12-2015

Health assessment of two reintroduced populations of American martens (Martes americana) in Michigan

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To the Graduate Council:

I am submitting herewith a dissertation written by Maria Catherine Spriggs entitled "Health assessment of two reintroduced populations of American martens (Martes americana) in Michigan." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Natural Resources.

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Health assessment of two reintroduced populations of American martens (*Martes americana*) in Michigan

A Dissertation Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Maria Catherine Spriggs
December 2015

DEDICATION

I dedicate this work to my husband, Nathan Roberts.

ACKNOWLEDGEMENTS

I am thankful for the following people who have helped me tremendously: my parents Ann & Ray Spriggs, Jill Witt, Dan Mulcahy, Bob Sanders, Tamara Hillman, Melissa Nichols, Chris Schumacher, Eric Clark, Brad Silet, Rusty Aikens, Lisa Muller, Paula & Kelli Gumpington, Dave Unger, Max Cox, Kristine VanHoosier, Amos Morris, Jeff Andrews, Rachel Thompson, Erik Klaphake, Ann Duncan, Kendal Harr, Pam Nankervis, Ari Cornman, and my committee members Rick Gerhold, Becky Wilkes, Paul Keenlance, and especially Deb Miller.

I gratefully acknowledge the following for financial support: Evansville Zoological Society, Pittsburgh Zoo, Minnesota Zoo, and Detroit Zoo.

ABSTRACT

The American marten (*Martes americana*) was extirpated from Michigan during the early-20th century due to loss of vast areas of mature conifer forest and unregulated trapping. The species was reintroduced into the Upper Peninsula (UP) and Northern Lower Peninsula (NLP) during the mid-20th century. While the American marten population in the UP has grown and is doing well, the population in the NLP has been less successful. The reasons for the limited success of the NLP population are unknown, but may include lack of suitable habitat, limited reproductive success, poor genetic diversity, disease, or negative environmental impacts. American marten were live-trapped from 2011-2015 in the Manistee National Forest (NLP) and the Hiawatha National Forest (UP) of Michigan concurrent with a large-scale habitat and genetic study to evaluate the health of these two reintroduced populations. Parameters assessed included blood chemistry and complete blood counts, fecal parasite exams, hair stable isotope ratios, and serological evidence of disease. In addition, carcasses from trapper-harvested American marten in the UP were collected during 2012-2014 for hair stable isotope ratios and *Toxoplasma* serology. This is the first report of an assessment of general health and exposure to pathogens in American marten in Michigan and will be used to inform future management decisions including additional reintroductions of the species to the NLP.

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INTRODUCTION

The American marten (*Martes americana*) is a mesocarnivore ranging from the boreal forests of Canada into coniferous and mixed coniferous/deciduous forests of the northern United States including the Great Lakes region. The species was extirpated from Michigan during the early-20th century due to loss of vast areas of mature conifer forest and unregulated trapping. American martens were taken from the Chapleau Game Preserve in Ontario, Canada for reintroduction to the Upper Peninsula (UP) in the 1950's and 1970's and to the Northern Lower Peninsula (NLP) in the 1980's. While the American marten population in the UP has grown, the population in the NLP remains limited and the American marten is listed as a Forests' Sensitive Species in the Huron-Manistee National Forest of the NLP. The presence of American martens has been used as an indicator of forest structural quality. To better manage for American martens and other species with similar habitat requirements, the U.S. Forest Service and the Michigan Department of Natural Resources have expressed an immediate need for additional American marten population and habitat research. American martens are a culturally important species to Native Peoples of the region, and tribal, federal and state agencies are invested in this research for guidance on population and habitat management for this regionally rare species.

Data is needed in order to incorporate disease transmission risks in management decisions such as additional reintroductions of American martens to the NLP. As such, I aimed to answer the following questions: 1) How can we minimize risk associated with live-trapping and anesthesia? 2) If a future reintroduction were to take place from the UP to the NLP, what diseases or parasites may be of concern? 3) How will we determine if an individual marten is healthy? 4) What are the causes of mortality of radio-collared American martens in Michigan? 5) Using stable isotope analysis, is there any difference in foraging ecology between the UP and NLP?

This study is the first report of blood gas values, lactate and physical exam findings of wild American martens under isoflurane anesthesia. Similar to other members of Mustelidae, American marten are susceptible to infectious diseases including canine distemper, toxoplasmosis, parvovirus and leptospirosis but there is no information about the exposure of American marten to these pathogens in Michigan. This study is the first report of serologic evidence of pathogen exposure, and also of endoparasitism in American martens in Michigan based on non-invasive fecal analyses from live-trapped animals. Carbon and nitrogen stable isotope data is used in this report to elucidate differences in the feeding ecology of American martens in UP versus the NLP.

CHAPTER I
Effects of live-trapping and isoflurane anesthesia on free-ranging American martens (*Martes americana*)

Co-researchers for this part include Paul Keenlance, Lisa Muller, Ari Cornman, Bob Sanders, Becky Wilkes, Rick Gerhold and Debra Miller. Co-researchers assisted in field-work, data collection, statistical analysis, and/or manuscript review. We gratefully acknowledge the Evansville Zoological Society, Minnesota Zoo, Pittsburgh Zoo, and Detroit Zoo for financial support and the Sault Tribe of Chippewa Indians Natural Resource Department and the Keweenaw Bay Indian Community Natural Resource Department for their contributions to this work. This article has not been published.

Abstract

Seventy-two free-ranging American marten in Michigan were live-trapped and anesthetized using isoflurane in the field from 2011-2015. A total of 129 anesthetic procedures were performed with no mortalities. Hypo- and hyperthermia were the most common anesthetic complications and rectal temperature was significantly higher during summer months than winter. Dental abnormalities were common in wild American martens; the majority of abnormal findings were broken or discolored teeth attributed to previous dental trauma and were not trap-induced. Blood was analyzed during 64 anesthetic events from 49 American martens for venous blood gas, lactate, hematocrit and/or selected serum biochemistry analytes. Using domestic feline reference ranges, the acid-base status and oxygen saturation of anesthetized American martens in this study were normal as determined by blood pH and pulse oximetry, respectively. Lactate values have not been reported in martens but have been used to assess exertion associated with capture and handling of other free-ranging animals including the wolverine (*Gulo gulo*), another mustelid species (Fahlman et al. 2008). Serum biochemistry parameters, multiple environmental parameters, and American marten-specific attributes were evaluated for their influence on lactate in American martens using an information theoretic approach. The most important serum biochemistry value for predicting lactate was blood urea nitrogen. The most important environmental or marten-specific variables for predicting lactate included initial body temperature and time from when an American marten was discovered in a trap until the immobilization started. Lactate was measured by two different devices (VetScan i-STAT 1 and Lactate Plus) and compared for clinical agreement. Both methods for lactate measurement gave similar results. Recommendations for the live-trapping and isoflurane anesthesia of free-ranging American martens include using caution during warmer summer months, minimizing disturbance to an American marten prior to induction, monitoring lactate in addition to vital rates, and being prepared to prevent or treat both hypo- and hyperthermia during any time of year.

Introduction

The American marten (*Martes americana*) is a mesocarnivore ranging from the boreal forests of Canada into coniferous and mixed coniferous/deciduous forests of the northern United States including the Great Lakes region (Powell et al. 2003). The species was extirpated from Michigan during the early-20th century due to habitat loss and unregulated trapping and reintroduced to the Upper Peninsula (UP) and northern Lower Peninsula (NLP) in the mid-20th century (Earle et al. 2001, Cooley 2004). While the American marten population in the UP has grown, the population in the NLP is limited and the American marten is listed as a Forests' Sensitive Species in the Huron-Manistee National Forest of the NLP (Marten Conservation Strategy 1996). Reasons for the limited population growth in the NLP may include population genetics, habitat fragmentation, or forest management practices. A project to assess the NLP population required the live-trapping and immobilization of American martens. We report the blood gas and lactate values of American martens under isoflurane anesthesia and expand on a previous report that found isoflurane to be safe in American martens immobilized in the field (Desmarchelier 2007). Isoflurane has advantages over injectable anesthetics including rapid induction and recovery, ability to adjust depth of anesthesia, no risk of miscalculations in dosage or erroneous weight estimations, and no requirement for controlled drug access. All anesthetics carry inherent risk however, and capture and handling can cause exertion and stress that may affect anesthetic risk. Lactate values have not been reported in American martens but have been used to assess exertion associated with capture and handling of other free-ranging animals including the wolverine (*Gulo gulo*), another mustelid species (Fahlman et al. 2008). Lactate production largely occurs under conditions of anaerobic cellular respiration or hypoxia (Allen and Holm, 2008). Causes of tissue hypoxia include exercise, low arterial oxygen, anemia, shock, and others (Allen and Holm 2008). Understanding the environmental and American marten-specific variables that may influence lactate can help in creating handling protocols that minimize morbidity or mortality associated with live-trapping and anesthesia of American martens.

Methods

American martens were live-trapped in the Manistee National Forest (n=43) in the NLP and the Ottawa National Forest (n=5) and the Hiawatha National Forest (n=24) in the UP using box live traps (Tomahawk Live Trap, LLC, Hazelwurst, Wisconsin, USA) from 2011-2015. A total of 121 anesthetic procedures were performed outdoors at ambient temperatures ranging from 10° F to 90° F. The majority of American martens were restrained in a fabric cone and induced with isoflurane (IsoFlo®, Abbott Laboratories, Abbott Park, Illinois, USA) via facemask with a portable anesthesia machine as described by Desmarchelier et al. (2007) (n=113). The portable anesthesia machine could be carried into the backcountry

when necessary by affixing the vaporizer and oxygen cylinder to a backpack (Figure 1). Some martens were chamber-induced by placing the live trap inside a large plastic bag or plastic container (n=5), or induced by placing an isoflurane-soaked cotton ball at the tip of the facemask but not in direct contact with the American marten's nose (n=3). American martens were maintained on isoflurane in oxygen using a nonrebreathing circuit. American martens were positioned on a foam pad covered with a disposable pad to reduce exposure to cold surfaces and were monitored by either a wildlife veterinarian or other trained personnel. Body weight was measured using a gram scale (My Weigh, Phoenix, Arizona, USA). Heart rate, respiratory rate, rectal temperature and oxygen saturation via pulse oximetry were recorded after induction and periodically throughout the anesthetic procedure. Induction time (time from onset of isoflurane to recumbency), length of procedure (onset to discontinuation of isoflurane), recovery time to standing, and time to release were recorded. Each American marten was implanted with a sterile microchip (AVID Identification Systems, Norco, California, USA) between the shoulder blades for permanent identification. Some American martens (n=55) were fitted with a radiotelemetry collar (Advanced Telemetry Systems, Isanti, Minnesota, USA) as part of a larger habitat and population study.

Methods used to prevent or treat hypothermia included the use of a foam pad between the American marten and the tailgate, towels to cover the American marten, instant hand warmers, warm air from a blow dryer, moving the anesthesia machine and American marten into a heated truck cab, and minimizing the use of isopropyl alcohol to prepare venipuncture sites. The heating pad and blow dryer were supplied with power by an inverter connected to a separate vehicle battery and were always available during summer months as well. Methods used to treat or prevent hyperthermia included application of 70% isopropyl alcohol (First Priority, Inc., Elgin, Illinois, USA) to footpads and jugular grooves, administration of subcutaneous fluids and/or fluids per rectum. Subcutaneous fluids, either warmed or at room temperature as appropriate, were used to prevent or treat dehydration when indicated (0.9% NaCl, Hospira, Inc., Lake Forest, Illinois, USA; 30 ml/kg SQ). Emergency supplies including endotracheal tubes and laryngoscope and emergency drugs were available to treat serious complications.

A total of 72 blood samples were collected from 53 American martens. American martens were sampled more than once if recaptured for radiocollar maintenance. Blood was collected from the jugular vein and did not exceed 1% body weight in volume. Sampling was not repeated in the same animal within 30 days to minimize risk of iatrogenic anemia. Blood was placed into lithium heparin anticoagulant (BD Microtainer ® Tubes, Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) and serum separator tubes (Covidien, Mansfield, Massachusetts, USA). The blood in the serum separator tubes was allowed to clot and was then centrifuged in the field for 10 minutes. Serum was transported in a cooler while in the field at a controlled temperature between 37-41°F.

Fresh whole or lithium heparinized venous blood was analyzed in the field within 10 min of collection using a portable blood gas analyzer (VetScan i-STAT 1, Abaxis, Union City, California, USA) and CG4+ cartridge (analytes: pH, pCO_2, HCO_3, tCO_2, pO_2, sO_2, lactate) and using a portable lactate meter (Lactate Plus, Nova Biomedical, Waltham, Massachusetts, USA). Serum samples were analyzed within 8 hours using the VetScan (Abaxis, Union City, California, USA) using the equine rotor [creatine kinase (CPK), aspartate aminotransferase (AST)] and small animal comprehensive rotor [blood urea nitrogen (BUN), creatinine (CREAT), glucose]. Whole blood was used to determine hematocrit (HCT) using microhematocrit capillary tubes (SafeCrit, Westwood, Massachusetts, USA) and the StatSpin® VT centrifuge (Iris, Westwood, Massachusetts, USA).

Statistical analysis was performed using JMP® Pro 10.0.02 (SAS Institute Inc., Cary, North Carolina, USA). Lactate values measured simultaneously by i-STAT and the lactate meter were compared for clinical agreement using the Bland-Altman technique, which is a correlation analysis to measure if two values are related and not if they provide similar clinical measurements (Bland and Altman, 1986). Blood parameters, environmental variables, and American marten-specific attributes were compared using *a priori* models from factors hypothesized to affect lactate values. We used mixed linear models (PROC MIXED; SAS Institute, Inc., Cary, North Carolina, USA) and Akaike Information Criteria for small sample sizes (AICc) for model selection (Burnham and Anderson 2002).

The capture and handling protocol was approved by the University of Tennessee Animal Care and Use Committee (protocol #2180) and American marten live-trapping and sample collection was an authorized tribal activity under the 2007 Inland Consent Decree between the State of Michigan and the Little River Band of Ottawa Indians.

Results

A total of 129 anesthetic procedures were performed on 72 individual American martens (40 males, 32 females) including 11 juveniles (<6 months old). On average, adult males weighed 1002±116 grams and females weighed 695±97 grams. American martens restrained in a fabric restraint cone were induced at 5% isoflurane in 1-2 L/min of oxygen on a non-rebreathing circuit. Induction time was short for facemask induction ($\overline{x}$=1.4 min, range 1-9 min, n=121). Induction using the isoflurane-soaked cotton ball method was also short ($\overline{x}$=1.7 min, range 1-2, n=3) while chamber induction was longer ($\overline{x}$=7.6 min, range 3-13, n=5). Regardless of induction method, all American martens were maintained via facemask between 0.5-5% isoflurane to effect. The average procedure length was 24±8 minutes (range 8-51 min, n=121). The average time between discontinuation of the isoflurane and recovery to standing was recorded for 112 procedures and averaged 7±4 minutes (range 1-20 min). The time between discontinuation of the isoflurane and release was recorded for 116

procedures and averaged 15±6 minutes (range 6-48 min). Average rectal temperature, heart rate, respiratory rate and oxygen saturation during summer and winter months are shown in Table 1. Rectal temperature was significantly higher during summer months than winter months (P<0.05) and there was a significant (P=0.03) correlation (r=0.27) between ambient temperature and initial rectal temperature. Heart rate was significantly higher in the first ten minutes of anesthesia during summer than winter (p<0.05). No American marten needed to be intubated due to apnea or treated with emergency drugs.

Venous blood gas, lactate and select serum biochemistry values results are shown in Table 2. However, two values for the i-STAT were deleted because of error messages for temperature of the machine at the time of analysis. Seventeen American martens had only a Lactate Plus value and no i-STAT data due to limited sample size or shortage of i-STAT cartridges. Bland-Altman analysis of 26 events found a mean difference of -0.13±0.49 mmol/L (range -0.76-1.38 mmol/L) between the Lactate Plus meter and i-STAT. All but two values were within ±2 SD of the mean and both of the outliers were at the lower end of the range for lactate (Figures 2 and 3). Therefore we considered both i-STAT and Lactate Plus meter readings as equivalent measurements of lactate. Where both readings were available, we used the mean of the two measurements for further analysis. If only one method was available, we used that value. Lactate was not normally distributed and was transformed using a natural log for analysis.

Blood values evaluated using *a priori* models for their influence on lactate included BUN, creatinine, glucose, CPK, AST, and hematocrit. We used Pearson correlation (PROC CORR) to evaluate relationships between variables. The only highly correlated variables were CPK and AST (r=0.91) and weight and sex (r=0.85). However, based on Burnham and Anderson (2002) we used these parameters in the analysis because the correlation was not extreme (i.e., r observed was <0.95) and each added additional information. The top model for predicting lactate with blood variables included BUN and creatinine (Table 3). However, all models with AICc < 3.0 included BUN (79% of model weights). Adding creatinine and glucose improved the model slightly, but CPK and AST appeared as uninformative parameters in the top models (Arnold 2010). The ß estimate for BUN was 0.01 (SE=0.00). Environmental and American marten-specific variables evaluated for their influence on lactate included the time from when a American marten was discovered in a trap until the immobilization started (TimeSince), number of broken nails (BrknNails), presence of fresh gingival or dental trauma (OralTrma), ambient temperature (AmbientT), initial body temperature (InitialT), season, sex, body-weight (weight), lactation status of females (Lactating), recovery time from discontinuation of isoflurane to standing (Recovery), and whether or not the American marten was wearing a radiotelemetry collar (Collar). The top models for American marten-specific and environmental variables (AICc < 3.0) included InitialT and TimeSince (Table 4). Adding AmbientT, broken nails, and oral trauma were also in the top models but

the strength of the models appeared to be related to InitialT (ß = 0.11, SE=0.05) and TimeSince (ß = 0.06, SE=0.06).

The most common physical exam finding was previously damaged dentition, which was seen in 43.1% of the 72 individual American martens examined. Of the affected American marten, 19.4% had one or more previously fractured but not yet devitalized (discolored) canines, 6.9% had one or more intact but devitalized canines, 9.7% had one or more canine that was both fractured and devitalized, and 6.9% had previously damaged incisors (discolored, fractured and/or excessively worn). Four American martens (5.6%) were noted to have tartar. There was no difference between males and females with dental damage (*P*=0.48). In contrast to the previously damaged teeth seen in individual American marten, minor recent gingival trauma presumably due to chewing at the trap was noted in 9.3% of 129 procedures. Evidence of a freshly fractured tooth was seen during two procedures (1.6%).

Other physical examination findings included superficial abrasions on chin, lips or nose (n=8), collar-associated dermatitis (n=5), hair mats (n=3), puncture or suspected bite wound on leg (n=2), crust on ear pinna (n=2), fleas (n=2), mild epistaxis (n=1), pinpoint ulcer on the anus (n=1), and thorn in skin of leg (n=1). Nail wear or breakage was recorded for 100 procedures. One or more nails was worn, frayed or broken in 80 procedures due to digging while in the trap.

American martens were treated for hyperthermia (rectal temperature ≥ 105°F) during 18 of 129 procedures, 16 of which occurred during summer months. All but three of these 18 American martens had a final rectal temperature of <104°F by the end of the procedure. American martens were treated for mild hypothermia (<98°F) during 12.4% of procedures (n=16), and severe hypothermia (<96°F) during 3.9% of procedures (n=5). Of the 21 procedures during which hypothermia occurred, only 9 were during winter months and occurred despite measures taken to prevent hypothermia such as heating pads, hand warmers, or blow dryer use. Subcutaneous fluids were administered during 47% of procedures and became routine practice during summer months for prevention or treatment of hyperthermia, dehydration, hyperlactemia and/or capture myopathy. Due to difficulty in keeping fluids warm during transport, they were not routinely administered during winter months.

Discussion

Restraint in the fabric cone for anesthetic induction as described by Desmarchelier (2007) was effective for almost all American martens as they readily entered the cone upon opening the trap door. Chamber induction was required for 5 procedures, and 4 of these procedures were for two American martens that had been previously trapped and anesthetized via restraint in the fabric cone. It is suspected that they were more wary of entering the cone than first-time captures. The use of an isoflurane-soaked cotton ball has been used

for anesthesia of small mammals including eastern grey squirrels (*Sciurus carolinensis*), Allegheny woodrats (*Neotoma magister*), three-banded armadillos (*Tolypeutus matacus*), and others (Parker et al. 2008; West et al. 2008). Isoflurane-soaked cotton ball anesthesia resulted in capture myopathy in armadillos probably due to overdose as isoflurane can vaporize to high concentrations with this method (West et al. 2008). A method was desired for a very brief sedation of American martens for placement of a microchip and thus the cotton-ball method was attempted for induction in 3 American martens. Subjectively the induction was very rapid and deep but concern for overdose and/or repeated use of the cotton-ball to maintain an anesthetic plane longer than intended precluded recommendation of this method in American martens without further research.

