### PHYSICAL CONDITION OF AMERICAN MARTENS, MARTES

### AMERICANA, FROM TWO FOREST REGIONS IN NORTHEASTERN

#### ONTARIO

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by

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Thesis submitted in partial fulfillment

of the requirements for the degree of

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#### ABSTRACT

To assess the effect of habitat at the landscape level, the physical condition of martens was compared between populations inhabiting two forest types. Three hundred and fourteen martens were collected from traplines near Cochrane, Ontario (49°N 81°W) and Sudbury, Ontario (46°N 81°W). In order to assess physical condition, the relationships between (a) macroscopic fat depots and body fat content, and (b) body mass and protein content were first tested for 77 martens.

Linear regressions found that most of the variation in percent body fat was explained by the perirenal dry mass, perirenal dry mass to headless dry mass ratio, omental dry mass and both the fresh and dry omental mass to headless mass ratios. Sex effects were found in the omental dry mass and the perirenal dry mass.

For male martens, all omental and perirenal dry mass indices were equally good predictors of percent body fat but age differences were found in the perirenal indices. For female martens, the omental dry mass, perirenal dry mass and perirenal dry mass to headless dry mass ratio were the best predictors of percent body fat but an age effect was found for the omental dry mass. It was concluded that the dry omental and perirenal masses, could be used to assess fat levels in male and female martens, respectively.

No index for percent protein was found in this study but body mass was strongly associated with protein mass. However, because of mass differences due to sexual dimorphism and age, comparative use would need to be limited to martens of similar size.

Condition parameters were compared between two marten populations inhabiting boreal and mixed forest regions. Both male and female juvenile martens from the boreal forest were heavier than their counterparts from the mixed forest. Juvenile females from the boreal forest also had more perirenal fat than those from the mixed forest. Ovulation rates for adult female martens did not differ between the two forest regions. These results suggest that winter habitat conditions in the mixed forest are not as favourable as those in the boreal forest, at least for juvenile female martens.

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### **TABLE OF CONTENTS**

ABSTRACT		i
ACKNOWLEDGEMENTS		iii
TABLE OF CONTENTS		iv
LIST OF FIGURES		vii
LIST OF TABLES		viii
LIST OF APPENDICES		x
GENERAL INTRODUCTION		1
STUDY AREAS		5
GENERAL METHODS		7
Source of Specimens Age Determination		7 7
PART I INDICES OF BODY FAT NORTHEASTERN ONT	AND PROTEIN FOR MARTENS IN	10
<ol> <li>INTRODUCTION         <ol> <li>INTRODUCTION</li> <li>Marten Physiole</li> <li>Condition Indice</li> <li>Condition Indice</li> <li>Fat Ind</li> <li>I.2.1 Fat Ind</li> <li>I.2.2 Protein</li> <li>Objectives and</li> </ol> </li> </ol>	ogy es ices Indices Hypotheses	10 10 12 12 15 15
2. METHODS		17
<ul> <li>2.1 Choice of Indice</li> <li>2.2 Lipid Extraction</li> <li>2.3 Protein Estimate</li> <li>2.4 Statistical Analy</li> <li>2.4.1 Fat Indice</li> <li>2.4.2 Protein</li> </ul>	and Indices and Indices e and Indices rses ex Development Index Development	17 17 19 19 19 20

# Page

	З.	RESU	LTS	•••••••				. 23
		3.1 3.2	Fat Indices Protein Indices					. 23 . 33
	4.	DISCL	ISSION	•••••			••••••	. 37
		4.1	Fat Index Deve 4.1.1 Percer 4.1.2 Fat Inc 4.1.3 Sex Ef 4.1.4 Age Ef	lopment ht Body Fat lices fects for Fat I fects for Fat I	ndices Indices			37 37 38 38 39 41
		4.2	Protein Index D 4.2.1 Percer 4.2.2 Proteir	evelopment nt Protein n Mass				42 42 43
	5.	CONC	LUSION			•••••		45
PART	' <b>    </b>	PHYSIC REGION	AL CONDITION		NS FRC	M TWO F	OREST	46
	1.	INTRO	DUCTION					46
		1.1 1.2 1.3 1.4	Habitat Suitabili Effect of Sex ar Effects of Habit Objectives and	ity nd Age on Ph at and Physic Hypotheses	ysical C al Conc	ondition dition on O	 vulation	46 47 48 48
	2.	METH	ODS	•••••				50
		2.1 2.2 2.3	Assessment of Corpora Lutea ( Statistical Analy 3.3.1 Compa 3.3.2 Reproc	Physical Con Counts vses urisons of Cor luctive Comp	dition ndition li arisons	ndices		50 50 51 51 52

vi

# Page

3.	RESULTS		•••••	53
	<ul><li>3.1 Variation in Ph</li><li>3.2 Variation in Fa</li><li>3.3 Variation in Ph</li><li>3.4 Reproductive A</li></ul>	ysical Condition between Years t within Regions ysical Condition between Regions Assessment		53 53 55 57
4.	DISCUSSION		•••••	60
	<ul> <li>4.1 Variations in Pl</li> <li>4.2 Effect of Habita</li> <li>4.3 Ovulation and I</li> <li>4.4 Limitations of S</li> <li>4.5 Management I</li> </ul>	hysical Condition within Forest Reg at on Physical Condition Physical Condition Study mplications	gions   	60 60 62 64 66
5.	SUMMARY		•••••	67
6.	LITERATURE CITED			6 <b>8</b>
7.	APPENDICES			79

### LIST OF FIGURES

		Page
Figure 1.	Forest inventory in Ontario and locations of the study sites. Redrawn by JF. Robitaille <sup>4</sup>	6
Figure 2.	Relationships of six fat indices (A - F) with percent body fat for 38 male martens. Ninety-five % confidence bands are shown around the line of best fit	29
Figure 3.	Relationships of six fat indices (A - F) with percent body fat for 36 female martens. Ninety-five % confidence bands are shown around the line of best fit	32
Figure 4.	Relationship between carcass fresh mass and protein mass for 20 male ( $\blacksquare$ ) and 22 female ( $\blacktriangle$ ) martens. A male marten with a CFM of 798.02 g and with a protein mass of 85.29 g was a significant outlier (jackknife residual = -6.10, p < 0.01) (Kleinbaum <u>et al</u> . 1988). Exclusion of this individual increased the amount of variance explained by CFM in protein mass to $R^2 = 0.82$ in male martens	36
		ψŪ

# LIST OF TABLES

		Page
Table 1.	Variables used to estimate percent body fat and protein content in martens	21
Table 2.	Percent body fat for marten groups	24
Table 3.	Correlations between percent body fat and index variables (n = 37). For the best correlations, linear regressions were generated. Slope coefficients, intercepts and standard errors (SE) of the regression terms are shown. Abbreviations for variable are described in Table 1	25
Table 4.	Percent body fat estimated for 40 martens using equations derived from selected index variables in Table 3. All regressions were significant at $p < 0.0001$ . Abbreviations for variables are described in Table 1	27
Table 5.	Comparisons of slopes and intercepts between male $(n = 39)$ and female $(n = 38)$ martens for selected fat indices in Table 4. Standard errors for slopes and intercepts are shown. Abbreviations for variables are described in Table 1	28
Table 6.	Comparisons of slopes and intercepts between age classes within each sex for selected fat indices. Standard errors for slopes and intercepts are given. Abbreviations for variables are described in Table 1	30
Table 7.	Percent protein and protein mass for marten groups	34
Table 8.	Relationships of index variables with percent protein and protein mass ( $n = 42$ ). Abbreviations for variables are described in Table 1	35
Table 9.	Condition indices and estimated body fat (%) for sex/age groups of martens from the mixed and boreal forest regions. Means and standard errors of the variables are given for each group. Abbreviations for variables are described in Table 1	54

# Page

Table 10.	Condition indices for male and female martens from the mixed and boreal forest regions. Estimated body fat (%) for each sex was pooled to compare fat between the two regions. Means and standard errors of the variables are given for each group. Abbreviations for variables are described in Table 1	56
Table 11.	Reproductive comparisons of adult female martens from the mixed and boreal forest regions, including results from other studies.	58
Table 12.	Comparisons of condition indices between ovulating and non- ovulating adult female martens. Abbreviations for variables are described in Table 1	59

### LIST OF APPENDICES

Page
------

Appendix 1.	Winter climate normals from Environment Canada (1996) for Kapuskasing, Ontario (49°N 82°W) and Sudbury, Ontario (46°N 81°W), representing the boreal and mixed forests respectively	79
Appendix 2.	Frequency distributions of pulp cavity ratios for male (A - B) and female (C - E) martens from the mixed and boreal forest regions, including females pooled from both regions	80
Appendix 3.	Age distributions of 187 male and 127 female martens from two forest regions. Estimated means, standard deviations (SD) and age class dividing points in pulp cavity ratios are shown for 275 martens. Standard deviations for pulp cavity ratios were determined using normal quantile plots for boreal forest males and pooled females, and the rankit method for all other groups	81
Appendix 4.	Mean fat content of three 20 g aliquots per homogenized marten ( $n = 32$ ), recalculated from Calford and Clark (1996).	82
Appendix 5.	Mean fat content of heads, headless carcasses, and whole carcasses of martens (n =16)	82
Appendix 6.	Fourteen variables ( $\overline{x} \pm 1$ SEM) used to develop fat indices for martens (n = 77). Variable abbreviations are described in Table 1	83
Appendix 7.	Fourteen variables ( $\overline{x} \pm 1$ SEM) used to develop fat indices for marten sex/age classes. Variable abbreviations are described in Table 1	84
Appendix 8.	Protein mass and percent protein of 10 g replicates from lean, homogenized martens ( $n = 42$ ). The number of samples for each aliquot was limited by the amount of homogenate available, and a repeated measures analysis was used only for martens with three aliquots ( $n = 23$ )	85

xi

Appendix 9.	Condition indices for martens from the mixed and boreal forest regions caught during the 95/96 and 96/97 trapping seasons. Means and standard errors of the indices are shown for each group. Abbreviations for variables are	
	described in Table 1	86

#### **GENERAL INTRODUCTION**

Under appropriate environmental conditions, animal populations are maintained through the survival and reproduction of individuals. Therefore, the viability of a population can be partly attributed to the physical condition or fitness of its inhabitants. From a management perspective, the health of an exploited population is of special interest, in order to ensure long-term sustainability.

The American marten, <u>Martes americana</u>, (hereafter marten) is a furbearing mustelid generally found in the boreal forests of North America. The marten belongs to the seven-member genus <u>Martes</u>, of which only <u>M. americana</u> and the fisher, <u>M. pennanti</u> are found in North America. The northern limit of the marten approximates the 10 °C July isotherm to the north, while historically the southern limit extended to the edges of the boreal forest. Several subspecies have been identified based on geographic distribution and physical traits (Strickland <u>et al</u>. 1982).

The marten is the size of a domestic cat, <u>Felis catus</u>, but has the elongated body type characteristic of other members in the weasel family. Martens are sexually dimorphic with males larger than females. In central Ontario, male martens range from 563 to 990 g in mass, and from 513 to 659 mm in body length, while female martens range from 400 to 605 g in mass and from 465 to 572 mm in body length (Strickland <u>et al</u>. 1982). Body mass can vary regionally. For example, martens from Minnesota and Michigan weighed more than those from Ontario (Strickland <u>et al</u>. 1982). Adult size is usually reached by

three months of age, but males in their juvenile year weigh less than adult males (Strickland et al. 1982, Nagorsen 1994).

Adult martens are territorial and solitary except during the breeding season. Home range size can change seasonally, presumably to adjust for variability in resource distribution (Thompson and Colgan 1987, Potvin and Breton 1997). Male home ranges are larger than those of females (Strickland <u>et</u> <u>al</u>. 1982, Buskirk and McDonald 1989) and overlapping of home ranges occurring frequently between members of the opposite sex (Strickland <u>et al</u>. 1982, Katnik <u>et</u> <u>al</u>. 1994).

