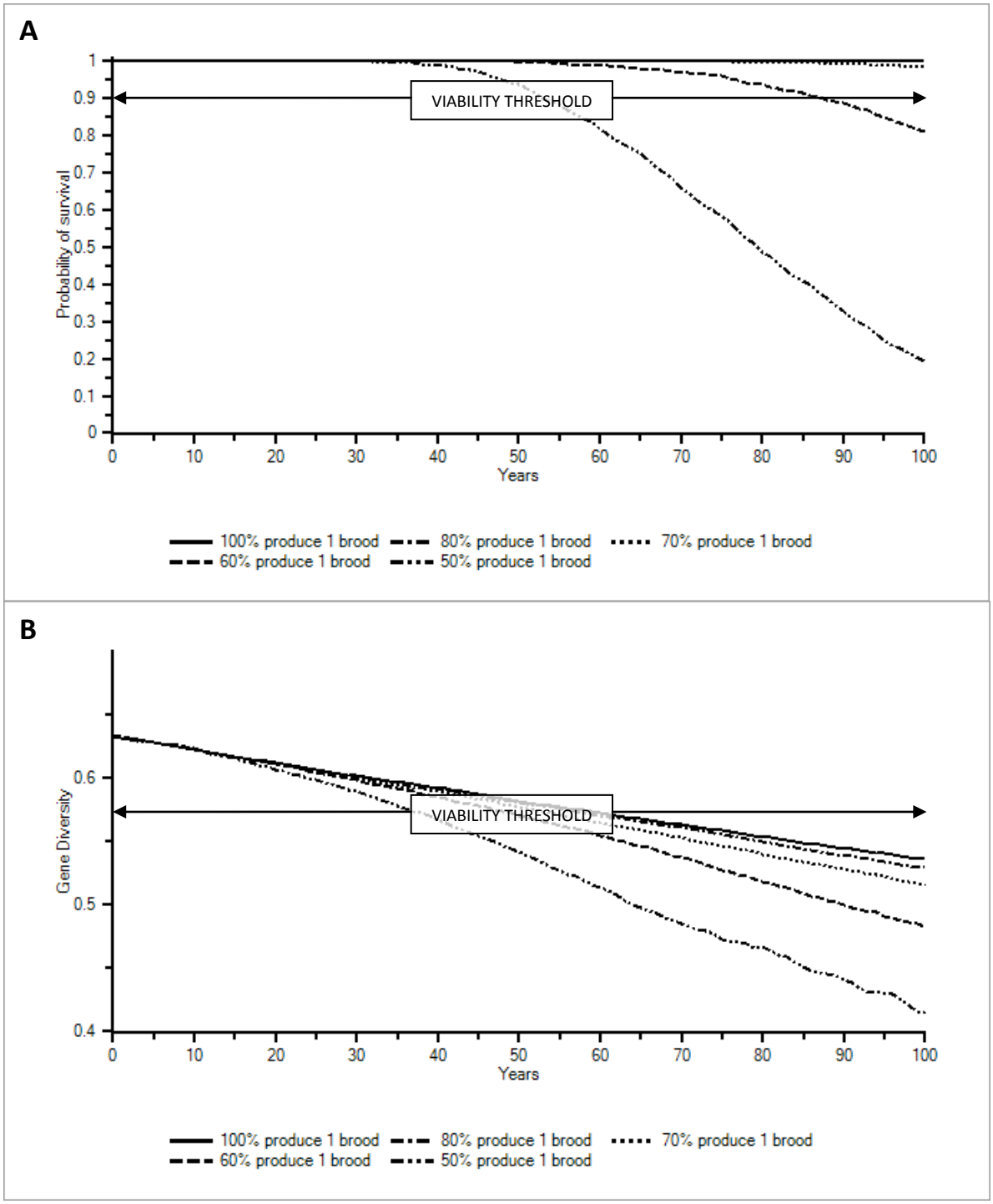


Figure 4. Predicted (A) probability of survival and (B) genetic diversity (heterozygosity) for American marten in Michigan's Manistee National Forest over 100 years with different levels of female reproductive success (percentage of females producing 1 litter of kits/yr vs. not reproducing). Simulations were run for 1000 iterations. Viability threshold indicates the critical level below which populations lose long-term viability (demographic viability = fewer than 10% of populations go extinct, genetic viability = less than 10% heterozygosity loss).

Catastrophes

The populations I modeled with a 50% reduction in survival due to canine distemper maintained demographic viability, but lost genetic viability with a 19.46% reduction in heterozygosity over 100 years (Figure 6). When I increased CDV susceptibility to >50% reduction in survival I saw populations lose demographic viability, and continue to lose heterozygosity. Populations with 70% survival reduction had a probability of extinction of 0.283 (0.014 SE), and lost 24.65% of heterozygosity. Populations with 90% survival reduction had a probability of extinction of 0.798 (0.013 SE), and lost over a quarter (28.44%) of original heterozygosity (Figure 6). I found increasing susceptibility to CDV resulted in extreme population size reductions, which rendered populations too small to remain demographically viable, and subsequently led to population extinction. I obtained the same results for simulations of CDV excluding inbreeding (results not presented), indicating that the demographic effects of CDV appear to trump any heightened effects that low diversity may have on disease susceptibility.

The toxoplasmosis exposure levels I tested did not appear to impact demographic viability or contribute additional heterozygosity loss beyond models that were not exposed to toxoplasmosis. Both of the toxoplasmosis levels of exposure were demographically viable, and genetic diversity loss ranged from 15.48% loss with 3% probability of toxoplasmosis occurrence to 15.66% loss with 6% probability of occurrence (Figure 6).

When I exposed populations to fire, demographic viability was maintained, and genetic diversity loss was only marginally greater than the loss I observed in models not exposed to fire (16.75% heterozygosity loss, Figure 6). When I combined all catastrophe

types (CDV survival reduction of 50%, toxoplasmosis occurrence probability of 3%, and fire) I observed a 21.01% loss of genetic diversity (Figure 6B), and although demographic viability was maintained, the probability of extinction was near the viability threshold at 0.073 (0.008 SE). Overall, it appeared that catastrophes either contributed little to a population's viability (toxoplasmosis, fire), or had severe demographic impacts on a population, to the extent that low genetic diversity played a small role on overall viability (CDV). At the time of this study I did not have any direct evidence of inbreeding depression, and PVA modeling provided little insight into how low diversity may be affecting my populations' abilities to withstand disease or environmental stochasticity. Without any further evidence, I found it difficult to make conclusions on how current genetic health of marten in Michigan may be affecting adaptive potential.

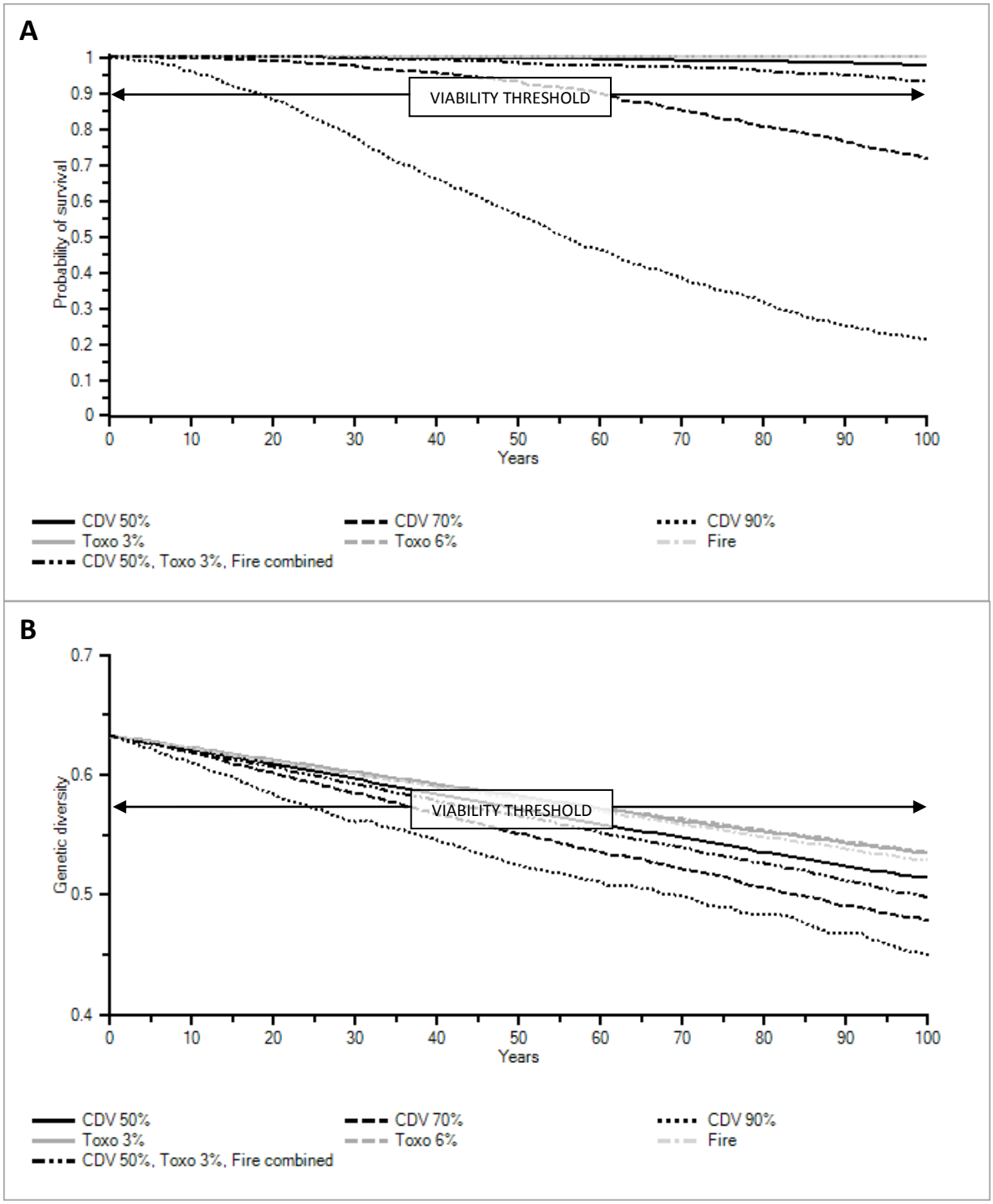


Figure 5. Predicted (A) probability of survival and (B) genetic diversity (heterozygosity) for American marten in Michigan's Manistee National Forest over 100 years with different levels of catastrophe exposure. CDV = Canine Distemper Virus (percentages indicate level of survival reduction), Toxo = Toxoplasmosis (percentages indicate probability of occurrence). Simulations were run for 1000 iterations. Viability threshold indicates the critical level below which populations lose long-term viability (demographic viability = fewer than 10% of populations go extinct, genetic viability = less than 10% heterozygosity loss).