On average, American martens recovered to standing 7 min after discontinuation of the isoflurane and were released 15 min after discontinuation of the isoflurane. American martens were released when they exhibited normal behavior such as vocalization and aggression and when field equipment was packed and personnel ready to depart; thus release times do not necessarily reflect the earliest possible time that an American marten could have been released based on normal behavior. Length of recovery as reported by Desmarchelier et al. (2007) was 6.3±2.8 min and was defined as the time between discontinuation of the isoflurane and American marten behavior considered ready for release. Recovery times reported here are shorter than those of reported using non-reversible injectable anesthetics in American martens (Desmarchelier et al. 2007). Recovery time, defined as return to normal behavior, of black-footed ferrets anesthetized with isoflurane was 16.3±1.4 min and was longer than that of injectable anesthetics (Kreeger et al. 1998). One male American marten in the current study had a prolonged recovery of 48 minutes during which time he was conscious but recumbent, vocalized only when stimulated, had a normal rectal temperature at the end of the procedure, and normal blood glucose. It is possible that this was an older animal based on subjective evaluation of tooth wear. The American marten was empirically treated with subcutaneous fluids, supplemental oxygen, and oral dextrose and was recaptured 18 months later and had a normal recovery.

Rectal temperature was significantly higher during the summer than the winter at all time points and were an average of 100.4-103.4°F during the summer and 98.0-102.3°F during the winter over 20+ minutes of anesthesia. Hyperthermia occurred most often during summer months as expected and there was a weak, positive but significant correlation between initial temperature and ambient temperature. In a previous report of American martens under isoflurane (Desmarchelier 2007) rectal temperatures ranged from 93.2-104.2°F at an average of 12 minutes post-induction but temperature at subsequent time points was not reported. Captive pine American martens (*Martes martes*) immobilized with medetomidine-ketamine had rectal temperatures ranging from 101.2-104.2°F (Arnemo et al. 1994). Free-ranging American martens immobilized with

a combination of tiletamine-zolazepam and xylazine had rectal temperatures ranging from 98.6-102.7°F (Belant 2005). While the normal body temperature of American martens is unknown, the reported body temperature for the related fisher (*Martes pennanti*) is 102.0±1.4°F (International Species Information System, 2002). Hyperthermia in anesthetized animals results in increased oxygen consumption and increased risk for serious complications such as capture myopathy (Arnemo and Caulkett 2008). Increased oxygen consumption associated with increased body temperatures may explain the significantly higher heart rates seen in the first ten minutes of anesthesia during summer months (April-September) compared to winter months (October-March). Anesthesia was discontinued for one American marten with an initial temperature of 107.2°F; active cooling measures included drenching footpads in isopropyl alcohol, cool water enema, subcutaneous fluids and placing an ice pack in the recovery box. This American marten was recaptured a week later and had an uneventful anesthesia.

While hypothermia occurred during winter months, it also occurred during summer months likely due to inhalation of cool oxygen, respiratory evaporative heat loss, reduction in thermoregulation associated with anesthesia, and a large body surface area to volume ratio (Heard 2008). Severe hypothermia can result in prolonged anesthetic recovery, depressed respiratory and heart rate and a reduced anesthetic requirement (Heard 2008). One hypothermic American marten in this report (final rectal temperature 94.7°F) had a longer than average time to standing and time to release of 18 and 27 minutes respectively. Frequent monitoring of body temperature and proactive efforts to prevent ongoing heat loss are indicated for American marten under isoflurane anesthesia.

Desmarchelier et al. (2007) reported heart rate, respiratory rate and oxygen saturation via pulse oximetry of American martens after an average of 10 minutes of isoflurane anesthesia; heart rate was 216±17.2 bpm (n=8), respiratory rate was 31±11.5 breaths per minute (n=18), and oxygen saturation values via pulse oximetry were >95% (n=not reported). These are similar to reported vital rates in this report after 10-19 minutes of isoflurane anesthesia during both summer and winter (Table 1).

Old dental damage including previously fractured, worn or discolored teeth were found in 43.1% of all American martens examined. In contrast to old or previous dental damage, recent oral trauma including gingival abrasions or freshly fractured teeth occurred in a minority of procedures (9.3% and 1.6%, respectively) and were presumably due to biting at the trap. Similar to other small mustelids, American martens use their canines to kill prey with precision by severing the spinal cord between vertebrae (Dayan et al. 1992). Damage could occur to teeth in this process or as a result of chewing bone. Dental damage was common in a review of large carnivore skull specimens, and individuals apparently functioned well despite damage to canine or carnassial teeth (Van Valkenburgh 1988). In a study of 155 North American river otters (*Lontra canadensis*) captured with leghold traps, all otters had injuries ranging from

superficial abrasions, missing claws, or digit luxations to open digit fractures (Tocidlowski et al. 2000). Seventy-four percent of river otters obtained from a supplier for a reintroduction program had fractured teeth and three were considered non-releasable due to severity of dental injury (Serfass et al. 1993). In contrast, trap-associated injury was uncommon in this study using Tomahawk live-traps for capture of American martens.

There were five instances of skin infection of the neck associated with collar wear. The collar was removed in all cases and a long-acting ceftiofur crystalline free acid injection (Excede®, 200 mg/ml, Zoetis, Florham Park, New Jersey, USA; 6.6 mg/kg, s.q.) was administered by a wildlife veterinarian to 3 of the 5 affected animals. All five American martens were subsequently reexamined and found to have fully recovered. Infections associated with the collar were attributed to weight gain resulting in the collar being too tight. Given the length of an American marten's neck and the excellent muscle relaxation achieved with isoflurane anesthesia, it is important to consider how the collar will ride and fit after recovery from anesthesia, return to normal neck posture, and after potential weight gain. Similar complications and the use of collar break-away mechanisms have been reported previously in American martens, and additional research into the optimal collar material, size and fit is warranted (Thompson et al. 2012).

Reference ranges for blood gas and serum biochemistry values are not available for the American marten. Using the domestic cat (another obligate carnivore) reference range provided by the manufacturer of the i-STAT analyzer (i-STAT User Manual, Abaxis, Union City, CA), pH, pCO_2, HCO_3, and TCO_2, were within normal range for American martens under isoflurane anesthesia in this study. Compared to venous blood gas values of polecats (*Mustela eversmanni*) anesthetized with isoflurane, the mean blood pH, HCO_3 and base excess were higher and pCO_2 lower in this report indicating that acid-base status and ventilation appear to be better in American martens under isoflurane compared to polecats in these two studies (Gaynor et al. 2011). A reference interval for venous (versus arterial) PO_2, sO_2 and BE has not been developed by the i-STAT manufacturer, but the lower values seen in American marten venous samples as compared to arterial samples from domestic cats is expected due to the de-oxygenation of venous blood relative to arterial blood. Using the domestic ferret and cat reference ranges provided by the manufacturer of the VetScan analyzer (VetScan User Manual, Abaxis, Union City, CA), the mean creatinine values for American martens in this report was within the reference range for domestic ferrets and cats. The mean BUN (35 mg/dL) was above the upper end of the reference range for cats (10-30 mg/dL) but within the reference range for ferrets (10-38 mg/dL). There was a large standard deviation for BUN with 32% of American martens having BUN values above the ferret reference range. An elevated BUN with normal creatinine may have been due to recent ingestion of raw meat bait or may be due to dehydration (Tripathi et al. 2011). Only one American marten was azotemic with an elevated BUN and creatinine, which is consistent with dehydration or renal dysfunction (Tripathi et al. 2011). Mean

blood glucose was higher in American martens than the ferret reference range and is attributed to transient excitement, catecholamine release and/or stress associated with handling (Evans 2011). Additionally, the mean values for enzymes CPK and AST were also above ferret and cat reference ranges and are attributed to enzyme release from skeletal muscle associated with exertion (Hall and Bender 2011).

The average lactate values for American martens reported here was 3.1 and 2.7 mmol/L as determined by the i-STAT and Lactate Plus meter, respectively, which are just above the i-STAT reference range for domestic cats. Lactate is cleared by the liver and kidneys and its half-life depends on the cause of hyperlactatemia (Pang and Boysen 2007; Allen and Holm 2008). It is possible that lactate values in some American martens may have been declining from peak values depending upon the time spent in the trap and time of exertion. The mean values for the muscle enzymes CPK and AST are 4 and 7 times the upper limit of the domestic cat reference range, respectively. CPK and AST take longer to rise and have a longer half-life compared to lactate; thus the relatively low lactate levels may be due to metabolism of lactate during the in-trap time prior to blood sampling (Hall and Bender, 2011). A slight increase in lactate was seen with longer times since initial human disturbance to the trapped American marten and blood sampling and this variable was included in the top models. American martens were noted to pull substrate material into the trap to form a nest and presumably rested in this material until disturbed by a person checking the trap. A delay between finding the American marten and inducing anesthesia may result in the American marten exerting itself and causing an increased lactate level. Initial body temperature was also included in the top model of environmental and American marten-specific variables and also likely reflects recent physical exertion. Of the serum biochemistry variables examined, BUN was included in the top 2 models. An increase in BUN may reflect recent ingestion of raw meat bait or may be increased due to dehydration. Urine specific gravity or other testing was not performed in this study to determine the cause of elevation in BUN.

Lactate values from the Lactate Plus meter were previously reported to be higher than the i-STAT for the same sample but correlation between each method and the reference method (Vitros LAC slide assay) was good (Karon et al. 2007). Comparison of the i-STAT and Lactate Plus meter using a Bland-Altman analysis showed both methods to provide similar clinical values. Average lactate values for live-trapped American martens in this study were just above the reference range for domestic cats and 12% of American martens sampled had lactate levels >5.0 mmol/L. Field measurement of lactate was found to be a useful objective measurement of a American marten's overall condition in addition to body temperature and physical exam, and the Lactate Plus meter was found to be a useful and suitable alternative to the more expensive i-STAT blood gas analyzer. Field personnel found lactate measurement easy to perform and interpret. American martens with elevated lactate levels were considered to be

at higher risk for complications and were given subcutaneous fluids, monitored closely during anesthesia, and procedures were completed quickly with efforts made to minimize stress upon recovery. In addition, American martens with markedly elevated lactate levels were found via radiotelemetry within a week after release to confirm survival and normal activity. Measuring lactate also helped to increase awareness about potential sublethal effects of handling American martens and trapping protocols were ultimately influenced: subcutaneous fluids were administered routinely to American martens anesthetized during the summer, the number of traps was limited to that which could be checked early in the day, females were anesthetized and released first, a trap was not carried out of the forest until all equipment was ready, and noise and visual stimulation was minimized prior to immobilization. Regardless of lactate level, no American marten in this report experienced mortality or overt signs of capture myopathy.

Restoration of a sustainable population of American martens in the NLP may require additional translocation of American martens or other management actions and knowledge of American marten anesthesia and physical examination findings may be useful to biologists and veterinarians. Future research could include urine or other analysis to assess hydration status of trapped American martens and further evaluation of the physiologic state of trapped American martens. Using trap timers would also be useful in assessing lactate levels as a function of overall time spent in a trap. We recommend isoflurane anesthetic for the field immobilization of American martens as it provides effective and safe anesthesia at a wide range of ambient temperatures as determined by vital signs, oxygen saturation and venous blood gas values. Although it requires the purchase of an isoflurane vaporizer and oxygen cylinder, the equipment can be transported via backpack into the backcountry and may be preferable to injectable anesthetics when an adjustable level of sedation and short recovery are desired or if one wants to avoid the use of controlled substances. When using isoflurane, one should be prepared to prevent and treat hypo- and hyperthermia at any ambient temperature. Measurement of lactate using a drop of blood and an inexpensive handheld lactate meter could provide useful information to guide the treatment and monitoring of individual American martens and the refinement of capture and handling protocols.

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Appendix

Table 1. Temperature, heart rate, respiratory rate and oxygen saturation (mean±SD) of 72 American martens during 121 anesthetic events using isoflurane. Significant differences ($p<0.05$) between seasons at each time are indicated by differing superscript.

Season	Time after induction (min)	Rectal temperature (°F)	Heart rate (bpm)	Respiratory rate (breaths/min)	Oxygen saturation (%)
Summer (n=94)	0-9	103.4±1.6[a]	256±43[a]	47±17	97±3
	10-19	101.7±1.8[a]	234±51	36±13	97±3
	20+	100.4±2.2[a]	234±31	27±11	96±3
Winter (n=35)	0-9	102.3±2.0[b]	236±47[b]	42±16	97±3
	10-19	99.5±2.1[b]	248±23	28±5	97±2
	20+	98.0±1.7[b]	211±40	N/A	97±1

Table 2. Venous blood gas, lactate and select serum biochemistry values for 53 American martens under isoflurane anesthesia. Values previously reported for polecats under isoflurane anesthesia are shown as well as ranges of other carnivores (domestic ferret and cat) for comparison.

Parameter	Mean	Range	SD	N[a]	Polecat Mean ± SE[b]	Other carnivore ranges[c]
pH	7.27	7.06-7.40	0.07	37	7.193±0.019	7.25-7.40
pCO_2 (mmHg)	47.4	30.4-112.0	13.1	37	55±3	33.0-51.0
HCO_3 (mmol/L)	24.4	14.7-155.0	22.3	37	20.8±0.7	13.0-25.0
TCO_2 (mmol/L)	21.9	16.0-28.0	3.0	37	NR[d]	16-25
PO_2 (mmHg)	77.1	29.9-166	29.9	36	NR	90-110
sO_2 (%)	89.7	69-99	7.6	36	NR	>90
Base excess (mmol/L)	-6.4	(-14)-0	3.4	37	-8.0±0.6	(-5)-(+2)
Lactate (i-STAT; mmol/L)	3.1	0.7-11.7	2.2	37	NR	0.5-2.7
Lactate (Lactate Plus; mmol/L)	2.7	0.8-11.8	1.8	57	NR	NR
BUN (mg/dL)	35	18-79	15	63	NR	10-38
Creatinine (mg/dL)	0.5	0.2-1.0	0.2	63	NR	0.2-0.7
Glucose (mg/dL)	167	87-282	38	63	NR	65-145
CPK (U/L)	1930	191-7358	1901	63	NR	50-450
AST (U/L)	298	98-773	142	63	NR	12-43
Hct (%)	43	30-56	6	72	NR	42-52

[a]Number reflects the total number of samples included as not all values were obtained for every American marten and some American marten were sampled more than once.
[b]Venous blood gas values reported for polecats (*Mustela eversmanni*) under isoflurane anesthesia (Gaylord et al. 2011).
[c]Reference range sources: VetScan i-STAT 1 User Manual (Abaxis, Union City, CA 94587; pH, pCO_2, HCO_3, TCO_2, lactate of feline venous whole blood; PO_2, BE, sO_2 of feline arterial whole blood), VetScan User Manual (Abaxis, Union City, CA 94587; BUN, creatinine, glucose, CPK, AST of ferret serum), Nieminen et al. 2007 (Hct of *M. americana*).
[d]Not reported.

Table 3. Blood values affecting lactate in blood collected during 64 anesthetic events of free-ranging American martens anesthetized using isoflurane under field conditions from 2011-2015 in Michigan. Models were ranked by decreasing Akaiake Information Criterion for small sample sizes (AICc).

Model	-2 Log Likelihood	K[a]	AICc	ΔAICc[b]	w_i[c]
Lactate[d] = $ß_0$[e] + BUN[f] + CREAT[f] + ε[g]	78.8	4	87.6	0	0.24
Lactate = $ß_0$ + BUN + CREAT + Glucose[f] + CPK[f] + AST[f]+ ε	71.5	7	88	0.4	0.20
Lactate = $ß_0$ + BUN + CREAT + Glucose+ ε	77	5	88.2	0.6	0.18
Lactate = $ß_0$ + BUN+ ε	81.8	3	88.3	0.7	0.17
Lactate = $ß_0$ + CREAT + ε	83.7	3	90.1	2.5	0.07
Lactate = $ß_0$ + GLUC + ε	84	3	90.5	2.9	0.06
Lactate = $ß_0$ + CREAT + GLUC + CPK + AST+ ε	77.3	6	91.1	3.5	0.04
Lactate = $ß_0$ + CREAT + GLUC + CPK + AST + HCT + ε	76.2	7	92.8	5.2	0.02
Lactate = $ß_0$ + AST+ ε	86.6	3	93.1	5.5	0.02
Lactate = $ß_0$ + CPK+ ε	86.8	3	93.3	5.7	0.01
Lactate = HCT[h] + ε	105.1	3	111.5	23.9	0.00

[a]Number of parameters.
[b]Relative difference between AICc of model and AICc of model with lowest AICc.
[c]Model weight.
[d]Lactate was measured by two different methods (VetScan i-STAT 1 and Lactate Plus) and compared for clinical agreement. Both methods for lactate measurement gave similar results. We used the mean of both measurements when available. Otherwise we used the measurements from either the i-STAT or Lactate Plus.
[e]Standardized regression coefficient.
[f]Serum samples including BUN (blood urea nitrogen), CREAT (creatinine), CPK (creatine kinase), AST (aspartate aminotransferase), and GLUC (glucose) were analyzed within 8 hours using the VetScan for biochemical analysis using the equine rotor and small animal comprehensive rotor.
[g]Regression error term.
[h]Whole blood was used to determine HCT (hematocrit) using micro capillary tubes and the StatSpin® VT centrifuge at 15,800

Table 4. American marten-specific and environmental values affecting lactate in blood collected during 67 anesthetic events of free-ranging American martens anesthetized using isoflurane under field conditions from 2011-2015 in Michigan. Models were ranked by decreasing Akaiake Information Criterion for small sample sizes (AICc).

Model	-2 Log Likelihood	K[a]	AICc	ΔAICc[b]	w_i[c]
Lactate[d] = $ß_0$[e] + InitialT[f] + TimeSince[g] + ε[h]	90.2	4	98.9	0	0.44
Lactate = $ß_0$ + InitialT + TimeSince + BrknNails[i] + OralTrma[j] + ε	86.5	6	100.1	1.2	0.24
Lactate = $ß_0$ + InitialT + AmbientT + TimeSince + ε	90.1	5	101.2	2.3	0.14
Lactate = $ß_0$ + InitialT + Recovery[k]+ ε	93.8	4	102.5	3.6	0.07
Lactate = $ß_0$ + TimeSince + ε	98.4	3	104.8	5.9	0.02
Lactate = $ß_0$ + InitalT+ ε	98.5	3	104.9	6	0.02
Lactate = $ß_0$ + BrknNails + OralTrma TimeSince + ε	94.9	5	105.9	7	0.01
Lactate = $ß_0$ + Season[l] + InitialT+ ε	97.3	4	105.9	7	0.01
Lactate = $ß_0$ + weight + ε	99.5	3	105.9	7	0.01
Lactate = $ß_0$ + AmbientT + TimeSince + ε	98.3	4	107	8.1	0.01
Lactate = $ß_0$ + weight + sex + Lactating + ε	96.7	5	107.8	8.9	0.01
Lactate = $ß_0$ + Recovery + ε	101.8	3	108.2	9.3	0.00
Lactate = $ß_0$ + BrknNails+ ε	103.8	3	110.2	11.3	0.00
Lactate = $ß_0$ + Season+ ε	106.8	3	113.2	14.3	0.00
Lactate = $ß_0$ + OralTrma + ε	107	3	113.4	14.5	0.00
Lactate = $ß_0$ + AmbientT + ε	107.2	3	113.5	14.6	0.00
Lactate = $ß_0$ + Collar[m] + ε	107.2	3	113.6	14.7	0.00
Lactate = $ß_0$ + sex + Lactating + ε	107	4	115.6	16.7	0.00

Table 4 Continued

[a]Number of parameters.

[b]Relative difference between AICc of model and AICc of model with lowest AICc.

[c]Model weight.

[d]Lactate was measured by two different methods (VetScan i-STAT 1 and Lactate Plus) and compared for clinical agreement. Both methods for lactate measurement gave similar results. We used the mean of both measurements when available. Otherwise we used the measurements from either the iSTAT or Lactate Plus.

[e]Standardized regression coefficient.

[f]Initial body temperature was the first rectal body temperature obtained in a American marten after induction with isoflurane.

[g]The time between trap discovery and induction of anesthesia was recorded to within the closest 0.25 hour.

[h]Regression error term.

[i]The total number of toenails that were broken, worn or frayed was counted.

[j]Recent or fresh oral trauma such as gingival abrasions or recently broken teeth was recorded as present or absent.

[k]Recovery time was recorded as the time from discontinuation of isoflurane to standing.

[l]Season was described as summer (April through September) or winter (October through March).

[m]American martens were categorized as collared if they had a previously placed radiocollar on at the time of the capture, anesthesia and lactate measurement.

Figure 1. An isoflurane vaporizer and E-tank oxygen cylinder are attached to a backpack frame for backcountry use. It is important that the vaporizer, if filled with isoflurane, does not tip over during storage, transport or use.

