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EFFECTS OF SEX AND AGE ON WINTER DIET IN THE AMERICAN MARTEN IN THE UPPER PENINSULA OF MICHIGAN

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BY

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ABSTRACT

EFFECTS OF SEX AND AGE ON WINTER DIET IN THE AMERICAN MARTEN IN THE UPPER PENINSULA OF MICHIGAN

By

Andrea L. Hales

The effect of size sexual dimorphism on diet in American martens (*Martes americana*) was investigated. Three hundred and eighteen gastrointestinal tracts were collected from trapped martens 2000–2004 in the Upper Peninsula of Michigan. Prey species were similar to those found throughout North America. Ten prey species were identified in 151 martens and classified into six prey groups based on body size. The proportion of prey groups to kilocalories consumed was similar between male and female marten diets, as well as between juvenile and adult diets. The average dietary breadth (0.49) was similar to other North American studies and dietary overlap (0.99) was high between sexes and between age groups. This suggests that the diet of males and females, and of adults and juveniles was identical and there was no prey partitioning.

The effect of unequal sample sizes was examined by reducing sample size (by 50%) of one sampled group and repeating the statistical analyses. Comparison of ten constructed datasets (half the number of randomly selected stomach samples and all intestinal samples) showed that stomach and intestinal contents were similar, indicating that using either stomachs only, intestines only, or a combination (in equal proportions) of stomach and intestinal contents would not change test results. Ten identically designed comparisons of stomach samples (half the number of randomly selected females and all males) showed no differences between male and female stomach contents. Ten comparisons of stomach contents between half of the juveniles and all of the adults were

also similar; indicating that unequal sample sizes of sex and age classes did not bias diet estimates of stomach contents.

ACKNOWLEDGMENTS

This project would not have been possible without the support of numerous individuals. I thank my thesis advisor Dr. Jackie Bird for her encouragement and support throughout this project. Her enthusiasm in the research lab inspired me to remain steadfast while examining some very smelly marten guts. I extend a special thank you to one of my committee members, Dr. Jerry Belant, whose suggestion of this project helped make it a reality, and whose support, suggestions, and eagerness helped improve this project. I thank Dr. John Bruggink for reviewing and suggesting improvements for this project.

I thank my lab assistants Alex Buck for volunteering to go through stomach and intestinal contents; Jessica Kustin for assisting me with cleaning separated contents; Lauren Johnson for her assistance in separating stomach and intestinal contents from organs; Jing-Fang Niu for her assistance with supplies; the faculty and staff of the Biology Department whose help was immeasurable; the graduate students of the Biology Department for their support and encouragement despite the smell, especially Eileen Oppelt and Jessie Simmon whose friendships I will cherish always; and most of all, I thank my family for their love and support, for instilling in me an appreciation of the outdoors, for helping me throughout my master's career and a difficult time away from home.

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CHAPTER ONE

EFFECTS OF SEX AND AGE ON WINTER DIET IN THE AMERICAN MARTEN

INTRODUCTION

Sexual dimorphism in average body length and weight is seen in many animal species (Shine 1989, Dayan and Simberloff 1994, Fairbairn 1997, Karubian and Swaddle 2000, McDonald 2002). When there is potential for one sex to be exposed to predation through defense of territories (e.g., male martens [*Martes* spp.]) or nests (e.g., female raptors, some male fish), selection may favor larger individuals of that sex (Shine 1989).

Predator body size in part also determines the size of it's prey and influences where the predator will forage (Shine 1989, Dayan and Simberloff 1994). Consequently, differences in male and female diets within a species are expected when they differ in body size, which may decrease competition for food (Dayan et al. 1989, Shine 1989, McDonald 2002, Begg et al. 2003).

There are two hypotheses for why sexual dimorphism occurs. The first hypothesis is that dimorphism reduces dietary overlap which in turn reduces intraspecific competition (Shine 1989, Dayan and Simberloff 1994, Begg et al. 2003). Evidence that predators partition prey species according to body mass has been provided by mustelid species (McDonald 2002). In the British Isles, Dayan and Simberloff (1994) found strong evidence of prey partitioning between sexes of mustelid species based on predator size, despite seasonal fluctuations in prey species abundance.

The second hypothesis is that the difference in size between males and females is attributed to other selection pressures (Begg et al. 2003). Smaller females require less

energy than larger females for daily maintenance and thus direct more energy into reproduction or reproductive-related activities such as caring for young (Begg et al. 2003). In contrast, larger males require more energy to defend territories (Begg et al. 2003).

Adult male American martens (*Martes americana*) are 20–40% longer and heavier than females (Strickland et al. 1982, Buskirk and MacDonald 1989, Holmes and Powell 1994). Dimorphism of this magnitude would be expected to result in prey partitioning between sexes (Dayan and Simberloff 1994). However, several studies have reported high dietary overlap between male and female martens, suggesting little partitioning of prey species (Nagorsen et al. 1989, 1991; Andruskiw 2003). Where differences between male and female diets have been reported, males ate larger prey such as ruffed grouse (*Bonasa umbellus*) and snowshoe hares (*Lepus americanus*) while females most commonly consumed mice and voles (Poole and Graf 1996, Bull 2000).

As animals mature, foraging skills are refined (Raven 2005). It is expected that juveniles are less skilled hunters and their diets should different from adults. Few studies have compared juvenile (<1 year old) and adult ($\geq$1 year old) marten diets. Thompson and Colgan (1987) found no differences between juvenile and adult diets in north central Ontario. Surprisingly, in the Northwest Territories, juveniles consumed more large prey items (e.g., snowshoe hare) than adults (Poole and Graf 1996).

Prey switching is a method of maintaining body condition as prey populations fluctuate (Hawley and Newby 1957, Weckwerth and Hawley 1962, Thompson and Colgan 1987, Begg et al. 2003). Mustelids such as fishers (*M. pennanti*), stone martens (*M. foina*), and honey badgers (*Mellivora capensis*) switch prey species as their relative

abundance changed (Kuehn 1989, Genovesi et al. 1996, Begg et al. 2003). Similarly, American martens switch prey species depending on species abundance (Hawley and Newby 1957, Weckwerth and Hawley 1962, and Thompson and Colgan 1987, Begg et al. 2003).

Identifying stomach contents and scat remains has been used to estimate importance of prey species and dietary breadth in carnivore diets (Buskirk and MacDonald 1984, Thompson and Colgan 1987, Nagorsen et al. 1989, Nagorsen et al. 1991, Poole and Graf 1996, Bull 2000, Cumberland et al. 2001). The importance of a prey species is often based on the relative abundance or frequency of occurrence of a prey species in samples (Buskirk and MacDonald 1984, Thompson and Colgan 1987, Nagorsen et al. 1989, Nagorsen et al. 1991, Poole and Graf 1996, Bull 2000, Cumberland et al. 2001). After a meal, a larger proportion of identifiable parts from small prey may remain in marten gut contents than that of large prey, which may have been predominantly tissue. Thus, percent frequency may misrepresent the relative contribution of prey species to predator fitness. Cumberland et al. (2001) suggested that because larger prey species provide more calories to a predator, estimated percent caloric intake may be a better indicator of the value of prey species in diet.

My objectives were: 1) to estimate marten diet in Michigan's Upper Peninsula (UP) during early winter, 2) to compare prey species composition between sexes and age classes, and 3) to compare the relative contribution of prey species using percent frequency of occurrence and estimated caloric intake.

METHODS

Study Area

The Upper Peninsula of Michigan is located on the southern shore of Lake Superior and covers an area of 42,610 km^2 (Latitude: 454442–464917 N, Longitude: 841437–893001 W; Figure 1.1). December temperatures range from average lows of -8° C to highs of -1° C and average precipitation is 49.5 cm (National Weather Service, Weather Underground 2007).