Martens reach sexual maturity at 15 months of age, but do not usually produce their first litter until their second year. After the breeding season in midsummer, implantation of blastocysts is delayed for 190 to 250 days. The parturition period extends from mid-March to late April with litter sizes averaging nearly three young per female (Strickland <u>et al</u>. 1982). Dispersal occurs in the fall, prior to the commercial harvest season in Ontario.

Martens are generalist feeders that may feed on small mammals, birds, insects, and fruits depending on location, season and availability (Strickland <u>et al</u>. 1982, Martin 1994). Birds, eggs, insects and berries are used in the summer months, while small mammals such as the red-backed vole, <u>Clethrionomys</u> <u>gapperi</u>, and meadow vole, <u>Microtus pennsylvanicus</u>, are more common in winter diets. Snowshoe hare, <u>Lepus americanus</u>, may become an important food source when rodent densities are low (Strickland and Douglas 1987, Poole and Graf 1996) or in deep snow conditions (Martin 1994). The marten hunts primarily on the ground, and the slim body profile of the marten is thought to be an evolutionary adaptation to forage more efficiently in subnivean and subterranean environments (Harlow 1994).

Martens have little subcutaneous fat and usually contain less than 5 % body fat (Buskirk and Harlow 1989). Body fat percentages do not differ between the sexes, but there is evidence of geographical variation in body fat percentages among populations (Buskirk and Harlow 1989). For each marten, fat content may also vary from year to year (Thompson and Colgan 1987). Low lipid levels and a slim body profile raise the question of how thermoregulatory challenges are met in the winter season. It is thought that through a combination of behavioural and physiological tactics, martens can survive the winter with minimal energy reserves. Such techniques may include optimizing time budgeting between foraging and rest (Robitaille and Baron 1987, Harlow 1994), a reduction in body-core temperature (Buskirk <u>et al</u>. 1988, Harlow 1994), utilizing forest structures that minimize heat loss (Buskirk <u>et al</u>. 1988), and catabolizing alternative energy reserves in addition to fat (Harlow and Buskirk 1991).

Martens are found in a variety of habitats across their North American range, but are generally associated with mature conifer cover and are thought to be dependent on the availability ground structure such as coarse woody debris (CWD) (Buskirk and Powell 1994). In Ontario, martens inhabit a variety of forest conditions from the boreal forest to deciduous forests, as well as logging clear cuts, illustrating that not all marten habitats provide the same amount of conifer content (Rowe 1972). Moreover, Giroux (1997) found that second-growth mixed

3

forests contained relatively less CWD than more mature forests. Because we do not know the exact amount of conifer cover and structure needed by martens, it was assumed that differences in forest attributes at the landscape level would correspond to differences in their suitability. In this thesis, the effect of habitat on marten physical condition was tested through the comparison of study sites in contrasted forest regions.

The primary purpose of this study was to compare the physical condition of martens from boreal and mixed forest regions. To reach this objective, the study was divided into two parts. In the first part, fat and protein reserves were tested as potential indices of marten physical condition. In the second part, the condition of marten populations from two regions in northeastern Ontario were compared. While the focus remained on fat and protein reserves, the reproductive status of adult females was also assessed by comparing ovulation rates.

4

#### STUDY AREAS

Martens were harvested along five traplines near Cochrane<sup>1</sup>, Ontario (49°N, 81°W), and four traplines near Sudbury<sup>2</sup>, Ontario (46°N, 81°W) (Figure 1). The forest around Cochrane lies along the southern edge of the Boreal forest and was classified by Rowe (1972) as the Northern Clay forest section, characterized by dominant stands of lowland black spruce, <u>Picea mariana</u>, and areas of spruce-cedar swamps. The forest around Sudbury is part of the Great Lakes-St. Lawrence forest region and was classified by Rowe (1972) as the Sudbury-North Bay forest section. This area is a large transitional zone with a relatively large occurrence of deciduous trees, particularly white birch, <u>Betula papyrifera</u>, and poplar, <u>Populus</u> spp., with some coniferous species present in mixed stands. For this study, the Northern Clay and Sudbury-North Bay forest were simply referred to as the boreal and mixed forests, respectively.

Historical daily mean temperatures and snowfall for the forest regions<sup>3</sup> during the months when the martens were trapped (November to January) are shown in Appendix 1.

<sup>&</sup>lt;sup>1</sup> Abbotsford, Adair, Clive, Findlay, Fournier, Hanna, Hepburn, Iroquois Falls, Lamarche, Marathon, Scapa, Sherring, Singer, Sweatman, and Sydere townships.

<sup>&</sup>lt;sup>2</sup> Athlone, Aylmer, Clement, Ellis, Haentschel, Macbeth, Mackelcan, McLeod, McNish, Pardo, Rathbun, and Selkirk townships.

<sup>&</sup>lt;sup>3</sup> Environment Canada. 1996. Canadian Climate Normals: 1961-1990. <u>http://www.cmc.ec.gc.ca/climate/normals/eprovwmo.htm</u>. © Environment Canada.

Figure 1. Forest cover in Ontario and the location of study sites. Redrawn by J.-F. Robitaille<sup>4</sup>.

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<sup>&</sup>lt;sup>4</sup> Natural Resources Canada. <u>http://www.pfc.cfs.nrcan.gc.ca/landscape/inventory/maps/map4.html</u>.





#### **GENERAL METHODS**

#### Source of Specimens

Skinned marten carcasses (n = 314) were collected from nine registered traplines in northeastern Ontario (Figure 1) after the 1995-1996 and 1996-1997 trapping seasons. Martens were frozen at -15 °C for periods up to eight months. Location of capture was recorded as well as the date of capture, if available. Sex was determined by the presence or absence of a baculum and by examining the genitalia. A ventral incision from the sternum to the pelvis was made to open the body cavity. Ingesta from the gastro-intestinal tract were removed and stored in 10 % formalin. The carcass was weighed on a Sartorius digital scale ( $\pm$ 0.01 g). The baculum was removed from each male marten and frozen. In females, the ovaries were severed from the uterine horns and stored in Bouin's fluid (70 % picric acid, 25 % formalin. 5 % acetic acid) (Culling 1963). Heads were removed from each marten util time of age determination.

#### Age Determination

All martens were aged as either adults or juveniles using a combination of two techniques, where possible. The temporal muscle coalescence on the dorsal part of the cranium was measured for all marten (Poole <u>et al</u>. 1994) with dial calipers ( $\pm$ 0.1 mm). Males were classified as adults if the length of the temporal muscle coalescence from the lamboidal crest to the point where the temporal ridges separated was over 20 mm. Females were classified as juveniles if the width between the temporal muscles at the lamboidal crest was greater than 4

mm. Total closure of the muscles at the crest indicated an adult female. Females were unaged if the width of the closure was between 0 - 4 mm. With this method, almost all male martens and 80 % of the females were expected to be aged reliably (Poole <u>et al.</u> 1994).

The lower jaws were removed from 275 martens and placed in boiling water for 15 minutes to facilitate removal of the left canine. Teeth were dried, labeled, and taped in series to 8" x 11" pieces of paper for radiographing. X-rays were viewed with a microfiche reader at 19 X magnification. Tooth width and pulp cavity width were measured ( $\pm$ 1 mm) and the pulp cavity ratio was calculated as pulp cavity width / tooth width (Poole <u>et al</u>. 1994).

Because dividing points in pulp cavity ratios for aging martens may vary geographically (Poole <u>et al</u>. 1994), dividing points were determined separately for each forest region. The frequency distributions of pulp cavity ratios for each sex were examined, and it was assumed that bimodal distributions represented adult and juvenile age groups. The modes were used to approximate the median value within each age group. Tentative age classes were assigned to martens by splitting the bimodal pulp cavity distribution into two groups. If the estimated sample size of an age group was more than 50 individuals, pulp cavity ratios were plotted against a cumulative percent probability scale to determine the range of values within two standard deviations from the assumed mean (Sokal and Rohlf 1981). If the sample size of an age group was fewer than 50, a rankit method was used instead (Sokal and Rohlf 1981). The dividing point was defined as (a) a discrete pulp cavity ratio separating 95 % of adults and juveniles

from one another, or (b) a range of overlapping ratios between adults and juveniles where the age of an individual was unknown.

Bimodal distributions in pulp cavity ratios were observed for marten populations from both forest regions (Appendix 2A-E). Normal quantile plots were used to age male martens from the boreal forest and the rankit method was used for female martens from the boreal forest and all martens from the mixed forest region. Dividing points in the pulp cavity ratios were found for both male populations, but the upper 95 % percentile limit in adult females and the lower 95 % limit in juvenile females overlapped for both forest regions, resulting in a range of indeterminate ages (Appendix 3). By pooling all females and using a normal quantile plot, the range of indeterminate age class was narrowed (Appendix 3).

Each marten was assigned an age class using the temporal muscle closure and/or pulp cavity ratio. When disagreements between the two techniques occurred, a second pulp cavity ratio measurement was taken and an age class was assigned according to the two results that concurred with each other. If the temporal muscle coalescence and two readings of the pulp cavity ratio resulted in an unknown age, no age class was assigned. Age classes for female martens were further assessed using the occurrence of corpora lutea, assuming that only adults ovulated (Strickland <u>et al</u>. 1982).

The age for one female could not be determined using radiography or temporal muscle coalescence, and was consequently omitted from any analyses involving age class comparisons.

9

# PART I INDICES OF BODY FAT AND PROTEIN FOR MARTENS IN NORTHEASTERN ONTARIO

#### **1. INTRODUCTION**

#### 1.1 Marten Physiology

Animals that experience periods of high energy demands depend on energy stored as carbohydrates, proteins or fats. Because lipids are stored in non-aqueous environments, they appear in the form of dense adipose tissue. Lipid content in the subcutaneous or internal adipose tissues can reach 90 % by weight and yield close to the theoretical limit of 39.7 kJ/g of triglyceride; greater than the 16.9 kJ/g from dry carbohydrates. Because proteins are stored in aqueous environments, the catabolization of protein-rich tissues can result in considerable water loss (Cahill 1979). Based on energetic efficiencies, fat is considered the major source of energy storage in animals.

The catabolization of energy reserves during fasting is characterized by three general phases (Harlow and Buskirk 1991). Phase I of fasting in mammals is generally short, lasting from several hours to a few days, and involves a rapid depletion of glycogen to retain blood glucose levels. Phase II is characterized by a drop in blood glucose and amino acid levels, with an increase in blood ketone levels. Ketones are produced by the lipolyzation of triglycerides from adipose tissue in the liver, and act to conserve proteins. Urea excretion is also reduced during this phase to retain water and nitrogen. In phase III, fat reserves are depleted and gluconeogenesis of proteins is used to elevate blood glucose levels.

Throughout fasting, animals are subject to dehydration. Adipose tissue releases very little water through the oxidation of fat, but tissues with high protein content such as muscle can serve as important sources of water (Bintz <u>et al</u>. 1979). While gluconeogenesis is not the major source of energy during phases I and II, some protein is also catabolized continuously to maintain water balance.

Fasting martens enter each of the three phases, but phase II is not as pronounced as in similar-sized mammals with greater fat reserves, such as the white-tailed prairie dog, <u>Cynomys leucurus</u>, (Harlow and Buskirk 1991). Ketone levels in martens during phase II are lower than in prairie dogs, resulting in a higher level of protein catabolism and an early transition to phase III. During a five-day fast, mean body mass lost by martens was 24 %, contrasted to a 7 % loss by prairie dogs. More than twice as much energy was derived from protein in martens than in prairie dogs to maintain water balance, with martens catabolizing fat and protein in a 1.56:1 ratio compared to 3.85:1 ratio in prairie dogs.