Supplementation

All of the supplementation methods I tested resulted in similar patterns of demographic and genetic viability as base models not incorporating supplementation, and thus had little effect on mitigating genetic diversity loss (Figure 7). My supplementation methods maintained demographic viability, but still lost approximately 15% of heterozygosity in the long-term. Although genetic viability was lost over the 100 year period, I did observe a slight plateau in heterozygosity loss in the years immediately following supplementation, indicating that an introduction of new individuals does have a small effect on genetic diversity loss in the short-term (Figure 7B). It is important to note that due to high model sensitivity to carrying capacity increases, I ran supplementation models with a K of 100, even when the supplementation of new individuals would bring the population above carrying capacity. I did this in an attempt to partition the effects of supplementation from the effects of increased carrying capacity on population viability, but capping carrying capacity at 100 could have dampened the effects of supplementation. Furthermore, inaccurate estimations of other key population parameters (i.e. reproduction and survival rates), may have impacted the results of my supplementation simulations, but until PVA modeling is improved, I cannot say for certain if a translocation would serve to increase genetic health in the MNF.

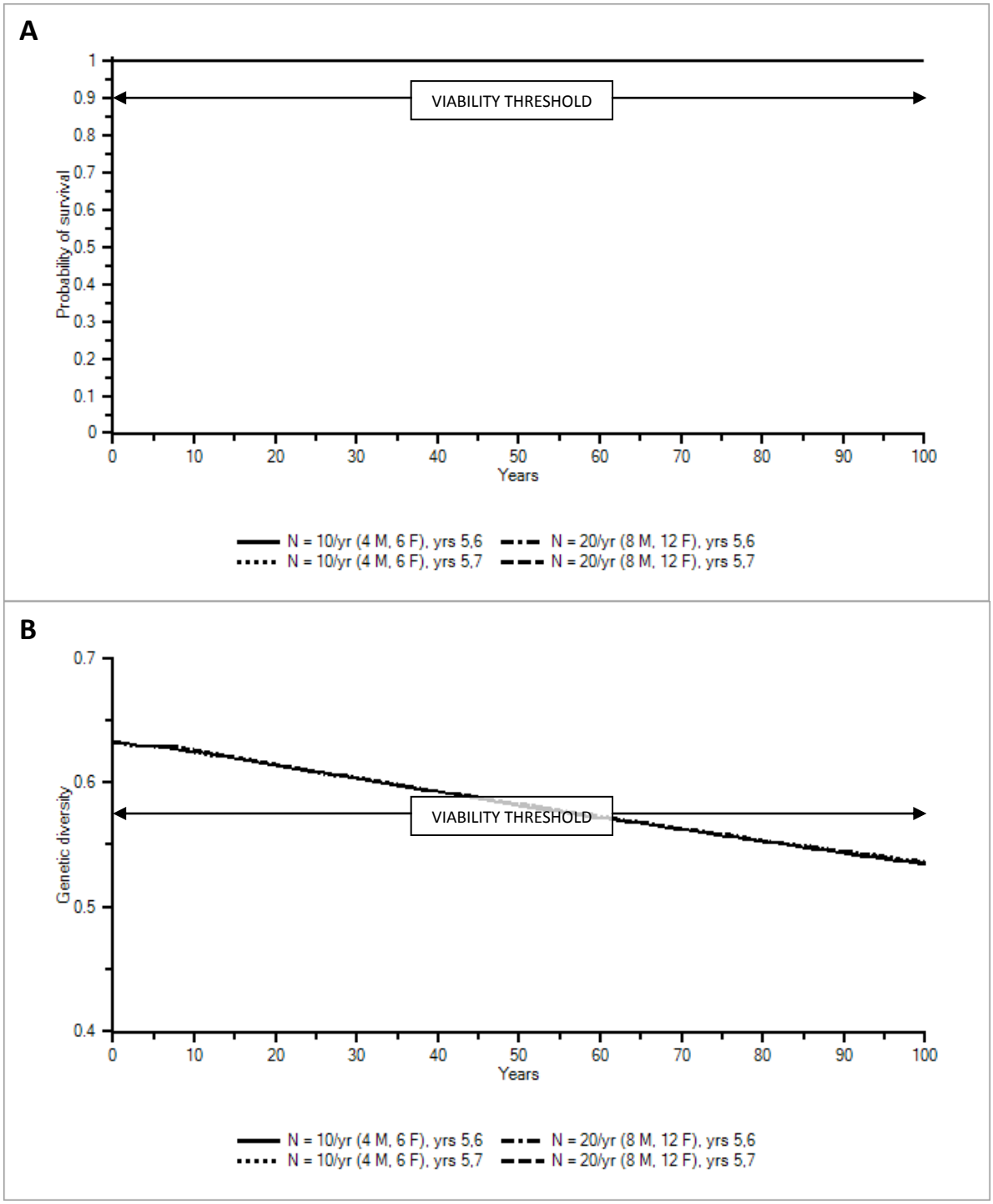


Figure 6. Predicted (A) probability of survival and (B) genetic diversity (heterozygosity) for American marten in Michigan's Manistee National Forest over 100 years with different supplementation methods. Simulations were run for 1000 iterations. Viability threshold indicates the critical level below which populations lose long-term viability (demographic viability = fewer than 10% of populations go extinct, genetic viability = less than 10% heterozygosity loss).

# Chapter IV

## Discussion

The primary objective of my study was to estimate the current genetic health, and long-term population viability of reintroduced marten populations in Michigan's LP and eastern UP. I sought to update and extend upon previous research of marten populations in Michigan in order to formulate management recommendations focused on the preservation of genetic health of marten in the LP populations. I also sought to measure genetic health in the eastern UP to determine the feasibility of utilizing this area as a source population for marten in the LP, in the case a translocation was deemed necessary for genetic health preservation.

### Genetic diversity estimates

Overall, I found evidence of reduced genetic diversity in all of the reintroduced marten populations in this study. My findings of reduced allelic diversity and increased levels of inbreeding in the reintroduced marten populations of Michigan's LP and eastern UP align with, and add support to, the large collection of studies across taxa documenting declines in genetic diversity of reintroduced populations as a result of small founding sizes (Maudet *et al.* 2002, Mock *et al.* 2004, Stockwell *et al.* 1996, Vernesi *et al.* 2003, Williams *et al.* 2000). Erosion of genetic diversity has been well documented in reintroduced and bottlenecked populations for the Florida panther (Roelke *et al.* 1993),

wolves on Isle Royale (Wayne *et al.* 1991), bighorn sheep (Berger 1990), and the Greater Prairie Chicken (Bouzat *et al.* 2008).

Compared to previous studies of marten diversity, I found the reintroduced marten populations of Michigan to have decreased allelic diversity, but to have maintained levels of heterozygosity. I found my estimates of allelic diversity to be lower than those reported for mainland Canadian populations (Kyle *et al.* 2000, Kyle & Strobeck 2003, Williams & Scribner 2010), and similar to diversity estimates reported for peninsular and island populations in the Pacific Northwest (Small *et al.* 2003). Marten populations across mainland Canada (N = 24) had mean allele counts ranging from 4.82 to 6.64 alleles per locus (mean = 5.89, Kyle & Strobeck 2003), and only three of the 24 Canadian populations sampled had allele counts lower than the highest allele count observed in this study (Pembroke, ON, N = 30, 5.09 alleles/locus; Fort McMurray, AB, N = 23, 4.82 alleles/locus; Fort Resolution, NWT, N = 30, 5.27 alleles/locus; compared to MNF 2011-13, N = 35, 5.36 alleles/locus).

Caution must be taken when comparing raw allele counts, as this measure can be highly sensitive to sample size. A comparison using only the Canadian populations with sample sizes of 35 or smaller (N = 12, see Table 1 of Kyle & Strobeck 2003) to my populations with sample sizes of 35 or larger (MNF 2011-13 and eastern UP) allows for a more accurate assessment of allelic diversity. By adjusting for sample size in this way, I am more likely to have an equal or greater probability of detecting alleles in my sampled populations compared to the Canadian populations. Using this method, the allele counts of the reintroduced Michigan populations still fall below 75% of the mainland Canadian marten populations with similar sample sizes (Kyle & Strobeck 2003). A comparison of

the populations with the smallest sample sizes (N=17) from both studies reveals a similar pattern, with the MNF (2005-06) mean allele count of 3.73 alleles/locus falling well below the mean allele count of the Edson, AB population (5.45 alleles/locus, Kyle & Strobeck 2003).