Collection

American martens were extirpated from Michigan in the early 1900s (Earle et al. 2001). Subsequent successful reintroductions in Michigan's UP resulted in removal of the marten from the state's threatened species list in March 1999 (Frawley 2002, Cooley et al. 2003). A marten trapping season was established in the Upper Peninsula of Michigan from 1–10 December 2000 and expanded to 1–15 December in 2002, with a season limit of one marten per trapper (Frawley 2001, Cooley et al. 2003). Submission of all legally and incidentally harvested marten carcasses is required by the Michigan Department of Natural Resources (MDNR).

Gastrointestinal (GI) tracts of trapped martens were collected by the MDNR from the 2000–2004 trapping seasons and frozen until analysis. Sex, age, date, and location trapped were recorded for each marten. I used martens with complete information that were registered as harvested during the marten trapping season for analysis.

All martens harvested during 2000–2001 were used for analysis. Due to large numbers of carcasses and time constraints, not all carcasses were analyzed during 2002–2004. In 2002 and 2003, more males were trapped than females; in these years all

females were sampled and an equal number of males were randomly selected for analysis. Because high numbers of both sexes were trapped in 2004, male and female martens from that year were randomly selected to equal numbers sampled during 2003.

Gastrointestinal Content Analysis

Because stomachs and intestines of an individual marten may contain remains from different meals (Nagorsen et al. 1989), stomach and intestinal contents were analyzed separately per marten to reduce that source of variability. Contents of GI tracts from martens caught during 2000–2001 were analyzed for parasites prior to diet analysis (Veine 2004). Examined contents from GI tracts were placed in plastic bags, labeled, and frozen for diet analysis. Gastrointestinal tracts from martens caught during 2002–2004 were opened and scraped of their contents. I rinsed all marten gut contents with 75% ethyl alcohol, separated hair samples into labeled containers and air dried hairs for identification (Weingart 1973).

I made negative impressions of hairs sampled from each marten's GI tract (Weingart 1973). I used a compound microscope at 40–400x magnification, hair identification guides (Adorjan and Kolenosky 1969, Moore et al. 1997, Andruskiw et al. 2003), and reference slides I made from museum specimens to identify prey species.

I designated six prey groups for analysis based primarily on body size: shrew, mice/vole, bird, chipmunk, squirrel, and grouse/hare. The shrew group included *Sorex* spp. and short-tailed shrews (*Blarina brevicauda*). The mice/vole group consisted of deer mice (*Peromyscus maniculatus*) and red-backed voles (*Clethrionomys gapperi*). The bird group was all bird species excluding ruffed grouse. The chipmunk group comprised eastern chipmunks (*Tamias striatus*), and the squirrel group included red squirrels

(*Tamiasciurus hudsonicus*) and eastern gray squirrels (*Sciurus carolinenesis*). Ruffed grouse and snowshoe hares comprised the grouse/hare group.

Martens eat carrion when available, particularly during winter (Strickland et al. 1982, Strickland and Douglas 1987, Thompson and Colgan 1987). White-tailed deer (*Odocoileus virginianus*) occurred in a high proportion (47%) of the gastrointestinal tracts sampled. The overlap of the marten trapping season and deer hunting season made it impossible to separate what was carrion and what was used as bait at marten trap sites. Therefore, white-tailed deer was excluded from analysis. Similarly, marten hair and vegetation present in the food contents was considered incidental and was excluded from analysis (Buskirk and MacDonald 1984, Nagorsen et al. 1989, Poole and Graf 1996, Bull 2000).

Statistical Analysis

I combined stomach and intestinal contents (Chapter 2) and used chi-square analysis to compare frequency of occurrence of prey groups between males and females, and between juveniles (<1 year old) and adults (≥1 year old; Bull 2000, Murakami 2003).

I calculated biomass of prey groups following Poole and Graf (1996). Poole and Graf (1996) reported that stomachs can contain up to 120 g of contents; all stomach contents in this study weighed <120 g. Therefore, for each marten I converted occurrences of prey species to biomass by multiplying the number of occurrences by the mean body mass of each prey species, to a maximum of 120 g (Kurta 1995, Poole and Graf 1996). I calculated mean ingested biomass for prey groups identified in the stomach and intestinal contents of individual martens (Brewer 1991, Kurta 1995). I used a three-

way analysis of variance (ANOVA) to estimate the effects of gut site, sex, and age on prey biomass and the interactions among these factors (Analytical Software 2003).

I calculated kilocalories (kcal) for prey groups using mean body mass of prey species (Brewer 1991, Kurta 1995, Cumberland et al. 2001). Unknown kcal values for prey species were estimated using linear regression based on species kcal values from Cumberland et al. (2001). I used three-way ANOVA to compare kcal consumption of prey groups between stomach and intestines, between males and females, and between juveniles and adults. All analyses were conducted using STATISTIX 8 (Analytical Software 2003) with significance accepted when $P \leq 0.05$.

I calculated dietary breadth as $(1/\sum P_i^2)/N$ where P_i equals the proportion of prey species *i* in the diet of martens in a particular sex and age class and N is the number of prey groups (Nagorsen et al. 1989). Values range from 0 to 1, where 1 = prey species consumed in identical proportions. Dietary overlap was calculated using:

$$\text{dietary overlap} = \frac{\sum P_{ij} * \sum P_{ik}}{(\sum P_{ij}^2 * \sum P_{ik}^2)^{1/2}}$$

where P_{ij} is the proportion of prey group *i* in the diet of group *j* and P_{ik} is the proportion of prey group *i* in the diet of group *k*, and reflects similarity of diets (Nagorsen et al. 1991). Values range from 0 to 1, where 0 = no overlap and 1 = identical diets.

RESULTS

I sampled 318 martens of which 151 contained stomach and intestinal contents used for analyses. I excluded 157 individuals because either stomach or intestines were empty and 10 individuals because they were not collected during the marten trapping season. I found 433 prey items representing ten species in the stomachs and intestines of

151 martens of known sex (68 female and 83 males; Table 1.1) and age (47 juveniles and 104 adults; Table 1.2). The percentage of martens containing 1–7 prey items was 9%, 32%, 33%, 19%, 3%, 3%, and 1%, respectively. Mean number of prey items per marten was 2.87 (standard deviation ±1.52).

Red-backed voles were the most frequently identified prey species, occurring in 147 (97%) GI tracts (Figure 1.2). Deer mice were the second most abundant (57%), followed by short-tailed shrews (47%), *Sorex* species (21%), eastern gray squirrels (17%), red squirrels (16%), bird species (15%), eastern chipmunks (12%), snowshoe hares (3%), and ruffed grouse (1%).

The proportions of all prey groups used by male and female martens was similar ($\chi^2 = 7.57$, 5 df, $P = 0.180$; Figure 1.3). Males and females acquired similar amounts of kcals from all prey groups (Table 1.3). Dietary breadth was 0.46 for males and 0.50 for females. Dietary overlap between males and females was 0.98.

The proportions of prey groups consumed by juvenile and adult martens was similar ($\chi^2 = 4.45$, 5 df, $P = 0.487$; Figure 1.4). Juveniles and adults acquired similar amounts of kcals ($P \geq 0.13$) from all prey groups except mice/vole ($P = 0.01$; Table 1.3). Dietary breadth was 0.56 for juveniles and 0.47 for adults. Dietary overlap between juveniles and adults was 0.99.

DISCUSSION

Diet composition

Mice and voles were the most frequently consumed prey, consistent with other marten winter diet studies (Lensink et al. 1955, Weckworth and Hawley 1962, Buskirk and MacDonald 1984, Strickland and Douglas 1987, Thompson and Colgan 1987, Slough

et al. 1989, Buskirk and Ruggiero 1994, Poole and Graf 1996, Bull 2000, Cumberland et al. 2001).

There are few resident winter bird species in the UP (Brewer 1991). Where winter avifauna consists of few resident species, birds are usually not a major winter food of martens (Nagorsen et al. 1989). I found that birds were an infrequent food source for martens in the UP. It is possible that the birds were opportunistic feedings.