Because of the reliance on protein as an important energy source, captive martens can endure fasting longer than predicted by the metabolic rate and mean fat content of martens (Buskirk and Harlow 1989). However, extensive use of body protein can lead to muscle impairment, immunity deficiencies, and death. Martens exhibited an uncharacteristic increase in blood amino acid levels in phase II of fasting (Harlow and Buskirk 1996) that suggested protein is being

11

conserved, possibly through the use of urealytic microbes in the lumen of the small and large intestine (Harlow and Buskirk 1991) or seasonally stored labile protein reserves in the skin, viscera and blood albumins (Harlow 1994).

#### **1.2 Condition Indices**

Methods for determining physical condition range from metabolic assays to the direct examination of energy reserves. Changes in the levels of amino acids, enzymes, blood cell types, hormones and other metabolites sampled from urine and blood can indicate changes in nutritional quality or quantity (Seal <u>et al</u>. 1978, Kie <u>et al</u>. 1983, DelGiudice <u>et al</u>. 1987a,b). However, many metabolic indicators vary with age and sex, making interpretations difficult (Seal <u>et al</u>. 1978). Kie <u>et al</u>. (1983) found that body weight and fat indices were better indicators of physical condition than blood assays in white-tailed deer, <u>Odocoileus virginianus</u>.

Body mass is a commonly used index of physical condition because a drop in weight indicates a loss of adipose and muscle tissue (Torbit <u>et al</u>. 1985). Since different species may not catabolize fat and protein in the same ratio (Harlow and Buskirk 1991), the amount of each type of tissue lost can not be inferred from the loss in body mass alone. Fat and protein content can be determined separately through the extraction of lipids from tissues with a lipophilic solvent (Nelson 1975), and by burning the lipid-free tissue to ash (Huot and Picard 1988).

#### 1.2.1 Fat Indices

The measurement of fat as an energy source requires the extraction of lipids from the whole body since several sites may be metabolized for energy purposes. However, since this process is not logistically feasible for large animals or numerous animals, studies of fat dynamics often include an investigation of practical (i.e. accurate and time-efficient) indices of fat content for the whole animal. Ideally, a fat index takes the shape of a macroscopic depot with well-defined boundaries that requires little effort to extract and measure, but accurately reflects change in overall fat contents.

Several fat depots have been used to estimate body fat but limitations exist for a single depot. For example, subcutaneous fat is only a good index for animals in good condition (Riney 1955). Conversely, indices using bone marrow fat are unreliable for animals in good condition (Lochmiller <u>et al</u>. 1985, Holand 1992). The kidney fat index that combines perirenal fat and kidney weight has been used to estimate fat content and physical condition (Cothran <u>et al</u>. 1987), but kidney weight may vary seasonally and allometrically (Van Vuren and Coblentz, 1985). Moreover, the kidney fat index was considered unreliable when the physical condition of an animal was poor (Ransom 1965, Holand 1992). The limitations of a fat depot as a index may depend on the sequence of fat mobilization. Riney (1955) found that fat depots were catabolized in order of subcutaneous fat, omenta, intestinal mesentery, renal depots and bone marrow, during starvation in roe deer, <u>Cervus elaphus</u>. Therefore, a single fat deposit will not necessarily provide a good estimate of fat content over a range of physical

13

conditions. Thus, some researchers may use a combination of fat deposits to determine condition (Litvaitis <u>et al</u>. 1986, Winstanley <u>et al</u>. 1998).

Index reliability may also be species-specific. Pond and Ramsay (1992) found that in Carnivora, subcutaneous fat deposits become disproportionately larger and the intra-abdominal deposits smaller, with increasing body mass. Thus, martens should have a relatively high proportion of adipose tissue stored intra-abdominally. In addition, martens have less fat than the mean 6.8 % found in carnivores (Buskirk and Harlow 1989, Pond and Ramsay 1992). Selection for a slender, lean body profile may have resulted in a reduction in the amount of total fat as well as selection for more fat to be stored internally rather than in the subcutaneous region.

Fat depots such as the omental mass (Thompson and Colgan 1987, Poole and Graf 1996), omental mass to body mass ratio (Buskirk and Harlow 1989, Hénault and Renaud 1993), and perirenal mass (Thompson and Colgan 1987) have all been used to assess fat conditions in martens. However, only Buskirk and Harlow (1989) tested the relationship of a fat depot to relative body fat in martens, and found that the ratio of omental mass to carcass mass was a better predictor of fat than the omental mass alone.

Sex differences in the distribution and utilization of fat depots have been documented in humans (Vasselli <u>et al</u>. 1983, Hattori <u>et al</u>. 1991) and other animals (Morton <u>et al</u>. 1994), and are believed to a result of contrasting reproductive strategies. In addition, slim body profiles and sexual dimorphism in martens may also lead to sex differences in energy demands (Harlow 1994), and

warrant investigation into sex-based differences in fat indices. Fat dynamics may also have an developmental component (Steen 1988), leading to age-related differences in the distribution and utilization of fat resources.

#### **1.2.2 Protein Indices**

Because muscle tissue represents a greater percentage of body mass than adipose tissue (Munro 1969), a significant decrease in body mass may be attributed to the loss of muscle tissue, and consequently the loss of protein (Kendall <u>et al</u>. 1973). Virgl and Messier (1993) found that body mass was a good predictor for protein mass in muskrats, <u>Ondatra zibethica</u>. Several studies have used body mass as an index of physical condition in martens (Weckwerth and Hawley 1962, Thompson and Colgan 1987, Hénault and Renaud 1993) although the relationship between mass and protein content was not examined.

#### **1.3 Objectives and Hypotheses**

The main objective in this part of the study was to develop indices that would best assess physical condition in martens. Relationships between an index and the condition parameter examined may vary between sexes and possibly among age classes, hence leading to the selection of different indices that are best in describing segments of the whole population. The established indices would further be used to compare two populations of martens in the second part of this study.

The objectives of the first part of the study were to:

1) develop and test one or several index(ices) that will explain the most variation in percent body fat of martens.

2) test for sex differences regarding selected fat indices.

3) test for age differences regarding selected fat indices.

4) develop a protein index that will explain the most variation in protein content.

Based on the above, the following hypotheses were tested:

 $H_1$ : The omental mass to body mass ratio will explain the most variation in percent body fat for a population of martens.

H<sub>2</sub>: Sex differences in the suitability of fat indices will exist.

H<sub>3</sub>: Differences in the suitability of fat indices will exist between age

classes within each sex.

H<sub>4</sub>: Protein content will be estimated by body mass.

#### 2. METHODS

#### 2.1 Choice of Indices

Based on the available evidence regarding the leanness of martens (Buskirk and Harlow 1989) and the distribution of fat depots in the order Carnivora (Pond and Ramsay 1992), we selected two intra-abdominal depots to test their relationship with total fat content in the body. Preliminary examination revealed that the greater omentum and perirenal fat depots were well-defined, easily extracted and measurable, and therefore repeatable for all animals. The liver was chosen as a control variable because little fat is stored in the mammalian liver (Munro and Fleck 1969). To control for variability in mass due to sex and age, the variables were divided by body mass. The relationships of the liver, fat depots, percent body fat and body mass with protein content were also examined. Because the indices were needed to determine all energy resources available to a marten, the variables were included in the estimate for total body fat content.

#### 2.2 Lipid Extraction and Fat Indices

Procedures for the study were consistent with those of Calford and Clark (1996) who initiated the study with 32 martens. The liver, greater omentum and perirenal fat depots were excised from 77 marten carcasses. The liver was removed by severing the falciform ligament, hepatic portal vein and common bile duct. The greater omentum was removed by cutting the omental mesentery along the curvature of the stomach. Perirenal fat depots were cut above and

along the dorsal aorta and posterior cava, removing both the kidneys and fat depots. The kidneys, renal veins and arteries were removed by making an incision in the renal capsule and squeezing the kidney out. The liver and fat depots were placed on pre-weighed labeled cupules, weighed on a Sartorius digital scale (±0.01 g) and then dried to constant mass at 60°C to remove the water content. Headless carcasses were weighed, dried to constant mass at 90°C and reweighed again. The carcass was dipped into liquid nitrogen, placed into a mortar and crushed into smaller pieces. The pieces along with the dried liver and fat depots were placed in a blender and ground to a fine homogenate. Three 20-g samples were taken from each homogenate and placed into preweighed 250-ml Erlenmeyer flasks. One hundred ml of petroleum ether was poured into each flask (Dobush et al. 1985). The flasks were agitated periodically over 24 h. Petroleum ether was decanted with a nylon mesh (sieve opening < 0.25 mm) and another 100 ml of petroleum ether was added. The procedure was repeated until the fluid in the flask was transparent. The flasks were left in a fumehood to dry. If no differences in percent fat were found among the aliguots for each marten, they were pooled and percent body fat for the fresh mass of a marten<sup>5</sup> was calculated as:

#### Fat (%) = total dry mass (g) of combined aliquots - lean dry mass (g) x 100 x 0.3 the total dry mass (g)

<sup>&</sup>lt;sup>5</sup> The results were multiplied by 0.3 to account for an initial water content of 70 % in the animal (Buskirk and Harlow 1989).

To determine if the removal of the heads affected the overall estimate of percent body fat, lipid was extracted from 16 heads, following the procedure and calculations outlined above.

#### 2.3 Protein Estimate and Indices

Three 10-g aliquots of lean dry homogenate from 42 martens were placed in pre-weighed 100-ml Erlenmeyer flasks and capped. The flasks were heated in a furnace at 500 °C for 6 h (Huot and Picard 1988). The residual ash was weighed. Ash content was considered to be mostly bone tissue (Spector 1956). Protein percent of a fresh headless carcass<sup>5</sup> was calculated as (Waterlow 1969): Protein (%) = total lean dry mass (g) of the combined aliquots - ash mass (g) x 100 x 0.3 total lean dry mass (g)

Protein mass for a headless fresh carcass (HFM) was

calculated as:

Protein mass (g) = Protein (%) x HFM (g) 100

#### 2.4 Statistical Analyses

All analyses were performed using SAS JMP version 3.2.2.

#### 2.4.1 Fat Index Development

To test for consistency in the extraction method, percent fat estimates from the three aliquots per homogenized animal for 32 martens were compared using a repeated measures analysis. The effect of head removal on total fat content was analyzed. Percent body fat for each of the 16 heads and that of the corresponding headless bodies were converted into grams, added together and expressed as a percent fat for the entire carcass. Percent body fat for headless carcasses was compared to that of the entire carcasses using a paired t-test.

Fourteen variables (excluding carcass fresh mass) were used to assess the performance of indices for estimating percent body fat (Table 1) and included the mass of the liver, omentum and perirenal fat depots and their ratio to headless body mass to adjust for body size. Martens were split into two groups to test the index models. For 37 martens, Pearson correlations with percent body fat were compared with paired t-tests after Fisher's z – transformations (Kleinbaum <u>et al</u>. 1988). Variables with the highest correlations were used to develop model equations from least squares linear regressions. The models were tested with the remaining 40 martens by comparing the correlations as well as observed and predicted percent fat means for each index with t-tests.

Unpaired t-tests and analyses of covariance (ANCOVA) were used to examine the effects of sex on selected fat indices. For each sex, Pearson correlations for selected indices found in the first test group of 37 martens were compared. Unpaired t-tests and ANCOVAs were also used to examine the effects of age on selected fat indices.

Data series with heterogeneous variances were normalized with either  $log_{10}$  transformations, or with truncations if outlier values were more than three standard deviations away from the mean.

Table 1. Variables used to estimate percent body fat and protein content

in martens.