I also observed reduced allelic diversity using a comparison of allelic richness estimates, which correct for sample size differences. The populations in my study displayed lower allelic richness estimates than the putative source populations for the Michigan reintroductions (Williams & Scribner 2010). Allelic richness in the Ontario and Minnesota marten populations ranged from 5.0 to 5.4, which far exceeded the allelic richness values observed in the LP and eastern UP populations of my study, which ranged from 3.6 to 4.3 alleles/locus (Williams & Scribner 2010). A comparison of only the LP populations and their source population reveals a clear decline in allelic diversity since reintroduction (MNF 2005-06, A = 3.6; MNF 2011-13, A = 4.3; PGR, A = 3.8; compared to Crown Chapleau Game Preserve, ON, A = 6.0; Williams & Scribner 2010).

I found the reintroduced marten populations of Michigan to be most similar in allelic diversity to the peninsular and island marten populations in Alaska and British Columbia. These populations had allele counts ranging from 3.64 to 5.57 (mean = 4.51 alleles/locus), and allelic richness values ranging from 2.50 to 4.04 (mean = 3.30 alleles/locus, Small *et al.* 2003). The allelic richness values I observed in this study were actually higher than those found in many of the Pacific Northwest populations, although it should be noted that those estimates were adjusted to a smaller sample size (n = 5) than the estimates in this study. In this case, the raw allele counts may be a better comparison as the sample sizes for most of the Pacific Northwest populations were similar to the

sample sizes of my study. The similarity in allelic diversity between the reintroduced marten populations of Michigan, and populations in the Pacific Northwest is a reflection of the genetic effects of population isolation. Long-term isolation and fragmentation due to glacial advance and retreat in the Pacific Northwest has resulted in many small populations with allelic diversity comparatively low to mainland populations (Small *et al.* 2003). In Michigan, reintroductions using small founding sizes, and lack of population connectivity have also resulted in comparatively low allelic diversity. With continued isolation, it is possible that marten populations in Michigan and *americana* populations in the Pacific Northwest will continue to decline in allelic diversity, as has been seen in the long-term isolated populations of the *caurina* subspecies in the Pacific Northwest (Small *et al.* 2003).

In contrast to allelic diversity, heterozygosity levels in the Michigan marten populations were comparable to previous marten studies. Expected heterozygosity levels across mainland Canada and the Pacific Northwest ranged from 0.390 to 0.680 (Kyle *et al.* 2000, Kyle & Strobeck 2003, Small *et al.* 2003, Williams & Scribner 2010), which fully encompassed the range of values seen in this study (0.565 to 0.636). This pattern of allelic diversity decline and maintenance of heterozygosity is often seen in bottlenecked populations due to heterozygosity estimates being relatively insensitive to the loss of allelic variation (Allendorf 1986). During a drastic population size reduction, rare alleles are lost quickly, which ultimately has a larger impact on allelic diversity than on overall heterozygosity, as the rare alleles were present in few individuals prior to population size reduction (Allendorf 1986). Due to this pattern, it is not surprising that I observed a decline of allelic diversity in the reintroduced Michigan populations, but no strong

decline in heterozygosity. A similar trend has also been observed in previous studies of reintroduced populations (Larson *et al.* 2002, DeYoung *et al.* 2003, Tarr *et al.* 1998).

Comparison to past Michigan marten research

The findings of my study are congruent with the previous findings of Bicker (2007) on the LP marten populations, indicating a decline in genetic diversity since reintroduction. I detected no significant differences in diversity measures (allelic richness, heterozygosity, and inbreeding coefficient) between samples collected by Bicker in the MNF (2005-06), and the samples I collected between 2011-13. Although not significant, I did see trends indicating a continued decline in genetic diversity in the MNF population. This was most evident in the increase in inbreeding coefficient (MNF 2005-06, $F_{IS} = 0.016$; MNF 2011-13, $F_{IS} = 0.238$), and development of significant heterozygote deficiency in the 2011-13 sample. I also observed an increase in loci out of HWE, and detected significant genetic differentiation between the two sampling periods ($F_{ST} = 0.068$, $P < 0.001$), which gives evidence that possible underlying population structure and increased inbreeding is causing the MNF to further depart from HWE. The observations of Bicker (2007) and the subsequent trends I have observed in the recent MNF sample, provide support to the possibility the LP marten populations are experiencing accelerated genetic drift, resulting in genetic diversity declines since reintroduction.

Compared to the samples collected in 2005-06 in the MNF, I observed a large number of private alleles in the 2011-13 sample, and an increase in effective breeding

size, both of which are likely an effect of large differences in sample size between collection periods. I collected two-times the number of samples in 2011-13 (N = 35) than were collected in 2005-06 (N = 17), which most likely resulted in alleles being detected in my sample, that were missed in the earlier sampling period. It is possible that private alleles in the MNF may have arisen through mutation or dispersal, but I find both of these alternatives unlikely given the circumstances of this study.

In small populations, genetic drift is acting at an equal, or more likely, greater rate than mutation, which would make gaining private alleles through mutation difficult in the MNF (Allendorf *et al.* 2013). Even in the absence of drift, typical mutation rates of microsatellites ($10^{-4}$) are too slow to explain the large gain of private alleles I observed in the MNF (Allendorf *et al.* 2013). If private alleles were introduced to the MNF through dispersal, it would most likely be through individuals from the next closest population, which in the case of the MNF, would be the PGR. However, dispersal between the PGR and MNF has been documented to be very low (Nelson 2006), and for any alleles in dispersing individuals to become private in the MNF, the alleles would have to be subsequently lost from the PGR population. Together, successful dispersers bringing in new alleles to the MNF, and for those alleles to also be lost from the PGR are highly unlikely to occur concurrently, and even more unlikely to occur often enough to explain the magnitude of differences I observed in private allele counts. Furthermore, I detected a reduction in observed heterozygosity and an increase in inbreeding coefficient in the MNF since 2005-06. These are both indicators of further diversity decline, which I would not expect to observe if the MNF population were regularly receiving immigrants from the PGR, or if mutation were exceeding genetic drift in this small population. I think it is

therefore, most likely that large sample size differences between sampling periods provides the best explanation for the high number of private alleles observed in the recent MNF sample.

The increase in effective breeding size I detected between the MNF sampling periods may also be due to sample size differences. Effective population size estimators are known to be highly sensitive to sample size, and tend to give large or infinite confidence intervals for calculations using small sample sizes (Beebee 2009). This sensitivity to sample size can be seen in the large discrepancy between the Bayesian, linkage disequilibrium, and temporal method estimates used in my study (Table 2). Although it is possible the effective breeding size has increased in the MNF since 2005-06, I find it unlikely the increase has been substantial or effective in mitigating further genetic diversity loss based on the increase observed in the inbreeding coefficient. Furthermore, even with the increase estimated, the effective breeding size of the MNF (27, 95% CI: 22-42) is below the recommended size of 50 individuals for mitigating negative genetic effects in a reintroduction (Slough 1994). I think it is most likely that small population size in combination with isolation from nearby marten populations is the primary cause for the sustained or continued genetic diversity decline I observed in the MNF population.

Previous research concerning marten populations in Michigan's UP have had discordant results concerning levels of genetic diversity, and population structure across the peninsula (Swanson *et al.* 2006, Williams & Scribner 2010). My genetic diversity estimates for the eastern UP population align best with the findings of Williams and Scribner (2010). In their comparison of the UP marten populations to their putative

source populations, Williams and Scribner (2010) found the eastern UP cluster to have the lowest allelic richness and heterozygosity compared to other marten clusters in the central and western UP, and compared to all putative source populations. The eastern UP also displayed a significant level of inbreeding ($F_{IS}$ = 0.024, Williams & Scribner 2010). My allelic richness and heterozygosity estimates for the eastern UP in this study were similar to the findings of Williams and Scribner (2010), and my estimate of inbreeding showed a further reduction of genetic diversity than what was found in the 2010 study (This study: A = 4.1, $H_O$ = 0.517 (0.078 SE), $F_{IS}$ = 0.142; Williams & Scriber 2010: A = 4.2, $H_O$ = 0.546 (0.068 SE), $F_{IS}$ = 0.024). In contrast, I found much lower estimates of allelic richness and heterozygosity in the eastern UP than were found by Swanson *et al.* (2006) when samples collected across the UP were pooled into a single panmictic population (This study: A = 4.1, $H_O$ = 0.517 (0.078 SE), Swanson *et al.* 2006: A = 7.4, $H_O$ = 0.63). The similarity of my results to Williams and Scribner (2010) provide support for their finding of multiple distinct genetic clusters reflective of multiple source populations for marten in Michigan's UP. The differences I observe between my findings and those of Swanson *et al.* (2006) does not lend support to their finding of a single panmictic marten population in the UP. I find it most likely based on reintroduction history, and the similarities between my findings and those of Williams and Scribner (2010), that marten in the UP exist in multiple distinct clusters, and of those clusters, the eastern UP displays the lowest genetic diversity. Furthermore, I would not recommend using the eastern UP as the single source population for a translocation to the LP, until further research can determine the cause(s) of low diversity, and how the removal of 20 to 40 animals would impact the genetic health of this cluster. Rather I would recommend

obtaining individuals for translocation from all clusters across the UP in order to maximize the diversity introduced to the LP populations while minimizing the impact of removal on each of the UP population clusters.