This is the second study to document gray squirrel in marten diet. Overlap in geographic ranges of these species is limited (Stokes and Stokes 1986, Whitaker and Hamilton 1998, Foresman and Pearson 1999). Weckwerth and Hawley (1962) found gray squirrel in <0.5% of the summer and fall diets of marten in Montana. Gray squirrels were found in considerably higher proportions (17%) in this study. Winter activity patterns of martens and gray squirrel coincide, increasing the chances of martens encountering gray squirrels while foraging (Stokes and Stokes 1986, Whitaker and Hamilton 1998, Foresman and Pearson 1999).

Eastern chipmunks occurred in 12% of martens sampled at a time of year when it should be difficult for martens to access them. Chipmunks use burrow systems up to 10 meters long and in northern climates, spend the winter months in burrows 60 to 74 cm below ground surface (Whitaker and Hamilton 1998). In Michigan, chipmunks may leave their burrows during warm periods in winter; however, most remain in burrows for extended periods limiting their vulnerability to predators (Baker 1983, Whitaker and Hamilton 1998). The apparently high occurrence of chipmunks may be a consequence of chipmunks leaving their burrows during warm periods and being consumed or of some

martens being captured before the legal trapping season and registered as captured during this season.

In contrast to other studies (Thompson and Colgan 1987, Poole and Graf 1996, Cumberland et al. 2001), snowshoe hare and ruffed grouse were uncommon in the marten diet. Snowshoe hares and ruffed grouse populations cycle, peaking every 10–11 years (Brewer et al. 1991, Kurta 1995). Abundance estimates of ruffed grouse and snowshoe hare in Michigan's UP were greatest in 2000 and decreased through 2004 (Minzey 2004). The few occasions these prey species were consumed were during years of low population levels.

The Upper Peninsula of Michigan is near the southern limit of the current geographic range for the snowshoe hare, and densities of animals at the edge of their range most often do not reach the densities of those at the center (Raven et al. 2005). Lower relative abundance may account for the infrequent occurrence of hares in the diet of martens in my study because hares would be an unpredictable and scarce food source. Therefore, it may not be beneficial for martens to hunt snowshoe hares specifically but rather take them opportunistically.

Ruffed grouse distribution extends across the northern United States and southern Canada (Gough et al. 1998). Michigan is situated at the center of the geographic range and although ruffed grouse appeared to be at least as abundant as snowshoe hares during spring surveys (Minzey 2004), they were not an important food item in early winter.

It is more energetically beneficial for martens to take larger prey. In late winter when snow depths make it difficult to locate small mammals, and larger prey become more vulnerable to predation due to decreased muscle mass (Slough et al. 1989) larger

prey may be more frequent in marten diet. Under these conditions, energetic benefits of capturing larger prey outweigh the costs of capture (Cumberland et al. 2001). Martens in my study were trapped in early December before increased snow depths made searching for larger prey more energy cost efficient. If martens were sampled later in the winter (January–March), ruffed grouse and snowshoe hare may have occurred more frequently in their diet.

Male and female diets

Large male martens are probably more capable of killing larger prey species than females (Dayan et al. 1989, Poole and Graf 1996, McDonald 2002), suggesting dietary breadth in males would be greater than in females. Dietary breadth for males and females was similar for martens in Michigan's UP in early winter. These values are at the high end of the range in studies of marten winter diet in North America where dietary breadths ranged from 0.19–0.69 (Nagorsen et al. 1989; Table 1.4).

Poole and Graf (1996) suggested that low dietary breadth during winter may reflect low prey species abundance and diversity. The number of prey species available in the UP in early winter is low (Baker 1983) compared to that found in other studies (Cowan and MacKay 1950, Weckwerth and Hawley 1962, Buskirk and MacDonald 1984, Hargis and McCullough 1984, Nagorsen et al. 1989, Nagorsen 1991, Poole and Graf 1996, Bull 2000, Cumberland et al. 2001). However, many studies used different classification schemes for prey groups (Cowan and Mackay 1950, Quick 1955, Weckwerth and Hawley 1962, Koehler and Hornocker 1977, Zielinski et al. 1983, Nagorsen et al. 1989). To address variation in prey groupings across studies, I reclassified data from other studies (Cowan and Mackay 1950, Quick 1955, Nagorsen et

al. 1989) into my six prey groups and recalculated dietary breadth to estimate if the method of grouping species influenced dietary breadth results. Recalculated dietary breadths changed minimally (0.05–0.19) and reinforced that prey group classification may not be as important as the proportions of prey species within the groups.

Dietary overlap between sexes was high (0.98), suggesting little partitioning of prey (Nagorsen et al. 1989). My data do not support intraspecific competition as a selective pressure for sexual dimorphism. However, as martens were only sampled during early winter, low prey species abundance and diversity may increase intraspecific competition and dietary overlap regardless of sexual dimorphism.

Frequency of occurrence is useful to determine which species are most often consumed by predators. However, this technique may underestimate the relative contribution of larger prey species to predator fitness (Cumberland et al. 2001). Several small mammals may be completely consumed and indigestible parts (e.g., bones, teeth, and hair) can be identified in scat or contents in the GI tract (Strickland and Douglas 1987). However, marten may fill their stomach with one large prey species without ingesting identifiable parts, resulting in their underestimation in diet studies (Strickland and Douglas 1987). In New Brunswick, three large prey species (grouse, snowshoe hare, and squirrel) comprised only 31% frequency of occurrence in martens' diet (Cumberland et al. 2001). When minimum caloric intake was calculated for those same marten diets, these three prey species comprised about 95% of total calories consumed.

I found similar use of the six prey groups, based on kcals, by male and female martens (Figure 1.3). Small prey species (voles, mice, shrews) represented a small amount of the total ingested kcals and the importance of larger prey species (squirrels,

grouse, hares) was more pronounced. For example, squirrels were not consumed as frequently as small mammals. However, when occurrence values were converted to kcals, squirrels supplied more kcal than all other prey groups.

Age related diets

Juvenile and adult diets were similar, with low dietary breadth indicating martens were using a comparatively low number of species in early winter. As martens mature, improved hunting skills should decrease competition with juveniles (Raven et al. 2005). High dietary overlap of prey species between age classes suggests little partitioning of prey likely due to low diversity of prey species (Nagorsen et al. 1989).

Juveniles and adults acquired similar kcals from all prey groups except the mice/voles group (Figure 1.4 bottom). Factors influencing this are unknown; a comparative study of prey abundance was not performed. Hunting skills may be a factor, adults may have learned that it is more cost efficient to hunt abundant smaller prey than large prey that are less abundant whereas juveniles may be trying to capture larger prey (e.g., squirrels). Alternately, if juveniles were dispersing and had not established territories at the time of the trapping season, the difference in the mice/voles group may be attributed to adults having defended territories with abundant prey.

As with other studies of marten winter diets throughout North America, small mammals were consumed most frequently by the martens in my study (Strickland and Douglas 1987, Thompson and Colgan 1987, Nagorsen et al. 1989, Buskirk and Ruggiero 1994, Poole and Graff 1996, Bull 2000, Cumberland et al. 2001). Because the relative abundance of small mammals is usually greater than larger prey species, martens have an increased chance of encountering small mammals. However, kilocalories consumed were

a better indicator of the importance of large prey species. Squirrels provided the largest amount of kcals to martens in early December in Michigan's UP. This was unexpected as squirrels were not a frequent prey group in other winter diet studies. Similar to other studies, martens are using the largest available prey species (Buskirk and MacDonald 1984, Cumberland et al. 2001). The exception is that in other studies the largest available prey species were grouse and hares and in Michigan's Upper Peninsula it is squirrels.

CHAPTER TWO

POTENTIAL FOR SAMPLING BIAS IN DIET STUDIES OF AMERICAN MARTEN

INTRODUCTION

Numerous techniques have been used to estimate carnivore diets including direct observations, following tracks to kill sites, scat collection, stable isotope analysis, animal capture, and carcass collection (Murie 1961, Weckwerth and Hawley 1962, Spencer and Zielinski 1983, Buskirk and MacDonald 1984, Hargis and McCullough 1984, Raine 1987, Nagorsen et al. 1989, Nagorsen et al. 1991, Poole and Graf 1996, Ben-David et al. 1997, Simon et al. 1999, Bull 2000, Cumberland et al. 2001, Trites and Joy 2005). However, because many of these approaches are labor intensive or costly, sample sizes are often small.