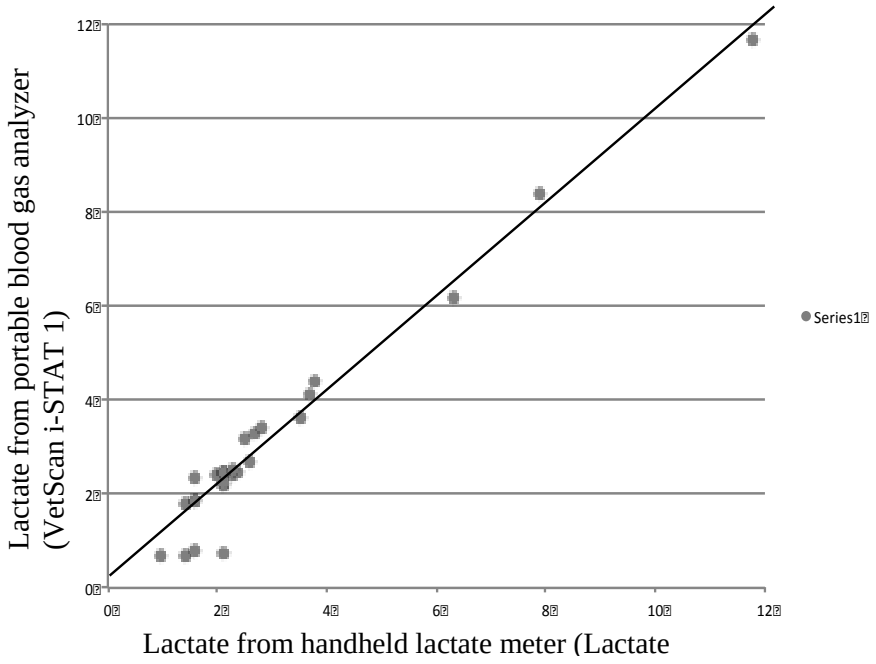

Figure 2. The Bland-Altman analysis to compare lactate measured by two methods from free-ranging American marten live-trapped and anesthetized using isoflurane under field conditions. Lactate was measured using a portable blood gas analyzer (VetScan i-STAT 1) and CG4+ cartridge (analytes: pH, pCO_2, HCO_3, tCO_2, pO_2, sO_2, lactate) and a portable lactate meter (Lactate Plus). The line of identity is where y=x and is indicated by a solid black line.

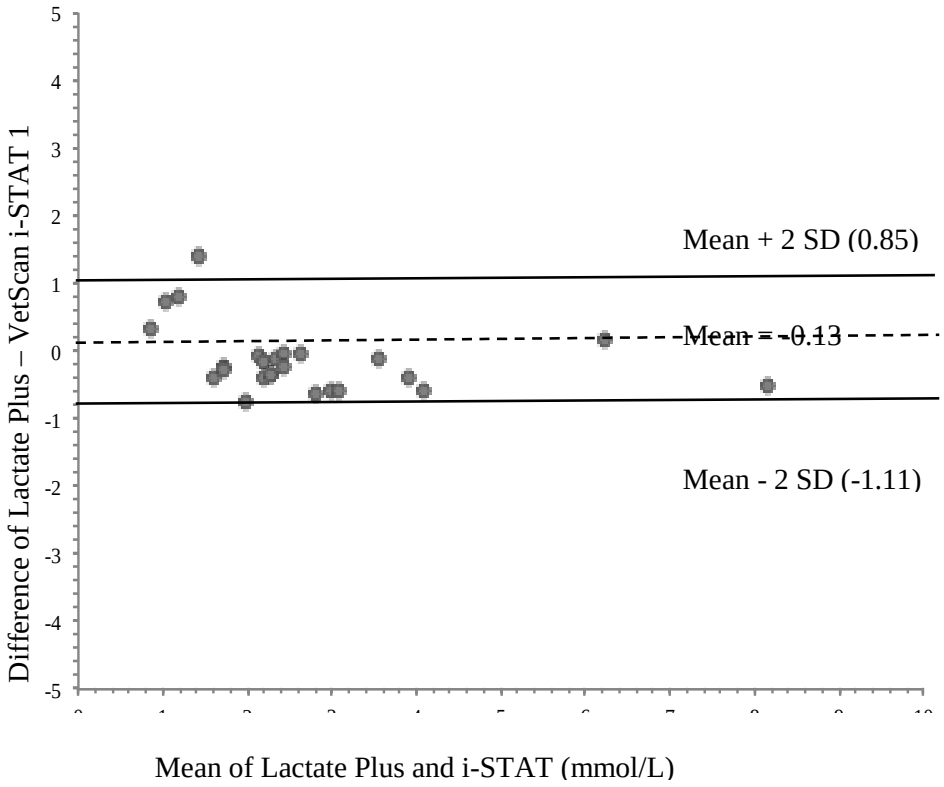

Figure 3. The Bland-Altman analysis to compare lactate measured by two methods on free-ranging American marten live-trapped and anesthetized using isoflurane under field conditions. Lactate was measured using a portable blood gas analyzer (VetScan i-STAT 1) and CG4+ cartridge (analytes: pH, pCO_2, HCO_3, tCO_2, pO_2, sO_2, lactate) and a portable lactate meter (Lactate Plus). The solid black lines represent the upper and lower 95% confidence limits. Only 2 values (n=26) were outside of the confidence limits and were when mean lactate < 2.

CHAPTER II
SEROSURVEY, HEMATOLOGY, AND CAUSES OF MORTALITY OF FREE-RANGING AMERICAN MARTENS IN MICHIGAN

Co-researchers for this part include Paul Keenlance, Ari Cornman, Bob Sanders, Becky Wilkes, Rick Gerhold and Debra Miller. Co-researchers assisted in field-work, data collection, sample analysis, statistical analysis, and/or manuscript review. We gratefully acknowledge the Evansville Zoological Society, Minnesota Zoo, Pittsburgh Zoo, and Detroit Zoo for financial support and the Sault Tribe of Chippewa Indians Natural Resource Department and the Keweenaw Bay Indian Community Natural Resource Department for their contributions to this work. This article has not been published.

Abstract

American martens (*Martes americana*) were reintroduced into Michigan during the mid-20th century but little is known about the clinical pathology of American martens, what diseases may impact American martens in Michigan, or causes of mortality. Samples were obtained from live-trapped American martens (n=58) and from carcasses of American martens harvested for fur (n=34) and were tested for exposure to one or more select pathogens considered to have potential to cause disease. Antibodies were detected to toxoplasma and canine distemper virus with prevalence of 58% and 3.4%, respectively. There was no evidence of exposure to *Leptospira* or *Dirofilaria immitis*. Urine samples (n=16) were tested via IFA or PCR for *Leptospira* and were negative. Parvoviral DNA was detected via PCR in 5.5% of fecal and small intestine samples. Cell blood counts (n=64) and serum biochemistries (n=63) were obtained from 49 live-trapped American martens. American martens seropositive for *T. gondii* were found to have significantly higher eosinophil and globulin levels than seronegative animals. Biochemical parameters found to be significantly different between sexes were calcium, creatinine, glucose and phosphorus. There was no significant difference between sexes in any of the hematologic parameters. Differences between summer and winter seasons were found in several hematologic and biochemical parameters. There was no significant difference in cell blood count or serum biochemistry values between radio-collared (n=17) and non-collared (n=47) American martens. The primary cause for mortality of radio-collared American martens was predation. Histologic examination of natural and harvest mortalities revealed a high percentage of American marten with verminous pneumonia.

Introduction

The American marten (*Martes americana*) was reintroduced into the Upper Peninsula (UP) and Northern Lower Peninsula (NLP) during the mid-20th century but there was no assessment of health of the reintroduced American martens conducted at the time (Cooley 2004). Since the reintroduction, the American marten population in the UP has grown and sustains an annual fur harvest while the NLP population is limited in size and the American marten is listed as a

Forests' Sensitive Species in the Huron-Manistee National Forest of the NLP (Marten Conservation Strategy 1996). There is no harvest of American marten in the NLP. Additional translocation of American martens in the future may be considered to augment genetic diversity in the NLP and the UP may be considered as a source population. This study was undertaken to obtain information about pathogen exposure in both locations, to evaluate clinical pathology findings in live-trapped American martens to assess general health of the animals and to identify the causes of the mortality of radio-collared American martens, which have not previously been reported in Michigan. While disease screening was not performed as part of the original reintroduction, current data regarding pathogen exposure will be useful to minimize risk of disease transmission in any future translocation of American martens to or within Michigan.

American martens are susceptible to infectious diseases that affect other members of Mustelidae including canine distemper virus (CDV), toxoplasmosis, leptospirosis, *Dirofilaria immitis*, and parvovirus (Frolich et al. 2000; Gabriel et al. 2012; Moinet et al. 2010; Tocidlowski et al. 2000). CDV causes mortality in a wide range of carnivores and isolated populations of American marten, such as the NLP population, are at risk (Gabriel et al. 2012). Clinical signs reported in naturally infected beech American martens (*Martes foina*) included abnormal behavior such as loss of fear, respiratory signs, conjunctivitis, convulsions, pruritus and death (Tavernier et al. 2012). Ferrets (*Mustelo putorius furo*), American martens, and other mustelids are particularly susceptible to the virus and high levels of mortality are seen during outbreaks (Williams et al. 1988; Frolich et al. 2000; Philippa et al. 2008). *Toxoplasma gondii* has been reported to cause mortality in the related black-footed ferret (*Mustela nigripes*), captive-raised American mink (*Neovison vison*), and southern sea otters (*Enhydra lutris*) (Jones et al. 2006; Cole et al. 2000; Burns et al. 2003b). Animals are more susceptible to clinical signs due to *T. gondii* when coinfected with CDV due to immunosuppression (Dubey and Lappin 2012). Leptospirosis was a potential factor in the decline of the endangered European mink (*Mustela lutreola*) as chronic infection can decrease reproductive success, cause abortion, and damage kidneys thus limiting lifespan (Moinet et al. 2010). *Dirofilaria immitis*, commonly known as the canine heartworm, has been reported in other mustelids including the North American river otter (*Lontra canadensis*) in the eastern United States (Tocidlowski et al. 2000). The natural host range for canine parvovirus (CPV) are canids, and members of the families Felidae, Mustelidae, Procyonidae, Ursidae and Viverridae are susceptible to infection (Frolich et al. 2005). Parvovirus can be clinical or subclinical in susceptible species and disease can depend on the infecting strain (Brown et al. 2006). In a serosurvey of small carnivores in Germany, there was a 31% exposure of stone American marten (*Martes foina*) (n=4) to CPV and 1 of 2 pine American martens (*Martes martes*) tested was positive. Follow-up polymerase chain reaction (PCR) testing

for the virus in tissue and feces from the seropositive animals was negative presumably due to short viral persistence in the tissues (Frolich et al. 2005).

There are no previous reports of hematologic or serum biochemistry values in free-ranging American martens. Clinical pathology findings have been used in other species to assess the health of animals during reintroduction programs and are used in a wide range of other studies that evaluate health of wild animals (Tocidlowski et al. 2000). The primary causes of mortality in radio-collared American martens have been previously reported to include predation, trapping, and hypothermia and this is the first report of causes of mortality in American martens in Michigan (Bull and Heater 2001; Hodgman et al. 1994; McCann et al. 2010; Woodford et al. 2013).

Methods

American martens (n=58) were live-trapped during 2011-2015 in the Manistee National Forest of the NLP and the Hiawatha and Ottawa National Forests of the UP of Michigan concurrent with telemetry and habitat studies. American marten were immobilized using isoflurane (IsoFlo®, Abbott Laboratories, Abbott Park, IL, USA) and a portable anesthetic machine and were monitored by a wildlife veterinarian or other trained personnel (Desmarchelier et al. 2007). Each American marten was implanted with a sterile microchip (AVID Identification Systems, Norco, California, USA) for permanent identification and some were fitted with a radiotelemetry collar (Advanced Telemetry Systems, Isanti, Minnesota, USA). Radio-collared American martens were regularly monitored as part of a separate telemetry and habitat study. If a telemetry mortality signal was obtained, the carcass was retrieved and submitted for necropsy as soon as possible. Fourteen percent (n=10) of American marten were trapped and sampled more than once during the course of the study. Age was determined as juvenile or adult based on size, pelage and dental eruption. Feces was collected and frozen between -20° and -80°C until analysis. Urine was collected via manual expression of the bladder and was stored under refrigeration if submitted for analysis within 5 days or stored frozen between -20° and -80°C until analysis.

Blood was collected from the jugular vein and did not exceed 1% body weight in volume. Sampling was not repeated in the same animal within 30 days due to minimize risk of iatrogenic anemia. Whole blood was placed into lithium heparin anticoagulant (BD Microtainer ® Tubes, Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) and serum separator tubes (Covidien, Mansfield, Massachusetts, USA). Blood from the lithium heparin tube was used to determine hematocrit using microhematocrit capillary tubes (SafeCrit, Westwood, MA, USA) and the StatSpin® VT centrifuge (Iris, Westwood, Massachusetts, USA). Blood smears were made using fresh whole blood or anticoagulated blood, fixed with methanol (Diff-Quick® fixative, Siemens Healthcare, Washington, D.C., USA) and examined by a veterinary pathologist for an

estimated white blood cell count and differential (Urika, LLC, Mukilteo, Washington, USA).

Blood in the serum separator tubes was allowed to clot and then centrifuged in the field using the Leading Edge Centri-V 806 centrifuge (Leading Edge Veterinary Equipment, Centennial, Colorado, USA). Serum was transported in a cooler between 37-41°F and protected from direct contact with ice. Serum was stored frozen between -20° and -80°C until analysis for serosurvey.

For biochemistry analysis, serum samples were not frozen and were analyzed within 8 hours of collection using the VetScan biochemistry analyzer (Abaxis, Union City, California, USA) and the Equine rotor [bicarbonate, creatine kinase (CK), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT)] and the Small Animal Comprehensive rotor [sodium, potassium, glucose, calcium, blood urea nitrogen (BUN), creatinine, total bilirubin, albumin, total protein, globulin, alkaline phosphatase (ALP), alanine-aminotransferase (ALT), amylase, phosphorus].

Microscopic agglutination was used to detect serum antibodies to 6 serovars of *Leptospira* (*L.* Pomona, *L.* Grippotyphosa, *L.* Hardjo, *L.* Canicola, *L.* Icterohemorrhagiae, and *L.* Bratislava) at DCPAH. An agglutination titer >200 was considered positive. Urine from 10 American martens was tested using immunofluorescent antibody staining (IFA) for leptospires at DCPAH. Urine from 9 American martens was examined for leptospiral organisms via polymerase chain reaction (PCR) at the University of Tennessee's Clinical Virology Laboratory, Veterinary Medical Center (UT; Knoxville, Tennessee, USA).

Serum IgG antibodies to *T. gondii* were detected via a modified agglutination test (MAT) at UT. The MAT antigen consisted of whole formalin-fixed tachyzoites and a dilution titer ≥32 was considered positive. Antigen testing for *D. immitis* was conducted at UT using an ELISA-based test (SNAP® Heartworm RT Test, Idexx Laboratories Inc., Westbrook, Maine, USA). Serum antibodies to CDV were detected via virus neutralization at Michigan State Diagnostic Center for Population and Animal Health (DCPAH; Lansing, Michigan, USA). A neutralization titer greater than 32 was considered positive.

Carcasses from trapper-harvested American marten in the UP were collected during 2012-2014 and stored frozen at -20°C until the time of sampling. Ages of harvested American martens were determined by cementum annuli and categorized as juvenile (<1 year old) or adult (>1 year old). Tongue, small intestine and serum samples were collected from the carcasses. Additional tissue samples were collected from a subset of carcasses for histopathologic examination based on carcass condition. Radio-collared American martens that died during the course of the study underwent gross and histopathologic examination by one of the coauthors (Miller). Small intestine, tongue and fecal samples were tested via real-time PCR at UT for parvovirus and sequencing was performed (UT Molecular Biology Core Facility, The University of Tennessee, Knoxville, Tennessee, USA) to identify the viral strain in positive samples.

Statistical analysis was performed with JMP® Pro 10.0.02 (SAS Institute Inc., Cary, North Carolina, USA). For American marten sampled more than once, the first positive sample was used for prevalence calculations. One American marten was excluded from the calculations for hematologic parameters due to a collar-associated dermatitis. The Shapiro-Wilk test was used to test for normalcy of data distribution. Pearson's chi-square test was used to evaluate differences between ages and locations and pathogen exposure. A *P*-value <0.05 was considered significant. The Wilcoxon test was used to determine significant differences in hematology parameters between males and females, collared and non-collared American martens, and summer and winter. Statistical comparisons could not be made between juveniles and adults due to the small number of juvenile blood samples obtained (n=3). Juveniles were excluded from comparisons of clinical pathology values between seasons and between sexes.

The capture and handling protocol was approved by the University of Tennessee Animal Care and Use Committee (protocol #2180) and American marten live-trapping and sample collection was an authorized tribal activity under the 2007 Inland Consent Decree between the State of Michigan and the Little River Band of Ottawa Indians.

Results

Of the 58 (31M, 27F) live-trapped American martens, four were determined to be juveniles. There were 35 American martens sampled from the NLP and 23 from the UP. The number of American martens tested, the sample type, testing methodology and results are shown in Table 5. There was no significant difference in prevalence of any pathogen between study sites (NLP versus UP) or sexes. A total of 58 American martens were tested for CDV and 3.4% (n=2) were seropositive. Positive titers ranged from 32 to >512. Two seropositive American martens were resampled at a later time; one remained seropositive 4 months later and the other was seropositive 8 months later, but then seronegative 20 months after the original test date. Six seronegative American martens were retested 2-14 months later and none seroconverted. A limited number of juveniles sampled for CDV precluded statistical comparison between juveniles and adults.

Of 81 samples tested for antibodies to *T. gondii*, 25 were from carcasses and 56 were from live-trapped American martens and 58% (n=47) were seropositive. There was no significant difference in the seroprevalence between the UP and NLP (*P*=0.2). Titers for *T. gondii* ranged from <32 to >8192. Twelve American marten were resampled: three seronegative American martens remained so 5-11 months later, six seropositive American martens remained so 2-12 months later, and three American martens seroconverted between 12-32 months. There was no significant difference in seroprevalence between juveniles and adults (*P*=0.4).

Two American martens were seropositive for both CDV and *T. gondii*. Both were adult females that had successfully reproduced at least once during the

course of the study; both were believed to be alive at the time of manuscript submission.

Two American martens had low titers (100 and 200) considered negative for *Leptospira interrogans* serovar Grippotyphosa. Urine from 16 seronegative American martens were negative for *L. interrogans* via IFA or PCR. There was no evidence of infection with *D. immitis* in either study location. Three American marten were retested 2-7 months later for *D. immitis* antigen and remained negative.

Tongue tissues from harvested American martens were initially used for PCR testing for parvovirus. Tongue tissue was advantageous because some trappers submitted only the skull and not the remainder of the carcass. Tongue samples (n=34) were divided into 7 batches and tested via PCR for parvovirus and were negative. Testing was repeated with small intestinal samples (n=22) including small intestine from 9 of the American marten included in the tongue sample testing. Small intestinal samples were tested in batches of 4-5 and one batch was positive. Individual samples from the positive batch were tested and 2 were positive for canine parvovirus. These 2 positive American martens were previously found to be negative when testing was conducted using tongue samples. Sequencing of the positive samples confirmed canine parvovirus type 2c. These two American martens were also seropositive for *T. gondii* but were not tested for exposure to CDV. Small intestine from 6 American marten mortalities (non-harvest) were tested individually and were negative. Feces from 27 live-trapped American marten were tested individually and one juvenile American marten, approximately 4 months old, was positive for CPV. This juvenile was seronegative for both *T. gondii* and CDV. There was no significant difference between juveniles and adults (P=0.9). Only the results of the small intestine and fecal PCR testing are included in the reported prevalence (tongue samples excluded) due to the apparent failure to detect CPV in tongue samples.

Cell blood count and differentials (n=64) are reported from 48 individual American martens (Table 6). Platelet counts were not performed but platelets were estimated to be adequate and had normal morphology in all samples. Three American marten were noted to have rare doehle bodies. Other morphologic comments included reactive lymphocytes (n=1), toxic neutrophils 1+ (n=1) and slight echinocytosis (n=2). Microfilaria were not identified in any of the blood smears. Serum biochemistry (n=63) are reported from 49 individual American martens (Table 7). Hemolysis (1-2+) was reported in 68% of the serum samples by the VetScan analyzer. There was no significant difference in any of the hematologic or biochemistry parameters between collared (n=17) and non-collared American martens (n=47). American martens seropositive for *T. gondii* had significantly higher eosinophil counts ($\bar{x}$=0.06 x10^3/uL) than seronegative animals ($\bar{x}$=0.015 x10^3/uL, P<0.05) but there was no difference in estimated total white blood cell count between the two groups. Seropositive American martens also had significant higher globulin levels ($\bar{x}$=2.87 g/dL) than seronegative animals ($\bar{x}$=2.49 g/dL, P<0.05).

There was no significant difference between the sexes in any of the hematologic parameters. The estimated total WBC count was lower in the winter ($\overline{x}$=3.00x10^3/uL) compared with summer ($\overline{x}$=5.91x10^3/uL) (P<0.05) due to lower counts of neutrophils, lymphocytes and monocytes in the winter (Table 8). Significant differences were found between summer and winter seasons in ALP, calcium, phosphorus, creatinine, glucose, sodium, potassium, total protein, globulins, and bicarbonate (Table 8). Significant differences were found between adult males and females in calcium, creatinine, glucose and phosphorus (Table 9).