Abbreviations	Variable description
MASS	
HFM	headless fresh mass (g)
HDM	headless dry mass (g)
CFM	carcass fresh mass (g)
LIVER	
LFM	liver fresh mass (g)
LDM	liver dry mass (g)
LFM / HFM	(liver fresh mass / headless fresh mass) x 100
LDM / HDM	(liver dry mass / headless dry mass) x 100
OMENTUM	
OFM	omental fresh mass (g)
ODM	omental dry mass (g)
OFM / HFM	(omental fresh mass / headless fresh mass) x 100
ODM / HDM	(omental dry mass / headless dry mass) x 100
PERIRENAL	
PRFM	perirenal fresh mass (g)
PRDM	perirenal dry mass (g)
PRFM / HFM	(perirenal fresh mass / headless fresh mass) x 100
PRDM / HDM	(perirenal dry mass / headless dry mass) x 100

#### 2.4.2 Protein Index Development

Mass and liver variables (Table 1) as well as percent body fat were used to develop indices of percent protein and protein mass for 42 martens. Percent protein estimates from the three aliquots per homogenized animal for 23 martens were compared using a repeated measures analysis. For protein mass, Pearson correlations and Fisher's z - transformations were compared with t - tests. Because the distribution of percent protein could not be normalized with a transformation, Spearman correlations were used to compare relationships between the variables and percent protein. Unpaired t-tests and ANCOVAs were used to examine the effect of sex on the selected index(ices).

Data series with heterogeneous variances were normalized using either log<sub>10</sub> transformations or truncations.
## 3. **RESULTS**

## 3.1 Fat Indices

No significant variability was found in percent fat among the three aliquots of homogenate for each marten (Wilks'  $\lambda = 0.87$ , F = 2.16, p = 0.13) so the aliquots were pooled to determine percent body fat based on 60 g of homogenate (Appendix 4). Percent fat in the whole carcass was not significantly different from percent fat in the headless carcass (t = 2.02, p = 0.06) (Appendix 5) and most of the variation in percent body fat of the whole carcass was explained by percent fat in the headless carcass (r = 0.999, p < 0.0001).

Mean percent body fat ( $\pm$ 1 SEM) for 77 martens was 2.75  $\pm$  0.17 % (range: 0.23 - 8.15 %). There were no significant differences in percent body fat between male and female martens (t = 0.43, p = 0.67) or among any of the sex/age classes (F = 0.08, p = 0.97) (Table 2).

Means and standard errors of the fat index variables are shown in Appendix 6. For the first group of martens (n = 37), the OFM, OFM/HFM, ODM, ODM/HDM, PRDM, and PRDM/HDM had the strongest relationships with percent body fat (Table 3). In order to develop geometric regression models for comparing fat relationships in the second test group of martens, outlier variables greater than three standard deviations were truncated. A female marten with perirenal dry mass of 5.25 g was omitted from the development of model equations involving the perirenal fat depots, and a male marten with an omental fresh mass of 17.76 g and a perirenal dry mass of 4.82 g was excluded from all models. In the second group of martens (n = 40) the PRDM/HDM explained

Age class	Males x ±1 SEM (%)	Females <sup>a</sup> T±1 SEM (%)
Both age classes	2.67±0.24 (n = 39)	2.82±0.24 (n = 38)
Adults	2.61±0.33 (n = 21)	2.84±0.39 (n = 15)
Juveniles	2.76±0.36 (n = 18)	2.76±0.32 (n = 22)

Table 2. Percent body fat for marten groups.

<sup>a</sup> one female unaged.

Table 3. Correlations between percent body fat and index variables (n = 37). For the best correlations, linear regressions were generated. Slope coefficients, intercepts and standard errors (SE) of the regression terms are shown. Abbreviations for variables are described in Table 1.

Variable <sup>a</sup>	Pearson's r	Slope Coefficient (p < 0.0001)	SE	Intercept (p < 0.0001)	SE
HFM (g)	0.36*				
HDM (g)	0.47**				
LFM(g)	0.34*				
LDM (g)	0.35*				
LFM/HFM	0.03				
LDM/HDM	-0.07				
OFM (g)	0.86*** <sup>b</sup>	0.53 <sup>c</sup>	0.08	0.91	0.27
ODM (g)	0.91*** <sup>b</sup>	0.98 <sup>c</sup>	0.12	1.01	0.22
OFM/HFM	0.82*** <sup>b</sup>	2.84 <sup>c</sup>	0.37	0.69	0.26
ODM/HDM	0.90*** <sup>b</sup>	1.68 <sup>°</sup>	0.19	0.84	0.22
PRFM (g)	0.67***				
PRDM (g)	0.83*** <sup>b</sup>	1.68 <sup>d</sup>	0.26	1.25	0.18
PRFM/HFM	0.64***				
PRDM/HDM	0.84*** <sup>b</sup>	3.23 <sup>d</sup>	0.45	1.02	0.20

<sup>a</sup> Variables log<sub>10</sub> transformed.

<sup>b</sup> Correlations not significantly different (t < 1.96, p > 0.05).

<sup>c</sup> 
$$n = 36$$
; <sup>d</sup>  $n = 35$ 

\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.0001

most of the variance in percent body fat but was not statistically better than the PRDM, OFM/HFM, ODM/HDM and ODM (Table 4). Paired t-tests showed no significant differences between observed and predicted means (Table 4). When the five fat indices were compared between male and female martens using all 77 martens, no significant differences were found in the slopes of the regressions, but female martens had significantly higher intercept values for the ODM and PRDM (Table 5). There were no differences in the means between males and females for ODM, OFM/HFM, ODM/HDM, PRDM and PRDM/HDM (Appendix 6).

For male martens, the ODM explained most of the variance in percent body fat but not more significantly than the other five index variables (Figure 2). A male marten with large omental and perirenal fat depots (see above) was excluded from the analysis but including the individual did not change the relationships significantly ( $R^{2}_{OFM} = 0.68$ ,  $R^{2}_{OFM/HFM} = 0.66$ ,  $R^{2}_{ODM} = 0.72$ ,  $R^{2}_{ODM/HDM} = 0.71$ ,  $R^{2}_{PRDM} = 0.78$ ,  $R^{2}_{PRDM/HDM} = 0.78$ ). Slope and intercepts did not differ between adult and juvenile males (Table 6) but unpaired t-tests showed that juveniles had a significantly larger PRDM (log<sub>10</sub> PRDM, t = 2.10, p < 0.05) and PRDM/HDM (log<sub>10</sub> PRDM, t = 2.66, p < 0.05) than adults (Appendix 7).

The PRDM explained the most variation in percent body fat for females but it was not statistically better than the ODM and PRDM/HDM (Figure 3). One female marten with 8.15 % body fat was excluded from the analysis but including the individual did not change the relationships significantly ( $R^2_{OFM} = 0.72$ ,  $R^2_{OFM/HFM} = 0.68$ ,  $R^2_{ODM} = 0.73$ ,  $R^2_{ODM/HDM} = 0.70$ ,  $R^2_{PRDM} = 0.79$ ,  $R^2_{PRDM/HDM} =$  Table 4. Percent body fat estimated for 40 martens using equations derived from selected index variables in Table 3. All regressions were significant at p < 0.0001. Abbreviations for variables are described in Table 1.

Index Variable	Body fat x ±1 SEM (%)	R <sup>2</sup>	F-ratio	
OFM (g)	2.86±0.19	0.50	37.69	
ODM(g)	3.02±0.25	0.65 <sup>a</sup>	71.44	
OFM/HFM	2.83±0.20	0.57 <sup>a</sup>	50.67	
ODM/HDM	2.95±0.25	0.69 <sup>a</sup>	82.86	
PRDM (g)	3.01±0.21	0.55 <sup>a</sup>	47.13	
PRDM/HDM	3.02±0.20	0.78 <sup>a</sup>	131.07	

Note: Estimated mean body fat from lipid extraction =  $2.93 \pm 0.23$  %.

<sup>a</sup> Regression coefficients not significantly different (t < 1.96, p > 0.05).

Table 5. Comparisons of slopes and intercepts between male (n = 39) and female (n = 38) martens for selected fat indices in Table 4. Standard errors on the slopes and intercepts are shown. Abbreviations for variables are described in Table 1.

Index variable <sup>a</sup>	Regression terms	Males	Females	F - statistic
	slope	0.72±0.08	0.78±0.08	0.20
	intercept	0.54±0.03	0.56±0.03	0.22
0014	slope	0.56±0.05	0.56±0.04	0.002
ODM	intercept	0.25±0.02	0.37±0.01	19.24**
ODM/HDM	slope	0.58±0.05	0.60±0.05	0.13
	intercept	0.41±0.02	0.43±0.02	0.26
PRDM	slope	0.62±0.07	0.54±0.05	0.97
	intercept	0.41±0.03	0.55±0.02	13.52*
PRDM/HDM	slope	0.67±0.08	0.60±0.05	0.48
	intercept	$0.60 \pm 0.04$	0.62±0.03	0.27

<sup>a</sup> Variables were log<sub>10</sub> transformed.

\* p < 0.001, \*\* p < 0.0001

Figure 2. Relationships of six fat indices (A - F) with percent body fat for 38 male martens. Ninety-five % confidence bands are shown around the line of best fit.



Table 6. Comparisons of slopes and intercepts between age classes within each sex for selected fat indices. Standard errors for slopes and intercepts are given. Abbreviations for variables are described in Table 1.

Index variable	Regression terms	Adults	Juveniles	F - statistic
Males <sup>a</sup>				
	slope	0.44±0.09	$0.37 \pm 0.09$	0.27
OFM	intercept	1.07±0.35	1.01±0.43	0.02
OFM/HFM	slope	2.76±0.55	2.10±0.48	0.80
	intercept	1.02±0.36	0.90±0.42	0.05
ODM	slope	0.77±0.11	0.71±0.12	0.16
	intercept	1.21±0.24	0.94±0.31	0.48
ODM/HDM	slope	1.56±0.26	1.39±0.20	0.27
	intercept	1.21±0.27	0.81±0.29	0.98
PRDM	slope	1.14±0.19	1.45±0.28	0.85
	intercept	1.48±0.24	0.91±0.40	1.85
	slope	2.74±0.42	3.22±0.47	0.58
FRUM/HUM	intercept	1.30±0.24	0.56±0.32	3.33

Continued

Table 6. (continued)

Index variable	Regression terms	Adults	Juveniles	F - statistic
Females <sup>b</sup>				
ODM	slope	0.60±0.06	$0.56 \pm 0.05$	0.27
	intercept	$0.41 \pm 0.02$	0.35±0.02	5.37*
PRDM	slope intercept	0.60±0.06 0.54±0.03	0.50±0.07 0.55±0.04	0.92 0.03
PRDM/HDM	slope intercept	0.64±0.07 0.62±0.04	0.58±0.08 0.62±0.04	0.29 0.001

<sup>a</sup> n = 38, one juvenile male with an omental fresh mass of 17.76 g and a perirenal dry mass of 4.82 g was excluded from the analyses.

<sup>b</sup> n = 37, one female unaged, variables  $log_{10}$  transformed.

\* p < 0.05.

Figure 3. Relationships of six fat indices (A - F) with percent body fat for 37 female martens. Ninety-five % confidence bands are shown around the line of best fit.



0.80). The slopes did not differ between adult and juvenile females but adult females had significantly higher intercepts for the ODM (Table 6). There were no age differences in the means of these three variables (Appendix 7).

## 3.2 **Protein Indices**

No significant variability was found in percent protein among the three aliquots for each marten (Wilks'  $\lambda = 0.99$ , F = 0.15, p = 0.86), so they were pooled to determine protein content based on 30 g of samples (Appendix 8).