Carcasses collected during established trapping seasons can offer advantages when studying diet of carnivores including potentially large sample sizes and decreased time spent obtaining samples because trappers collect specimens (Buskirk and MacDonald 1984, Thompson and Colgan 1987). A limitation associated with this method is that sampling typically is restricted to the authorized period of harvest.

It is usually advantageous to design studies with equal samples sizes by groups to be compared (i.e., a balanced design; Zar 1999); however, this can be difficult in field studies. When examining GI tracts from carcasses, it is common to find intestinal contents paired with empty stomachs because food spends more time in the intestine than in the stomach (Randall et al. 2000). In addition, trapping bias frequently results in

unequal samples of groups to be compared (e.g., more males than females, more juveniles than adults; Strickland and Douglas 1987).

Nagorsen et al. (1989) suggested keeping stomach and intestinal contents separate for analysis because they may contain prey items from two different meals, adding an unwanted source of variation. However, diet analyses in previous studies were conducted on combined data sets consisting of contents of the stomach, intestine, or both stomach and intestine (Nagorsen et al. 1989, Nagorsen et al. 1991, Poole and Graf 1996, Cumberland et al. 2001). My objectives were to: 1) compare dietary contents of the stomach and the intestines; and 2) estimate the effects of unequal sample sizes using diet comparisons between stomach and intestines and between sex and age classes.

METHODS

Gastrointestinal (GI) tracts of trapped martens were collected from the Upper Peninsula of Michigan (Latitude: 454442N–464917N, Longitude: 841437W–893001W) by the MDNR from the 2000–2004 trapping seasons and frozen until analysis. Sex, age, date, and location trapped were recorded for each marten. I used martens with complete information that were registered as harvested during the marten trapping season for analysis.

All martens harvested during 2000–2001 were used for analysis. Due to large numbers of carcasses and time constraints, not all carcasses were analyzed during 2002–2004. In 2002 and 2003, more males were trapped than females; in these years all females were sampled and an equal number of males were randomly selected for analysis. Because high numbers of both sexes were trapped in 2004, male and female martens from that year were randomly selected to equal numbers sampled during 2003.

Because stomachs and intestines of an individual marten may contain remains from different meals (Nagorsen et al. 1989), stomach and intestinal contents were kept separate and analyzed separately per marten to reduce that source of variability. Contents of GI tracts from 2000–2001 were analyzed for parasites prior to diet analysis (Veine 2004). Examined contents from GI tracts were placed in plastic bags, labeled, and frozen for diet analysis. Gastrointestinal tracts from 2002–2004 were opened and scraped of their contents. I rinsed all marten gut contents with 75% ethyl alcohol, separated hair samples into labeled containers and air dried hairs for identification (Weingart 1973).

I made negative impressions of hairs sampled from each marten's GI tract (Weingart 1973). I used a compound microscope at 40–400x magnification, hair identification guides (Adorjan and Kolenosky 1969, Moore et al. 1997, Andruskiw et al. 2003), and reference slides I made from museum specimens to identify prey species.

I designated six prey groups for analysis based primarily on body size: shrew, mice/vole, bird, chipmunk, squirrel, and grouse/hare. The shrew group included *Sorex* spp. and short-tailed shrews (*Blarina brevicauda*). The mice/vole group consisted of deer mice (*Peromyscus maniculatus*) and red-backed voles (*Clethrionomys gapperi*). The bird group was all bird species excluding ruffed grouse. The chipmunk group comprised eastern chipmunks (*Tamias striatus*), and the squirrel group included red squirrels (*Tamiasciurus hudsonicus*) and eastern gray squirrels (*Sciurus carolinenesis*). Ruffed grouse and snowshoe hares comprised the grouse/hare group.

I compared the proportion of occurrence of prey groups within the stomach and within the intestines using a chi-square test. I calculated kilocalories (kcal) for prey groups using mean body mass of prey (Brewer 1991, Kurta 1995, Cumberland et al.

2001). Unknown kcal values for prey species were estimated using linear regression based on species kcal values from Cumberland et al. (2001). I compared kcal's of prey groups between stomachs and intestines using analysis of variance. To determine if unequal sample sizes of these groups (e.g., contents from stomach and contents from intestines) influenced results, I generated ten data sets where half the stomachs were removed randomly. A chi-square test was used to compare frequencies of prey groups in each of the ten generated data sets of 75 stomachs' contents with the original 151 intestinal contents.

I conducted ten similarly designed analyses using male (n = 83) and female (n = 68) diets with half of the females (n = 34) randomly removed, and ten analyses using juvenile (n = 47) and adult (n = 104) diets with half of the juveniles (n = 23) randomly removed. Chi-square tests were used to compare each of the ten generated data sets of female contents with the original male contents, and each of the ten generated data sets of juvenile contents with the original adult contents. All analyses were conducted using STATISTIX 8 (Analytical Software 2003) with significance accepted when $P \leq 0.05$.

RESULTS

Proportions of prey groups in the stomach and in the intestine of 151 martens were similar ($\chi^2 = 2.13$, 5 df, $P = 0.83$; Figure 2.1), as were comparisons when half the number of the stomach content samples were removed from analyses ($\chi^2 = 1.56$–4.80, 5 df, $P = 0.44$–0.91). Estimated kcals present in stomachs and intestines by prey group were similar ($F = 0.00$–1.06, df = 1, $P = 0.30$–0.98).

Proportions of prey groups consumed by male and female martens were similar

($\chi^2 = 7.57$, 5 df, $P = 0.18$; Figure 1.3), similar to comparisons when half the number of female samples were removed from analyses ($\chi^2 = 2.87–10.12$, 5 df, $P = 0.07–0.72$). Proportions of prey groups consumed by juvenile and adult martens were similar ($\chi^2 = 4.45$, 5 df, $P = 0.487$; Figure 1.4), similar to comparisons when half the number of juvenile samples were removed from analyses ($\chi^2 = 2.06–5.06$, 5 df, $P = 0.41–0.84$).

DISCUSSION

Often, collecting samples for research is opportunistic. However, small sample sizes increase the probability of variability due to sampling error (Trites and Joy 2005). Diversity of individuals sampled (sexes and ages) must also be considered (Trites and Joy 2005). Being able to increase sample sizes and confidence in data can strengthen the results of a study.

Most often the stomach was the empty organ (Chapter 1). Traps typically used to capture marten in Michigan are body gripping and foot–hold traps. Body gripping traps kill captured animals quickly, preventing further digestion and passage of contents through the GI tract. In contrast, foot hold traps leave the marten alive until the trapper returns to collect the animal. The time between trapping and collection may be enough for the trapped marten to partially or completely empty its gastrointestinal tract. Thus, stomachs and intestines from different martens may both be used to estimate diet if they are sampled in equal proportions within each group to be compared (e.g., males: 50 stomachs and 200 intestines to females: 20 stomachs and 80 intestines). These results suggest that if unequal numbers of stomachs only and intestines only are sampled, random samples of stomachs and/or intestines can be selected from martens with contents

in both stomachs and intestines to make sample sizes of each group being compared equal, a considerable time-saving practice.

Martens in Michigan's UP during early winter used a small number of prey species which may explain in part the similarity between organ content. Regardless, there was no difference in dietary content when analyzing the contents of stomach or intestines. Therefore, all marten specimens (e.g. stomach, intestine) may be included in a diet study, increasing sample size. However, for studies conducted under different conditions, this method of analysis may not work. As animals consume more food, gut transit (the speed at which prey travels through the stomach) increases (Randall et al. 2000). For example, if martens are sampled at a time or place when prey is more abundant and more types of prey are present then gut transit would presumably increase. Under these conditions, the probability of organ contents differing increases.