The causes and histopathologic findings for known mortalities of radio-collared American martens are shown in Table 10. The most common histopathologic finding was granulomatous pneumonia, often associated with intralesional parasites, and present in 60% (n=9) of carcasses examined microscopically (Figure 4). Affected American martens had findings that included focal pulmonary granuloma, mild to moderate granulomatous pneumonia, interstitial pneumonia, and mild inflammatory infiltrates. Eosinophilic gastritis was present in 20% (n=3) of American martens examined. The right kidney of one American marten was replaced by fibrous tissue with no identifiable normal architecture seen on histopathologic examination (Figure 5). The tissue section included surrounding fat and small tubular structures lined by columnar epithelium. There was a semblance of a cortex and medulla but this appeared to be fibrosis and necrosis with mineralization. In some areas there was presumptive parasite remnants but current parasite infection could not be confirmed. These findings are consistent with presumed previous *Dictyocaulus renale* infection.

The age, sex and histologic findings of non-collared harvested American martens from the UP are shown in Table 11. Only carcasses that were considered to be in good condition were included for histologic examination. The most common cause for mortality of radio-collared American martens during the course of this study was predation with 38.9% of mortalities due to known or highly suspected predation attributed to coyotes, raptors and other American marten. While limited sample size precludes statistical analysis, more females (n=5) died due to predation than males (n=2). Other causes for mortality were vehicular collision (16.7%, n=3) and confirmed or possible collar entrapment (11.1%, n=2). A total of 54 individual American martens were radio-collared for the concurrent habitat and telemetry study and thus collar entrapment occurred in 3.7% of the collared American martens overall. Two American marten were trapped in the UP (there is no harvest of American marten in the NLP) and the cause for three mortalities could not be determined due to the poor condition of the carcass. One male American marten kit less than 40 days old from the NLP was apparently abandoned and died shortly thereafter. Necropsy and histopathology revealed hemorrhage in the brain and also likely the lung, most suggestive of trauma (which could include a fall) as the immediate cause of death. There were also changes in the lungs consistent with an inflammatory

process that may have contributed to the subsequent trauma (e.g., ill thrift resulting in falling). These changes included multifocal, sub-acute, moderate to severe bronchiolar pneumonia and eosinophilic infiltrate that may have been a response to parasites or perhaps foreign material such as aspirated material or amniotic fluid. Pancreatic atrophy was consistent with not eating however signs of emaciation such as serous atrophy of fat were absent and brown fat reserves were considered adequate.

Discussion

This is the first report of clinical pathology, pathogen exposure and causes of mortality of American marten in Michigan. At the time of the original reintroduction of American marten into Michigan, American marten were not vaccinated or quarantined prior to release and there was no knowledge of the diseases affecting American marten in the source population (Spriggs, unpublished data). Two American marten, both from the NLP population, had evidence of exposure to CDV. The overall seroprevalence of 3.4% was low as American marten are highly susceptible to CDV and thus few may survive with an antibody titer to be detected in a serosurvey. Potential reservoirs of CDV in Michigan include raccoons (*Procyon lotor*) and domestic dogs (Michigan Department of Natural Resources, 2012a). One of the seropositive American marten was resampled 20 months later and found to be seronegative (titer=8) per the stated cut-off (≥32 considered seropositive). A fresh serum sample was submitted and thus sample storage did not contribute to low titer detection. Another seropositive American marten was retested 4 months later and remained seropositive. Domestic dogs vaccinated against CDV have long-lasting immunity but the protective antibody titer or duration of immunity in naturally exposed American marten is not known (Schultz et al. 2010). Cell-mediated immunity also plays a role in protection against CDV and thus a low or waning humoral antibody titer may not indicate loss of immunity (Wimsatt et al. 2003). Quarantine and vaccination for CDV have been used in reintroduction programs of endangered black-footed ferret, free-ranging sea otters, and Siberian polecat and could be considered for future translocation or reintroduction of American marten (Williams et al. 1996; Wimsatt et al. 2003; Jessup et al. 2009).

Leptospirosis affects wildlife in Michigan, but no American marten in the current study had evidence of exposure to *Leptospira* nor were any found to be shedding the bacterium as determined by IFA and PCR testing of urine (Michigan Department of Natural Resources, 2012b). PCR has been shown to be sensitive in detecting renal leptospiral shedding in urine of humans and dogs early in the course of disease prior to seroconversion and thus the combined testing of American martens with urine PCR and serology was conducted for thoroughness (Bal et al. 1994; Harkin et al. 2003). A survey of several species of *Mustelidae* in New Zealand found that they were not important in the maintenance or transmission of leptospirosis however there are reports of leptospirosis in

domestic and fur-trade ferrets (Powers 2009; Hathaway and Blackmore 1981). A survey in southwestern France revealed a high seroprevalence to leptospira in both stone American martens (*Martes foina*) and pine American martens (*Martes martes*) but there was no evidence of renal infection in either species and thus they were considered to have limited potential for leptospiral shedding (Moinet et al. 2010).

There was a 58% prevalence of *Toxoplasma gondii* infection in American marten in this study and no difference in seroprevalence between study sites or sexes. Despite a relatively high prevalence, the effect of infection with *T. gondii* on the American marten population is not known. The subclinical effects of this parasite are not well understood, but are likely significant and could include an increased susceptibility to predation, neurologic disease, and decreased reproductive success (McAllister 2005; Larkin et al. 2011; Gabriel et al. 2012). Toxoplasmosis is also a threat to human health and risk factors include consumption of undercooked meat and gardening or exposure to soil that has been contaminated with cat feces. Immunosuppressed people and pregnant women are at highest risk. A study of pregnant Inuit women revealed that participation in skinning animals including American marten were a risk factor for seroconversion during pregnancy (McDonald et al. 1990).

Two adult American martens (1.5 and 3.5 yr old) harvested in the UP were infected with canine parvovirus type 2c as determined by PCR of small intestinal samples. One juvenile American marten from the NLP was also positive via fecal PCR. Small intestinal and fecal samples appeared to be better than tongue samples for detection of the virus in this study. The low prevalence of parvovirus detected via PCR in feces and tissue samples in the current study may underestimate the true prevalence of infection in American marten in Michigan given an unknown duration of tissue infection and fecal shedding of the virus in American martens. Whether CPV infection causes disease in American marten is not known, but clinical or subclinical infections are possible in other carnivores (Brown et al. 2006). Parvovirus is transmitted primarily through feco-oral transmission and an animal that recovers from CPV is likely immune for life (Frolich et al. 2005). CPV persists in the environment and animals do not need to come in direct contact to become infected, hence solitary animals like American martens can become infected through viral exposure at latrines and disinfection of field and capture equipment with a suitable disinfectant is recommended (Brown et al. 2006).

All American martens tested were negative for *D. immitis* using an ELISA snap test for the antigen and microfilaria were not identified in blood smears. False negatives could occur if the infection was unisex (male worms only), immature worms, or very low worm burden as occurs in cats (SNAP Heartworm RT Test package insert; Idexx Laboratories Inc.,Westbrook, Maine, USA). American martens have not previously been reported with *D. immitis* but the related North American river otter has been affected (Tocidlowski et al. 2000). Because Michigan is considered to be a moderate risk state for heartworm in

dogs (Figure 6), ongoing surveillance is warranted as infection rates are increasing in northern states (Companion Animal Parasite Council 2015). Screening of American martens that are part of a reintroduction program could be considered in endemic areas.

American martens seropositive for *T. gondii* had significantly higher eosinophil and globulin levels than seronegative animals, although the absolute difference in these values were small and may not be clinically significant. Infection with *T. gondii* induces an immune response that includes the production of eosinophils, cytokines, and gloublins (Filisetti and Candolfi 2004). There were no significant differences seen in any of the hematology or biochemistry parameters between radio-collared versus non-collared American martens, suggesting there is no difference in the systemic health between the two groups. One radio-collared American marten had a collar-associated dermatitis and had a normal WBC count (5.0 x 10^3/uL) with a mild monocytosis (0.70 x 10^3/uL) as compared to mean values reported here. This American marten was included in the comparisons between collared and non-collared American martens. Additional sampling of collared American martens is warranted, as the sample size in this report is unequal and small. To our knowledge there are no other reports of hematology comparisons between radio-collared and non-collared wild mammals.

Significant differences were found in several biochemistry parameters between summer and winter. Phosphorus and ALP levels were higher in summer, which could be due to inclusion of young American martens during these months. Young, growing mammals are expected to have higher levels of phosphorus and ALP associated with skeletal development (Bain 2011; Ferguson and Hoenig 2011). Since age was not definitively determined, it is possible that some American martens sampled during late summer months may have been born in the spring and acquired adult characteristics by the time of sampling. Calcium, globulin, sodium, and total protein values were also significantly higher during the summer than winter and the difference may be due to varying hydration status of American martens live-trapped during warmer ambient temperatures. Dehydration can cause an increase in all of these values (Evans 2011; Ferguson and Hoenig 2011; George and Zabolotzky 2011 p 157). Potassium and creatinine were higher and bicarbonate was lower in summer compared to winter but these differences were small and not likely clinically significant.

The estimated total white blood cell count and absolute values of neutrophils, lymphocytes and monocytes were slightly but significantly higher during summer months than winter. Seasonal variation in hematology values have been reported in other species and attributed to seasonal changes in metabolism, stress, nutrition, and environmental conditions (Beechler et al. 2009; Tocidlowski et al. 2000). Increased contact between American martens associated with courtship, breeding, or territorial behaviors could increase risk of disease or parasite transmission and result in an increase in white blood cell production but

more research is needed to understand what factors may affect the hematology of American martens in different seasons.

The most common cause of death of radio-collared American martens was predation, accounting for 38.9% (n=7) of known mortalities. This is consistent with previous reports of causes for mortality in American marten. Other causes of mortality of radio-collared American martens in this report included vehicular collision (n=3), confirmed or possible collar entrapment (n=2), and trauma and maternal abandonment of a kit (n=1) and unknown causes (n=3). In northwestern Oregon, 62.8% of 35 radio-collared American martens died during the course of a 3-year study. Predation by bobcats, raptors, American martens and coyotes was the most common cause of mortality in this area. Three American martens died of hypothermia and one American marten died due to collar entrapment in this study (Bull and Heater, 2001). Reintroduced American marten in Wisconsin were monitored for survival and 84.6% of 13 known mortalities were due to predation (Woodford et al. 2013). In a separate study of survival of radio-collared American martens in Wisconsin, the majority of mortalities was due to predation by raptors, fisher and other mammalian predator (75% of 12 known mortalities) and one of 40 radio-collared American martens died due to collar entrapment (McCann et al. 2010). In Maine, the majority of mortalities of radio-collared American martens was due to trapping (89.8%), and predation, starvation and gunshot accounted for only a minority of mortalities (Hodgman et al. 1994). We believe that American marten may be predisposed to collar entrapment due to their use of tight spaces such as tree snags and subnivean sites, and also due to difficulty in properly fitting the collar given the marked neck muscle relaxation associated with isoflurane anesthesia and the length and flexibility of the American marten's neck. The use of spacers and break-away collars has been reported in American martens and should be considered to minimize risk for collar-associated mortality (Thompson et al. 2012). Additional research into the optimal collar material, size and fit is warranted. Juvenile American martens are not frequently radio-collared, thus causes for juvenile mortality are not well-known. A single kit mortality is reported here and was attributed to cerebral hemorrhage (possibly from a fall). The kit was left in place by field personnel for several hours in hopes that the dam would return but was apparently abandoned. An underlying pneumonia may have contributed to the condition. Because the kit was alive when first spotted and was not emaciated, this may have been an acute event. American marten females move their kits periodically from one maternal den to another and it is not known if this kit fell and/or was abandoned during that process.

The most common histologic finding was verminous pneumonia, which was confirmed or presumed to be present in 60% of the American martens examined. The causative parasite was not identified at the time of this writing but parasites with the potential for pulmonary migration previously identified in fecal examination of the same populations of American martens included *Alaria* sp., *Crenosoma* sp., *Strongyloides*, *Filaroides* and hookworms (Chapter 4). Wild

animals are typically considered able to compensate for parasitism, however the subclinical effects on fitness or risk of predation are often not known. While verminous pneumonia was an incidental finding, the associated pulmonary pathology may have caused mild respiratory compromise. Other histologic findings included eosinophilic gastritis present in 20% (n=3) and presumably due to endoparasitism. Two American martens were found to have protozoal cysts within skeletal muscle consistent with *T. gondii* and both were seropositive for *T. gondii.* One young female American marten was found to have granulomatous pneumonia and mild lymphohistiocytic steatitis of the pericardial fat with presumed intracytoplasmic inclusion bodies. Canine distemper virus forms intracytoplasmic inclusion bodies in infected animals and American marten are considered to be highly susceptible to the virus (Tavernier et al. 2012). One American marten in this report had destruction of the right kidney or verminous nephritis consistent with presumed past infection with *Dioctophyme renale. D. renale* was reported in 2% of 405 American martens from Ontario but has not previously been reported from American martens in Michigan (Seville and Addison 1995).

The restoration of American marten to Michigan began with the reintroduction of American martens to both the UP and NLP, however the NLP population is believed to remain limited in size. Baseline data regarding the causes of mortality, pathogen exposure and expected clinical pathology findings of American marten in Michigan will be useful in assessing the potential role of disease in population dynamics and in planning for any future translocations of American marten in Michigan.

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Appendix

Table 5. Results of testing for select pathogens in American martens in Michigan.

	Canine distemper virus	*Toxoplasma gondii*	*Leptospira* serovars	*Leptospira* sp.	*Dirofilaria immitis*	Canine parvovirus
No. Pos./No. tested	2/58	47/81	0/55	0/16	0/35	3/55
Prevalence	3.4%	58.0%	0.0%	0.0%	0.0%	5.5%[b]
Sample type	Serum	Serum	Serum	Urine	Serum	Small intestine, feces
Test method†	SN	MAT	MA	IFA, PCR[a]	ELISA	PCR
Detection	Antibody	Antibody	Antibody	Antigen	Antigen	Antigen

†SN=serum neutralization, MAT=modified agglutination test, MA=microscopic agglutination, IFA=immunofluorescent antibody staining, PCR=polymerase chain reaction, ELISA=enzyme-linked immunosorbent assay

[a]Urine samples were tested by IFA alone (n=7), IFA at initial sampling and PCR at subsequent sampling of same American marten (n=3), or PCR alone (n=6).

[b]Prevalence was determined as the number of positive samples overall in the combined small intestine (n=28) and fecal (n=27) samples

Table 6. Hematology values (n=64) in 48 free-ranging American martens.

Parameter (units)	Mean ± SD	Median	Mean ± SD reported by Nieminen et al., 2007	Reference range, ferret
Hematocrit (%)*†	43 ± 6	44	47 ± 5	40-70
WBC estimate (10^3/uL)	4.94 ± 3.25	4.50	8.36 ± 0.95	3-16.7
Neutrophils (10^3/uL)	3.93 ± 2.83	3.70	2.68 ± 0.47	0.9-7.4
Lymphocytes (10^3/uL)	0.68 ± 0.56	0.53	5.04 ± 0.44	0.6-10.5
Monocytes (10^3/uL)	0.21 ± 0.14	0.18	0.08 ± 0.04	0.0-0.5
Eosinophils (10^3/uL)	0.04 ± 0.08	0.00	0.53 ± 0.13	0.0-0.7
Basophils (10^3/uL)	0.00 ± 0.01	0.00	Not reported	0.0-0.2
Band neutrophils (10^3/uL)	0.00 ± 0.02	0.00	Not reported	0.0-0.1

*Data distributed normally
†n=62

Table 7. Serum biochemistry values (n=63) in 49 free-ranging American martens.

Parameter (units)	Mean ± SD	Median	Range (central 95%)	Reference range, cat	Mean ± SD reported by Nieminen et al., 2007	Reference range, ferret
Albumin (g/dL)*	4.1 ± 0.4	4.1	3.2-5.1	2.2-4.4	3.6 ± 0.1	1.9-3.8
Alkaline phosphatase (U/L)	192.5 ± 114.5	172	56.4-578.2	10-90	122 ± 11	8-72
Alanine-aminotransferase (U/L)	131.3 ± 47.3	124	57-257	20-100	623 ± 154	65-346
Amylase (U/L)	318.6 ± 90.3	293	181.2-530	300-1100	Not reported	4-50
Aspartate aminotransferase (U/L)	297.6 ± 141.6	252	113.6-711.8	12-43	246 ± 56	Not available
Bicarbonate (mmol/L)*	23.1 ± 3.3	23	16-30.4	20-33	Not reported	Not available
Blood urea nitrogen (mg/dL)	34.8 ± 14.7	32	18-77.2	10-30	42.0 ± 3.6	10-38
Calcium, total (mg/dL)*	9.0 ± 0.6	8.9	8-10.5	8-11.8	8.5 ± 0.2	8-10.4
Creatinine (mg/dL)	0.5 ± 0.2	0.4	0.2-0.9	0.3-2.1	0.7 ± 0.1	0.2-0.7
Creatine kinase (U/L)	1930.4 ± 1900.6	1314	216.2-7292	50-450	280 ± 61	Not available
Globulin (g/L)*	2.7 ± 0.6	2.6	1.7-4	1.5-5.7	2.2 ± 0.1	2.3-4.5
Glucose (mg/dL)	166.9 ± 38	162	99-271.2	70-150	186.7 ± 7.9	65-145
Phosphorus (mg/dL)†	5.4 ± 1.8	5.2	2.7-10.1	3.4-8.5	4.9 ± 0.3	3.6-7.3
Potassium (mmol/L)	4.9 ± 0.5	4.8	4.1-6.3	3.7-5.8	4.2 ± 0.1	4.1-5.5
Sodium (mmol/L)	148.8 ± 3.8	148	141.8-159.2	142-164	150 ± 1	146-156
Total bilirubin (mg/dL)	0.3 ± 0.1	0.3	0.2-1.0	0.1-0.6	0.3 ± 0.03	0.3-0.6
Total protein (g/dL)*	6.8 ± 0.6	6.9	5.7-7.9	5.4-8.2	5.8 ± 0.2	5-7.6

*Data distributed normally
†n=61

Table 8. Significantly different hematologic and biochemistry parameters of adult American martens between summer (April-September) and winter (October-March).

Parameter (units)	Summer[a]		Winter[b]		
	Mean ± SD	Min-Max	Mean ± SD	Min-Max	P-value
WBC estimate (10^3/uL)	5.91 ± 3.60	0.60-15.0	3.00 ± 1.45	1.00-5.00	0.0006
Neutrophils (10^3/uL)	4.78 ± 3.13	0.33-13.95	2.42 ± 1.35	0.50-4.50	0.0011
Lymphocytes (10^3/uL)	0.82 ± 0.64	0-3.06	0.40 ± 0.25	0.12-1.00	0.0016
Monocytes (10^3/uL)	0.24 ± 0.16	0-0.60	0.14 ± 0.08	0.01-0.35	0.0058
Alkaline phosphatase (U/L)	205.2 ± 85.3	58.0-356.0	117.5 ± 56.2	54.0-253.0	0.0002
Bicarbonate (mmol/L)	22.1± 3.1	16.0-30.0	25.4 ± 2.7	22.0-31.0	0.0003
Calcium, total (mg/dL)	9.1 ± 0.5	8.0-10.1	8.7 ± 0.4	8.0-9.4	0.0041
Creatinine (mg/dL)	0.5 ± 0.2	0.2-1.0	0.4 ± 0.1	0.2-0.6	0.0085
Globulin (g/L)	2.9 ± 0.5	1.9-4.1	2.4 ± 0.5	1.6-3.7	0.0002
Glucose (mg/dL)	161.4 ± 39.0	87.0-282.0	182.1 ± 32.5	123.0-243.0	0.0154
Phosphorus (mg/dL)*	6.0 ± 1.7	3.1-10.4	4.1 ± 1.0	2.6-6.1	<0.0001
Potassium (mmol/L)	5.0 ± 0.5	4.1-7.0	4.6 ± 0.4	4.0-5.3	0.0008
Sodium (mmol/L)	149.7 ± 4.1	143.0-161.0	147.3 ± 2.9	140.0-153.0	0.0313
Total protein (g/dL)	7.0 ± 0.6	5.6-8.0	6.5 ± 0.3	6.0-7.0	0.0006

[a]n=40 unless otherwise noted.
[b] For hematologic parameters, n=21. For biochemistry parameters, n=20 unless otherwise noted.
*Winter (n=20) and summer (n=38)

Table 9. Significantly different biochemistry parameters between adult male and female American martens.