## Population viability analysis of the MNF

I found the majority of population viability analyses of marten in the MNF predicted the population would remain demographically viable, but the population would lose genetic viability (lose ≥ 10% heterozygosity) within the next 100 years. PVA models were most sensitive to changes in carrying capacity, and the percentage of females successfully reproducing.

### Sensitivity to carrying capacity

The simulations in which I modeled carrying capacities of 250 individuals or larger, showed quick population expansion to carrying capacity, and this mitigated genetic diversity loss. As a result, these models were the only simulations which retained genetic viability through the 100 year time frame. Quick population expansion may lessen the negative genetic effects of a bottleneck, because populations which grow to a large enough size after a bottleneck may limit the loss of heterozygosity (Allendorf *et al.* 2013, Nei *et al.* 1975). In contrast, a population that remains at a small size for generations after a bottleneck is more likely to continue to lose heterozygosity, as drift acts more strongly on the small population (Allendorf *et al.* 2013, Nei *et al.* 1975).

Several conditions of reintroduced populations may promote high population growth rates. Marten released into the MNF would likely experience a reduction in population density, and accompanying release from competition. Female marten in better body condition may reproduce more frequently, or produce larger litter sizes, as reproduction may be food-related (Clark *et al.* 1987, Hawley & Newby 1957, Weckwerth & Hawley 1962). I would also expect survival rates to increase for marten released in the MNF due to the absence of fisher (*Martes pennanti*), which have been documented to predate on marten (McCann *et al.* 2010). Additionally, low density in a reintroduced population may increase juvenile survival by reducing antagonistic interactions between juvenile and adult male marten competing for home range habitat, or for female marten during the breeding season (Bull & Heater 2001). It is therefore possible that marten reintroduced to the MNF would be capable of quick population expansion, and under these conditions I would expect to see the results predicted by the models in which quick growth mitigates genetic diversity loss.

However, my genetic diversity estimates for marten in the MNF are not fully supportive of the pattern of quick population expansion and slowing diversity loss. I detected the development of significant heterozygote deficiency in the MNF in 2011-13, that was not present in 2005-06. I also measured greater levels of inbreeding and loci out of HWE in the recent MNF sample. Furthermore, my $N_b$ estimates indicate only a slight increase in population size in the MNF, which may be an artifact of sample size differences, and overall, population sizes are still small. Although differences between the sampling periods were not significant, the trend towards continued genetic diversity decline, and small $N_b$ size are not supportive of a quickly expanding population, and

rather are more reflective of the pattern observed when a population remains small for generations post bottleneck or small founding event (Allendorf *et al.* 2013, Nei *et al.* 1975). The discordance I see between my PVA modeling estimates with high carrying capacities, and my genetic diversity estimates indicate the PVA is most likely overestimating the growth capabilities of the MNF population, and the sensitivity of the model to this parameter is likely linked to at least one other factor effecting population growth in the MNF.

Sensitivity to female reproductive success

PVA models were also highly sensitive to female reproductive success. Long-term population viability (both demographic and genetic) drastically declined when fewer than 70% of females successfully produced one litter per year. Based on the sensitivity of my PVA models to this parameter, it is probable that low reproductive success alone, or in combination with other factors such as litter sizes and survival, may be limiting population growth in the MNF. If low reproduction rates, small litter sizes, or low survival are limiting population growth the MNF, this could explain why my genetic diversity estimates do not match those predicted by the PVA when carrying capacities are high.

I had only limited data for the MNF population on the percentage of females breeding, the percentage of females successfully producing litters, litter sizes, juvenile survival, or adult survival. In order to supplement my missing or limited data for these parameters, I used values obtained from the literature, and often used literature

maximums for values such as age of last reproduction. By using literature values in my PVA models for the MNF, I may have overestimated the population growth abilities of marten in the MNF. For instance, Strickland *et al.* (1982) found few wild marten live over 4 years of age. So although marten have been documented to survive and reproduce at up to 12 years of age (Strickland & Douglas 1987), the average age of reproduction in the MNF may be much lower, and by using the maximum of 12 years I may have drastically altered my PVA simulations to assume the MNF marten population is capable of much greater reproductive output than it may realistically be producing.

Furthermore, estimates of adult marten survival rates vary widely depending on habitat, presence of harvest, and predator assemblages (survival rates: 0.64 in Bull & Heater 2001; 0.81 in McCann *et al.* 2010; 0.63 in a logged forest in Thompson 1994; 0.91 in an unlogged forest in Thompson 1994), which made it difficult to generalize a survival parameter value for use in my PVA simulations. I did not have a current survival estimate for marten in the MNF, and the use of a generalized survival estimate in my PVA may have also contributed to overestimated or inaccurate results of population viability for marten in the MNF.

If reproduction, survival, or a combination of these parameters are much reduced in the MNF population, than I would expect the demographic and genetic viability results of the PVA simulations to also decrease, which may result in a loss of demographic viability and a more severe decline in genetic viability over the long-term. Although marten are secretive, and it is often difficult to obtain accurate values of reproduction and survival in a wild population, I believe performing additional research to calculate these parameters for the MNF would enable for a much more accurate PVA, than what I have

obtained using literature values. At this time, I think my current PVA results have overestimated the population viability of marten in the MNF, and that the parameters of reproduction and survival must be further investigated to improve PVA accuracy.

Supplementation

The supplementation methods I proposed for the MNF population were not effective in mitigating genetic diversity loss, according to my PVA modeling results. My supplementation methods may not have been effective due to the overwhelming effects of genetic drift in a small, isolated population, or it is possible environmental and demographic factors are having a stronger impact on population growth than genetic factors. Lastly, it is also possible my PVA model produced an inaccurate representation of the effects a translocation would have on the MNF, due to model sensitivity to carrying capacity, and overestimation of reproduction and survival parameters (see above).

It is possible that the isolation of the MNF population would result in continued genetic diversity decline despite a translocation, if genetic drift continued to be a strong driver of diversity loss. In this case, a translocation may only mitigate genetic diversity loss in the years immediately following the release of new individuals, and in the long-term genetic diversity would continue to decline. This is similar to what I observed in my PVA simulations, in which I saw a plateau of genetic diversity loss in the years immediately after I implemented a supplementation, but by the end of the 100 year period, genetic diversity had continued to decline to levels similar to simulations not

receiving a supplementation. Similarly, if demographic and environmental factors are driving population expansion more strongly than genetic factors (i.e. inbreeding or overall genetic variation), then I would also expect a translocation to do little to improve population viability. In this case, a translocation may serve to slow genetic diversity loss, but ultimately does little to improve the population's long-term viability, because the underlying demographic or environmental factors (i.e. low survival or poor habitat) that caused the population's initial decline have not been resolved. In this case I would also expect to see a short-term improvement in population viability, but ultimately the same results in the long-term as a population not receiving a supplementation.

Although isolation, or demographic and environmental factors are capable of dampening the genetic benefits of a supplementation, I think the primary explanation for the ineffectiveness of my supplementation methods is PVA sensitivity and parameter inaccuracies causing underestimation of translocation benefits to genetic diversity. There is overwhelming evidence across taxa of the benefits of translocations, and the positive effects only a few individuals can have on the loss of genetic diversity in small or isolated populations. Evidence exists for the benefits of translocations, and successful genetic rescue events for the Florida panther (Pimm *et al.* 2005, Hedrick & Fredrickson 2010), bighorn sheep (Hogg *et al.* 2006), Isle Royale and Scandanavian wolves (Adams *et al.* 2011, Vilá *et al.* 2002), Mexican wolves (Fredrickson *et al.* 2007, Hedrick & Fredrickson 2010), the Greater Prairie Chicken (Bouzat *et al.* 2009), adders (Madsen *et al.* 2004), and creeping spearwort (Willi *et al.* 2007). Furthermore, in previous studies in which isolation and demographic or environmental factors were believed to be drivers of population growth, a translocation still served to increase genetic diversity for a longer

period than what I observed in my PVA models of a supplementation in the MNF (Bouzat et al. 2009, Adams et al 2011). Given the large amount of evidence supporting the benefits of translocations, and with considerations of my previously mentioned PVA sensitivities, I believe my supplementation results are an inaccurate representation of how a translocation would impact the MNF. I think it is likely my supplementation methods of an introduction of 20 to 40 individuals would do much more to increase genetic diversity than my model predicts. Without additional information on current demographic (i.e. survival and reproductive rates) and environmental (i.e. habitat/resource quality) factors impacting the MNF population, I cannot say with certainty that a translocation alone would improve population viability in the long-term. I do, however, think my supplementation methods would serve to improve genetic diversity in the MNF population more so, and for a longer time period than my PVA results suggest.

## Conclusions and management recommendations

Overall, the findings of my study are congruent with previous marten research in Michigan, indicating a decline in genetic diversity since reintroduction (Bicker 2007, Nelson 2006, Williams & Scribner 2010). The sustained or worsened genetic diversity estimates in my study are still most likely a result of the singular, small founding events used to reestablish marten in the LP, as well as very low population connectivity, and slow population growth post reintroduction (Bicker 2007, Nelson 2006). The small change in effective breeding size I observed in the MNF is an indication the population is likely remaining at a small size, which is subjecting the population to continued genetic

drift. Furthermore, lack of connectivity between populations is amplifying the effects of genetic drift by preventing dispersers from providing much needed allelic exchange (Nelson 2006). In the UP, I observed similar levels of diversity as were detected by Williams and Scribner (2010), and support their finding of multiple genetic clusters of marten in the UP. I observed increased levels of inbreeding in the eastern UP, and significant heterozygote deficiency, which may be an indication this cluster is declining in genetic health. This decline may be a product of overharvest (Skalski *et al.* 2011) in the area, and further research should be performed to determine potential causes for genetic health decline in the eastern UP.