Mean percent protein ( $\pm$ 1 SEM) was 17.40  $\pm$  0.14 % (range: 13.69 - 19.18 %). Percent protein did not differ between sexes (t = 0.55, p = 0.58) nor among the sex/age classes (F = 1.41, p = 0.26) (Table 7). Mean protein mass ( $\pm$ 1 SEM) was 85.89  $\pm$  3.07 g (range: 49.72 - 122.73 g). Males had a greater protein mass than females (Table 7) but no age differences existed within male (t = 1.42, p = 0.17) or female (t = 0.18, p = 0.86) groups.

Percent protein was significantly correlated only with protein mass (Table 8). The HFM and CFM had the highest correlations with protein mass (Table 8). By sex, protein mass was strongly correlated with the CFM (p < 0.0001) in both males (r = 0.78) and females (r = 0.84). An ANCOVA comparing the relationships of protein mass with CFM for male and female martens (Figure 4) revealed no significant differences in the slopes (F = 0.16, p = 0.69) or intercepts (F = 0.19, p = 0.67).

	Percent Protein $\overline{x} \pm 1$ SEM (%)	Protein mass $\overline{x} \pm 1$ SEM (g)
Males (n = 20)	17.48±0.21	103.57±2.31 <sup>a</sup>
Adults (n = 9)	17.00±0.30	108.81±3.32
Juveniles (n = 11)	17.87±0.21	99.28±3.01
Females <sup>b</sup> (n = 22)	17.32±0.20	69.78±2.21 <sup>a</sup>
Adults (n = 9)	17.33±0.23	70.72±3.32
Juveniles (n = 12)	17.38±0.20	70.09±2.88

 Table 7. Percent protein and protein mass for marten groups.

<sup>a</sup> p < 0.0001

<sup>b</sup> One female unaged

Variable	x ±1 SEM	Spearman p correlations with percent protein	Pearson r correlations with protein mass <sup>a</sup>
Percent protein	17.40±0.14	-	0.52*
Protein mass (g)	85.87±3.07	0.52*	-
Percent body fat	3.36±0.34	0.02	-0.25
CFM (g)	622.22±20.46	0.18	0.95** <sup>b</sup>
HFM (g)	493.15±16.90	0.23	0.97** <sup>b</sup>
HDM (g)	161.99±5.57	0.22	0.92**
LFM	25.41±1.03	0.08	0.68**
LDM	7.62±0.32	0.05	0.67**
LFM/HFM	5.20±0.16	-0.04	-0.15
LDM/HDM	4.76±0.16	-0.03	-0.09

 Table 8. Relationships of index variables with percent protein and protein

mass (n = 42). Abbreviations for variables are described in Table 1.

<sup>a</sup> Variables log<sub>10</sub> transformed.

<sup>b</sup> Correlations not significantly different (t < 1.96, p > 0.05).

\* p < 0.001, \*\* p < 0.0001

Figure 4. Relationship between carcass fresh mass and protein mass for 20 male ( $\blacksquare$ ) and 22 female ( $\blacktriangle$ ) martens. A male marten with a CFM of 798.02 g and with a protein mass of 85.29 g was a significant outlier (jackknife, residual = - 6.10, p < 0.01) (Kleinbaum <u>et al.</u> 1988). Exclusion of this individual increased the variance explained by the CFM in protein mass to R<sup>2</sup> = 0.82 in male martens.



#### 4. **DISCUSSION**

#### 4.1 Fat Index Development

#### 4.1.1 Percent Body Fat

In one other study on the fat content in martens, Buskirk and Harlow (1989) determined that the percent body fat for 27 skinned martens from Wyoming averaged 4.6 % (range: 2.3 - 8.8 %), while that of 12 skinned martens from Alaska averaged 2.4 % (range: 1.2 - 4.8 %). With an average of 2.75 %, the fat content of martens in this study was closer to the values reported for martens from Alaska. Martens in this study had a similar range of fat values (0.2 - 8.2 %) to those reported by Buskirk and Harlow. As with Buskirk and Harlow, no sex difference in percent body fat was found in this study (Table 2).

Any differences in the fat estimates found in this study and those by Buskirk and Harlow (1989) were probably not due to differences in experimental techniques. Little variation in fat content was found among aliquots for each marten (Appendix 4) and suggested the technique used was consistent among samples. An analysis of lipid content in the heads revealed no bias due to the study of headless rather than whole carcasses (Appendix 5).

The skinning process probably did not affect the estimate of percent body fat. Martens are lean animals and little or no subcutaneous fat was observed on the animals. Moreover, trappers try to remove as little fat as possible with the pelt so it was assumed that little fat would be lost. Buskirk and Harlow (1989) found that there was no significant difference in the fat content between skinned martens and martens with their pelt, and that the fat content of skinned carcasses was found to explain 98 % of the variation in the fat content of whole carcasses.

Although the liver, omentum and perirenal depots were not tested independently against the fat content in the rest of the animal, including them in the total fat content of the headless carcass probably did not result in any biased correlations. The fat depots were relatively small (Appendix 6), so the amount of fat they contained was considered to be trivial compared to total fat in the animal. Although the liver was relatively large (Appendix 6) it has been reported to contain little fat (Spector 1956) and therefore to have little or no role in fat storage.

# 4.1.2 Fat Indices

In agreement with findings of Buskirk and Harlow (1989), the liver proved to be an inadequate predictor of total body fat (Table 3). The omental indices (OFM, ODM, OFM/HFM, ODM/HDM) and dry perirenal indices (PRDM and PRDM/HDM) explained the most variation in percent body fat (Table 3). The fresh perirenal fat depots (PRFM and PRFM/HFM) were not as good predictors of body fat as the dry perirenal fat depots (Table 3). Possibly because the boundaries of the perirenal depots were not as defined as the omenta, the fresh samples excised may have contained some non-adipose tissue high in water content that contributed more variability to its mass. Also, the fresh omental mass (OFM) did not perform well for the test group of 40 martens (Table 4). Because the initial group of martens were processed earlier, the extended freezing times for the second group of martens may have contributed more variability in the OFM due to variability in water content (Buskirk and Harlow 1989), and resulted in a weaker correlation with percent body fat. Greer (1968) found that freezing durations can affect the estimated fat content in bone marrow by as much as 10 % for a single animal. Drying the carcass and fat depots should reduce any variability in water content.

Because of the marten's relative leanness compared to other carnivores (Pond <u>et al</u>. 1992), including its close relative the fisher, <u>Martes pennanti</u> (Guérin 1999), few alternative macroscopic fat depots could have been examined in the marten.

# 4.1.3 Sex Effects for Fat Indices

The dry mass of the omentum and perirenal depots (ODM, ODM/HDM, PRDM, and PRDM/HDM) were good predictors of percent body fat when all martens were grouped, but females had higher intercept values than males for both the ODM and PRDM (Table 5). There are two implications for this observation. First, a single index for both sexes would have underestimated fat content in females and overestimated it in males. Second, this finding suggested that females have less intra-abdominal fat than males at similar body fat levels. This may be a sex-related difference in fat distribution and utilization, but when dry fat depots were corrected for body mass, the sex differences in the intercepts were removed (Table 5). Thus, the sex differences in the omental and perirenal fat depots may also have been due to body size. In sexually dimorphic species, such as the marten, there may be a need to correct for body mass if the same index is used for both sexes. A critical threshold of fat content in the body has been associated with the occurrence of ovulation in females although the physiological interaction between fat and ovulation is not fully understood. Ovulation depends on the availability of glucose and fatty acids (Bronson and Manning 1991), and the proximity of perirenal fat to the ovaries provides a ready supply of energy. Sudgen <u>et</u>. <u>al</u>. (1994) found that lipoprotein lipase activity was greater in perirenal fat than in other fat depots for recently fasted adult female rats. The strong relationship of the perirenal fat depot with percent body fat in female martens in this study (Figure 3) further underscores the possible role of perirenal fat as a energy source in reproduction.

One objective of this study was to find a simple geometric relationship between fat content and an index(ices) so that fat condition could be easily assessed for a population of martens assumed to have a range of normally distributed fat values. Therefore, outliers in some the regression analyses were truncated as opposed to transforming the data set so that transcendental functions could be avoided. At extreme values, the association between two variables can change so that including the outlier(s) in an analysis may result in less variation explained due to the leverage effect of large residuals (Sokal and Rohlf 1981). However, when outliers were included in the fat indices for male and female martens, the relationships did not change significantly (Figures 2 and 3), and suggested that the indices proposed here were adequate to assess fat content over a wide range of values.

40

## 4.1.4 Age Effects for Fat indices

Juvenile males contained more dry perirenal fat than adult males (Appendix 7) although there was no significant difference in percent body fat between the two age groups (Table 2), and this may have indicated an agedependant effect in fat distribution.

No significant differences between male age classes were found for the absolute weights of the omental indices (OFM and ODM) although juvenile males weighed less than adult males (Appendix 7). Therefore, the effect of dividing the OFM and ODM by mass could result in greater ratio values for juveniles. Thus, these ratios would falsely overestimate percent body fat in juveniles as being higher than in adult males.

This situation illustrates the problem of correcting for body size using a simple ratio or proportion because it is based on the assumption that the part (e.g. fat depot) is directly proportional to the whole (e.g. body). However, excluding muscle, the relative size of most tissues decreases with an increase in body size of the organism (Schmidt-Nielsen 1984). Heroux and Gridgeman (1958) suggested that the coefficients of variation could be used to quickly assess the relationship between the size of an organ and body mass. For example, in this study the difference in the coefficients of variation calculated for the OFM and HFM in male martens (Appendix 6) were 106 % and 11 % respectively, and suggested that no linear relationship existed between them. Other techniques of removing variability in an index variable due to body size or mass such as an analysis of residuals or an allometric equation of mass with the

index variable, would provide more powerful corrections for body size than a ratio (Reist 1985).

For female martens in this study, adults had higher intercept values than juveniles for the dry omental indices (ODM and ODM/HDM) (Table 6). This suggests that (a) omental indices estimating percent body fat for all females would underestimate fat content in adults and overestimate it in juveniles, and (b) there is more omental fat in juvenile females than in adult females at similar body fat levels. This difference in fat distribution may be age-related when the distribution of fat changes with reproductive maturity.

## 4.2 Protein Index Development

## 4.2.1 Percent Protein

Mean percent protein for martens (17.40%) approximated values found for rabbits and rats (Munro and Fleck 1969). Although percent protein was not correlated with body mass (Table 8), dividing mass by some function of size such as length probably would not result in a significant correlation with percent protein. Virgl and Messier (1993) did not find an index for percent protein when mass was corrected with size measurements in muskrats (<u>Ondatra zibethica</u>). Although protein mass can be lost during fasting (Torbit <u>et al</u>. 1985, Harlow and Buskirk 1991), the relative protein content may not change. Gluconeogenesis can result in the release of a relatively large quantity of water from skeletal muscle that contributes to the total mass lost from the body, so that protein concentrations remain relatively stable even when protein mass is lost (Waterlow 1969, Bintz <u>et al</u>. 1979). Therefore, lack of difference in percent protein between the sexes and among sex/age groups (Table 7), as well as its poor correlation with percent body fat (Table 8), was not unexpected. A significant decrease in percent protein may not occur until late in a prolonged period of starvation when structural or more inert proteins such as collagen are catabolized (Neuberger and Richards 1969).

A low, outlying value of 13.69 % protein for one marten in this study indicated that the animal was in poor physical condition despite having 2.76 % body fat, in contrast to the mean body fat (2.75 %) found for martens in this study. If percent protein obtained for this marten was not due to experimental error, then it indicates that fat and protein dynamics in martens are drastically different, where fat and protein are short-term and long-term processes, respectively. Omission of this individual narrowed the range of percent protein values for 41 martens to less than 4 % (range: 15.85 – 19.18 %). This observed range supported the ideas that (a) most of the martens in this portion of the study were healthy and (b) low fat levels are normal and acceptable in martens.