Parameter (units)	Males (n=31)		Females (n=29)		
	Mean ± SD	Min-Max	Mean ± SD	Min-Max	P-value
Calcium, total (mg/dL)	9.2 ± 0.4	8.4-10.1	8.8 ± 0.5	8.0-10.0	0.0033
Creatinine (mg/dL)	0.5 ± 0.2	0.3-1.0	0.4 ± 0.1	0.2-0.7	0.0133
Glucose (mg/dL)	158.8 ± 32.6	117.0-235.0	178.3 ± 41.2	87.0-282.0	0.0302
Phosphorus (mg/dL)*	5.7 ± 1.6	3.4-9.9	4.9 ± 1.8	2.6-10.4	0.0293

*Males (n=30) and females (n=28)

Table 10. Age, sex, cause of death and other histopathologic findings of radio-collared American marten mortalities from the northern Lower Peninsula and Upper Peninsula of Michigan from 2011-2014.

ID	Month and year of first capture	Month and year of death	Age at death	Sex	Cause of death	Other histopathologic findings when applicable
1 (314)	May 2011	May 2014	Adult	M	Vehicular collision	
2 (290)	May 2011	Aug 2011	Adult	M	Presumptive vehicular collision - Collar found smashed on road	
3 (599)	Jan 2015	Jan 2015	Adult	F	Collar entrapment- Found with mandibular jaw entrapped in collar.	Verminous pneumonia, pulmonary edema, bile duct hyperplasia (presumptive)
4 (6552)	June 2013	May 2015	Adult	F	Possible collar entrapment – carcass found wedged in tree root	
5 (6002)	June 2013	Dec 2013	Adult	F	Legal harvest	
6 (367)	May 2013	May 2013	Juv.	M	Presumptive fall from tree – young kit (<40 days old) found alive initially but apparently abandoned by dam	Bronchiolar pneumonia, cerebral hemorrhage, pancreatic atrophy, vascular congestion
7 (798)	May 2012	May 2014	Adult	F	Predation – found decapitated	Verminous pneumonia, mild eosinophilic gastritis, enteritis and pancreatitis, focal exocrine pancreatic nodular hyperplasia
8 (554)	May 2014	Sept 2014	Juv.	M	Presumptive predation – collar found with bite marks	

Table 10 Continued

ID	Month and year of first capture	Month and year of death	Age at death	Sex	Cause of death	Other histopathologic findings when applicable
9 (365)	May 2012	June 2013	Adult	F	Predation (suspect raptor)	Granulomatous and eosinophilic pneumonia
10 (627)	May 2013	June 2014	Adult	F	Unknown	Verminous pneumonia, eosinophilic gastritis, heavy tick infestation
11(635)	Jan 2012	June 2012	Adult	M	Vehicular collision	
12 (078)	July 2013	June 2014	Adult	M	Unknown – carcass found decomposed	
13 (155)	May 2014	Sept 2014	Adult	M	Unknown – carcass found decomposed	
14 (090)	May 2011	Apr 2013	Adult	F	Predation (suspect coyote)	
15 (009)	Jan 2012	Apr 2013	Adult	F	Predation (suspect coyote)	Pulmonary hemorrhage (trauma-induced), protozoal skeletal muscle cysts (presumed *Toxoplasma gondii* cysts)
16 (822)	May 2011	May 2012	Adult	F	Presumed predation – collar found on goshawk nest	
17 (333)	July 2011	July 2011	Adult	M	Predation (suspect American marten)	
18 (1030)	Jan 2014	Jan 2014	Adult	M	Incidental harvest	Pulmonary granuloma with presumptive intralesional parasite

Table 11. Age, sex and histopathologic findings of American martens harvested for fur in the Upper Peninsula of Michigan from 2011-2014.

ID	Age at time of death (years)*	Sex	Histopathologic findings
1 (1217)	0.5	M	Right renal destruction (presumed previous *Dioctophyme renale* infection), eosinophilic gastritis
2 (2553)	0.5	F	Pulmonary granuloma with intralesional parasite
3 (1207)	0.5	F	Granulomatous pneumonia, mild lymphohistiocytic steatitis of pericardial fat with presumed intracytoplasmic inclusion bodies
4 (1194)	0.5	M	Intestinal parasitism
5 (2535)	1.5	M	Focal protozoal skeletal muscle cyst (presumed *Toxoplasma gondii* cysts)
6 (2538)	1.5	F	Granulomatous pneumonia
7 (2550)	1.5	M	Multifocal hepatic mineral tophi (presumed artifact)
8 (2554)	0.5	F	Granulomatous pneumonia

*Age was determined by cementum annuli for harvested American martens.

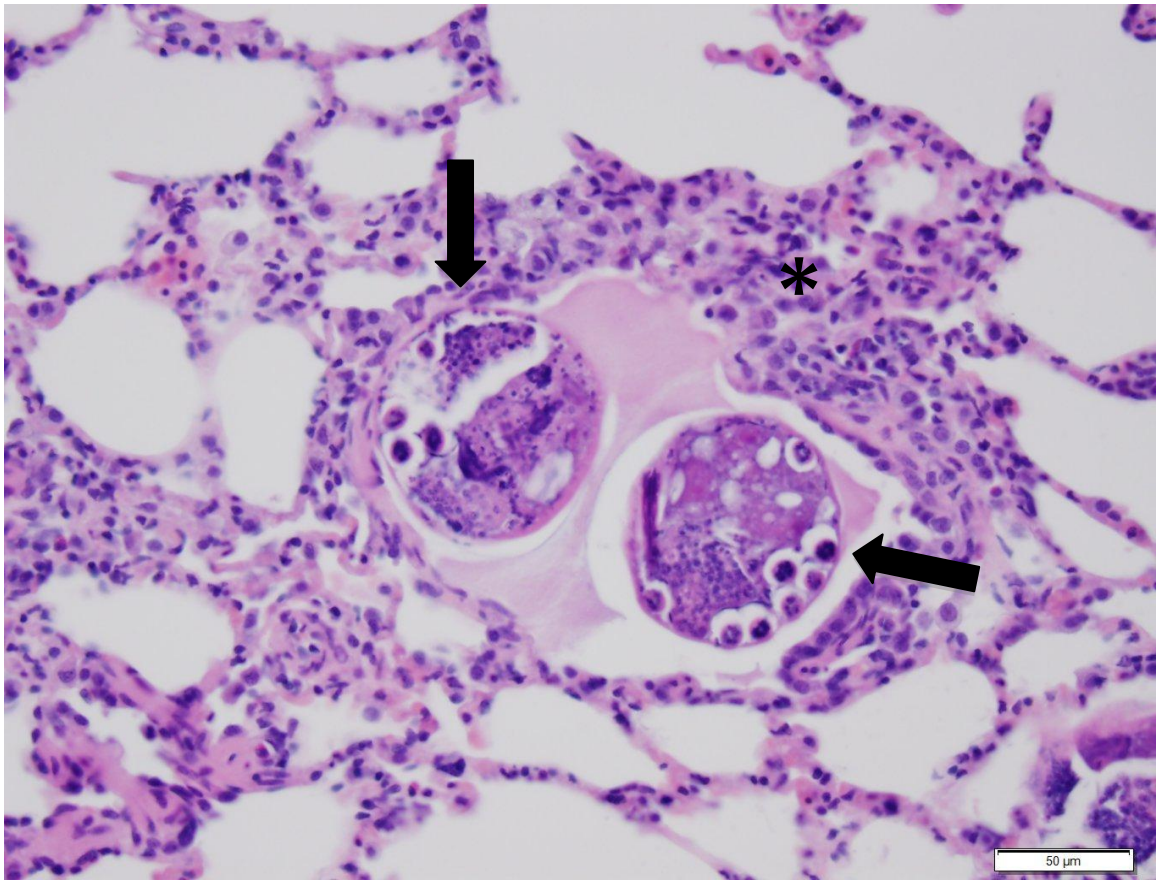

Figure 4. Parasites (arrows) found within lung alveoli of an American marten (ID #627) from Michigan. Mild interstitial pneumonia is present and characterized by diffuse, mild interstitial infiltrates of mixed inflammatory cells and mild type II pneumocyte hyperplasia (asterisk). H&E stain. Original magnification 400X. Scale = 30 µm.

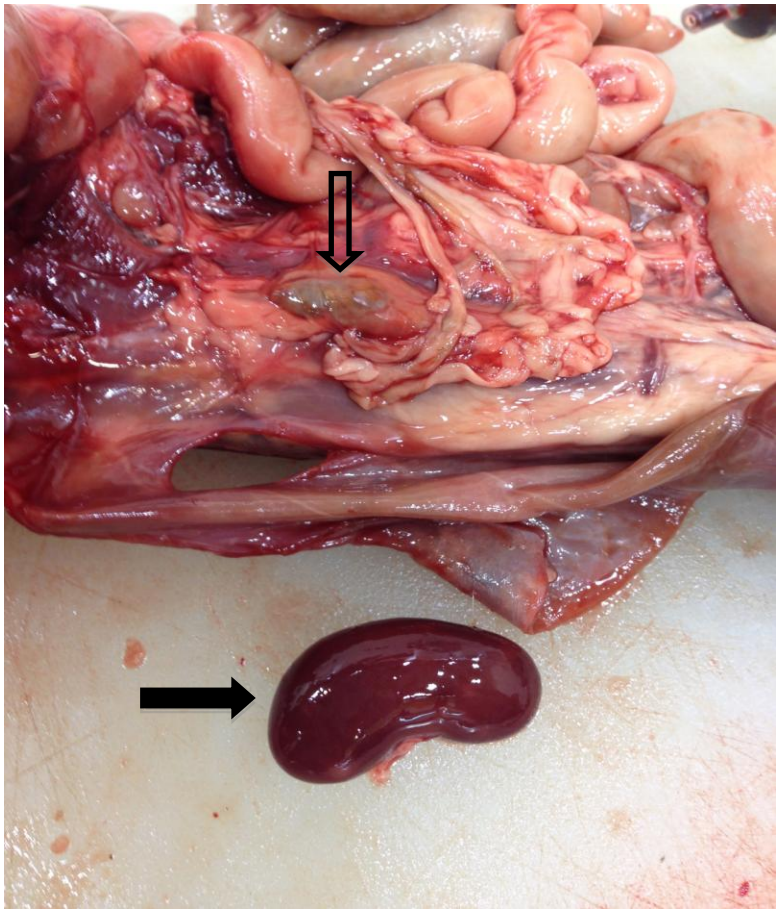

Figure 5. Destruction of the right kidney (open arrow) of an American marten due to presumed previous parasitic infection with *Dioctophyme renale*. The left kidney has been removed from the body for comparison of size (closed arrow).

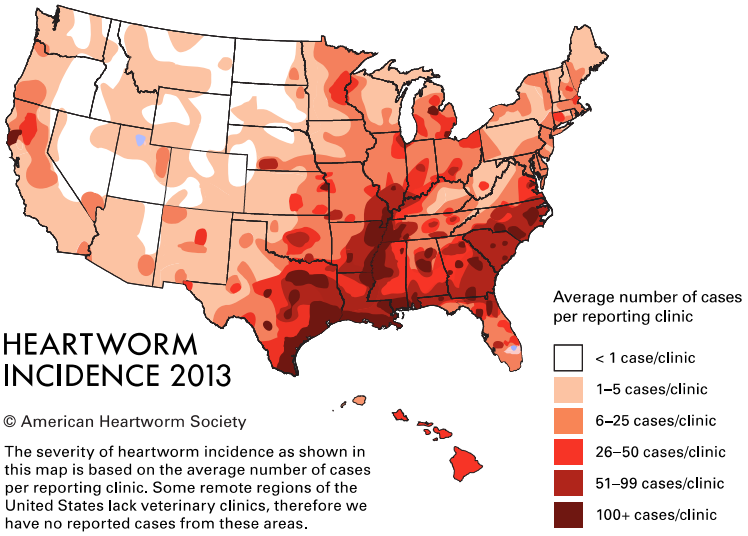

Figure 6. Michigan is considered to be a moderate risk state for canine heartworm infection as seen on the map of heartworm incidence in the United States in 2013 (Companion Animal Parasite Council 2015).

CHAPTER III
INSIGHTS INTO AMERICAN MARTEN FEEDING ECOLOGY IN MICHIGAN BASED ON STABLE ISOTOPE ANALYSIS

Co-researchers for this part include Lisa Muller, Joseph Bump, Paul Keenlance, Becky Wilkes, Rick Gerhold and Debra Miller. Co-researchers assisted in field-work, data collection, sample analysis, statistical analysis, and/or manuscript review. We gratefully acknowledge the Evansville Zoological Society, Minnesota Zoo, Pittsburgh Zoo, and Detroit Zoo for financial support and the Sault Tribe of Chippewa Indians Natural Resource Department and the Keweenaw Bay Indian Community Natural Resource Department for their contributions to this work. This article has not been published.

Abstract

The American marten was reintroduced into Michigan and while the population in the Upper Peninsula (UP) has grown and sustains an annual harvest, American martens in the northern Lower Peninsula (NLP) are regionally rare. Little is known about whether there are differences in feeding ecology between the NLP and UP, and whether limited prey availability may be contributing to the poor growth of the NLP population. Nitrogen stable isotope ($\delta^{15}N$) values change with trophic level or the amount of meat consumed by an animal; carbon stable isotope ($\delta^{13}C$) values are affected by the type of plants consumed. To gain insight into whether a reduction in prey availability or other difference in foraging ecology is limiting population growth in the NLP, we sought to determine whether location (NLP versus UP) would be influential in predicting $\delta^{15}N$. We measured carbon and nitrogen stable isotope ratios of hair from live-trapped and harvested American marten from the NLP and UP. We used Akaike Information Criterion to determine the top model for predicting $\delta^{15}N$ values from variables including location, season, age, sex, body weight and $\delta^{13}C$. Age and body weight were most influential in predicting $\delta^{15}N$. American martens <1-year old had higher $\delta^{15}N$ than adult American martens and those with higher body weights had higher $\delta^{15}N$ values. Adult body weights of male American martens were higher in the summer than winter while there was no significant difference in female body weights between the seasons. The decline in body weight of male American martens during the winter is attributed to short periods of fasting during the winter rather than season-long changes in prey availability, as season was not influential in predicting $\delta^{15}N$. In addition, we conclude that there is unlikely to be significant difference in trophic level or prey availability between the UP and NLP because location also did not appear to be influential in predicting $\delta^{15}N$. Other factors that may be limiting population growth in the NLP such as loss of genetic diversity or habitat availability warrant further investigation.

Introduction

The American marten (*Martes americana*) is a mesocarnivore ranging from the boreal forests of Canada into coniferous and mixed coniferous/deciduous forests of the northern United States including the Great Lakes region (Powell et

al. 2003). The species was extirpated from Michigan during the early-20th century due to habitat loss and unregulated trapping and reintroduced to the state's Upper Peninsula (UP) and northern Lower Peninsula (NLP) in the mid-20th century (Earle et al. 2001, Cooley 2002). While the American marten population in the UP has grown and supports an annual fur-harvest, the population in the NLP is limited possibly due to habitat fragmentation, forest management practices, or population genetics. The American marten is listed as a Forests' Sensitive Species in the Huron-Manistee National Forest of the NLP (Marten Conservation Strategy 1996). Additionally, there is no season for trapping of American marten in the NLP. Lack of prey availability may have been a limiting factor in the recovery of American marten in Wisconsin but it is not known if differences in feeding ecology or prey availability exist between the NLP and UP of Michigan (Carlson et al. 2014).

American marten are considered generalist predators and voles are reported to be a main dietary prey item in North America (Ben-David et al. 1997), but little is known about the diet of American marten in Michigan. American marten in the UP have been documented to prey on shrews including *Sorex* spp. and northern short-tailed shrews (*Blarina brevicauda*), mice (*Peromyscus maniculatus*), southern red-backed voles (*Clethrionomys gapperi*), ruffed grouse (*Bonasa umbellus*) and other bird species, eastern chipmunk (*Tamias striatus*), American red squirrel (*Tamiasciurus hudsonicus*), eastern grey squirrel (*Sciurus carolinensis*), and snowshoe hare (*Lepus americanus*) (Hales et al. 2007). In this study, shrew, mice and vole represented the highest proportion of gastrointestinal contents identified from 151 trapped American martens (Hales et al. 2007). There are no reports of diet of American marten in the NLP.

Stable isotope analysis has been used to infer aspects of feeding ecology in animals that are difficult or impossible to directly observe. Other methods of dietary analysis are labor intensive such as collection of scat from radio-collared American marten or via snow-tracking individual American marten. Examination of gastrointestinal contents for diet analysis is not practical in a population in which natural mortalities are difficult or impossible to detect or in a population of American martens that are not harvested for fur. In addition, scat and gastrointestinal content analysis provides information about diet consumed in the past day while stable isotope analysis allows for an integrated glimpse into diet consumed over a longer period of time, depending upon the turnover rate of the tissue analyzed. Because the heavier isotope of nitrogen is incorporated in a predator relative to prey consumed, inferences can be made about trophic level based on the $\delta^{15}N$ (Ben-David and Flaherty 2012). There are few studies examining stable isotope values in American marten but none have been conducted in American marten in Michigan (Ben-David et al. 1997; Carlson et al. 2014). We report the stable carbon and nitrogen isotope ratios from hair of harvested and live-trapped American marten from the reintroduced populations of American marten in Michigan's Upper Peninsula and northern Lower Peninsula.

Methods

American martens were live-trapped in the Manistee National Forest (n=23) in the NLP and the Hiawatha National Forest (n=17) in the UP using box live traps (Tomahawk Live Trap, LLC, Hazelwurst, Wisconsin, USA) from 2012-2014. American martens were anesthetized with isoflurane (IsoFlo®, Abbott Laboratories, Abbott Park, Illinois, USA) with a portable anesthesia machine as described by Desmarchelier et al. (2007). Body weight was recorded for live American martens using a gram scale (My Weigh, Phoenix, Arizona, USA). Hair was collected from the tail using scissors cutting close to the skin. Some individual American martens were resampled in a different season or year resulting in more total samples than individual American martens. Hair was also collected from the tail or limbs of carcasses that were legally trapped for fur in the UP (n=47) and from incidentally trapped and natural mortalities in the UP (n=1) and NLP (n=4) from 2012-2014. Sex was determined by visualization of external genitalia, palpation of baculum, and/or inspection of internal gonads (carcasses only). Age category was determined as <1-year old (cementum = <1) or adult (cementum= ≥ 1) by cementum annuli aging (carcasses only) or via reproductive monitoring of radio-collared American martens. The season and year of sample collection was recorded. Summer included the months of April through September while winter included October through March. Year 1 included sampled collected from October 2012-September 2013; year 2 included samples collected from October 2014-September 2014. The capture and handling protocol was approved by the University of Tennessee Animal Care and Use Committee (protocol #2180) and American marten live-trapping and sample collection was an authorized tribal activity under the 2007 Inland Consent Decree between the State of Michigan and the Little River Band of Ottawa Indians.

Hair samples were analyzed for carbon and nitrogen stable isotopes at Michigan Technological University. Statistical analysis was performed using JMP® Pro 10.0.02 (SAS Institute Inc., Cary, North Carolina, USA). Tukey-Kramer HSD test was used detect differences in body weights among locations and seasons within each sex with $P<0.05$ considered significant. Age, weight, sex, season, location and $\delta^{13}C$ were compared using *a priori* models from factors hypothesized to affect $\delta^{15}N$ values. We used mixed linear models (PROC MIXED; SAS Institute, Inc., Cary, North Carolina, USA) and Akaike Information Criteria for small sample sizes (AICc) for model selection (Burnham and Anderson 2002).

Results

Body weights of adult American martens live-trapped in the NLP and UP and during summer and winter are shown in Table 12. Body weights of male American martens were significantly higher during the summer than winter ($P<0.05$). There was no significant difference in weights between summer and winter of female American martens or between locations for either sex.

Variables evaluated for their influence on $\delta^{15}N$ included age, weight, sex, season, location and $\delta^{13}C$. The top model for predicting $\delta^{15}N$ included age and weight (Table 13). Adding location, $\delta^{13}C$, sex, and year were also in the top models but the strength of the models appeared to be related to age. The ß estimate for age (<1-year-old compared to adult) was -1.515 (95% CI: -2.65 to -0.38) and for body weight was 0 (95% CI: 0-0) (Table 14).

Discussion

AICc modeling was used to assess the influence of location, season, age, sex, weight and $\delta^{13}C$ on $\delta^{15}N$. Age and weight were included in the top model while location and season were not influential in predicting $\delta^{15}N$. The ß estimate for age (<1-year-old compared to adult) was -1.515 (95% CI: -2.65 to -0.38) thus American martens <1 year-old are expected to have higher $\delta^{15}N$ values than adults. The ß estimate for weight was 0 (95% CI: 0-0) thus larger American martens are expected to have higher $\delta^{15}N$ values than smaller ones. Differences in feeding ecology (e.g., increased trophic level) could explain elevated δ15N values in the <1-year old group as compared to adults and in larger martens compared with smaller ones (Ben-David et al. 1997; Ben-David and Flaherty 2012). Because location was not included in the top model, we conclude that there is unlikely to be a significant difference in prey availability in the NLP compared to the UP to explain the limited growth of the NLP population. This information may be useful for managers if future translocation were to be considered to the NLP and in prioritizing research needs for this limited population.