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LITERATURE CITED

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Table 1.1. Number of prey items in gastrointestinal tracts from male (83) and female (68) American martens trapped in the Upper Peninsula of Michigan, December 2000–2004.

Prey items	2000		2001		2002		2003		2004	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
No. of martens analyzed	12	12	12	9	11	8	27	16	21	23
No. of prey items	31	42	29	23	29	22	80	44	66	67
Sorex spp.	3	5	4	0	2	4	3	2	5	4
Short-tailed shrew	7	6	5	11	4	8	13	6	5	6
Deer mouse	7	9	2	4	5	1	19	5	19	15
Red-backed vole	8	9	8	7	10	6	32	13	28	27
Bird	0	4	1	0	4	0	4	3	3	4
Eastern chipmunk	2	6	3	0	2	0	0	0	2	3
Red squirrel	2	3	3	0	0	0	7	6	0	3
Gray Squirrel	2	0	2	1	2	3	2	9	1	4
Ruffed grouse	0	0	0	0	0	0	0	0	1	0
Snowshoe hare	0	0	1	0	0	0	0	0	2	1

Table 1.2. Number of prey items in gastrointestinal tracts from juvenile (47) and adult (104) American martens trapped in the Upper Peninsula of Michigan, December 2000–2004.

Prey items	2000		2001		2002		2003		2004	
	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
No. of martens analyzed	6	18	8	13	8	11	15	28	10	34
No. of prey items	13	60	20	32	21	30	40	84	27	106
Sorex spp.	2	6	1	3	3	3	0	5	1	8
Short-tailed shrew	4	9	4	12	6	6	8	11	2	9
Deer mouse	0	16	2	4	1	5	3	21	6	28
Red-backed vole	2	15	7	8	7	9	22	23	9	46
Bird	2	2	0	1	2	2	3	4	1	6
Eastern chipmunk	1	7	2	1	0	2	0	0	3	2
Red squirrel	0	5	2	1	0	0	2	11	1	2
Gray squirrel	2	0	2	1	2	3	2	9	1	4
Ruffed grouse	0	0	0	0	0	0	0	0	0	1
Snowshoe hare	0	0	0	1	0	0	0	0	3	0

Table 1.3. Results of ANOVA of prey group kilocalories in stomach and intestine of male (83) and female (68) American martens of two age classes (47 juveniles and 104 adults) in the Upper Peninsula of Michigan, December 2000–2004.

Variables	Shrew		Mice/Vole		Bird		Chipmunk		Squirrel		Grouse/Hare	
	F	P	*F*	P	*F*	P	*F*	P	*F*	P	*F*	P
GI	0.00	0.98	1.06	0.30	1.04	0.31	0.11	0.74	0.38	0.54	0.01	0.91
SEX	2.75	0.10	1.35	0.25	0.58	0.45	0.03	0.86	1.57	0.21	1.71	0.19
AGE	0.14	0.71	6.37	0.01	0.14	0.70	0.02	0.89	0.54	0.46	2.36	0.13
GI*SEX	0.02	0.88	0.00	0.98	0.28	0.60	0.00	0.95	0.56	0.45	1.30	0.26
GI*AGE	1.06	0.30	0.10	0.76	0.03	0.86	0.11	0.74	0.46	0.50	1.30	0.26
SEX*AGE	1.66	0.20	0.18	0.67	2.17	0.14	2.34	0.13	2.71	0.10	0.08	0.78
GI*SEX*AGE	0.30	0.59	1.73	0.19	0.45	0.50	0.00	0.95	0.02	0.90	0.01	0.91

Table 1.4. Standardized dietary breadth of martens from studies across North America (from Nagorsen et al. 1989).

Study Area	Dietary breadth	Source
Upper Peninsula, Michigan	0.49	This study
Vancouver Island, B.C.	0.69	Nagorsen et al. 1989
Fort Nelson area, B.C.	0.31	Quick 1955
Rocky Mountains, Alberta and B.C.	0.19	Cowan and Mackay 1950
Eastern Cascades, Washington	0.24	Newby 1951
North central Idaho	0.22	Koehler and Hornocker 1977
Glacier national Park, Montana	0.36	Weckwerth and Hawley 1962
Sierra Nevada, California	0.22	Zielinski et al.1983

Table 2.1. Number of prey items in stomachs (n=151) and intestines (n=151) from American martens trapped in the Upper Peninsula of Michigan, December 2000–2004.

	2000		2001		2002		2003		2004	
	stomach	intestine	stomach	intestine	stomach	intestine	stomach	intestine	stomach	intestine
No. of martens analyzed	24	24	21	21	19	19	43	43	44	44
No. of prey items	40	33	33	19	24	27	64	60	68	65
Sorex spp.	2	6	3	1	2	4	2	3	5	4
Short-tailed shrew	7	6	8	8	6	6	10	9	4	7
Deer mouse	7	9	4	2	2	4	13	11	18	16
Red-backed vole	12	5	11	4	8	8	20	25	30	25
Bird	3	1	1	0	2	2	6	1	2	5
Eastern chipmunk	5	3	1	2	1	1	0	0	3	2
Red squirrel	3	2	2	1	0	0	7	6	2	1
Gray Squirrel	1	1	2	1	3	2	6	5	2	3
Ruffed grouse	0	0	0	0	0	0	0	0	1	0
Snowshoe hare	0	0	1	0	0	0	0	0	1	2

Figure 1.1. Michigan and the Upper Peninsula.

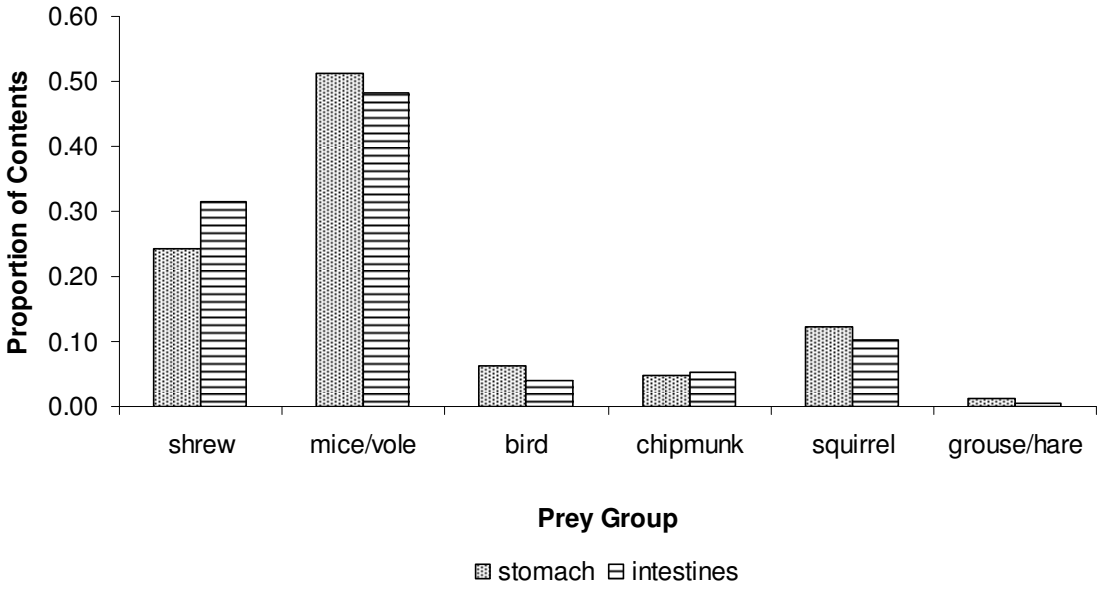


Figure 1.2. Proportion of prey groups in the stomachs and intestines of 151 trapped American martens in the Upper Peninsula of Michigan, December 2000–2004.