#### 4.2.2 Protein Mass

It was expected that protein mass would be correlated with carcass mass (Table 8) since protein-based muscles represent a significant portion of total body mass (Munro 1969). Since body mass can vary with other measurements of size such as length (Virgl and Messier 1993), differences among individuals may not necessarily reflect differences in physical fitness, but rather differences in sex and age. Therefore, the use of body mass as an index of protein content, and hence physical condition, would need to be restricted to animals of similar size. Martens are sexually dimorphic, so males were expected to contain more protein mass, which was confirmed (Table 8). In addition, adult male martens generally weigh more than juvenile males and would also be expected to contain more protein. However, in this study protein mass did not differ between juvenile and adult males (Table 9) although adult males weighed more than juvenile males (Appendix 7). Weight differences between the male age groups may originate from weight differences in other tissues other than muscle, such as bone mass (Orimo <u>et al.</u> 1992). Therefore, only similar-sized animals should be compared when mass is used an index of condition, unless corrected for variability in size (Bailey 1968, Winstanley <u>et al.</u> 1998).

# 5. CONCLUSION

The absolute and relative dry masses of the omentum and perirenal fat were found to be equally good indicators of percent body fat when martens were examined as a whole. Sex differences in the dry omentum and perirenal fat depots were found and suggested limitations in the ability of a single index to accurately estimate body fat for a population of martens. Age differences in the perirenal depot of males and in the omentum of females were found. For male martens, the ratio of the omentum to body mass was not considered a good fat index due to the lack of a linear relationship between the omentum and body mass. Because of the potential variability of water content in fresh tissue, the dried omentum is recommended as an index of body fat for male martens. For female martens, dried perirenal fat indices are recommended as the best fat indices.

Percent protein could not be estimated by body mass but protein mass was strongly correlated with fresh carcass mass. It is recommended that comparisons of protein mass be limited to sex/age groups of similar body size because of sexual dimorphism and age-related differences.

45

# PART II PHYSICAL CONDITION OF MARTENS FROM TWO FOREST REGIONS

## 1. INTRODUCTION

#### **1.1 Habitat Suitability**

Martens have relatively low fat reserves, apparently as an adaptation for retaining a slim body profile for subnivean access (Harlow 1994). Despite low fat levels and a high surface area to volume ratio, the marten remains active throughout the winter months, and this imposes high energetic demands in thermoregulatory expenditure (Buskirk and Powell 1994).

Martens are habitat specialists, with a strong dependence on mature conifer or conifer-dominated mixed forests where tall trees, abundant coarse woody debris, and high canopy closure (Buskirk and Powell 1994, Bowman and Robitaille 1997) likely fulfill marten requirements, especially during the winter (Soutiere 1979). The overhead canopy closure provided by conifers can result in relatively low snow depths and warm micro-climates (Buskirk and Powell 1994). Structure provided by coarse woody debris creates hollows that favour easier access points for martens to subnivean areas to forage for small mammals (Thompson and Colgan 1994) or to resting sites where heat loss can be reduced (Buskirk <u>et al</u>. 1989, Taylor and Buskirk 1994). Because of low energy reserves, martens in habitats deficient in these attributes may exhibit poor physical and reproductive conditions, as well as greater mortality rates. However, there is little evidence to support the hypothesis that martens from suboptimal habitats experience lower fitness, but the link between prey abundance and reproductive output has been documented in martens (Thompson and Colgan 1987, Poole and Graf 1996).

In Ontario, martens can be found from central Ontario (45°N) to the northwest tip of the province (57°N), and inhabit a variety of forest types that can be generalized as the conifer-dominated Boreal forest region in the north and the hardwood-dominated Great Lakes-St. Lawrence forest region to the south. Populations of martens, inferred by annual harvest rates, are less dense in the Great Lakes-St. Lawrence region (Strickland and Douglas 1987), which suggests that survival rates and physical conditions are relatively poor in deciduousdominated forests.

## **1.2 Effect of Sex and Age on Physical Condition**

Other factors besides habitat condition, such as sex and age, can influence the physical conditions of martens. Because of their role in the production and care of young, females are subjected to greater energy demands than males (Bronson and Manning 1991). A larger surface-area-to-volume ratio in females can also result in greater thermoregulatory costs (Harlow 1994). Females that lack essential habitat elements will not meet their energetic demands and demonstrate lower physical fitness and reproductive performances. Thompson and Colgan (1987) found that in years of low prey abundance in northcentral Ontario, male body weights did not change while female body weights declined. Hawley and Newby (1957) observed that the ratio of adult males to adult females increased during a food shortage, suggesting greater mortality rates for female martens. In territorial species such as the marten, aggressive encounters with dominant individuals can lead to the dispersal of young animals into suboptimal habitats (Van Horne 1983). Evidence of this has been found in an Alaskan postfire sere (Paragi <u>et al</u>. 1996) and in logged forests in northcentral Ontario (Thompson 1994) where greater frequencies of younger marten cohorts were found than in nearby mature conifer forests. At a geographic scale, Hénault and Renaud (1993) found that the weights of juvenile martens in deciduous forests were less than the weights of juvenile martens in coniferous forest. Also, experiential hunting ability may lead to differences in physical condition between age groups, as seen with bobcats, Lynx rufus, (Litvaitis <u>et al</u>. 1986).

## **1.3 Effects of Habitat and Physical Condition on Ovulation**

At the onset of reproduction, ovulation may be regulated by the energy budget of females dependent on critical threshold values of body fat and body weights (Bronson and Manning 1991). The inability to acquire sufficient resources during the breeding season can prevent or reduce the number of ova released, and this may in turn reflect habitat quality. During decreased prey abundances, reduced ovulation rates were observed in yearling females in the Northwest Territories (Poole and Graf 1996) and in martens under the age of three in Ontario (Thompson and Colgan 1987). However, the effect of forest type on ovulation has not been examined.

## **1.4 Objectives and Hypotheses**

In the second part of the study, the main objective was to examine the possible effect of habitat on the physical condition of martens and reproductive

output in adult females, since it was assumed that the suitability of a habitat was correlated with the amount of energy reserves. Forest regions different in composition were used to study the effect of habitat on physical condition because it was assumed that the movement of individuals (i.e. home ranges, dispersal) would be confined within the boundaries of the large-scale study areas. Thus, differences in physical or reproductive condition would be attributed to an overall contrast at the landscape level without consideration for habitat structure at a smaller scale (e.g. stand level).

The objectives of this part of the study were to:

1) compare the physical condition among sex and age classes within each forest region, and of respective groups between forest regions, and

2) compare adult female reproductive performances between two forest regions.

The following hypotheses were tested:

 $H_1$ : Male martens will have relatively more fat than female martens. Within each sex, adult martens will have relatively more fat than juvenile martens.

H<sub>2</sub>: Martens from boreal forest will weigh more and have more fat than corresponding sex/age groups from the mixed forest.

 $H_3$ : Female martens from the boreal forest region will have a greater ovulation rate than those from the mixed forest.

## 2. METHODS

## 2.1 Assessment of Physical Condition

Physical condition was assessed for 314 martens obtained from the 1995/96 and 1996/97 trapping seasons. Dissection protocol and age determination are outlined in the general methods in Part I. The perirenal dry mass (PRDM) and omental dry mass (ODM) were used as indices of percent body fat in female and male marten respectively, as concluded in section 5 of Part I. Removal and drying of the perirenal fat depots and omenta are outlined in section 2.1 of Part I. The carcass fresh mass (CFM) was also used as an index of physical condition.

# 2.2 Corpora Lutea Counts

Reproductive performance can be best measured by the direct count of offspring born, but observing litter sizes in mammalian species is difficult. Ovulation, fertilization and implantation rates can be estimated by counting corpora lutea, blastocysts, and placental scars respectively, during necropic examinations of mammalian reproductive tracts. Blastocysts can be flushed out of the uterine horns with water, but blastocyst integrity depends on the condition of the carcass (Strickland and Douglas 1987). Placental scarring results from embryonic implantation and can be distinguished by sites of discolouration within the uterine wall. This technique works well for some species such as canids (Strand <u>et al</u>. 1995) and felids (Mowat <u>et al</u>. 1996), but the scars fade away within a few months after gestation in martens (Strickland and Douglas 1987). The number of corpora lutea indicates how many ova have been released and has

been used extensively to assess the reproductive condition of female martens (Strickland and Douglas 1987, Thompson and Colgan 1987, Poole and Graf 1996).

Ovaries were excised from marten carcasses, immersed in Bouin's fluid for a minimum of two weeks, and then rinsed with two 24-hr washes of 70 % ethanol (Culling 1963). The ovaries were removed from the ovarian bursa and sliced into four longitudinal sections with a razor blade. Sections were examined for the occurrence of corpora lutea with a dissection microscope at 66X magnification. Corpora lutea were identified by size, colour and texture of the tissue (Mathews 1986). A preliminary examination determined that a crosssection of the ovaries ranged from 1 to 2 mm in width and that the corpora lutea ranged from 1 to 1.5 mm in diameter. Therefore, it was expected that all corpora lutea would be observed with the described method. The number of corpora lutea for each ovary were added to give the total number of corpora lutea per female.

Ovaries were sectioned for 27 adult female martens from the boreal forest and 16 adult female martens from the mixed forest, obtained from the 95/96 trapping season.

#### 2.3 Statistical Analyses

## 2.3.1 Comparisons of Condition Indices

For each sex/age class within either forest region, mass and fat index variables were pooled from both years if unpaired t-tests showed no differences between years. For each forest region, unpaired t-tests were used to compare fat indices between age classes within each sex, and to compare the condition indices of each sex/age class between regions. Percent body fat was estimated using regression equations generated for the ODM (male) and PRDM (female), from Figures 1C and 2E, respectively. An ANOVA was used to compare percent body fat among sex/age classes with each forest region, and t-tests were used to compare percent body fat between sexes within each region and to compare populations by region. Log<sub>10</sub> transformations were used if Bartlett's test for the homogeneity of variance was violated (p < 0.05) for any data set. In cases where transformations could not normalize the variance, two-tailed Wilcoxon Z tests were used.

#### 2.3.2 Reproductive Comparisons

A Chi-square test was used to compare the frequency of ovulation between adult female martens from the boreal and mixed forest regions. Because of the small sample sizes involved, Yates correction for continuity was applied (Sokal and Rohlf 1981). An unpaired t-test was used to compare the mean number of corpora lutea in ovulating females between the two forest regions. The CFM and PRDM for ovulating and non-ovulating adult females were compared with unpaired t-tests. Pearson correlations were used to determine the relationship between the number of corpora lutea and the condition variables, CFM and PRDM.

## 3. **RESULTS**

## 3.1 Variation in Physical Condition Between Years

Within each forest region, no significant differences were found between trapping years in the CFM and ODM for adult and juvenile male martens, and in the CFM and PRDM for juvenile female martens, so data for both years were pooled (Appendice 9). Due to small sample sizes, adult females from the 1996/97 trapping season (Appendix 9) were added to the martens from the previous season without testing for differences.

# 3.2 Variation in Physical Condition Within Regions

Means and standard errors of condition indices and estimated body fat for sex/age classes within each forest region are shown in Table 9.

There were no significant differences in the ODM between male age classes in either the mixed ( $log_{10}$  ODM, t = 0.59, p = 0.56) or boreal ( $log_{10}$  ODM, t = 0.05, p = 0.96) forests. Adult males were larger than juvenile males from the mixed forest (t = 5.06, p < 0.0001) but no significant difference in mass was detected between male age groups from the boreal forest (t = 1.63, p = 0.11).

For females, the PRDM did not differ between age classes in either the mixed ( $log_{10}$  PRDM, t = 1.60, p = 0.12) or the boreal ( $log_{10}$  PRDM, t = 0.83, p = 0.41) forests. No significant differences in carcass mass between age groups were found in either the mixed (t = 1.69, p = 0.10) or boreal forests (Z = 0.75, p = 0.45).