Our results suggest that age and body size may be important in determining the trophic level based on $\delta^{15}N$ of American marten. There are few other studies evaluating the difference in diet between juveniles and adults and none use stable isotope analysis. There was no significant difference in diets between juveniles and adult American martens in Ontario, Canada using scat and carcasses examination (Thompson and Colgan 1990). In addition, the authors found no support for any difference in diets based on body size (Thompson and Colgan 1990). In another study of diet from Vancouver Island, British Columbia, juvenile female martens consumed less small mammal prey than adults but no significant differences were seen in other components of the diet between juveniles and adults (Nagorsen et al. 1989). Further research is warranted to determine the effect of age and body size on diet in American marten in Michigan.

Male American martens in our study weighed significantly less during the winter than summer consistent with the potential for nutritional stress and fasting during winter conditions; however, season was not influential in predicting $\delta^{15}N$ in the AICc model selection. The $\delta^{15}N$ value of hair reflects the diet consumed during the entire growth of the hair sampled, thus while short periods of fasting may exist during winter, there is not likely a significant difference in trophic level

between summer and winter to suggest that significant prey limitations are occurring during winter. No difference was seen in body weight of females between winter and summer which we consider may be due to equally high summer energetic demands as winter for females given that summer is the time for parturition, nursing, provisioning young, and being pursued and bred again (Clark et al. 1987). American martens have relatively low body fat and cannot rely solely on fat stores for energy during winter or periods of fasting. American martens have a low body fat percentage between 2.1-5.6% and do not reportedly have seasonal fluctuations in overall body fat (Buskirk and Harlow1989; Harlow and Buskirk 1991; Nieminen et al. 2007). Because of their low body fat, American martens must continually forage to survive but they can tolerate short periods of fasting (Nieminen et al. 2007). In order to survive periods of fasting, American marten burn both body stores of protein and fat (Harlow and Buskirk 1991; Nieminen et al. 2007). When American marten were experimentally fasted for 5 days, they were found to have a 13.4% decrease in body mass, a significant decrease in BMI and total body fat, depletion of muscle and liver carbohydrate reserves, and changes in clinical pathology parameters (Nieminen et al. 2007).

The results of this stable isotope analysis will be useful for monitoring trends over time and could be used to compare trophic level of American martens in Michigan versus elsewhere in North America. American martens are known to expand diet breadth when resources are limited, which could occur if climate change or other events impact environmental conditions in Michigan. American martens in southeastern Alaska were found to consume salmon carcasses during periods when small mammal prey was limited (Ben-David et al., 1997). There was no difference in body condition between American martens in Alaska consuming small mammals versus those consuming salmon; however, body weights were significantly lower in the autumn compared with spring (males) or summer (females) (Ben-David et al. 1997). Diet breadth of American martens in Ontario, Canada expanded during years of limited prey availability and there was no difference in diet between males and females in this study (Thompson and Colgan, 1990). There was a seasonal difference in diet with American martens eating more insects and berries in the summer and more hare and ermine in the winter (Thompson and Colgan, 1990). American martens in Oregon were found to consume larger prey species such as lagomorphs, woodrats, and squirrels in the winter than summer and authors suggest that consuming larger prey in the winter is energetically efficient and an adaptation to stress from winter temperatures (Bull 2000). In an analysis of stomach contents of 701 American martens from Vancouver Island, Canada, there was little difference in winter diet between males and females with females consuming more small mammals overall than males and females consuming more smaller birds (less than 10 grams) than males (Nagorsen et al. 1989). In a study of diet of European pine martens (*Martes martes*) in Poland, both males and females were found to seek alternative prey items when rodent populations were low.

Males ate more ungulate carrion, amphibians and fruits while females ate mostly small rodents and squirrels (Zalewski 2007).

Stable isotope analysis of prey items and plants could be analyzed using mixing models to approximate the percentage of each item in the American marten's (Ben-David and Flaherty 2012). For example, deer carrion has been reported to be an important component in the diet of American martens elsewhere in North America and Europe but further research is needed to understand its role in the feeding ecology of American martens in Michigan. Zalewski reviewed diet studies of the European pine American marten and in all studies males were reported to consume more ungulate carrion than females and females were found to consume a higher proportion of squirrels than males (2007). Deer carrion has been reported to account for up to 32% of American marten diets in southeastern Alaska during the winter and spring (Ben-David et al. 1997). Deer carrion was shown to be a predominant part of the winter diet of American martens in Wisconsin and the authors suggest that this may lead to an increased risk of predation and that prey availability may be a limiting factor in the recovery of American marten in the state (Carlson et al. 2014). European pine martens as well as American martens on Vancouver Island, Canada have also been reported to consume significant amounts of ungulate carrion (Nagorsen et al. 1989; Zalewski, 2007). There are no reports of the relative importance of deer carrion for American martens in Michigan however they have been observed to scavenge deer carcasses in both locations (Spriggs, unpublished data).

American martens have a remarkable capacity to survive harsh winter conditions. More information about American marten overwintering physiology could shed light on the observed difference in male body weights between winter and summer. Additional research is warranted to better understand variation in stable isotope signatures in American martens from the NLP and UP of Michigan. This data can be used to monitor trends over time or to compare American martens in Michigan to elsewhere in North America. Because location was not influential in predicting $\delta^{15}N$ in this analysis, we conclude that limitations in prey availability and/or major differences in foraging ecology do not appear to be contributing to the suspected limited growth of the population of American martens in the NLP.

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Appendix

Table 12. Body weights (gm) of live-trapped adult American martens from the northern Lower Peninsula (NLP) and Upper Peninsula (UP) of Michigan during summer (April-September) and winter (October-March). Significant differences (*P*<0.05) are indicated by corresponding superscripts.

Sex	Location	Season	Body weight, gm (mean±S.D)	Range
Male	Combined	Combined (n=25)	1031±107	860-1223
	Combined	Winter (n=12)	951±66[a]	873-1077
		Summer (n=13)	1071±106[a]	860-1223
	NLP	Winter (n=8)	938±63[b]	873-1077
		Summer (n=9)	1086±90[b]	946-1223
	UP	Winter (n=4)	977±74	885-1065
		Summer (n=4)	1038±147	860-1218
Female	Combined	Combined (n=22)	680±114	550-1002
	Combined	Winter (n=13)	664±143	550-1102
		Summer (n=9)	702±49	658-805
	NLP	Winter (n=5)	664±143	550-1102
		Summer (n=5)	702±49	658-805
	UP	Winter (n=8)	663±181	550-1102
		Summer (n=4)	699±34	662-739

Table 13. Factors affecting nitrogen stable isotope values ($\delta^{15}N$) from hair collected from 85 free-ranging American martens collected during 2012-2014 from the Upper Peninsula and northern Lower Peninsula of Michigan. Models were ranked by decreasing Akaiake Information Criterion for small sample sizes (AICc).

Model	-2 Log Likelihood	K[a]	AICc	ΔAICc[b]	w_i[c]
$\delta^{15}N = \beta_0$[d] + age[e] + weight[f] + ε[g]	24.3	4	38	0	0.81
$\delta^{15}N = \beta_0$ + age + weight + location[h] + $\delta^{13}C$[i] + ε	14	6	42.8	4.8	0.07
$\delta^{15}N = \beta_0$ + age + weight + sex[j] + ε	24	5	44	6	0.04
$\delta^{15}N = \beta_0$ + age + weight + year[k] + ε	24.2	5	44.2	6.2	0.04
$\delta^{15}N = \beta_0$ + age + weight + location + ε	24.3	5	44.3	6.3	0.03
$\delta^{15}N = \beta_0$ + age + weight + sex + season[l] + ε	22.9	6	51.7	13.7	0.00
$\delta^{15}N = \beta_0$ + age + weight + sex + location + ε	24	6	52.8	14.8	0.00
$\delta^{15}N = \beta_0$ + age + weight + location + season + $\delta^{13}C$ + ε	13.3	7	55.3	17.3	0.00
$\delta^{15}N = \beta_0$ + age + weight + location + year + $\delta^{13}C$ + ε	13.5	7	55.5	17.5	0.00
$\delta^{15}N = \beta_0$ + weight + location + season + ε	94.2	5	106.6	68.6	0.00
$\delta^{15}N = \beta_0$ + weight + ε	100.3	3	107.2	69.2	0.00
$\delta^{15}N = \beta_0$ + weight + sex + ε	98.4	4	107.9	69.9	0.00
$\delta^{15}N = \beta_0$ + weight + location + ε	99.2	4	108.8	70.8	0.00
$\delta^{15}N = \beta_0$ + age + weight + year + sex + location + season + d13C + ε	8.8	9	116.8	78.8	0.00
$\delta^{15}N = \beta_0$ + age + ε	181.2	3	187.6	149.6	0.00
$\delta^{15}N = \beta_0$ + sex + ε	225.2	3	231.6	193.6	0.00
$\delta^{15}N = \beta_0$ + location + year + season + ε	237	5	247.7	209.7	0.00
$\delta^{15}N = \beta_0$ + season + ε	251	3	257.3	219.3	0.00
$\delta^{15}N = \beta_0$ + location + ε	253.5	3	259.8	221.8	0.00
$\delta^{15}N = \beta_0$ + year + ε	253.9	3	260.2	222.2	0.00
$\delta^{15}N = \beta_0$ + location + season + $\delta^{13}C$ + ε	250.2	5	261	223	0.00

Table 13 Continued

[a]Number of parameters.
[b]Relative difference between AICc of model and AICc of model with lowest AICc.
[c]Model weight.
[d]Standardized regression coefficient.
[e]Age was categorized as <1-year old or >1-year old based on cementum annuli aging of carcasses or known birth year of radio-collared American martens.
[f]Body weight measured in grams.
[g]Regression error term.
[h]Location was either the Upper Peninsula (UP) or northern Lower Peninsula (NLP).
[i]Carbon stable isotope value of hair from American martens.
[j]Sex was determined as male or female by examination of external genitalia.
[k]Year 1 was October 2012-September 2013 and year 2 was October 2013-September 2014.
[l]Summer season included the months of April-September and winter included the months of October-March.

Table 14. Parameter estimates of d15N stable isotopes from hair collected from 85 American martens from 2012-2014. Values are from model averaging of the top models (model weights from 0.03-0.81).

Variable	Model Averaged Parameter Estimate	95% LCI	95% UCI
Intercept	6.25	3.00	9.50
Age (< 1-year-old compared to adults)	-1.515	-2.65	-0.38
Body weight (grams)	0.00	0.00	0.00
NLP compared to UP	0.63	-0.21	1.46
$\delta^{13}C$ (carbon stable isotope value)	-0.97	-1.53	-0.40
Females compared to males	0.58	-1.77	2.93
Year 1 (Oct. 2012-Sept. 2013) compared to Year 2 (Oct. 2013-Sept. 2014)	0.22	-1.13	1.56

CHAPTER IV
ENDOPARASITES OF AMERICAN MARTENS: REVIEW OF THE LITERATURE AND PARASITE SURVEY OF REINTRODUCED AMERICAN MARTENS IN MICHIGAN

Co-researchers for this part include Rick Gerhold, Jill Witt, Becky Wilkes, Paul Keenlance, Ari Cornman, Bob Sanders, and Debra Miller. Co-researchers assisted in field-work, data collection, sample analysis, statistical analysis, and/or manuscript review. We gratefully acknowledge the Evansville Zoological Society, Minnesota Zoo, Pittsburgh Zoo, and Detroit Zoo for financial support and the Sault Tribe of Chippewa Indians Natural Resource Department and the Keweenaw Bay Indian Community Natural Resource Department for their contributions to this work. This article has not been published.

Abstract

The American marten was reintroduced to both the Upper (UP) and northern Lower Peninsula (NLP) of Michigan during the 20th century. This is the first report of endoparasites of American martens from northern Lower Peninsula (NLP). Feces from live-trapped American martens were examined for the presence of parasitic ova and blood samples were obtained for hematocrit. The most prevalent parasites were *Capillaria* sp. and trematodes (*Alaria* sp. and *Echinostome* sp.). Helminth parasites reported in American marten for the first time include *Echinostome* sp., *Eucoleus bohemi*, hookworm, *Hymenolepis* sp., *Spirocerca* sp., and *Strongyloides* sp. This is the first report of shedding of *Sarcocystis* sp. sporocysts in an American marten and identification of two coccidian parasites, *Cystoisospora* sp. and *Eimeria* sp. The pathologic and zoonotic potential of each parasite species is discussed, and previous reports of endoparasites of American marten in North America are reviewed.

Introduction

The American marten (*Martes americana*) is an arboreal mesocarnivore that ranges from the boreal forests of northern North America into coniferous and mixed coniferous/deciduous forests of the northern and northeastern United States including the Great Lakes region (Clark et al. 1987). The American marten requires a complex understory and high density of dead trees for foraging, denning, and resting (Clark et al. 1987). These habitat types are sensitive to changing climate and human development. Parasites and their host relationships have been of increasing interest in the field of climatology and biogeography, as these relationships can shed light on historical distributions of mammals or changing environmental conditions (Koehler et al. 2009). Some parasites are of economic or zoonotic importance and may be introduced with illegal or inadvertent animal translocations. Reintroduction programs should take into account the presence of parasites which are pathogenic or to which the species of concern is not adapted (Kimber and Kollias, 2000).

Martes americana was reintroduced to the Upper Peninsula (UP) and northern Lower Peninsula (NLP) in the mid-20th century (Cooley 2004). Reintroduced American marten were not examined for parasitic or infectious

diseases at the time (Spriggs, unpublished data). While the American marten population in the UP has grown and sustains an annual harvest for fur, the population in the NLP is limited and there is no trapping of American marten in the NLP. The American marten is listed as a Forests' Sensitive Species in the Huron-Manistee National Forest of the NLP (Marten Conservation Strategy 1996). A survey of parasites was conducted concurrent with telemetry, habitat and health studies that required live-trapping of American martens in the NLP and UP. Limited information is available about the prevalence of endoparasitism in American marten in North American or in Michigan (Poole et al. 1983; Veine-Smith et al. 2011). This study reviews previous reports from North America, presents data from the parasitological examination of live-trapped American marten in Michigan, and identifies an association between hookworm infection and anemia in affected American marten.

Methods

American martens (n=49) were sampled from three of the four national forests in Michigan: Manistee National forest in the NLP, Hiawatha National Forest in the UP and Ottawa National Forest in the UP. American martens were live-trapped from 2011-2015 for a concurrent telemetry, habitat and health study. American marten were trapped using box live traps (Tomahawk Live Trap, LLC, Hazelwurst, WI, USA), immobilized using isoflurane (IsoFlo®, Abbott Laboratories, Abbott Park, IL, USA), and were monitored by a wildlife veterinarian or other trained personnel (Desmarchelier et al. 2007). Each American marten was implanted with a sterile microchip (AVID Identification Systems, Norco, California, USA) for permanent identification and some were fitted with a radiocollar (Advanced Telemetry Systems, Isanti, Minnesota, USA). Feces were collected either via a clean, lubricated fecal loop from the rectum or from the trap. Feces were stored under refrigeration, and examined within four days of collection. Nine American martens were recaptured and re-sampled.

Blood was collected from the jugular vein and did not exceed 1% body weight in volume. Sampling was not repeated in the same animal within 30 days due to minimize risk of iatrogenic anemia. Blood was placed into lithium heparin anticoagulant (BD Microtainer ® Tubes, Becton Dickinson and Company, Franklin Lakes, New Jersey, USA). Whole blood was used to determine hematocrit using microhematocrit capillary tubes (SafeCrit, Westwood, MA, USA) and the StatSpin® VT centrifuge (Iris, Westwood, Massachusetts, USA). Fecal float and sedimentation examinations were performed at Michigan State University's Diagnostic Center for Population and Animal Health using standard methods (Bowman et al. 2009a). Fecal flotation was performed using Sheather's sugar solution and zinc sulfate solution and examined by light microscopy.

Statistical analysis was performed with JMP® Pro 10.0.02 (SAS Institute Inc., Cary, North Carolina, USA). Prevalence was calculated as the number of infected hosts divided by the number of hosts examined. Because some

American marten were sampled more than once, all parasite species found in an individual were considered together for prevalence calculations. Differences between locations (UP and NLP) and sexes were examined with Pearsons's chi-square test with $P<0.05$ considered significant. Significant differences between presence or absence of anemia and hookworm infection was examined with Person's chi-square test. Parasite species richness by host was calculated as the number of parasite species present per host and species richness by sample was calculated as the number of parasite species present per sample. An unidentified trematode or capillaria species was not included in the host species richness if one or more species of trematode or capillaria was identified in other samples from the same host. Unidentified trematodes and capillaria were always included in the sample species richness calculations. Differences between sex and location in species richness were examined with Wilcoxon rank sums test with $P<0.05$ considered significant.

The capture and handling protocol was approved by the University of Tennessee Animal Care and Use Committee (protocol #2180) and American marten live-trapping and sample collection was an authorized tribal activity under the 2007 Inland Consent Decree between the State of Michigan and the Little River Band of Ottawa Indians.

Results

Sixty samples from 49 individual American marten (28 males, 21 females) were examined and results are shown in Table 15. Fifty-nine of the samples, representing 48 American marten, were sufficient in quantity for fecal sedimentation procedure. Two species of trematodes, eight species of nematodes, one species of cestode, and three species of protozoa were identified. Upon consultation with a wildlife veterinary parasitologist (Gerhold), two additional nematode species seen (*Syphacia muris* and *Aspicularis sp.*) were presumed to be pass-through from rodent prey and excluded from analyses.

Parasite species richness by host is shown in Table 16. Of 49 individual American marten examined, 91.7% were positive for one or more parasites and 61.2% were infected with two or more parasites. The mean sample species richness (±SD, range) of 60 samples was 1.7 (±1.1, 0-4). Mean species richness by host was 2.1 for males and 1.8 for females. There was no difference in mean species richness by host between the sexes ($P=0.4$). Trematode eggs were seen in over half (62.5%) of the hosts. Positive samples were identified as *Alaria* sp., *Echinostome* sp., or were unidentified. *Echinostome* sp. eggs were an average of 119.6x71.3 µm (n=12) and *Alaria* sp. eggs were an average of 112.2x71.8 µm (n=10). *Capillaria* eggs were seen in 79% and 78.1% of samples from the UP and NLP, respectively. *Capillaria* eggs were identified as *Eucholeus aerophila* ($\bar{x}_{size}$=58.9x23.1 µm; n=5), *E. bohemi* or *Aonchotheca putorii* ($\bar{x}_{size}$=61.5x25.7 µm; n=21). Hookworm eggs averaged 63.7x34.3 µm (n=5). There was no significant difference in prevalence of any of the identified

parasites between male and female American martens. Of 9 American martens that were sampled more than once, none had identical results for each time point.

The mean hematocrit was 45.6±8.1 (range 30-68; n=49). Hematocrit ranged from 42-52% in the only other report of American marten hematocrit (Nieminen et al. 2007). Using <42% as a cut-off, 24.5% of blood samples tested in this report were considered anemic. American martens infected with hookworms were significantly more likely to be anemic than non-infected American martens (*P*=0.01) with an odds ratio of 8.75 (95% CI: 1.4-56.4).

Discussion

Parasite species richness per host was similar to that reported by Veine-Smith et al. (2011) for American martens in the UP. We identified more parasite species in the NLP than the UP but this may be a function of the larger sample size from the NLP, and there was no significant difference in richness between the two locations. Foreyt and Langerquist (1993) found two or more parasites in 35% of American martens from Eastern Washington. In another survey from Washington, a total of nine helminth species were found and 48.4% of hosts had coinfection of two or more parasites with a maximum of 4 (Hoberg et al. 1990).

Helminth parasites reported in American marten for the first time include *Echinostome* sp., *E. bohemi*, hookworm, *Hymenolepis* sp., *Spirocerca* sp., and *Strongyloides* sp. This is also the first report of shedding of *Sarcocystis* sp. sporocysts in an American marten and the identification of two coccidia, *Cystoisospora* sp. and *Eimeria* sp., from this mustelid. Parasites identified as a concern for reintroduction of North American river otter included *Alaria canis*, *Strongyloides lutrae*, *Crenosoma goblei*, capillaria and coccidia due to potential for pathogenic effects or high prevalence (Kimber and Kollias, 2000). River otter reintroduction programs tested and treated newly captured otters for parasites and similar methods could be considered for reintroduction of American marten (Griess, 1987; Hoover et al. 1985; Serfass et al. 1993). Of 9 American martens that were sampled more than once, none had identical results in subsequent sampling, indicating either a change in infection status or inconsistent shedding of ova. Therefore multiple fecal parasitological examinations are warranted when screening American martens in a reintroduction program. Endoparasites previously reported in North American martens are presented in Table 17.

1.1 Trematodes of American martens in Michigan

Alaria sp. are flukes found in the small intestine of definitive hosts including felids, canids, and mustelids and do not typically cause disease in the definitive host. *Alaria mustelae* is known to infect mink (*Mustela vison*) and short-tailed weasels (*Mustela erminea*) as well as American martens (Veine-Smith et al, 2011). *Alaria taxidae* was identified from 25% (n=6) of American martens from the District of Mackenzie, Northwest Territories and was identified in Manitoba, Canada at prevalences ranging from 36-73% depending on the area

(Holmes 1963; Poole et al. 1983). Collection and speciation of adult flukes from carcasses was not performed in this report and infections are reported as genus only.