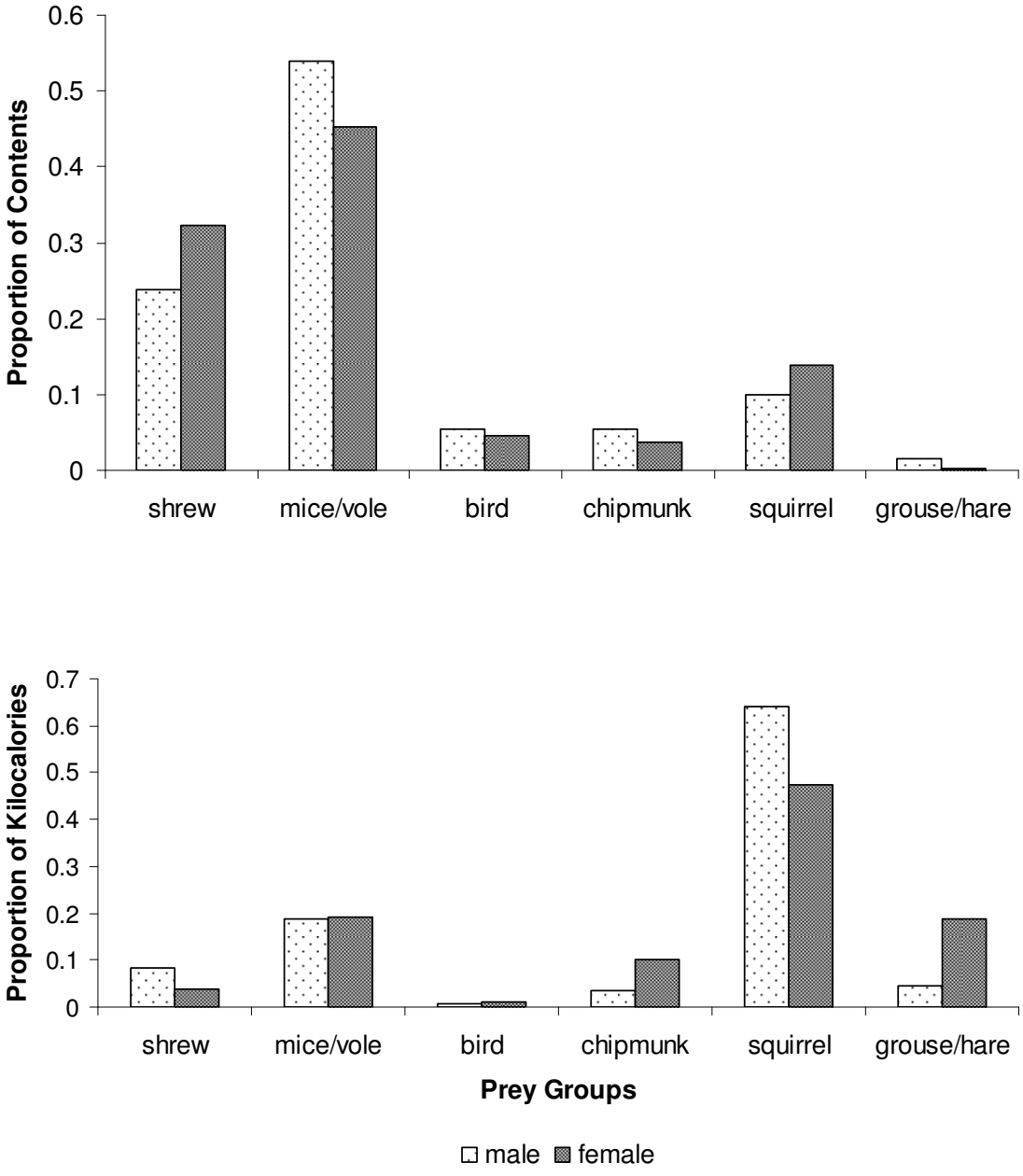


Figure 1.3. Proportion of prey groups in male (83) and female (68) American marten gastrointestinal tracts and proportion of kilocalories of prey groups in male (83) and female (68) American marten gastrointestinal tracts in the Upper Peninsula of Michigan, December 2000–2004.

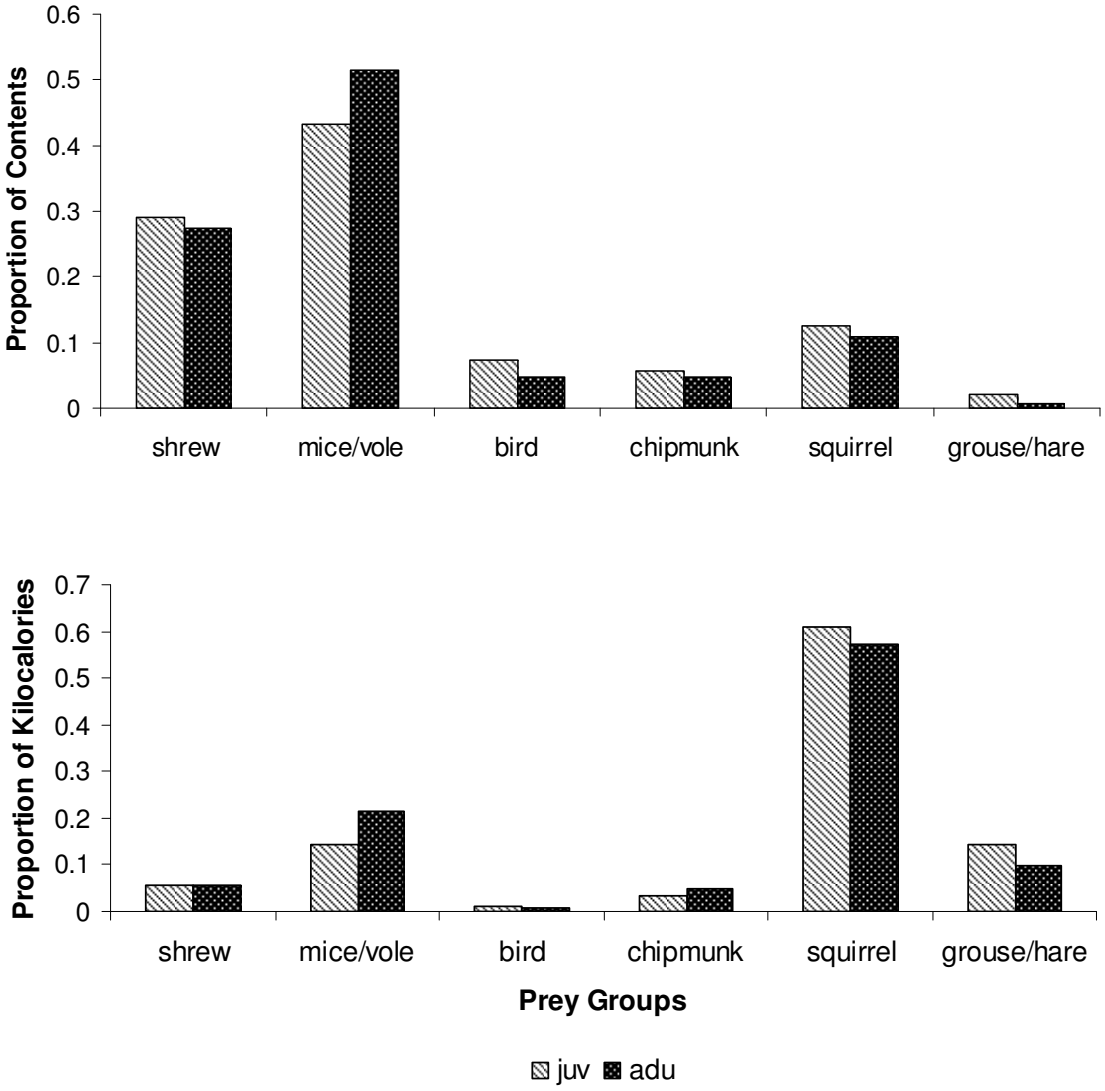


Figure 1.4. Proportion of prey groups in juvenile (47) and adult (104) American marten gastrointestinal tracts and proportion of kilocalories of prey groups in juvenile (47) and adult (104) American marten gastrointestinal tracts in the Upper Peninsula of Michigan, December 2000–2004.