Table 9. Condition indices and estimated body fat (%) for marten sex/age groups from the mixed and boreal forest regions. Means and standard errors of the variables are given for each group. Abbreviations for variables are described in Table 1.

	Mixed forest		Boreal forest	
Index	Adults Juveniles		Adults	Juveniles
Males				
CFM (g)	$742.06 \pm 14.15^{a}$	$631.31 \pm 16.70^{a,b}$	740.79±10.02	$716.46 \pm 11.10^{b}$
	(n = 39)	(n = 28)	(n = 65)	(n = 53)
ODM (g)	2.11±0.39	2.88±0.46	3.39±0.43	2.80±0.47
	(n = 39)	(n = 28)	(n = 66)	(n = 54)
Estimated	2.65±0.32	3.20±0.37	3.57±0.29	3.14±0.32
body fat (%)	(n = 39)	(n = 28)	(n = 66)	(n = 54)
Females				
CFM (g)	474.93±9.12	453.93±8.39 <sup>c</sup>	504.53±12.73	507.05±6.57 <sup>c</sup>
	(n = 22)	(n = 26)	(n = 27)	(n = 49)
PRDM (g)	0.87±0.20	$0.62 \pm 0.19^{d}$	0.83±0.15	0.93±0.12 <sup>d</sup>
	(n = 20)	(n = 22)	(n = 28)	(n = 50)
Estimated body fat (%)	3.28±0.44	2.64±0.42	3.16±0.44	3.42±0.33
	(n = 20)	(n = 22)	(n = 28)	(n = 50)

<sup>a,c,d</sup> p < 0.0001.

<sup>b</sup> p < 0.05.

No significant differences in estimated percent body fat (PBF) were found among the sex/age classes in the mixed ( $\log_{10}$  PBF, F = 0.88, p = 0.46) and boreal ( $\log_{10}$  PBF, F = 0.28, p = 0.84) forests.

# 3.3 Variation in Physical Condition Between Regions

Martens from the boreal forest had a higher percent body fat ( $log_{10}$  PBF, t = 2.23, p < 0.05) than those from the mixed forest (Table 10).

No significant difference in the ODM was found when all males from mixed and boreal forests were compared ( $log_{10}$  ODM, t = 1.39, p = 0.17) (Table 10). There were no significant differences between adult males for both the ODM ( $log_{10}$  ODM, t = 1.39, p = 0.17) and the CFM (t = 0.07, p = 0.94) (Table 9). Between juvenile males, no difference in the ODM was found ( $log_{10}$  ODM, t = 0.56, p = 0.58) but juvenile males from the boreal forest were heavier than those from the mixed forest region (t = 4.64, p < 0.0001) (Table 9).

For female martens, the PRDM did not differ significantly ( $log_{10}$  PRDM, t = 1.87, p = 0.06) between the mixed and boreal forests, but females from the boreal forest were heavier than those from the mixed forest (Z = 3.97, p < 0.0001) (Table 10). The PRDM ( $log_{10}$  PRDM, t = 0.17, p = 0.87) and the CFM (Z = 1.18, p = 0.24) were not significantly different between adult females but juvenile female martens from the boreal forest had a heavier PRDM ( $log_{10}$ PRDM, t = 2.51, p < 0.05) and CFM (t = 4.78, p < 0.0001) than those from the mixed forest region (Table 9).
Table 10. Condition indices for male and female martens from the mixed and boreal forest regions. Estimated body fat (%) for each sex was pooled to compare fat between the two regions. Means and standard errors of the variables are given for each group. Abbreviations for variables are described in Table 1.

	Mixed forest		Boreal forest		
Index	Males	Females	Males	Females	
CFM (g)	695.78±10.99 (n = 67)	463.56±8.40 <sup>a</sup> (n = 48)	729.56±8.28 (n = 118)	505.39±6.63 <sup>a</sup> (n = 77)	
ODM (g)	2.43±0.38 (n = 67)	na	3.12±0.29 (n = 120)	na	
PRDM (g)	na	0.74±0.13 (n = 42)	na	0.90±0.09 ( n = 79)	
Estimated body fat (%)	$2.91 \pm 0.21^{b}$ (n = 109)		3.36± (n =	.0.16 <sup>b</sup> 199)	

<sup>a</sup> p < 0.0001.

<sup>b</sup> p < 0.05. Estimated body fat  $log_{10}$  transformed.

## 3.4 **Reproductive Assessment**

There were no significant differences between the two regions in the proportion of adult females with corpora lutea (G = 0.29, p = 0.28) or in the mean number of corpora lutea per ovulating female (t = 0.42, p = 0.68) (Table 11). There were no significant differences in the CFM (t = 0.26, p = 0.80) or PRDM (t = 0.38, p = 0.71) between ovulating and non-ovulating females (Table 12). For females with corpora lutea (n =16), there were no significant relationships between the number of corpora lutea found and the CFM (r = 0.28, p = 0.20) or PRDM (r = 0.07, p = 0.73).

Table 11. Reproductive comparisons of adult female martens from the mixed

and boreal forest regions, including results from other studies.

	This study		Other studies	
Reproductive variable	Mixed forest (n = 16)	Boreal forest (n = 27)	Strickland and Douglas <sup>a</sup> (n = 880)	Fortin and Cantin <sup>b</sup> (n = 108)
Percentage of adult females that ovulated	50%	33.3%	87%	79%
Mean number of corpora lutea (±SEM) per ovulating female	2.86±0.45	3.11±0.40	3.46±0.07	4.25±0.09

<sup>a</sup> Algonquin Region, Ontario 1973-1985

<sup>b</sup> Laurentides Wildlife Reserve, Quebec 1984-1986

Table 12. Comparison of condition indices between ovulating andnon-ovulating female martens. Abbreviations for variables are describedin Table 1.

Condition index	Ovulating	Non-ovulating	
PRDM ±1 SEM (g)	0.94±0.26 (n = 17)	0.81±0.21 (n = 25)	
CFM ±1 SEM (g)	486.30±15.43 (n = 16)	481.23±12.34 (n = 25)	

# 4. **DISCUSSION**

#### 4.1 Variations in Physical Condition within Forest Regions

Although it was hypothesized that males would have relatively more fat than females and that adults would have more fat than juveniles, no differences in the fat indices or estimated percent fat were found among adult and juvenile age groups in either forest region (Table 9). The effects of sex and age on body fat in martens may only become evident on a local scale when a deterioration in habitat quality such as logging (Thompson 1994) or a decline in prey abundance (Thompson and Colgan 1987, Poole and Graf 1996) has occurred.

### 4.2 Effect of Habitat on Physical Condition

While no regional differences in physical condition were found between adult groups (Table 9), juvenile martens from the boreal forest region were heavier than those from the mixed forest region and juvenile females from the boreal forest region had more perirenal fat (Table 9). It is likely that the juvenile female group from the mixed forest region was responsible for the differences in estimated body fat between the two populations (Table 10). Hénault and Renaud (1993) also reported that juvenile martens from conifer forests were significantly heavier than those from deciduous forests in Quebec, although no differences in fat content were found in their study.

Differences in the physical condition of the juveniles from the two regions are a possible indication of habitat differences within the forest types. Adult martens are dominant over juveniles and presumably establish home ranges in the best habitats within different forest types. Martens may be transient in their juvenile year (Francis and Stephenson 1972), or establish ranges in sub-optimal habitats (Latour <u>et al</u>. 1994). In areas where the availability of suitable habitat is limited, a juvenile marten may be unable to secure adequate resources. This may be why juvenile martens of the mixed forest, where resources are presumed to be sparse, were found in relatively poor physical condition. A multi-scale analysis of habitat composition and population demographics would be needed to test this hypothesis.

Less body fat in juvenile females from the mixed forest (Table 9) was consistent with the prediction that juveniles and females would be more prone to a degradation in habitat. An approximated 1 : 1 ratio of juvenile females to adult females trapped in the mixed forest, compared to a 2 : 1 ratio in the boreal forest further suggested that juvenile females from the mixed forest region suffered higher mortality rates. The juvenile females were not expected to bear any reproductive costs, but thermoregulatory costs and the relative inability to secure resources may have contributed to the relatively poor physical condition of juvenile females in the mixed forest.

The differences in mass between juveniles of the two forest regions may also be a result of developmental differences rather than habitat differences, as suggested the dividing points of the percent pulp cavity for male martens (Appendix 3). Poole <u>et al</u>. (1994) noted that dividing points in pulp cavity ratios increased with increasing latitude. Parturition dates may vary with latitude in relation to photoperiods (Mead 1994), whereby martens in the southern part of their range would be born earlier in the year. Thus, a longer maturation period would result in lower dividing points for martens at lower latitudes. However, juvenile martens born relatively early in the season would be expected to be larger than those born later, but in this study, juveniles from the boreal forest were heavier than those from the mixed forest (Table 9). The weight differences between the juveniles may be due to different growth rates. In the boreal forest, where winter arrives earlier and temperatures are lower than in the mixed forest (Appendix 1), a rapid increase in body size would help minimize heat loss. It was found that the mass difference between male age classes in the boreal forest was not significant (Table 9) and indicated that the juveniles approached adult weight more quickly in this region than juveniles in the mixed forest. Morphometric and genetic variability among populations of martens (Mitton and Raphael 1990, Fortin et al. 1997) may contribute to developmental differences.

## 4.3 Ovulation and Physical Condition

The ovulation rate of adult female martens did not differ between forest regions, but the proportion of adult females that ovulated and the mean number of corpora lutea in martens from this study were lower than those reported for martens in other studies (Table 11). The low reproductive performance of adult female martens found in this study may be due to several factors. The reproductive conditions of female martens may have been poor for the year in study. The results in this study are similar to those Thompson and Colgan (1987) reported during two years of a decline in prey abundance where less than 50 % of adult females younger than three years old ovulated and there were fewer than 2.37 corpora lutea per ovulating female. A long term study of

62

ovulation in martens from the study regions may reveal that 1995 was a year of low reproductive performance in females. The ovulation rate has also been found to increase with age in martens (Strickland and Douglas 1987, Aune and Schladweiler 1997), and the low ovulation rates obtained in this study could be due to a greater frequency of younger adult female cohorts, characteristic of exploited populations.

Experimental error may have contributed to a low reproductive assessment. Incorrect age classifications for females may have overestimated the number of adult females in this study by assigning juvenile females an adult status. Results in this study (Appendix 3) concurred with Poole <u>et al</u>. (1994) that age determination using the pulp cavity ratios or temporal muscle coalescences are less accurate for female than for male martens, and result in ranges where females can not be aged confidently. The use of a more precise technique such as cementum annuli counts would be preferable to ascertain female age cohorts (Poole <u>et al</u>. 1994). In addition, ovaries in this study were not sectioned with a microtome and stained (Gilbert 1987), but due to the large size and distinctive texture of a corpus luteum, as well the thinness of the sections, it was unlikely that any corpora lutea were missed upon the examination of hand-sliced, rather than microtomed sections.

Ovulation parameters in adult females were not correlated with physical condition (Tables 11 and 12). This was probably due to the delay between trap dates and the breeding season, when the relationships with physical condition should preferably be studied. Embryonic development resumes in February

63

(Strickland <u>et al</u>. 1982) when any adverse effect of gestation on physical condition may be observed (Thompson and Colgan 1987).

## 4.4 Limitations of Study

The assessment of marten physical condition was considered satisfactory for an analysis at the landscape level. The strong correlation of the perirenal dry mass with percent body fat in females allowed a reasonable degree of accuracy in analyzing fat differences (Figure 3E), while the predictive ability of the omental dry mass was good in males (Figure 2C). Therefore, these indices were concluded to be valid indices for assessing fat levels for martens in Ontario. The application and accuracy of these indices for martens from other regions, as well as other species in the genus <u>Martes</u> would need to be examined due to possible morphometric differences.