Adult *Alaria* sp. produce eggs in the intestines of the definitive host which are shed approximately 21-35 days after infection in the host feces. After two weeks in wet soil or water, the eggs hatch producing a miracidium. The miracidium invades a freshwater snail, at which point it develops into a cercaria. The cercaria invades a tadpole and develops into a mesocercaria. When the tadpole is ingested by an amphibian, reptile, or rodent, the mesocercariae remain in the tissues of the paratenic host. When an American marten or other carnivore consumes a paratenic host, the parasite is able to complete its life cycle. The mesocercariae migrate through the stomach, across the diaphragm, and into the lungs. Once in the lungs, the mesocercariae develops into metacercariae. The metacercariae are able to travel up the trachea and are then swallowed, at which point they develop into adult trematodes in the small intestines. While infection with *Alaria* sp. typically does not cause disease in the definitive host, there are reports of the mesocercariae causing neurologic disease due to aberrant migration through the central nervous system and of respiratory illness due to migration through the lungs in domestic dogs (Kazacos 2001; Kimber and Kollias 2000). Other species of trematodes have caused disease in North American river otter but the pathogenicity of *Alaria* and *Echinostome* sp. in American marten is not known (Kimber and Kollias 2000).

American martens in this report were found to have a significantly higher prevalence of infection with *Alaria* sp. in the UP than the NLP and prevalence in the UP is similar to that reported by Veine-Smith et al. (2001). The difference in prevalence between the UP and NLP may be a result of unequal sample sizes or may reflect a higher likelihood for American martens in the UP to ingest the intermediate or paratenic host species. If a pregnant female rodent or carnivore becomes infected with *Alaria*, the mesocercariae can migrate to the mammary glands and can be transmitted to nursing young (Bowman 2009b). An Ameircan American marten kit, not yet weaned, died in the NLP and histopathology revealed infection with *Alaria* sp. in the duodenum, confirming the potential for transmammary migration of mesocercariae in this mustelid.

In the report by Veine-Smith et al. (2011), feces from the large intestines of 140 American marten carcasses from the UP were examined for flukes using a sedimentation technique. *Alaria* sp. eggs were detected in 39% of the samples and there was a significantly higher prevalence of infection in the western UP compared to the eastern UP. Juvenile males had a higher prevalence of *Alaria* sp. than females and prevalence of *Alaria* sp. in males decreased with age (Veine-Smith et al. 2001).

The life cycle of members of the family Echinostomatidae is similar to that of *Alaria* sp. in that there are two intermediate hosts required, freshwater snails and frogs or fish (Taylor et al. 2007a). Echinostomes are known to infect a wide range of birds and mammals and can also infect humans. American martens

were infected with *Echinostome* sp. at similar prevalence in both the UP and NLP. This is the first report of echinostome infection in American martens.

1.2 Other reported trematodes of American martens in North America

Euryhelmis squamula has been reported in raccoons (*Procyon lotor*), mink, and American martens in Washington and uses amphibian intermediate hosts in this region including red-legged frogs (*Rana aurora*), cascades frogs (*R. cascade*) and tailed frogs (*Ascaphus truei*) (Hoberg et al. 1990). American martens from Washington were documented with 6% prevalence from the southern Cascades, confirming that American martens are ingesting anuran prey in this area (Hoberg et al. 1990). *E. squamula* has been reported in mink in North America and is a common parasite of the polecat in Europe (Ameel 1938; Miller and Harkema, 1964). A related parasite, *Euryhelmis monorchis* has been reported in mink in Michigan (Ameel 1938). The mink is the natural host for *Euparyphium beaveri* in Michigan, while *Euparyphium inerme* has been reported to infect river otters in the Pacific Northwest (Hoberg et al. 1997; Miller and Harkema 1964).

2.1 Cestodes of American martens in Michigan

A single American marten from the NLP was shedding *Hymenolepis* sp. ova. *Hymenolepis nana* is a zoonotic cestode of rodents, carnivores and humans found worldwide, but other species of *Hymenolepis* infect galliformes including potential American marten prey species. The parasite may use intermediate hosts or paratenic hosts including dung beetles, stable flies and fleas (Drew 2003; Joslin 2003; Loomis 2003; Sainsbury 2003). Without knowing the species of *Hymenolepis* found from the American marten in the NLP, it is not known whether this may have been a pass-through finding or was truly an infection of the American marten and the zoonotic potential is not known.

2.2 Other reported cestodes of American martens in North America

Taenia sp. are cestodes (commonly known as tapeworm) that maintains a completely sylvatic life cycle. The definitive hosts for *T. mustelae* and *T. martis americana* are primarily mustelids. Adult parasites live in the small intestine and eggs are passed in the feces of the host. Larvae hatch in the environment and are ingested by herbivores, the intermediate hosts. The ingested larvae form cysticercus in skeletal muscle and viscera and the life cycle is completed when a carnivore consumes the infected intermediate host. While the definitive host typically shows no signs of disease, the intermediate host may suffer morbidity or mortality including liver damage as a result of infection (Jones and Pybus 2001).

T. mustelae has a wide distribution across the Northern Hemisphere and has been reported in North American mustelids including American martens, short-tailed weasel (*Mustela erminea*), mink (*Neovison vison*), and least weasel (*M. nivalis*). The cestode has been found in two sciurid definitive hosts, *Marmota broweri* and *M. caligata* (Jones and Pybus 2001). American martens from the southern Cascades in Washington were reported with a 30% (n=19) prevalence of *T. mustelae* (Hoberg et al. 1990) whereas 12.5% (n=24) from the District of Mackenzie in the Northwest Territories were infected with the parasite (Holmes

1963). From the Duck Mountain area of Manitoba, there was a prevalence of 15% (n=9) of *T. mustelae* (Poole et al. 1983).

Taenia martis americana infects mustelids including American martens, fisher (*M. pennanti*), and the ringtail (*Bassariscus astutus*, Family: Procyonidae) as definitive hosts. Experimentally infected definitive hosts started to shed eggs 43 days post-infection. Rodents in North America reported with the larval stage of infection include *Lemmus sibricus*, *Microtus xanthognathus*, *Mus musculus*, and *Ondatra zibethicus* (Jones and Pybus 2001). American martens from Washington, USA were documented with the parasite at a prevalence of 16% (n=10) from the southern Cascades and 14% (n=2) from the northern Cascades. Two American martens from the southern Cascades were coinfected with both *T. mustelae* and *T. martis americana* (Hoberg et al. 1990). From the Northwest Territories of Canada, 29% (n=7) were found with *T. martis* (Holmes 1963) while from the Southern Indian Lake region of Manitoba, Canada there was a 23% (n=16) prevalence of *T. martis* (Poole et al. 1983).

Mesocestoides sp. are cestodes with a more complicated life cycle. Adult parasites are found in the small intestine of definitive hosts, which include canids, felids, and mustelids (Bowman 2009b). Larval or adult parasites can also infect birds, reptiles and other mammals (Wardle and McLeod 1952). The pre-patent period is two weeks. Eggs, or gravid proglottids, are suspected to be ingested by a first intermediate host, a coprophagic insect or a mite (Chowdhury and Aguirre 2001; Bowman 2009b). The insect is consumed by a second intermediate host, which may include birds, mammals, reptiles and amphibians. Lastly, the definitive host becomes infected by ingesting the second intermediate host (Bowman 2009b). Definitive hosts may have clinical signs of infection including anorexia, low serum albumin, and vomiting (Chowdhury and Aguirre 2001). Humans can be incidental definitive hosts and become infected by consuming undercooked game (Chowdhury and Aguirre 2001; Fuentes et al. 2003). American martens from western Washington were documented with 59% (n=38) prevalence of *Mesocestoides* sp. in the southern Cascades Mountain range and 21% (n=3) prevalence in the northern Cascades (Hoberg et al. 1990). *Mesocestoides lineatus* was identified in the small intestine of 33% (n=14) of American martens from eastern Washington and was significantly higher in juveniles compared to adults. Current taxonomy however suggests that this species may have actually been *M. variabilis* which occurs in North America, while *M. lineatus* is an Old World species (Fuentes et al. 2003). Coinfections with *M. lineatus* and *C. putorii* occurred in 35% of parasitized American martens and juveniles had statistically higher rates of coinfection than adults (Foreyt and Lagerquist 1993).

3.1 Nematodes of American martens in Michigan

Hookworms are zoonotic nematode parasites infecting carnivores (Taylor et al. 2007b). Depending upon the species of hookworm, a carnivore may be infected via ingestion of infective larvae or eggs, percutaneous infection, ingestion of paratenic hosts, or lactogenic transmission. Ingested or

percutaneously acquired larvae migrate to the lungs, moult to the next larval stage, are coughed up and swallowed to lay eggs in the small intestine. Ingested larvae may bypass the pulmonary migration. Larvae may also migrate out of the lungs into the muscle and remain in an infected female mammal until pregnancy occurs, at which point the larvae migrate to the mammary gland leading to lactogenic transmission (Taylor et al. 2007b). Hookworm infection in dogs and foxes can result in bloody diarrhea, anemia, poor hair-coat, poor growth in puppies and respiratory signs (Taylor et al. 2007b). We found that the odds of having anemia (hematorcrit<42%) were 8.75 higher for American martens infected with hookworms than uninfected American martens (Nieminen et al. 2007). While there was a significant association between presence of hookworm infection and presence of anemia in this report, other clinical signs related to hookworm infection were not seen. Because other causes of anemia were not investigated, the association does not prove causation and warrants further investigation. Determining the intensity of infection via carcass examination and histopathology may help in elucidating the role of hookworm infection in anemia in American martens. Because of the potential for anemia to affect fitness of an individual American marten or the growth of kits infected via transmammary transmission, treatment of hookworm infected American martens destined for relocation may be warranted.

Capillaria is a genus encompassing many species. *Aonchotheca putorii* (previously known as *Capillaria putorii*) infects the gastrointestinal tract of mustelids and others while other capillarids infect the respiratory tract, bladder, or liver of their respective definitive hosts (Bowman 2009b). *A. putorii* can have a direct or indirect life cycle in which adult parasites shed eggs in the gastrointestinal tract of the mammalian host. The eggs are capable of infecting other susceptible hosts directly or using an earthworm as an intermediate host (Segovia et al. 2007; Taylor et al. 2007b). *A. putorii* was found in 61.1% (n=11) of American martens from the UP in the current report, which was significantly higher than the 22.6% (n=7) prevalence in the NLP. However, during the initial stage of this parasite survey, some capillarid ova were not identified to species and these are represented as "unidentified *Capillaria*" in Table 15. If the 54.8% (n=17) prevalence of unidentified *Capillaria* found in the NLP were *A. putorii*, then there would not be a significant difference in prevalence of *A. putorii* between the UP and NLP. The prevalence of *A. putorii* from the UP is higher than the 47% (n=66) previously reported from American marten carcasses from the UP (Veine-Smith et al. 2011). *A. putorii* has also been reported in the stomach of ferret, mink, short-tailed weasel, raccoon, fisher, and striped skunk (*Mephitis mephitis*) and in the small intestine of bobcats, bears, raccoons, swine, hedgehogs, and the domestic cat (Foreyt and Lagerquist 1993; Bowman 2009b). *A. putorii* was found in 86% (n=36) of American martens from northeastern Washington. In affected American martens, the parasite was typically found in the stomach and less frequently in the large or small intestine. Using fecal flotation rather than

carcass examination, *Capillaria* sp. ova were found in 64% (n=21) of samples examined from the same population (Foreyt and Lagerquist, 1993).

Eucoleus aerophilus, previously known as *Capillaria aerophila*, infects the respiratory tract of its host (Bowman 2009b). These parasites have been reported in American martens as well as other carnivores including fisher, red fox (*Vulpes vulpes*), raccoon, coyote (*Canis latrans*), striped skunk, and badger (*Taxidea taxus*). *E. aerophilus* rarely infects humans (Lalošević et al. 2013). Prevalence of *E. aerophilus* was higher in the UP than the NLP but overall prevalence was low in both locations (10.5% and 2.4%, respectively). The life cycle of *E. aerophilus* may be direct as in other capillarids, or may be indirect with earthworms serving as intermediate hosts (Bowman 2009b, pp. 226-227). An American marten may become infected by ingesting eggs from the environment or by ingesting infected earthworms (Seville and Addison 1995). In farmed foxes, infection with *E. aerophilus* can lead to respiratory disease and clinical signs include coughing, wheezing, failure to thrive, pneumonia and even death. Cats and dogs have also been infected but typically do not suffer the same degree of clinical signs as foxes since their infections are not as intense as those in farmed foxes (Bowman 2009b, pp. 226-227). *E. aerophilus* was identified in 4% of the respiratory tracts of American martens from Ontario but it is unknown whether infection resulted in disease or increased risk of being trapped (Seville and Addison 1995). *Eucoleus bohemi* has been reported to infect the respiratory tract of foxes and dogs (Bowman 2009b, pp. 226-227). Its ova can be differentiated from the similar *E. aerophilus* by its pitted surface (Bowman 2009b, pp. 226-227). *E. bohemi* was found only in the NLP in a single American marten. The relative contribution of earthworms to American marten diet in Michigan is not known but may be significant given the high overall prevalence of capillarid parasites seen here.

Physaloptera spp. are found in the stomach of infected carnivores including raccoons, dogs, and cats, and eggs are shed intermittently (Chowdhury and Aguirre 2001). Crickets, beetles or other invertebrates act as intermediate hosts, while rodents and reptiles may be paratenic hosts (Chowdhury and Aguirre 2001). *Physaloptera* sp. was found in low prevalence in both the UP and NLP. While most infections do not cause disease in the host, severe ulcerative gastritis has been reported in a marsupial, the bandicoot (*Perameles* sp.) (Holz 2003). Of 64 American martens examined from the southern Cascades in Washington, 2% were infected (Hoberg et al. 1990). Of 140 American marten carcasses collected by Veine-Smith et al. (2011) from the Upper Peninsula of Michigan, *Physaloptera maxillaris* was found in 9% of American marten stomachs overall and there was a significantly higher prevalence in eastern UP (23%) compared to western UP (1%). *Physaloptera* sp. has been previously documented to infect mink and striped skunk as well (Veine-Smith et al. 2011).

Crenosoma sp. is a lungworm found within the respiratory tract of carnivores and insectivores (Craig and Anderson 1972). Adult parasites lay eggs in the lungs, which hatch into larvae that are coughed up and swallowed by

the host and passed in the feces. Infective larvae penetrate a gastropod, the intermediate host. When a carnivore consumes a snail or slug, the parasite life cycle is completed. Heavy infection with *Crenosoma* spp. can cause clinical signs such as coughing, sneezing, nasal discharge and difficulty breathing (Chowdhury and Aguirre 2001). *Crenosoma vulpis* larvae were identified in the feces of 2 American martens (10.5%) in the UP while no American martens were positive for the parasite from the NLP. The red fox is the typical host for *C. vulpis* and is sympatric with American martens in both the UP and NLP. Given the global distribution of *C. vulpis*, it is likely that this parasite exists in the NLP red fox population. *C. petrowi* has been reported from free-ranging Russian sable, a captive fisher in the United States and a badger from Canada (Craig and Anderson, 1972). A single American marten from Ontario was found to be infected with *Crenosoma petrowi* (<1% prevalence) but the reported prevalence in fisher from the same region was 15% (Seville and Addison 1995). Olsen (1952) examined 62 carcasses of *M. caurina* from Colorado and found 18 (29%) to be infected with *Crenosoma*, which he designated *C. coloradoensis*. *Crenosoma* sp. was found in the lungs of 2% (n=3) of American martens from the UP of Michigan (Veine-Smith et al. 2011).

Spirocerca lupi is a nematode primarily of dogs but was previously reported in *Martes martes* in Spain (Segovia et al. 2007). *Spirocerca* sp. was found in a single American marten from the NLP and was not identified to the species level. Parasites of this genus are typically found worldwide and throughout the United States but are more common in warm climates. *S. lupi* has an indirect life cycle with coprophagic beetles as an intermediate host and can also be transmitted via paratenic hosts such as mice or lizards (van der Merwe et al. 2008). When a carnivorous definitive host consumes the intermediate or paratenic host, the eggs hatch and larvae migrate through the stomach lining and along arteries to the esophagus. Nodules form around the parasite in the esophagus and infection in dogs can be subclinical or cause signs such as regurgitation, weight loss, difficulty swallowing, or result in aortic aneurysm (van der Merwe et al. 2008). Clinical signs associated with *Spirocerca* sp. have not been reported in a mustelid to our knowledge but pathology associated with the parasite is possible.

This is the first report of *Strongyloides* sp. in American marten. *Strongyloides martis* and *S. lutrae* have been reported in river otters (Hoberg et al. 1997). Parasites of this genus are generally species or host-specific and undergo both a direct life cycle and a free-living stage. Infective larvae or eggs in the soil are consumed by the host; larvae of some species can also enter the host through the skin (Morris and Shima 2003). Pathogenicity of *Strongyloides* sp. in mustelids is not known but disease could result from migration of the parasite through the lung (Kimber and Kollias 2000).

Dioctophyme renale, commonly known as the giant kidney worm, is one of the largest roundworms and can be found in wild and domestic species worldwide. It has been reported to infect carnivores including wolves, bears,

foxes, and mink as well as domestic dogs, cattle, horses and pigs. Humans have also been reported with *D. renale* (Chowdhury and Aguirre 2001). The adult worm is typically found in the right kidney because the infective larvae will exit the intestinal tract near the stomach, which is on the right side of the abdomen. Once the kidney is infected with the parasite, the parasite can live up to three years. When the parasite dies, the kidney is essentially destroyed and the host becomes reliant on the functionality of the remaining left kidney. Occasionally both kidneys are infected or the parasite is found elsewhere in the abdomen other than a kidney. The adult female worm lays eggs within the kidney, which are shed in the urine of the host mammal. It takes about six months for the egg to become infective, at which point it may be swallowed by the intermediate host, *Lumbriculus variegatus*, an aquatic annelid commonly known as blackworm. The egg develops within the intermediate host into an infective larvae. If the annelid is eaten by a American marten, or other mammalian host, the life cycle is completed when the larvae finds its way to a kidney. Fish, frogs and crayfish may act as paratenic hosts by consuming the infected annelid (Cheng 1986; Chowdhury and Aguirre 2001). A single American marten from the NLP/UP was reported with nephritis due to suspected prior infection with *D. renale* (Chapter 2). In an examination of 405 American martens from Ontario, *D. renale* was found in only 2% of American martens and only from districts with previous reports of infected mink. In 4 of the 5 infected American marten, there was only evidence of past infection such as the entire right kidney missing or just fibrous capsule remaining, while in 1 American marten the actual parasite was identified (Seville and Addison 1995).

Filaroides martis is a helminth parasite found in the trachea, bronchi and lungs and has been reported to infect mustelids including mink and American martens as well as canids (Chowdhury and Aguirre 2001). Aquatic and terrestrial snails are the intermediate hosts. A mink or American marten becomes infected when it ingests the intermediate host; the larva molts in the stomach mucosa of the definitive host and migrates to the thoracic cavity over the next month. Larvae increase in size over 100-fold during this timeframe (Ko and Anderson 1972). Infection with *F. martis* has been reported to cause pneumonia in other species, but its effect on American marten is not known (Chowdhury and Aguirre 2001). Of 405 American martens examined from Ontario, 8% had lung or aortic nodules associated with the parasite. Because yearlings had a significantly higher prevalence of infection than other ages, the authors suggest that infection could have an effect on survival of yearlings or that American marten are able to recover from infection at older age groups (Seville and Addison 1995). *F. martis* was found in the lungs of 4% (n=5) of American marten carcasses from the Upper Peninsula of Michigan (Veine-Smith et al. 2011). Histopathology revealed lesions consistent with verminous pneumonia in 60% (n=9) of American marten carcasses from Michigan (Chapter 2). While these were incidental findings and the causative parasite was not identified, it is possible that pneumonia may have

resulted in mild respiratory compromise in affected American martens (Chapter 2).

3.2 Other reported nematodes of American marten in North America

Pearsonema plica, previously known as *Capillaria plica*, was identified in the urinary bladder of 6% of the American martens from Ontario (Seville and Addison, 1995). *P. plica* has been reported embedded in the urinary tract of the domestic cat and dog, raccoon, red fox, coyote, wolf, striped skunk and fisher as well as American martens (Butterworth and Beverly-Burton 1980). This parasite has an indirect life cycle in which the earthworm acts as a paratenic host. After ingesting an earthworm, the definitive host begins to shed eggs of *C. plica* in the urine about 8 weeks after infection (Bowman 2009b). Infection usually does not cause disease for the host, but there is a suggestion that *P. plica* resulted in poor growth in fox kits (Bowman 2009b).