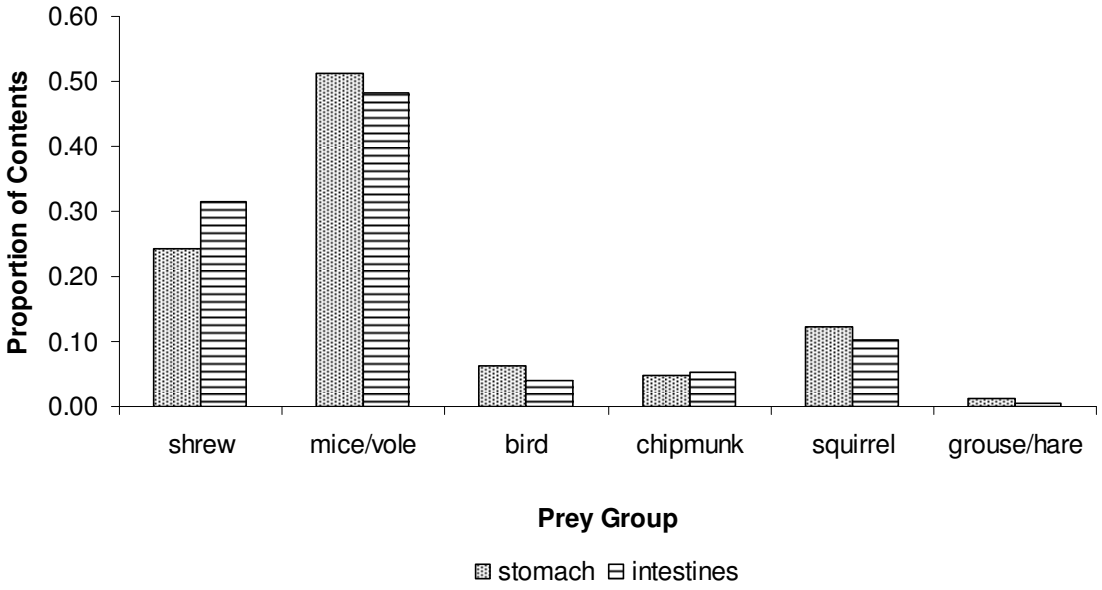


Figure 2.1. Proportion of prey groups in the stomachs and intestines of 151 trapped American martens in the Upper Peninsula of Michigan, December 2000–2004.

APPENDICES

APPENDIX A

American marten: protocol for processing specimens.

This protocol will remove stages of parasites from materials needed for diet analysis.

Process esophagus & stomach together, **AND** separately from large intestines (LI) & small intestines (SI).

Process LI & SI together.

DO NOT cross-contaminate cranial GIT with caudal GIT!!!

1. Freeze lungs, heart, kidneys, spleen, and liver for future parasitological examination.
2. **Stomach and esophagus**
   a. cut open and rinse contents into tray, scraping lining with tongue depressor.
   b. place gut wall in with rest of organs to be frozen (see 1 above).
   c. add enough water to examine thoroughly for adult worms. Use dissecting scope if necessary.
   d. place parasite specimens in labeled vial of 70% EtOH.

   Remaining stuff in tray, strain through tea strainer and empty strainer into labeled plastic bag and refreeze for diet analysis.

3. **Intestines**
   a. open intestines and rinse contents into tray, scraping lining with tongue depressor.
   b. add enough water to examine thoroughly for adult worms.
   c. strain through tea strainer into beaker (rinse strained contents well).
   d. allow beaker water column to sediment (1 minute per cm of water column).
   e. siphon off supernatant being careful to **NOT** disturb sediment at bottom.
   f. store sediments in labeled container of 70% EtOH for future parasite analysis.

   Place remaining contents in strainer into a labeled plastic bag and refreeze for diet analysis.

APPENDIX B

Marten gut content separation and parasite preservation protocol: used to separate stomach and intestinal contents for Marten diet project and preserve parasites for later projects.

Label 1 pint canning jars with pine marten identification (PM ID) number and stomach/intestine, keeping stomach and intestinal contents in separate jars. Next, place ID number, date and stomach/intestines on a piece of paper and place in the jar for later reference.

ONLY USE 70% EtOH TO RINSE INSIDE OF ORGANS AND CONTENTS!!! (this will preserve all parasite eggs and adults to be identified later)

1. Separate stomach from other organs and cut open. Scrape inside of stomach and rinse with 70% EtOH into a small bowl. Use more EtOH if needed to get contents floating.

2. Agitate contents with forceps to separate clumps. If there is bait or undigested flesh, separate and place into appropriate labeled 1 pint canning jar.

3. If liquid is too cloudy, strain the contents through small tea strainer into another bowl. Place liquid into appropriate labeled jar and dump strained contents back into bowl.

4. Add additional 70% EtOH till contents float and hairs can be separated. **DO NOT USE TOO MUCH EtOH OR IT WILL NOT FIT INTO THE JAR.**

5. Using forceps, sample the different hair types present using the protocol for negative impressions.

6. Place remaining contents and EtOH into the appropriate jar and place lid on securely.

7. Use a glass rod to push contents through the intestines and to facilitate cutting the intestines open. Repeat steps 1 – 6 for the intestinal contents.

8. Place labeled jars, with contents, into the jars original boxes and place to the side of the lab.

APPENDIX C

Negative Impressions Protocol: this protocol is for making negative impressions of hairs from gastrointestinal (GI) tracts of mammals to use in identifying prey species in American marten diet.

Label slide with marten identification number on upper surface. On the underside of the slide, label it with S (stomach) and I (intestines) with a line down the middle to separate the hairs found in the stomach and the intestines (see below). Labeling the S, I and line on the underside of the slide will keep the lines from smudging when the fingernail polish is added later.

<table>
<tr><td rowspan="2">PM04-01</td><td>S</td><td></td></tr>
<tr><td>I</td><td></td></tr>
</table>

1. Sample the contents of the stomach, taking several hairs of each type present, and place in a small beaker of 70% EtOH. This will remove oils, dirt and debris from hair cuticle.

2. Strain hair through 8 layers of cheese cloth and ring out the excess 70% EtOH.

3. Place cheese cloth, with hairs, in a beaker labeled stomach and repeat with the intestinal contents, keeping the content samples separate from each other.

4. Take labeled slide and using clear fingernail polish, cover the entire upper surface with a thin layer of polish.

5. Allow the polish to dry until tacky, ~30 seconds, and using forceps place the hairs on slide so there is little to no overlap. Samples from the stomach in the space labeled S, and samples from the intestines in the space labeled I.

6. Place a dry, clean slide on top of the sample slide. Be careful not to do this too quickly, the hairs will float away if not secured to the polish.

7. Place four spring clamps on the slides to ensure even pressure to entire slide. This step is important to ensure there are complete and even impressions of all the hairs on the slide.