The results of my PVA on the MNF population indicated long-term demographic viability, but loss of genetic viability, although it is likely the viability of this population has been overestimated. I found my model to be highly sensitive to modification of female reproductive success, and my use of literature values for this parameter may have caused growth rates to be magnified above actual levels. This magnification may have been costly in my estimation of viability for marten in the MNF, and it is pertinent that demographic factors (i.e. reproduction and survival rates) be further investigated in this population to improve PVA accuracy. Without population specific values for marten reproduction and survival in the MNF, it is difficult for me to assert the accuracy of the PVA results from this study, but I do believe the PVA served as an appropriate tool for highlighting factors pertinent to marten population growth and viability in the MNF. With the improvement of parameter accuracy, I believe PVA simulations can provide important information for future marten population viability in Michigan.

Based on the results of my genetic diversity estimates and PVA, I believe the populations of marten in Michigan's LP would benefit from a translocation of individuals to increase levels of genetic diversity, increase effective and census population size, and to decrease levels of inbreeding. My PVA simulations did not find my proposed supplementation methods effective in mitigating genetic diversity loss, but this result is likely an underestimation of the effects of a translocation due to potentially inaccurate parameters being incorporated into the model (see above). This is supported by many previous studies showing the benefits of translocations of even very few individuals (Adams *et al.* 2011, Bouzat *et al.* 2009, Fredrickson *et al.* 2007, Hedrick & Fredrickson 2010, Hogg *et al.* 2006, Madsen *et al.* 2004, Pimm *et al.* 2005, Vilá *et al.* 2002, Willi *et al.* 2007). I would recommend a translocation event of marten to the LP populations to begin with an introduction of 20 to 40 adult individuals obtained from diverse source locations across Michigan's UP or mainland Canada. Utilizing multiple source locations would maximize the diversity introduced to the LP populations, whilst minimizing the potentially detrimental effects of removing large numbers of individuals from source populations. Translocated individuals should be adults in order to introduce reproductively mature animals with lower mortality risk (Slough 1994), and releasing an equal or female biased ratio of marten could serve to increase reproduction rates, and also potentially increase the number of supplemented individuals indirectly through unborn young (Powell *et al.* 1994). Finally, I would recommend a translocation be spaced both temporally and spatially in order to reduce crowding and conflict at release sits, and to allow supplemented animals to establish territories in suitable habitat (Powell *et al.* 1994).

Future research on marten in Michigan should aim to estimate population specific reproductive and survival rates in order to improve PVA modeling, and to give insight into factors potentially effecting slow population growth. Further research should also be performed in the eastern UP to identify causes of low genetic diversity, and how harvest may be impacting the marten population throughout the UP.

## Literature Cited


Adams, J. R., L. M. Vucetich, P. W. Hedrick, R. O. Peterson, and J. A. Vucetich. 2011. Genomic sweep and potential genetic rescue during limiting environmental conditions in an isolated wolf population. Proceedings of the Royal Society B **278:**3339-3344.

Allen, A. W. 1982. Habitat suitability index models: Marten. United States Department of the Interior Fish and Wildlife Service. FWS/OBS-82/10.11. 9 pages.

Allendorf, F. W. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology **5:**181-190.

Allendorf, F. W., G. Luikart, and S. N. Aitken. 2013. Small populations and genetic drift. Pages 96-116 *in* Conservation and the Genetics of Populations. Wiley-Blackwell, Oxford, UK.

Ashbrook, F. G., and K. B. Hanson. 1930. The normal breeding season and gestation period of martens. U.S. Department of Agriculture Circular **107:**1-6.

Bach, L. A., R. B. F. Pedersen, M. Hayward, J. Stagegaard, V. Loeschcke, and C. Pertoldi. 2010. Assessing re-introductions of the African Wild dog (*Lycaon pictus*) in the Limpopo Valley Conservancy, South Africa, using the stochastic simulation program VORTEX. Journal for Nature Conservation **18:**237-246.

Banks, P. B., K. Norrdahl, and E. Korpimäki. 2002. Mobility decisions and the predation risks of reintroduction. Biological Conservation **103:**133-138.

Beebee, T. J. C. 2009. A comparison of single-sample effective size estimators using empirical toad (*Bufo calamita*) population data: genetic compensation and population size-genetic diversity correlations. Molecular Ecology **18:**4790-4797.

Beissinger, S. R. and M. I. Westphal. 1998. On the use of demographic models of population viability in endangered species management. The Journal of Wildlife Management **62:**821-841.

Berg, W. E. 1982. Reintroduction of fisher, pine marten, and river otter. Pages 158-173 *in* Sanderson, G.S., ed. Midwest furbearer management. The Wildlife Society, Central Mountains and Plains Section, Wichita, Kansas.

Berger, J. 1990. Persistence of different-sized populations: an empirical assessment of rapid extinctions in bighorn sheep. Conservation Biology **4:**91-98.

Bicker, S. A. 2007. Genetic bottleneck analysis in Michigan's Lower Peninsula reintroduced populations of American marten (*Martes americana*). M.S. Thesis, Central Michigan University, Mount Pleasant, Michigan.

Bouzat, J. L., H. H. Cheng, H. A. Lewin, R. L. Westemeier, J. D. Brawn, and K. N. Paige. 2008. Genetic evaluation of a demographic bottleneck in the Greater Prairie Chicken. Conservation Biology **12:**836-843.

Bouzat, J. L., J. A. Johnson, J. E. Toepfer, S. A. Simpson, T. L. Esker, and R. L. Westemeier. 2009. Beyond the beneficial effects of translocations as an effective tool for the genetic restoration of isolated populations. Conservation Genetics **10:**191-201.

Boyce, M. S. 1992. Population viability analysis. Annual Review of Ecology and Systematics **23:**481-506.

Buchanan, C. 2008. Multi-scale habitat selection by American marten (*Martes americana*) in Michigan's Lower Peninsula. M.S. Thesis, Central Michigan University, Mount Pleasant, Michigan.

Bull, E. L., and T. W. Heater. 2000. Resting and denning sites of American martens in northeastern Oregon. Northwest Science **74:**179-185.

Bull, E. L., and T. W. Heater. 2001. Survival, causes of mortality, and reproduction in the American marten in northeastern Oregon. Northwestern Naturalist **82:**1-6.

Buskirk, S. W., and R. A. Powell. 1994. Habitat ecology of fishers and American martens. Pages 283-296 *in* Martens, sables, and fishers: biology and conservation (S.W. Buskirk, A.S. Harestad, and M.G. Raphael, eds.). Cornell University Press, Ithaca, New York.

Clark, T. W., E. Anderson, C. Douglas, and M. Strickland. 1987. *Martes americana*. The American Society of Mammalogists **289:**1-8.

Davidson, M. 1986. Canine distemper virus infection in the domestic ferret. The Compendium on Continuing Education **8:**448-453.

Davis, C. S., and C. Strobeck. 1998. Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family *Mustelidae*. Molecular Ecology **7:**1776-1778.

Deem, S. L., L. H. Spelman, R. A. Yates, and R. J. Montali. 2000. Canine distemper in terrestrial carnivores: a review. Journal of Zoo and Wildlife Medicine **31:**441-451.

Desmarchelier, M., M. Cheveau, L. Imbeau, and S. Lair. 2007. Field use of isoflurane as an inhalant anesthetic in the American marten (*Martes americana*). Journal of Wildlife Diseases **43:**719-725.

DeYoung, R. W., S. Demarais, R. L. Honeycutt, A. P. Rooney, R. A. Gonzales, and K. L. Gee. 2003. Genetic consequences of white-tailed deer (*Odocoileus virginianus*) restoration in Mississippi. Molecular Ecology **12:**3237-3252.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size ($N_e$) from genetic data. Molecular Ecology Resources **14:**209-214.

Earle, R. D. 1996. Winter habitat preferences of marten. Michigan Department of Natural Resources job final report. Project W-127-R-14.

Earle, R. D., L. H. Mastenbrook, and T. F. Reis. 2001. Distribution and abundance of the American marten in Northern Michigan. Michigan Department of Natural Resources, Wildlife Report No. 3321.

Ebenhard, T. 1995. Conservation breeding as a tool for saving animal species from extinction. Trends in Ecology and Evolution **10:**438-443.

El Mousadik, A., and R. J. Petit. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic of Morocco. Theoretical and Applied Genetics **92:**832-839.

Fleming, M. A., E. A. Ostrander, and J. A. Cook. 1999. Microsatellite markers for American mink (*Mustela vison*) and ermine (*Mustela erminea*). Molecular Ecology **8:**1352-1354.

Frankham, R. 1995. Inbreeding and extinction: a threshold effect. Conservation Biology **9:**792-799.

Frawley, B. J. 2002. 2001 Marten harvest survey. Michigan Department of Natural Resources, Wildlife Division Report No. 3369.

Fredrickson, R. J., P. Siminksi, M. Woolf, and P. W. Hedrick. 2007. Genetic rescue and inbreeding depression in Mexican wolves. Proceedings of the Royal Society B **274:**2365-2371.

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. The Journal of Heredity, **86:**485-486.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. Science **245:**477-480.

Harden, J. 1998. Status of American marten in the Manistee National Forest of lower Michigan. M.S. Thesis, Central Michigan University, Mount Pleasant, Michigan.

Hargis, C. D., and D. R. McCullough. 1984. Winter diet and habitat selection of marten in Yosemite National Park. Journal of Wildlife Management **48:**140-146.

Hawley, V. D., and F. E. Newby. 1957. Marten home ranges and population fluctuations. Journal of Mammalogy **38:**174-184.