The potential ability of fat content in martens to assess habitat quality was an important factor in its choice as a condition index. Fat has been used for this purpose in a variety of mammalian species (Riney 1955, Lochmiller <u>et al</u>. 1985, Torbit <u>et al</u>. 1985, Litvaitis <u>et al</u>. 1986, Cothran <u>et al</u>. 1987, Winstanley <u>et al</u>. 1998), but because of the small amount of fat and lack of seasonal fat cycle in martens (Buskirk and Harlow 1989) it may be important only for the short-term balancing of energy budgets (Harlow 1994). Therefore, fat levels may be a insufficient criterion for assessing long-term condition and for gauging habitat quality.

Protein is a good alternative for assessing physical condition because of its importance as an additional energy source in martens, and may be better than

fat at reflecting long-term physical condition. However, no index for percent protein was found in this study (Table 8), and protein mass was correlated with body mass (Table 8), restricting comparisons to similar-sized groups. Possibly, a combination of body mass and a fat index can be used to classify martens into two broad categories of fitness, sufficient for assessing population health for large-scale management purposes.

Because the sample from the second year was small (Appendices 9 and 10), no temporal replication was available for the study of annual patterns. Physical condition would need to be assessed for a number of consecutive years to determine if the results found in this study reveal a more general pattern.

Contrast between habitat parameters could not be analyzed at the landscape level and analyses of smaller-scale stochastic characteristics (both temporal and spatial) were not monitored in any of the study sites. For instance, little difference in forest structure may have actually existed between the mixed and boreal forests, but it was assumed that the differences in forest cover would yield different conditions, especially in the amount of conifer available. The suitability of a forest type for martens may include other variables such as climatic conditions. For example, the intensity of selection and competition for optimal habitat may vary with snow cover (Bissonette and Broekhuizen 1995). Presumably, a lower content of conifer in the mixed forest results in greater snow depths, limiting access to coarse woody debris and subnivean sites. Furthermore, the mixed forest receives on average more snow than the boreal forest (Appendix 1). Other potential factors of importance such as trophic assemblages and logging intensity were also not examined. Since the assessment of these factors was beyond the scope of this project, the relationship between physical condition and habitat condition could only be partly verified in this study.

### 4.5 Management Implications

This study detected differences in the physical condition of juvenile martens from two distinct forest regions that supported our initial contention that martens from the mixed forest were not as fit as those from the boreal forest. Juveniles from the mixed forest were smaller, with females leaner, than those from the boreal forest, and this suggested that they experienced more difficult habitat conditions.

In view of sustainable habitat management, the relatively poor condition of juvenile females and the low juvenile females to adult females ratio suggested that some limiting habitat factor(s) did exist in the mixed forest. It is recognized that several other factors (e.g. vulnerability to predation) other than energy reserves may play a role in the survival rate of juvenile martens. A high natural mortality due to sub-optimal habitat will reduce breeding recruitment and decrease the sustainability of future harvest rates. A small-scale analysis contrasting habitat use by juvenile martens in mixed and boreal forest types would help determine the essential elements in sustaining viable juvenile populations. Also, a multi-annular study of physical condition in relation to winter conditions would help ascertain the role of seasonal habitat components on specific marten sex/age cohorts.

## 5. SUMMARY

Fat content did not differ among sex/age groups of martens within each forest region, but some regional differences in physical condition were detected. Percent body fat for martens from the boreal forest was higher than that of the mixed forest. Both juvenile males and females from the boreal forest were heavier than those from the mixed forest while juvenile females from the boreal forest also had more perirenal fat than those from the mixed forest. These differences were assumed to be related to differences in habitat quality.

The ovulation rates in adult female martens did not differ between forest regions but were lower than those reported in other studies. Condition indices examined from winter trapped marten did not predict the occurrence of ovulation or the number of corpora lutea.

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# 7. APPENDICES

Appendix 1. Winter climate normals from Environment Canada (1996) for Kapuskasing, Ontario (49°N 82°W) and Sudbury, Ontario (46°N 81°W) representing the boreal and mixed forests, respectively.

	Boreal forest <sup>a</sup>		Mixed forest <sup>b</sup>	
Month	Daily mean temperature (°C)	Snowfall (cm)	Daily mean temperature (°C)	Snowfall (cm)
November	-4.3	46.8	-1.3	32.6
December	-14.8	49.7	-9.9	61.2
January	-18.6	51.1	-13.5	59.5

<sup>a</sup> monthly averages from 1918 to 1990.

<sup>b</sup> montly averages from 1961 to 1990.

Appendix 2. Frequency distributions of pulp cavity ratios for male (A - B) and female (C - E) martens from the mixed and boreal forest regions, including females pooled from both regions.







Appendix 3. Age distributions of 187 male and 127 female martens from two forest regions. Estimated means, standard deviations (SD) and age class dividing points in pulp cavity ratios are shown for 275 martens. Standard deviations for pulp cavity ratios were determined using normal quantile plots for boreal forest males and pooled females, and the rankit method for all other groups.

		Pulp cavity ratios			
		N	Nales	Fer	nales
Forest region		Adults	Juveniles	Adults	Juveniles
Boreal	Mean	0.23	0.47	0.21	0.43
	SD	0.08	0.07	0.09	0.07
	n	66	54	29	50
	Dividing point	t 0.38 0.29 ·		- 0.39	
Mixed	Mean	0.18	0.43	0.21	0.43
	SD	0.07	0.07	0.1	0.05
	n	39	28	22	26
	Dividing point	C	0.32	0.33 - 0.41	
Pooled	Mean			0.2	0.42
	SD			0.09	0.05
	n			51	76
	Dividing point			0.32	- 0.38

Appendix 4. Mean fat content of three 20 g aliquots per homogenized

		Aliquot		
	1	2	3	Total fat content (60 g)
Fat ±1 SEM (%)	2.48±0.17	2.49±0.17	2.43±0.17	2.47±0.17

marten (n = 32), recalculated from Calford and Clark (1996).

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Appendix 5. Mean fat content of heads, headless  $\hat{e}$  arcasses, and whole carcasses of martens (n =16).

	Head	Headless carcass	Whole carcass	
Fat ±1 SEM (%)	2.99±0.16	2.98±0.51	2.99±1.82	 

Variable	All (n = 77)	Males (n = 39)	Females (n = 38)
HFM (g)	481.09±13.14	575.87±10.14	383.81±10.27
HDM (g)	157.36±4.48	190.15±3.34	123.72±3.38
LFM (g)	25.02±0.78	29.04±0.96	20.90±0.82
LDM (g)	7.48±0.24	8.96±0.27	5.97±0.21
LFM/HFM	5.27±0.13	5.10±0.18	5.44±0.18
LDM/HDM	4.8±0.11	4.76±0.16	4.84±0.16
OFM (g)	3.46±0.30	4.08±0.50	2.82±0.31
ODM (g)	1.93±0.23	2.30±0.39	1.54±0.23
OFM/HFM	0.72±0.06	0.72±0.09	0.72±0.07
ODM/HDM	1.18±0.12	1.19±0.17	1.17±0.17
PRFM (g)	1.89±0.16	2.27±0.22	1.50±0.21
PRDM (g)	0.94±0.11	1.13±0.15	0.75±0.14
PRFM/HFM	0.39±0.03	0.39±0.04	0.38±0.05
PRDM/HDM	3.44±0.06	0.57±0.08	0.56±0.08

Appendix 6. Fourteen variables ( $\overline{x} \pm 1$  SEM) used to develop fat indices for martens (n = 77). Variable abbreviations are described in Table 1.

	Ma	ales	Fen	nales
Variable	Adults (n = 21)	Juveniles (n = 18)	Adults (n = 21)	Juveniles (n =17)
HFM (g)	609.87±14.76 <sup>a</sup>	536.20±14.41 <sup>a</sup>	387.81±11.66	378.87±11.39
HDM (g)	199.12±5.18 <sup>b</sup>	179.68±4.96 <sup>b</sup>	125.12±3.79	121.98±3.81
LFM (g)	29.53±1.10	26.46±1.67	21.75±1.14	19.84±1.18
LDM (g)	9.06±0.29	8.83±0.46	6.10±0.28	5.80±0.33
LFM/HFM	4.91±0.23	5.32±0.29	5.60±0.23	5.24±0.28
LDM/HDM	4.60±0.18	4.95±0.27	4.90±0.20	4.77±0.26
OFM (g)	3.37±0.66	4.91±0.72	2.92±0.38	2.69±0.53
ODM (g)	1.71±0.52	2.99±0.56	1.51±0.26	1.57±0.41
OFM/HFM	0.56±0.12	0.90±0.13	0.73±0.09	0.70±0.13
ODM/HDM	0.86±0.25	1.58±0.27	1.14±0.17	1.21±0.28
PRFM (g)	2.07±0.29	2.49±0.33	1.74±0.35	1.21±0.15
PRDM (g)	0.84±0.20 <sup>c</sup>	1.46±0.22 <sup>c</sup>	0.89±0.24	0.59±0.13
PRFM/HFM	0.33±0.04	0.46±0.06	0.44±0.09	0.31±0.04
PRDM/HDM	0.41±0.09 <sup>d</sup>	0.77±0.10 <sup>d</sup>	0.65±0.16	0.45±0.08

Appendix 7. Fourteen variables ( $\overline{x} \pm 1$  SEM) used to develop fat indices for marten sex/age classes. Variable abbreviations are described in Table 1.

<sup>a</sup> p < 0.001, <sup>b</sup> p < 0.01, <sup>c,d</sup> p < 0.05

Appendix 8. Protein mass and percent protein of 10 g aliquots from lean, homogenized martens (n = 42). The number of samples for each aliquot was limited by the amount of homogenate available, and a repeated measures analysis was used only for martens with three aliquots (n = 23).

Protein content	1 (n = 42)	2 (n = 41)	3 (n = 24)	Total
x ±1 SEM (g)	6.42±0.23	6.55±0.23	5.67±0.31	16.06±0.41
x ±1 SEM (%)	15.22±0.27	15.66±0.26	15.60±0.34	15.49±0.27

Appendix 9. Condition indices for martens from the mixed and boreal forest regions caught during the 95/96 and 96/97 trapping seasons. Means and standard errors of the indices are shown for each sex/age group. Abbreviations for variables are described in Table 1.

		Mixed forest		Bo fo	oreal orest
Year	Index	Adults	Juveniles	Adults	Juveniles
Males					
95/96	CFM (g)	749.45±16.44 (n = 33)	616.94±18.83 (n = 17)	738.51±10.35 (n = 63)	712.42±11.46 (n = 47)
	ODM (g)	2.19±0.30 (n = 33)	2.75±0.77 (n = 17)	3.40±0.53 (n = 64)	2.92±0.31 (n = 48)
96/97	CFM (g)	701.39±38.55 (n = 6)	653.53±23.41 (n = 11)	812.41±58.08 (n = 2)	748.05±32.09 (n = 6)
	ODM (g)	$0.74 \pm 1.00$ (n = 3)	2.68±1.83 (n = 3)	3.12±3.01 (n = 2)	1.81±0.88 (n = 6)
Female	es				
95/96	CFM (g)	473.27±9.66 (n = 19)	455.31±10.88 (n = 17)	492.83±13.76 (n = 26)	505.31±7.43 (n = 40)
	PRDM (g)	0.91±0.26 (n = 19)	0.55±0.17 (n = 17)	0.81±0.18 (n = 27)	1.02±0.11 (n = 41)
96/97	CFM (g)	485.46±24.30 (n = 3)	451.34±14.96 (n = 9)	808.79±70.14 (n = 1)	514.76±15.67 (n = 9)
	PRDM (g)	0.22±1.13 (n = 1)	0.85±0.31 (n = 5)	1.38±0.95 (n = 1)	0.51±0.88 (n = 9)