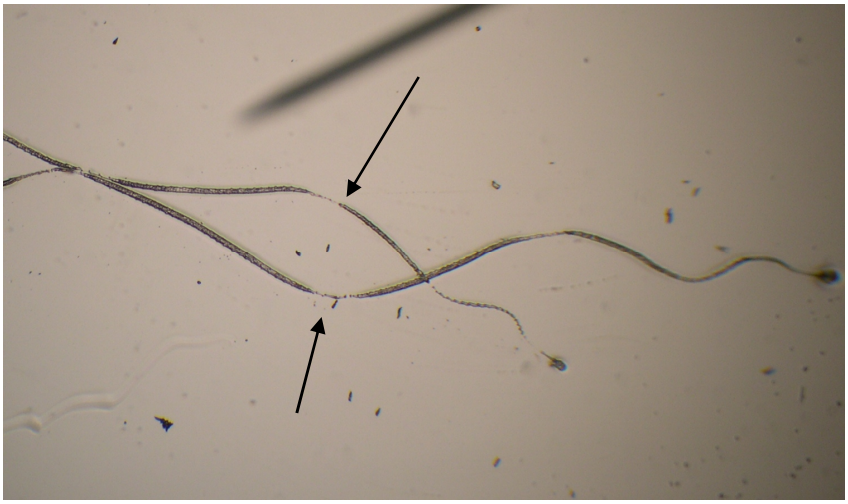
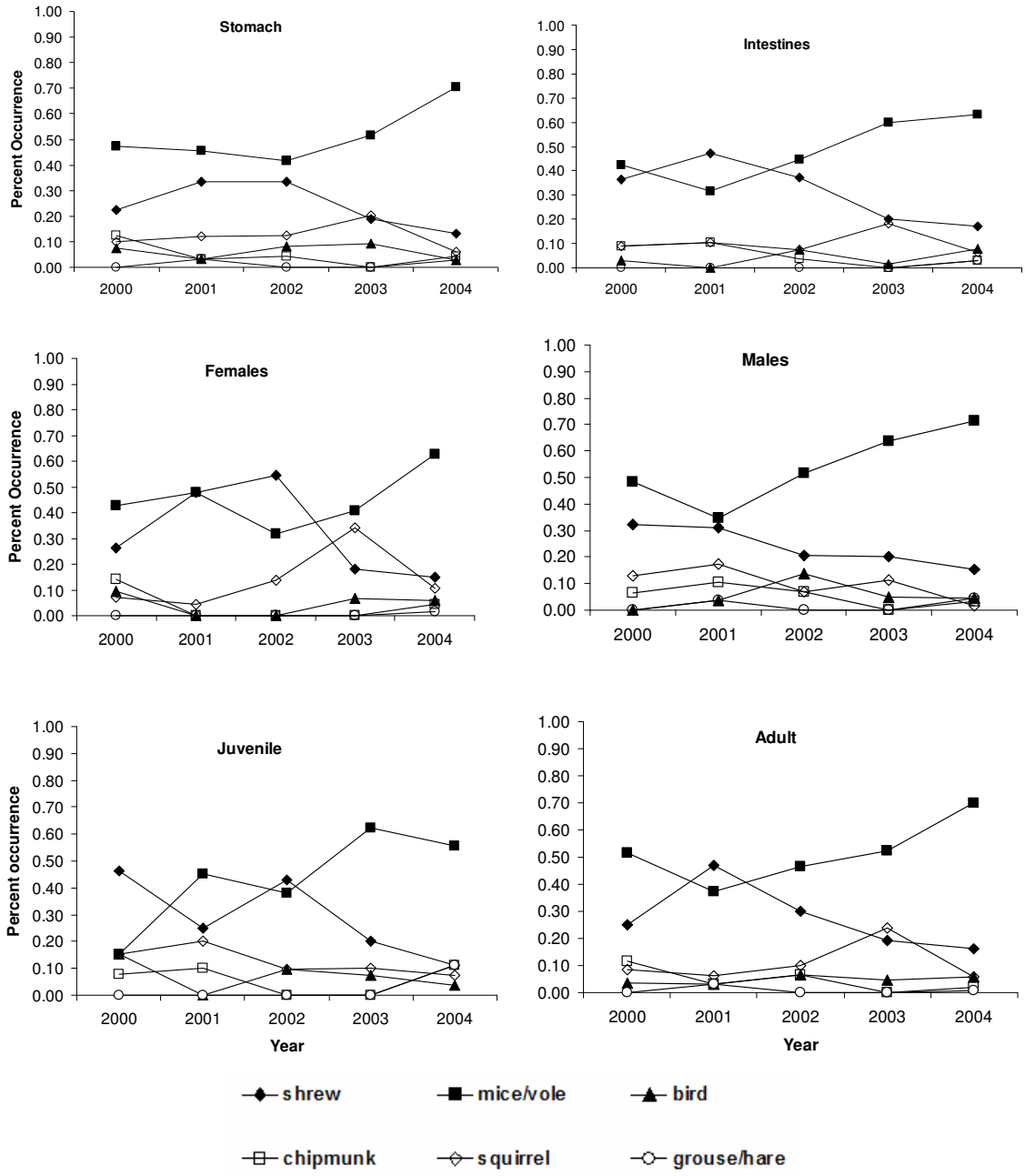
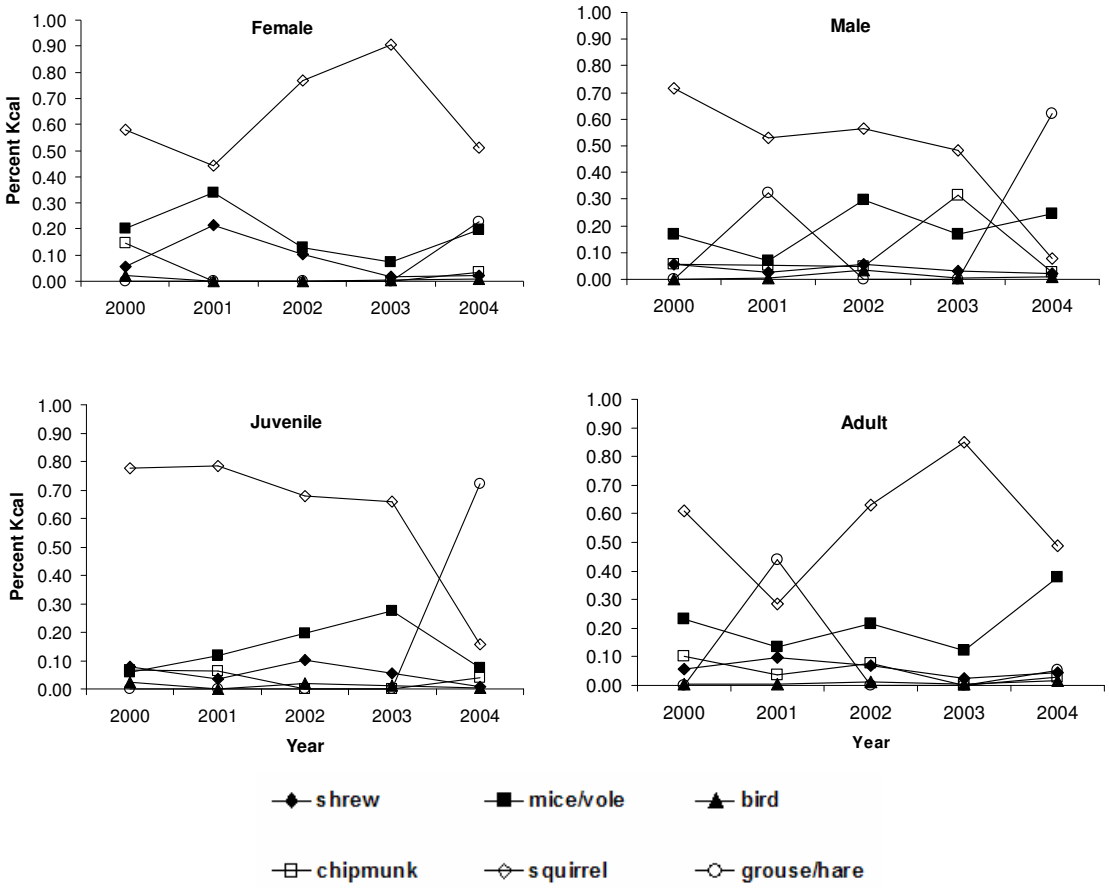


Figure C. 1. Example of short-tailed shrew hair negative impressions, 40x magnification. Strictures (indicated by the arrows) along the hair shafts are characteristic of shrew species.



Appendix D. Mean percent occurrence of six prey groups from stomach and intestinal contents of American martens from the Upper Peninsula of Michigan, December 2000 - 2004.



Appendix E. Mean percent kilocalories of prey groups from stomach and intestinal contents of American martens from the Upper Peninsula of Michigan, December 2000 - 2004.