Hedrick, P.W., and R. Fredrickson. 2010. Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. Conservation Genetics **11:**615-626.

Hill, W. G. 1981. Estimation of effective population size from data on linkage disequilibrium. Genetics Research **38:**209-216.

Hodgeman, T. P., D. J. Harrison, D. D. Katnik, and K. D. Elowe. 1994. Survival in an intensively trapped marten population in Maine. Journal of Wildlife Management **58:**593-600.

Hogg, J. T., S. H. Forbes, B. M. Steele, and G. Luikart. 2006. Genetic rescue of an insular population of large mammals. Proceedings of the Royal Society B **273:**1491-1499.

Holland, O. J., and D. M. Gleeson. 2005. Genetic characterization of blastocysts and the identification of multiple paternity in the stoat (*Mustela erminea*). Conservation Genetics **6:**855-858.

IUCN. 1998. Guidelines for re-introductions. Prepared by the IUCN/SSC Re-introduction Specialist Group, IUCN, Gland, Switzerland and Cambridge, UK. 10 pages.

Korbie, D. J., and J. S. Mattick. 2008. Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nature Protocols **3:**1452-1456.

Kyle, C. J., and C. Strobeck. 2003. Genetic homogeneity of Canadian mainland marten populations underscores the distinctiveness of Newfoundland pine martens (*Martes americana atrata*). Canadian Journal of Zoology **81:**57-66.

Kyle, C. J., C. S. Davis, and C. Strobeck. 2000. Microsatellite analysis of North American pine marten (*Martes americana*) populations from the Yukon and Northwest Territories. Canadian Journal of Zoology **78:**1150-1157.

Lacy, R. C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology **1:**143-158.

_____. 1993. Simulation modeling of American marten (*Martes americana*) populations: vulnerability to extinction. The Great Basin Naturalist **53:**282-292.

_____. 1997. Importance of genetic variation to the viability of mammalian populations. Journal of Mammalogy **78:**320-335

Larson, S., R. Jameson, J. Bodkin, M. Staedler, and P. Bentzen. 2002. Microsatellite DNA and mitochondrial DNA variation in remnant and translocated sea otter (*Enhydra lutris*) populations. Journal of Mammalogy **83:**893-906.

Madsen, T., B. Ujvari, and M. Olsson. 2004. Novel genes continue to enhance population growth in adders (*Vipera berus*). Biological Conservation **120:**145-147.

Manville, R. H. 1948. The vertebrate fauna of the Huron Mountains, Michigan. American Midland Naturalist **39:**615-640.

Markley, M. H., and C. F. Bassett. 1942. Habits of captive marten. American Midland Naturalist **28:**604-616.

Maudet, C., C. Miller, B. Bassano, C. Breitenmoser-Würsten, D. Gauthier, G. Obexer-Ruff, J. Michallet, P. Taberlet, and G. Luikart. 2002. Microsatellite DNA and the recent statistical methods in wildlife conservation management: applications in Alpine ibex [*Capra ibex (ibex)*]. Molecular Ecology **11:**421-436.

McCann, N. P., P. A. Zollner, and J. H. Gilbert. 2010. Survival of adult martens in northern Wisconsin. Journal of Wildlife Management **74:**1502-1507.

McFadden, L. M. 2007. Home-range dynamics and habitat selection of American martens (*Martes americana*) in Michigan's northern Lower Peninsula. M.S. Thesis, Central Michigan University, Mount Pleasant, Michigan.

Mock, K. E., E. K. Latch, and O. E. Rhodes Jr. 2004. Assessing losses of genetic diversity due to translocation: long-term case histories in Merriam's turkey (*Meleagris gallopavo merriami*). Conservation Genetics **5:**631-645.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution **29:**1-10.

Nelson, E. A. 2006. Analysis of dispersal in Michigan's northern Lower Peninsula reintroduced American marten (*Martes americana*). M.S. Thesis, Central Michigan University, Mount Pleasant, Michigan.

O'Grady, J. J., B. W. Brook, D. H. Reed, J. D. Ballou, D. W. Tonkyn, and R. Frankham. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biological Conservation **133:**42-51.

Peakall, R. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6:**288-295.

Pimm, S. L., L. Dollar, and O. L. Bass Jr. 2005. The genetic rescue of the Florida panther. Animal Conservation 2-8.

Pollak, E. 1983. A new method for estimating the effective population size from allele frequency changes. Genetics **104:**531-548.

Poole, K. G., A. D. Porter, A. de Vries, C. Maundrell, S. D. Grindal, and C. Cassady St. Clair. 2004. Suitability of a young deciduous-dominated forest for American marten and the effects of forest removal. Canadian Journal of Zoology **82:**423-435.

Powell, R. A., J. C. Lewis, B. G. Slough, S. M. Brainerd, N. R. Jordan, A.V. Abramov, V. Monakhov, P. A. Zollner, and R. Murakami. 1994. Evaluating translocations of martens, sables, and fishers. Pages. 93-137 *in* Martens, sables, and fishers: biology and conservation (S.W. Buskirk, A.S. Harestad, and M.G. Raphael, eds.). Cornell University Press, Ithaca, New York.

R Development Core Team. 2005. R: A language and environment for statistical computing, reference index version 2.15.3. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Reed, D. H., J. J. O'Grady, J. D. Ballou, and R. Frankham. 2003. The frequency and severity of catastrophic die-offs in vertebrates. Animal Conservation **6:**109-114.

Roelke, M. E., J. S. Martenson, and S. J. O'Brien. 1993. The consequences of demographic reduction and genetic depletion in the endangered Florida panther. Current Biology **3:**340-350.

Sarrazin, F., and R. Barbault. 1996. Reintroduction: challenges and lessons for basic ecology. Trends in Ecology and Evolution **11:**474-478.

Schwartz, M. K., G. Luikart, and R. S. Waples. 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution **22:**25-33.

Shaffer, M. L. 1990. Population viability analysis. Conservation Biology **4:**39-40.

Shands, W. E. 1991. The lands nobody wanted: the legacy of the eastern national forests. Pinchot Institute.

Sikes, R. S. and W. L. Gannon. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy **92:**235-253.

Skalski, J. R., J. J. Millspaugh, M. V. Clawson, J. L. Belant, D. R. Etter, B. J. Frawley, and P. D. Friedrich. 2011. Abundance trends of American martens in Michigan based on statistical population reconstruction. The Journal of Wildlife Management **75:**1767-1773.

Slauson, K. M., W. J. Zielinski, and J. P. Hayes. 2004. Habitat selection by American martens in coastal California. Journal of Wildlife Management **71:**458-468.

Slough, B. G. 1994. Translocations of American martens: An evaluation of factors in success. Pages 165-178 *in* Martens, sables, and fishers: biology and conservation (Buskirk, S.W., A.S. Harestad and M.G. Raphael, eds.). Cornell University Press, New York, New York.

Small, M. P., K. D. Stone, and J. A. Cook. 2003. American marten (*Martes americana*) in the Pacific Northwest: population differentiation across a landscape fragmented in time and space. Molecular Ecology **12:**89-103.

Soulé, M., M. Glipin, W. Conway, and T. Foose. 1986. The millenium ark: how long a voyage, how many staterooms, how many passengers? Zoo Biology **5:**101-113.

Stockwell, C. A., M. Mulvey, and G. L. Vinyard. 1996. Translocations and the preservation of allelic diversity. Conservation Biology **10:**1133-1141.

Strickland, M. A., C. W. Douglas, M. Novak, and N. P. Hunziner. 1982. Marten (*Martes americana*). Pages 599-612 *in* Wild mammals of North America (Chapman, J. A., and G. A. Feldhammer, eds.). John Hopkins University Press, Baltimore, Maryland.

Strickland, M. A., and C. W. Douglas. 1987. Marten *in* Wild furbearer management and conservation in North America (Novak, M., and J. Baker, eds.). Ontario Trappers Association, North Bay, Ontario.

Swanson, B. J., and C. J. Kyle. 2007. Relative influence of temporal and geographic separation of source populations in a successful marten reintroduction. Journal of Mammalogy **88:**1346-1348.

Swanson, B. J., L. R. Peters, and C. J. Kyle. 2006. Demographic and genetic evaluation of an American marten reintroduction. Journal of Mammalogy **87:**272-280.

Tallmon, D. A., A. Koyuk, G. Luikart, and M. A. Beaumont. 2008. ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. Molecular Ecology Resources **8:**299-301.

Tarr, C. L., S. Conant, and R. C. Fleischer. 1998. Founder events and variation at microsatellite loci in an insular passerine bird, the Laysan finch (*Telespiza cantans*). Molecular Ecology **7:**719-731.

Thompson, I. D. 1994. Marten populations in uncut and logged boreal forests in Ontario. Journal of Wildlife Management **58:**272-280.

Thompson, I. D., and P. W. Colgan. 1994. Marten activity in uncut and logged boreal forests in Ontario. Journal of Wildlife Management **58:**280-288.

Vernesi, C., B. Crestanello, E. Pecchioli, D. Tartari, D. Caramelli, H. Hauffe, and G. Bertorelle. 2003. The genetic impact of demographic decline and reintroduction in the wild boar (*Sus scrofa*): A microsatellite analysis. Molecular Ecology **12:**585-595.

Vilá, C., A. K. Sundqvist, O. Flagstad, J. Seddon, S. Björnerfeldt, I. Kojola, A. Casulli, H. Sand, P. Wabakken, and H. Ellegren. 2002. Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. Proceedings of the Royal Society B **270:**91-97.

Waples, R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics **121:**379-391.