Baylisascaris devosi is a nematode, or roundworm, reported in both American martens and fisher (Kazacos 2001). Eggs shed from the definitive host become infective after 11-14 days at which point small mammals such as rodents and squirrels become infected by ingesting the eggs while foraging. The larvae migrate throughout the tissues of these paratenic hosts and typically localize to the muscle of the forelimbs and thorax. Neural larval migrans is rare or nonexistent with *B. devosi* (Kazacos 2001). In contrast, the larvae of the related *B. procyonis*, or raccoon roundworm, frequently migrate to the CNS of non-adapted hosts and cause neurologic disease also known as neural larval migrans. Humans are susceptible to neural larval migrans caused by *Baylisascaris* sp., but *B. devosi* is less likely to cause disease in humans than *B. procyonis* (Kazacos 2001). Adult *B. devosi* inhabits the small intestine and the definitive host does not typically show signs but intestinal blockage is possible with a severe infection (Chowdhury and Aguirre 2001). American martens from the southern Cascades in Washington were infected with *B. devosi* with a prevelance of 2% while those from the northern Cascades were found with a prevalence of 21% (Hoberg et al. 1990). One American marten of 139 from Manitoba, Canada was found infected with *B. devosi* and in 1 of 42 *M. caurina* carcasses examined from Idaho was infected (Erickson 1946; Poole et al. 1983).

Soboliphyme baturini, commonly known as stomach worm, is a nematode parasite distributed from central Siberia across Beringia to the Pacific Northwest of the United States (Koehler et al. 2009). The primary definitive hosts are sable (*Martes zibellina*) and American martens. Other mustelids reported with the parasite include ermine (*M. erminea*), mink, and ferrets (*Mustelo putorius furo*) (Levine 1968; Swartz 1968; Koehler et al. 2009). *S. baturini* has been reported in other carnivores including the fox and domestic cat (Levine 1968). Mature female worms are found in the stomach or small intestine of the mustelid host and eggs are passed in the feces of the host. Earthworms are the intermediate host, while shrews become paratenic hosts when they ingest the earthworm. A American marten may be infected by consuming either infected shrews or earthworms (Koehler et al. 2009). Koehler et al. (2009) used genetic molecular

data of *S. baturini* to shed light on the expansion of the ancestral American marten across Beringia into N. America, its speciation during isolation in glacial refugia, and re-colonization in Alaska and reinfection with the *S. baturini*. American martens from the northern Cascades region of Washington were infected with a 7% (n=1) prevalence (Hoberg et al. 1990). *S. baturini* is of particular interest in biogeographical studies as it ranges from east of the Ural Mountains in Russia and along the western side of North America in line with the distribution of mustelid hosts. Clinical manifestation of infection in sable includes anemia and gastric ulceration (Thomas et al. 2008). There was a 55% (n=155) prevalence of infection with *S. baturini* in American martens from Prince of Wales Island in Alaska. There was no correlation between intensity of *S. baturini* infection and omental and mesentery fat deposits measured to assess nutritional condition (Thomas et al. 2008). American marten carcasses were collected over an 8-year time period from three locations in Alaska and stomachs were examined for *S. baturini*. The highest prevalence of 47% (n=1,430) was in the Southeast study area while the lowest prevalence of 19% (n=416) was in the Northern study area. The Southwestern study area had a prevalence of 30% (n=321). None of the American marten in this study had any sign of negative health impact from the parasite infection (Zarnke et al. 2004). A study conducted in Idaho examined 42 *M. caurina* carcasses and found one with *S. baturini* (Erickson 1946).

Trichinella spiralis has a very broad host range, infecting over 100 species of mammals. It is found worldwide except Antarctica and Australasia (Chowdhury and Aguirre 2001). Adult *T. spiralis* are found in the small intestine. Adult female parasites give birth to larvae which migrate through the body to become encysted in skeletal muscle. An American marten may become infected by consuming a rodent or other mammal with the encysted parasite. Once consumed, the larvae are freed and migrate to the small intestine of the host to complete its life cycle. Humans can acquire trichinellosis by eating undercooked meat (Taylor et al. 2007c). The first report of *T. spiralis* in American martens in N. America was from Manitoba, Canada where a single yearling was found infected of 139 American marten examined (Poole et al. 1983). Prevalence of *Trichinella* in American marten and other species may vary from year to year (Dick et al. 1986). American martens from the northern and southern Cascades of Washington were found to have Trichinella encysted in the diaphragm with a prevalence of 50% and 31%, respectively (Hoberg et al.1990). Another study from examined tongue muscle from 42 American martens from northeastern Washington and found only 5% prevalence, which the authors attribute to differences in technique and tissues examined (Foreyt and Lagerquist 1993). A study conducted in Ontario over found a 3.4% prevalence (n=68) of infection with *T. spiralis* in American martens (Dick et al. 1986). In this study, 4,773 carnivores of 18 species were examined. Only American martens, fishers and mink were found with the parasite. The prevalence in fishers in this study was slightly higher at 4.5% (n=83) and a single mink (of 12 tested) was positive. The authors

suggest that American martens and fishers are key in the sylvatic transmission of *Trichinella* in this part of Canada (Dick et al. 1986). A later study conducted by Seville and Addison (1995) in Ontario found a similar prevalence of 2% of 405 American martens examined. In another study, only one of 56 American martens examined in southwestern Quebec was positive (Bourque 1985). In the East Kootenay Bay area of British Columbia, Canada there was 61% prevalence (n=22) of *T. spiralis* in the diaphragm, rib, tongue or masseter muscle of American martens (Schmitt et al. 1976.) In contrast to the findings of Dick et al. (1986) in which only mustelids (American marten, fisher and mink) were found with *T. spiralis* despite testing 18 species of carnivores, this study found a high prevalence in coyote (61%, n=22) and confirmed infection in black bear, grizzly bear, lynx, skunk, cougar, bobcat, weasel and wolverine as well as small mammals including shrew, white-footed deer mouse, ground squirrel and red squirrel (Schmitt et al. 1976). A small number of American martens were collected from Montana, Idaho and Wyoming and found to have an 8.3% (n=2) prevalence of infection of tongue, diaphragm and thigh muscles (Worley et al. 1974).

Dracunculus insignis is a parasite known to infect raccoons, dogs, mink, fishers, and skunks in the United States east of the Rocky Mountains and in Ontario, Canada (Crichton and Beverly-Burton 1973; Cheng 1986). Experimental infections in ferrets are used as an experimental model for human drancunculosis (Eberhard et al. 1988). *D. lutrae* infects otters in North America (Crichton and Beverly-Burton 1973). Both species have a similar life cycle to the related the Old World parasite *D. medinensis*, commonly called the guinea worm, a zoonotic parasite. The adult worm is found in abdominal tissues of the host. Females *D. lutrae* migrate to the tissues under the skin of the animal to give birth to live larvae while secreting a substance that causes a blister to form. When the blister ruptures and is exposed to water the female releases the infective larvae into the water. The free-living larvae can survive without eating for several days until they are consumed by an aquatic coepod, the intermediate host. *D. insignis* develops through several more stages within the copepod over the next 3 weeks. When a definitive host such as a American marten ingests the copepod while drinking water, the larvae are freed in the stomach or small intestine and migrate to abdominal organs and tissues where it continues to develop into adult worms over the next 8-12 months (Cheng 1986). *D. insignis* was found in only a single American marten of 405 examined in Ontario despite the parasite being commonly found in raccoons of the same region. The authors suggest that there was a higher true prevalence but detection was low due to the pelt being removed for commercial reasons in most of the examined specimens (Seville and Addison 1995). This parasite is of economic concern as it can affect pelt quality in fur-bearer species.

4.1 Protozoa of American marten in Michigan

A single American marten from the NLP was found to be shedding *Sarcocystis* sp. sporocysts. *Sarcocystis* sp. is a protozoal parasite that has

infrequently been reported in carnivores such as domestic cats, dogs, raccoons, cougars, bobcats, mink, striped skunks, sea otters, fishers, and Pacific harbor seals (Foreyt and Lagerquist 1993; Gerhold et al. 2005; Larkin et al. 2011). *Sarcocystis* sp. require an intermediate host. Gerhold et al. (2005) reported a case of meningoenephalitis in a fisher caused by *S. neurona* in Maryland, USA. The Virginia opossum (*Didelphis virginiana*) is the definitive host for the parasite in N. America but the natural intermediate host for *S. neurona* has not been discovered (Gerhold et al. 2005). *Sarcosystis* sp. was found in the tongues of 10% (n=4) of American martens examined from northeastern Washington (Foreyt and Lagerquist, 1993).

Antibodies to *Toxoplasma gondii* were detected in 10.8% (n=15) of American martens in Ontario. American martens in Michigan were found to have a 58% seroprevalence (n=47) and there was no significant difference between the UP and NLP (Chapter 2). *T. gondii* has been reported to cause mortality in the related black-footed ferret (*Mustela nigripes*), captive-raised American mink (*Neovison vison*), and southern sea otters (*Enhydra lutris*) (Dubey et al. 2003; Burns et al. 2003b; Jones et al. 2006).

Coccidian parasites have been only rarely reported in American marten but this is likely because few studies have used fecal flotation and/or histopathology to detect parasites. All coccidian parasites are obligate intracellular parasites and undergo stages of asexual and sexual reproduction in the life cycle, which may be direct or indirect. Coccidian oocysts from five American martens in this report were sporulated to allow identification to the genus level. *Eimeria* sp. were identified in two of the five sporulated samples from American martens in our study, and *Cystoisospora* sp. in the remaining three. Taxonomy of coccidian parasites has been controversial and some have considered *Cystoisospora* sp. to be synonymous with *Isospora* sp. while others believe it to be a distinct genus based on molecular and morphological characteristics (Yi-Fan et al. 2012). *Cystoisospora* sp. were identified in American marten from Michigan in this report based on morphological features of the sporulated oocysts. Yi-Fan et al. (2012) reported *Cystoisospora* sp. in steppe polecats in China and suggested that previously reported *Isospora* sp. in various other mustelids including that of sable should be reassigned to *Cystoisospora* sp. Feces from 33 American martens from eastern Washington were examined and 6% were found with coccidian oocysts (Foreyt and Lagerquist, 1993). *Cryptosporidium* sp. and *Giardia* sp. cysts were detected in the feces of American martens from the Upper Peninsula of Michigan, both having a prevalence of 4% (n=5) (Veine-Smith et al. 2011).

Conclusion

North American martens are infected with a wide variety of endoparasites but information regarding the pathologic effects of parasitism remains limited. American martens infected with hookworms in our study were found to be at risk for anemia and this association warrants further investigation. Some parasites are infrequently reported in only a single location, while those reported in multiple

locations typically have a varying prevalence. Multiple ecological factors including habitat, prey availability, sympatric carnivore community, and host adaptation are likely involved in the variation of prevalence at different geographic locations. A number of parasites known to infect American martens have zoonotic potential. As American marten are frequently trapped as a furbearer species, this information could guide prevention of disease transmission to trappers and researchers working with the species.

Parasite infections that would have otherwise been innocuous could cause illness under conditions of stress and presumed immunosuppression associated with reintroduction programs (Kimber and Kollias, 2000). For these programs, non-invasive samples are desired for detecting endoparasitism. The majority of studies examined American marten carcasses for parasitism, which allows characterization and speciation of the adult parasite and information about intensity of infection but would not be acceptable for screening of live animals. In contrast, our results provide baseline information about parasites detected by non-invasive fecal examination from American martens in Michigan. Multiple fecal exams from individuals destined for relocation, including sedimentation and molecular techniques when available, are warranted to identify novel parasites and parasites with pathologic or zoonotic potential and to make appropriate treatment or management decisions.

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Appendix

Table 15. Prevalence of parasite species identified in fecal samples from live-trapped American marten in the Upper Peninsula and northern Lower Peninsula of Michigan. Significant differences between the locations are indicated by the same superscript (*P*<0.05).

Parasite	Prevalence UP (n=18)	Prevalence NLP (n=31)
Trematode overall	55.6%	66.7%
Echinostome sp.	16.7%	26.7%
Alaria sp.	38.9%[a]	13.3%[a]
Unidentified	0%[b]	26.7%[b]
Capillaria overall	77.8%	77.4%
Eucholeus aerophila	11.1%	3.2%
Eucholeus bohemi	0%	6.5%
Aonchotheca putorii	61.1%[c]	22.6%[c]
Unidentified	0%[d]	54.8%[d]
Coccidia*	27.8%	25.8%
Hookworm	11.1%	16.1%
Crenosoma vulpis	11.1%	0%
Physaloptera sp.	5.6%	3.2%
Sarcocystis sp.	0%	3.2%
Hymenolepis sp.	0%	3.2%
Spirocerca sp.	0%	3.2%
Strongyloides sp.	0%	3.2%

*Five samples were able to be sporulated and were identified as *Cystoisospora* sp. (n=3) and *Eimeria* sp. (n=2).

Table 16. Mean species richness by host for American martens by location and sex. There were no significant differences between the locations or sexes in richness.

Category	Mean species richness by host	SD	Range	n
Overall	2.0	1.3	0-6	49
UP	1.8	1.2	0-4	18
NLP	2.0	1.3	0-6	31
Males	2.1	1.3	0-6	28
Females	1.8	1.2	0-4	21

Table 17. Helminth parasites previously reported in American martens.

Species	Location	Prevalence (%)	Infected (n)	Study
Nematodes				
Baylisascaris devosi				
	Northern Cascades, Washington, USA	21.0	3	Hoberg et al. 1990
	Southern Cascades, Washington, USA	2.0	1	Hoberg et al. 1990
	Manitoba, CA	0.7	1	Poole et al. 1983
Capillaria putorii				
	Eastern Washington, USA	86.0	36	Foreyt and Lagerquist, 1993
	Upper Peninsula, Michigan, USA	47.0	66	Veine-Smith et al, 2011
Crenosoma sp.				
	Colorado, USA	29.0	18	Olsen, 1952
	Upper Peninsula, Michigan, USA	2.0	3	Veine-Smith et al, 2011
	Ontario, CA	0.2	1	Seville and Addison, 1995
Dioctophyme renale				
	Ontario, CA	2.0	8	Seville and Addison, 1995
Dracunculus insignis				
	Ontario, CA	0.2	1	Seville and Addison, 1995
Eucoleus aerophilus				
	Ontario, CA	4.0	16	Seville and Addison, 1995
Filaroides martis				
	Upper Peninsula, Michigan, USA	4.0	5	Veine-Smith et al, 2011
	Ontario, CA	8.0	37	Seville and Addison, 1995
Molineus patens				
	Southern Cascades, Washington, USA	9.0	6	Hoberg et al. 1990
Pearsonemia plica				
	Ontario, CA	6.0	24	Seville and Addison, 1995

Table 17 Continued

Species	Location	Prevalence (%)	Infected (n)	Study
Physaloptera sp.				
	Southern Cascades, Washington, USA	2.0	1	Hoberg et al. 1990
	Upper Peninsula, Michigan, USA	9.0	13	Veine-Smith et al, 2011
Soboliphyme baturini				
	Northern Alaska, USA	19.0	416	Zarnke et al. 2004
	Southeastern Alaska, USA	55.0	85	Thomas et al. 2008
	Southeastern Alaska, USA	47.0	1430	Zarnke et al. 2004
	Southwestern Alaska, USA	30.0	321	Zarnke et al. 2004
	Northern Cascades, Washington, USA	7.0	1	Hoberg et al. 1990
	Idaho, USA	0.9	1	Erickson, 1946
Trichinella spiralis				
	British Columbia, CA	61.0	22	Schmitt et al. 1976
	Northern Cascades, Washington, USA	50.0	7	Hoberg et al. 1990
	Southern Cascades, Washington, USA	31.0	20	Hoberg et al. 1990
	Eastern Washington, USA	5.0	2	Foreyt and Lagerquist, 1993
	Manitoba, CA	0.7	1	Poole et al. 1983
	Montana, Idaho and Wyoming, USA	8.3	2	Worley et al. 1974
	Ontario, CA	3.4	68	Dick et al. 1986
	Ontario, CA	2.0	8	Seville and Addison, 1995
	Quebec, CA	1.8	1	Bourque, 1985
Trematodes				
Alaria sp.				
	Duck Mountain, Manitoba, CA	73.0	45	Poole et al. 1983
	Porcupine Mountain, Manitoba, CA	57.0	4	Poole et al. 1983
	Southern Indian Lake, Manitoba, CA	36.0	32	Poole et al. 1983
	Upper Peninsula, Michigan, USA	39.0	54	Veine-Smith et al, 2011
	Northwest Territories, CA	25.0	6	Holmes, 1963

Table 17 Continued

Species	Location	Prevalence (%)	Infected (n)	Study
Euryhelmis squamula				
	Southern Cascades, Washington, USA	6.0	4	Hoberg et al. 1990
Cestodes				
Mesocestoides sp.				
	Northern Cascades, Washington, USA	21.0	3	Hoberg et al. 1990
	Southern Cascades, Washington, USA	59.0	38	Hoberg et al. 1990
	Eastern Washington, USA	33.0	14	Foreyt and Lagerquist, 1993
Taenia martis americana				
	Northern Cascades, Washington, USA	14.0	2	Hoberg et al. 1990
	Southern Cascades, Washington, USA	16.0	10	Hoberg et al. 1990
	Southern Indian Lake, Manitoba, CA	23.0	16	Poole et al. 1983*
Taenia mustelae				
	Southern Cascades, Washington, USA	30.0	19	Hoberg et al. 1990
	Duck Mountain, Manitoba, CA	15.0	9	Poole et al. 1983
	Northwest Territories, CA	29.0	7	Holmes, 1963
	Northwest Territories, CA	12.5	3	Holmes, 1963
Protozoa				
Cryptosporidium sp.				
	Upper Peninsula, Michigan, USA	4.0	5	Veine-Smith et al. 2011
Giardia sp.				
	Upper Peninsula, Michigan, USA	4.0	5	Veine-Smith et al. 2011
Sarcocystis sp.				
	Eastern Washington, USA	10.0	4	Foreyt and Lagerquist, 1993
Toxoplasma gondii				
	Ontario, CA	10.8	15	Tizard et al. 1976
	Michigan, USA	58.0	47	Chapter 2

Table 17 Continued

Species	Location	Prevalence (%)	Infected (n)	Study
Protozoa				
Cryptosporidium sp.				
	Upper Peninsula, Michigan, USA	4.0	5	Veine-Smith et al. 2011
Giardia sp.				
	Upper Peninsula, Michigan, USA	4.0	5	Veine-Smith et al. 2011
Sarcocystis sp.				
	Eastern Washington, USA	10.0	4	Foreyt and Lagerquist, 1993
Toxoplasma gondii				
	Ontario, CA	10.8	15	Tizard et al. 1976
	Michigan, USA	58.0	47	Poole et al. 1983
	Manitoba, CA	15.0	9	Poole et al. 1983
	Northwest Territories, CA	29.0	7	Holmes, 1963
	Northwest Territories, CA	12.5	3	Holmes, 1963

***Taenia* sp. (cf. *martis martis*)

CONCLUSION

The restoration of American martens to Michigan began with the reintroduction of American martens during the mid-20th century to both the UP and NLP. Research efforts are currently underway to identify factors that may be limiting growth of the population in the NLP and to make management recommendations. Based on analysis of stable isotope values, no significant differences appear to exist in foraging ecology between the UP and NLP and thus prey availability does not appear to be a limiting factor for population growth in the NLP. For the capture and immobilization of American martens for research and/or translocation, I recommend the use of isoflurane as a safe anesthetic and for precautions to be taken to minimize risk of complications from handling and anesthesia. This dissertation includes the expected ranges for cell blood count and serum biochemistry from live-trapped American marten in summer and winter which can be used to assess the systemic health of American martens marked for a translocation or that exhibit signs of disease. Further research is also needed in determining the optimal collar material and fit to minimize risk of complications or entrapment. Should additional translocation of American martens be considered, efforts should be made to prevent the introduction of novel pathogens as well. Rarely can all risk be removed as there may be pathogens that are not even known or described in the source or destination populations; this risk must be accepted by management and funding agencies and the general public when applicable. I did not identify any major disease risks should one consider translocating American martens from the UP to the NLP based on the available data. If American martens were to be translocated from the UP to the NLP, I would recommend: 1) continuing surveillance of UP and NLP American marten populations for pathogen exposure prior to translocation, 2) initiate surveillance of sympatric carnivores that may be reservoirs for CDV just prior to or at the time of the translocation to minimize risk of reintroduction during a CDV outbreak, 3) conduct pre-release screening or quarantine of translocated American martens with the purpose of detecting signs of disease, conducting screening diagnostics, and possibly vaccination or deworming, 4) place radiocollars on reintroduced American marten for post-release monitoring in order to evaluate the results.

VITA

Maria Spriggs completed her bachelor's degree at the Ohio State University with a major in Animal Science. She earned her Doctorate of Veterinary Medicine degree at OSU in June 2006. She completed a small animal rotating internship at Florida Veterinary Specialists in Tampa, Florida, followed by a zoo internship at Lowry Park Zoo also in Tampa. She worked as staff veterinarian at Mesker Park Zoo in Evansville, Indiana where she became involved in research of American martens in Michigan. She began her graduate studies at the University of Tennessee seeking a Ph.D. in Natural Resources with a concentration in wildlife health in 2013. She relocated back to Tampa, FL in 2014 to accept employment as a senior veterinarian at Busch Gardens and will continue her employment at Busch Gardens post-graduation.