_____. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conservation Genetics **7:**167-184.

Waples, R. S., and C. Do. 2010. Linkage disequilibrium estimates of contemporary $N_e$ using highly variable genetic markers: a largely untapped resources for applied conservation and evolution. Evolutionary Applications **3:**244-262.

Wayne, R. K., N. Lehman, D. Girman, P. J. P. Gogan, D. A. Gilbert, K. Hansen, R. O. Peterson, U. S. Seal, A. Eisenhawer, L. D. Mech, and R. J. Krumenaker. 1991. Conservation genetics of the endangered Isle Royale gray wolf. Conservation Biology **5:**41-51.

Weckwerth, R. P., and V. D. Hawley. 1962. Marten food habits and population fluctuations in Montana. Journal of Wildlife Management **26:**55-74.

Willi, Y., M. van Kleunen, S. Dietrich, and M. Fischer. 2007. Genetic rescue persists beyond first-generation outbreeding in small populations of rare plant. Proceedings of the Royal Society B **274:**2357-2364.

Williams, B. W., and K. T. Scribner. 2007. Demographic and genetic evaluation of an American marten reintroduction: a comment on Swanston et al. Journal of Mammalogy **88:**1342-1345.

Williams, B. W., J. H. Gilbert, and P. A. Zollner. 2007. Historical perspective on the reintroduction of the fisher and American marten in Wisconsin and Michigan. General Technical Report NRS-5. Newton Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. 29 pages.

Williams, B. W., and K. T. Scribner. 2010. Effects of multiple founder populations on spatial genetic structure of reintroduced American martens. Molecular Ecology **19:**227-240.

Williams, E. S., E. T. Thorne, M. J. G. Appel, and D. W. Belitsky. 1988. Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. Journal of Wildlife Diseases **24:**385-398.

Williams, R. N., O. E. Rhodes Jr., T. L. Serfass. 2000. Assessment of genetic variance among source and reintroduced fisher populations. Journal of Mammalogy **81:**895-907.

Wolf, C. M., B. Griffith, C. Reed, and S. A. Temple. 1996. Avian and mammalian translocations: update and reanalysis of 1987 survey data. Conservation Biology **10:**1142-1154.

Wood, N. A., and L. R. Dice. 1924. Records of the distribution of Michigan mammals. Michigan Academy of Science, Arts, and Letters **3:**425-469.

Yamaguchi, N., R. J. Sarno, W. E. Johnson, S. J. O'Brien, and D. W. Macdonald. 2004. Multiple paternity and reproductive tactics of free-ranging American minks, *Mustela vison*. Journal of Mammalogy **85:**432-439.

# Appendix A

## Summary of marten captures

List of all American marten captures utilized in this study. Sample locations consisted of two sites in Michigan's Lower Peninsula, the Manistee National Forest (MNF) and the Pigeon River Country State Forest (PGR), and a single sampling area in the eastern Upper Peninsula (East UP). Samples were collected by four agencies including Central Michigan University (CMU), Grand Valley State University (GVSU), the Little River Band of Ottawa Indians (LRBOI), and the Sault Ste. Marie Tribe of Chippewa Indians (SSMTCI).

| Capture date | ID | Sex | Sampling location | Collector/s | Capture type | Biological sample type |
|---|---|---|---|---|---|---|
| 3/25/2005 | 301 | M | MNF | CMU | Live | Tissue |
| 3/25/2005 | 302 | M | MNF | CMU | Live | Tissue |
| 3/26/2005 | 108 | F | MNF | CMU | Live | Tissue |
| 3/27/2005 | 116 | M | MNF | CMU | Live | Tissue |
| 4/3/2005 | 117 | F | MNF | CMU | Live | Tissue |
| 4/3/2005 | 304 | M | MNF | CMU | Live | Tissue |
| 4/5/2005 | 305 | M | MNF | CMU | Live | Tissue |
| 4/16/2005 | 118 | M | MNF | CMU | Live | Tissue |
| 1/17/2006 | 306 | M | MNF | CMU | Live | Tissue |
| 2/2/2006 | 307 | F | MNF | CMU | Live | Tissue |
| 2/11/2006 | 308 | F | MNF | CMU | Live | Tissue |
| 2/14/2006 | 309 | M | MNF | CMU | Live | Tissue |
| 2/15/2006 | 310 | F | MNF | CMU | Live | Tissue |
| 3/1/2006 | 311 | M | MNF | CMU | Live | Tissue |
| 3/11/2006 | 312 | F | MNF | CMU | Live | Tissue |
| 4/13/2006 | 313 | M | MNF | CMU | Live | Tissue |
| 6/5/2006 | H18 | UNK | MNF | CMU | Hair snare | Hair |
| 5/11/2011 | 090 | F | MNF | GVSU/LRBOI | Live | Blood |
| 5/11/2011 | 822 | F | MNF | GVSU/LRBOI | Live | Blood |
| 5/11/2011 | 884 | F | MNF | GVSU/LRBOI | Live | Blood |
| 5/12/2011 | 058 | M | MNF | GVSU/LRBOI | Live | Blood |
| 5/12/2011 | 522 | F | MNF | GVSU/LRBOI | Live | Blood |
| 5/12/2011 | 606 | M | MNF | GVSU/LRBOI | Live | Blood |
| 5/14/2011 | 290 | M | MNF | GVSU/LRBOI | Live | Blood |
| 5/15/2011 | 314 | M | MNF | GVSU/LRBOI | Live | Blood |
| 5/15/2011 | 609 | F | MNF | GVSU/LRBOI | Live | Blood |
| 5/17/2011 | 372 | M | MNF | GVSU/LRBOI | Live | Blood |
| 7/29/2011 | RKM | M | MNF | GVSU/LRBOI | Mortality† | Tissue |
| 7/30/2011 | 333 | M | MNF | GVSU/LRBOI | Live | Blood |
| 1/6/2012 | 550 | M | MNF | GVSU/LRBOI | Live | Blood |
| 1/7/2012 | 009 | F | MNF | GVSU/LRBOI | Live | Blood |
| 1/8/2012 | 600 | F | MNF | GVSU/LRBOI | Live | Blood |
| 1/8/2012 | 635 | M | MNF | GVSU/LRBOI | Live | Blood |
| 1/9/2012 | 521 | F | MNF | GVSU/LRBOI | Live | Blood |
| 1/10/2012 | 523 | M | MNF | GVSU/LRBOI | Live | Blood |
| 1/13/2012 | 124 | M | MNF | GVSU/LRBOI | Live | Blood |
| 5/7/2012 | 365 | F | MNF | GVSU/LRBOI | Live | Blood |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5/10/2012 | 317 | M | MNF | GVSU/LRBOI | Live | Blood |
| 5/10/2012 | 889 | M | MNF | GVSU/LRBOI | Live | Blood |
| 5/16/2012 | 367 | F | MNF | GVSU/LRBOI | Live | Blood |
| 5/17/2012 | 798 | F | MNF | GVSU/LRBOI | Live | Blood |
| 5/14/2013 | 367k | UNK | MNF | GVSU/LRBOI | Mortality* | Tissue |
| 5/16/2013 | 627 | F | MNF | GVSU/LRBOI | Live | Blood |
| 5/19/2013 | 581 | M | MNF | GVSU/LRBOI | Live | Blood |
| 6/30/2013 | 055 | M | MNF | GVSU/LRBOI | Live | Blood |
| 7/2/2013 | 601 | M | MNF | GVSU/LRBOI | Live | Blood |
| 7/3/2013 | 057 | M | MNF | GVSU/LRBOI | Live | Blood |
| 7/3/2013 | 100 | M | MNF | GVSU/LRBOI | Live | Blood |
| 7/3/2013 | 847 | F | MNF | GVSU/LRBOI | Live | Blood |
| 7/3/2013 | 857 | F | MNF | GVSU/LRBOI | Live | Blood |
| 7/6/2013 | 078 | M | MNF | GVSU/LRBOI | Live | Blood |
| 8/22/2013 | 619 | M | MNF | GVSU/LRBOI | Live | Blood |
| 2/21/2004 | 101 | M | PGR | CMU | Live | Tissue |
| 7/24/2004 | 102 | M | PGR | CMU | Live | Tissue |
| 1/17/2005 | 109 | M | PGR | CMU | Live | Tissue |
| 1/22/2005 | 103 | M | PGR | CMU | Live | Tissue |
| 2/4/2005 | 110 | F | PGR | CMU | Live | Tissue |
| 2/5/2005 | 111 | M | PGR | CMU | Live | Tissue |
| 2/6/2005 | 112 | F | PGR | CMU | Live | Tissue |
| 2/12/2005 | 113 | M | PGR | CMU | Live | Tissue |
| 2/25/2005 | 114 | M | PGR | CMU | Live | Tissue |
| 3/2/2005 | 115 | M | PGR | CMU | Live | Tissue |
| 3/16/2005 | 104 | F | PGR | CMU | Live | Tissue |
| 3/18/2005 | 105 | M | PGR | CMU | Live | Tissue |
| 3/18/2005 | 106 | F | PGR | CMU | Live | Tissue |
| 1/26/2006 | 120 | M | PGR | CMU | Live | Tissue |
| 2/14/2006 | 121 | M | PGR | CMU | Live | Tissue |
| 2/27/2006 | 122 | F | PGR | CMU | Live | Tissue |
| 3/3/2006 | 123 | M | PGR | CMU | Live | Tissue |
| 3/3/2006 | 124 | F | PGR | CMU | Live | Tissue |
| 3/3/2006 | 125 | M | PGR | CMU | Live | Tissue |
| 3/20/2006 | 126 | M | PGR | CMU | Live | Tissue |
| 3/21/2006 | 127 | F | PGR | CMU | Live | Tissue |
| 3/23/2006 | 128 | F | PGR | CMU | Live | Tissue |
| 3/24/2006 | 129 | M | PGR | CMU | Live | Tissue |
| UNK | 05294 | UNK | PGR | CMU | UNK | Tissue |
| 5/24/2013 | 128 | F | East UP | GVSU/SSMTCI | Live | Blood |
| 5/24/2013 | 180 | M | East UP | GVSU/SSMTCI | Live | Blood |
| 5/25/2013 | 247 | F | East UP | GVSU/SSMTCI | Live | Blood |
| 5/26/2013 | 038 | M | East UP | GVSU/SSMTCI | Live | Blood |
| 5/26/2013 | 192 | F | East UP | GVSU/SSMTCI | Live | Blood |
| 5/26/2013 | 599 | F | East UP | GVSU/SSMTCI | Live | Blood |
| 6/18/2013 | 002 | F | East UP | GVSU/SSMTCI | Live | Blood |
| 6/21/2013 | 512 | M | East UP | GVSU/SSMTCI | Live | Blood |
| 6/23/2013 | 994 | F | East UP | GVSU/SSMTCI | Live | Tissue |
| 6/24/2013 | 496 | M | East UP | GVSU/SSMTCI | Live | Blood |
| 6/28/2013 | 552 | F | East UP | GVSU/SSMTCI | Live | Blood |
| 8/13/2013 | 048 | M | East UP | GVSU/SSMTCI | Live | Blood |
| 8/13/2013 | 721 | F | East UP | GVSU/SSMTCI | Live | Blood |
| 8/14/2013 | 105 | M | East UP | GVSU/SSMTCI | Live | Blood |
| 11/1/2012 | 626 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 11/1/2012 | 1308 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 11/4/2012 | 1148 | UNK | East UP | SSMTCI | Harvest | Tissue |

| | | | | | | |
|---|---|---|---|---|---|---|
| 11/5/2012 | 627 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 11/7/2012 | 1131 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 11/9/2012 | 1222 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 11/16/2012 | 1203 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 11/20/2012 | 1226 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 11/24/2012 | 1156 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 11/30/2012 | 1225 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/2/2012 | 1224 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/3/2012 | 1139 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/3/2012 | 1142 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/3/2012 | 1192 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/4/2012 | 1143 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/4/2012 | 1223 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/5/2012 | 1157 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/6/2012 | 1158 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/6/2012 | 1159 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/6/2012 | 1208 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/6/2012 | 1229 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/8/2012 | 1145 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/9/2012 | 1137 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/9/2012 | 1138 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/9/2012 | 1144 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/9/2012 | 1150 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/10/2012 | 1141 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/11/2012 | 1201 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/12/2012 | 1230 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/13/2012 | 1209 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/14/2012 | 1202 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/14/2012 | 1205 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/17/2012 | 1136 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/20/2012 | 1227 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/20/2012 | 1228 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/26/2012 | 1147 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 12/26/2012 | 1309 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 1/15/2013 | 1211 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 1/15/2013 | 1212 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 1/26/2013 | 1193 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 2/20/2013 | 1216 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 2/24/2013 | 1214 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 3/1/2013 | 1215 | UNK | East UP | SSMTCI | Harvest | Tissue |
| 3/9/2013 | 1220 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 1 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 2 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 3 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 4 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 5 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 6 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 7 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 8 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 9 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 10 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 18 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 19 | UNK | East UP | SSMTCI | Harvest | Tissue |
| UNK | 20 | UNK | East UP | SSMTCI | Harvest | Tissue |

†Roadkill mortality

* Kit of female marten 367 found abandoned

## Appendix B

### Summary of population viability analysis runs

List of input parameters modified in each run of population viability analysis carried out using program VORTEX v. 10.0 (Lacy 1993)

| | | |
|---|---|---|
| Run 1-3 | Varying density dependence (DD) | |
| Run 1 | Mild DD | P(0) = 90, P(K) = 70, A = 1, B = 8 |
| Run 2 | Moderate DD | P(0) = 85, P(K) = 50, A = 2, B = 4 |
| | Run 2 serves as the base model for all subsequent simulations modeling effects of other parameter modifications. | |
| Run 3 | Strong DD | P(0) = 80, P(K) = 30, A = 4, B = 2 |
| Run 4-9 | Varying initial population sizes (N) and carrying capacities (K) | |
| Run 4 | N = 50, K = 50 | |
| Run 5 | N = 50, K = 100 | |
| Run 6 | N = 100, K = 100 | |
| Run 7 | N = 100, K = 250 | |
| Run 8 | N = 100, K = 500 | |
| Run 9 | N = 100, K = 1000 | |
| Run 10-14 | Varying brood distribution (percentage of females producing 1 litter) | |
| Run 10 | 100% of females produce 1 litter | 0% produce 0 litters |
| Run 11 | 80% of females produce 1 litter | 20% produce 0 litters |
| Run 12 | 70% of females produce 1 litter | 30% produce 0 litters |
| Run 13 | 60% of females produce 1 litter | 40% produce 0 litters |
| Run 14 | 50% of females produce 1 litter | 50% produce 0 litters |
| Run 15-18 | Varying supplementation methods (all adult marten, 1 M: 1.5 F) | |
| Run 15 | 10 marten released per year (4 M, 6 F) at years 5 & 6 | |
| Run 16 | 20 marten released per year (8 M, 12 F) at years 5 & 6 | |
| Run 17 | 10 marten released per year (4 M, 6 F) at years 5 & 7 | |
| Run 18 | 20 marten released per year (8 M, 12 F) at years 5 & 7 | |
| Run 19-25 | Varying catastrophes (CDV = Canine Distemper Virus, Toxo = Toxoplasmosis) | |
| Run 19 | CDV, 50% reduction in survival, 0% reduction in reproduction, 3% probability of occurrence. | |
| Run 20 | CDV, 70% reduction in survival, 0% reduction in reproduction, 3% probability of occurrence. | |
| Run 21 | CDV, 90% reduction in survival, 0% reduction in reproduction, 3% probability of occurrence. | |
| Run 22 | Toxo, 0% reduction in survival, 10% reduction in reproduction, 3% probability of occurrence. | |
| Run 23 | Toxo, 0% reduction in survival, 10% reduction in reproduction, 6% probability of occurrence. | |
| Run 24 | Fire, 30% reduction in survival, 10% reduction in reproduction, 3% probability of occurrence. | |
| Run 25 | CDV, Toxo, and Fire:<br>CDV, 50% reduction in survival, 0% reduction in reproduction, 3% probability of occurrence.<br>Toxo, 0% reduction in survival, 10% reduction in reproduction, 3% probability of occurrence.<br>Fire, 30% reduction in survival, 10% reduction in reproduction, 3% probability of occurrence. | |

## Appendix C

### Summary of population viability analysis results

Probability of extinction (P(E)), number of populations extinct of 1000 simulations over 100 years, percentage of heterozygosity ($H_e$) loss from year 0 to year 100, and final population size (N) of population viability analysis runs carried out using program VORTEX v. 10.0 (Lacy 1993). Bolded values indicate simulations which fail to meet demographic (P(E) < 0.10) and/or genetic viability ($H_e$ loss < 10%) requirements.

| Run‡ | P(E) (SE) | Num. of pop. extinct (of 1000) | $H_e$ loss (%) | Year 100 pop size (N) (SE) |
|---|---|---|---|---|
| 1 | 0 | 0 | **15.80** | 96.35 (0.21) |
| 2 | 0 | 0 | **15.64** | 90.91 (0.34) |
| 3 | 0.008 (0.003) | 8 | **19.46** | 63.56 (0.59) |
| 4 | 0.032 (0.006) | 32 | **31.32** | 37.46 (0.35) |
| 5 | 0 | 0 | **15.87** | 90.87 (0.31) |
| 6 | 0 | 0 | **15.51** | 90.90 (0.32) |
| 7 | 0 | 0 | 6.49 | 237.97 (0.51) |
| 8 | 0 | 0 | 3.32 | 479.19 (0.89) |
| 9 | 0 | 0 | 1.74 | 964.70 (1.59) |
| 10 | 0 | 0 | **15.48** | 93.40 (0.27) |
| 11 | 0.001 (0.001) | 1 | **16.30** | 85.43 (0.43) |
| 12 | 0.017 (0.004) | 17 | **18.51** | 67.88 (0.71) |
| 13 | **0.190 (0.012)** | 190 | **23.85** | 40.78 (0.84) |
| 14 | **0.806 (0.013)** | 806 | **34.44** | 14.88 (0.86) |
| 15 | 0 | 0 | **15.64** | 93.94 (0.25) |
| 16 | 0 | 0 | **15.20** | 94.83 (0.24) |
| 17 | 0 | 0 | **15.35** | 93.87 (0.26) |
| 18 | 0 | 0 | **15.35** | 94.69 (0.23) |
| 19 | 0.037 (0.006) | 37 | **19.46** | 77.34 (0.77) |
| 20 | **0.283 (0.014)** | 283 | **24.65** | 61.92 (1.21) |
| 21 | **0.798 (0.013)** | 798 | **28.44** | 54.74 (2.66) |
| 22 | 0 | 0 | **15.48** | 90.61 (0.33) |
| 23 | 0 | 0 | **15.66** | 90.80 (0.33) |
| 24 | 0 | 0 | **16.75** | 85.59 (0.48) |
| 25 | 0.073 (0.008) | 73 | **21.01** | 69.36 (0.87) |

‡For a detailed description of each run see Appendix B.