Potential for sampling bias in diet studies of American Marten *Martes americana*

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Abstract

Numerous techniques have been used to estimate carnivore diets. When examining gastrointestinal tracts from carcasses of trapped animals, it is common to find intestinal contents paired with empty stomachs. In addition, trapping bias frequently results in unequal samples of groups to be compared (e.g. more juveniles than adults). We investigated the effect of unequal sample sizes on American Marten *Martes americana* diet by reducing sample size (by 50%) of one sampled group and repeating the statistical analyses. Comparison of ten constructed datasets showed that stomach and intestinal contents were similar, indicating that using either stomachs only, intestines only, or a combination of stomach and intestinal contents would not change test results. Ten identically designed stomach sample comparisons by sex and age class showed no differences in diets between groups, indicating that unequal sample sizes did not bias diet estimates.

Keywords: age class, food habits, sample size, sex class, USA

Introduction

Numerous techniques have been used to estimate carnivore diets including direct observations, following tracks to kill sites, scat collection, stable isotope analysis and carcass collection (Murie 1961, Weckwerth & Hawley 1962, Spencer & Zielinski 1983, Buskirk & MacDonald 1984, Hargis & McCullough 1984, Raine 1987, Nagorsen *et al*. 1989, 1991, Poole & Graf 1996, Ben-David *et al.* 1997, Simon *et al.* 1999, Bull 2000, Cumberland *et al.* 2001, Trites & Joy 2005). However, because many of these approaches are labour 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 & MacDonald 1984, Thompson & Colgan 1987). A limitation of this method is that sampling typically is restricted to the authorised period of harvest.

It is usually advantageous to design studies with equal sample sizes by groups to be compared (i.e., a balanced design; Zar 1999); however, this can be difficult in field studies. When examining gastrointestinal (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 & Douglas 1987).

For American Marten, 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 American Marten studies were conducted on combined data sets consisting of contents of the stomach, intestine, or both stomach and intestine (Nagorsen *et al*. 1989, 1991, Poole & Graf 1996, Cumberland *et al.* 2001).

In this study, our objectives were to: 1) compare dietary contents of the stomach and the intestines in American Marten; and 2) estimate the effects of unequal sample sizes using diet comparisons between stomach and intestines and between sex and age classes.

Methods

The study was conducted in the Upper Peninsula of Michigan, USA (45°45′–46°49′N, 84°14′–89°30′W), located on the southern shore of Lake Superior and covering an area of 42,610 km².

Gastrointestinal (GI) tracts of trapped martens were collected by the Michigan Department of Natural Resources during the December trapping seasons from 2000 to 2004 and frozen until analysis. Sex, age, date, and location trapped were recorded for each marten. Only martens with complete information that were registered as harvested during the legal trapping season were used 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 analysed 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 analysed separately. Contents of GI tracts from 2000–2001 were analysed for parasites prior to diet analysis (Veine 2004). Examined contents from GI tracts were placed in plastic bags, labelled, and frozen for diet analysis.

Gastrointestinal tracts from 2002–2004 were opened and scraped of their contents. We rinsed all marten gut contents with 75% ethyl alcohol, separated hair samples into labelled containers and air-dried hairs for identification (see Weingart 1973).

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

We designated six prey groups for analysis based primarily on prey body mass: shrew, mice/vole, bird, chipmunk, squirrel, and grouse/hare. The shrew group included *Sorex* spp.

and Northern Short-tailed Shrews *Blarina brevicauda*. The mice/vole group consisted of North American Deer Mice *Peromyscus maniculatus* and Southern Red-backed Voles *Clethrionomys gapperi*. The bird group was all bird species excluding Ruffed Grouse *Bonasa umbellus*. The chipmunk group comprised Eastern Chipmunks *Tamias striatus*, and the squirrel group included American Red Squirrels *Tamiasciurus hudsonicus* and Eastern Grey Squirrels *Sciurus carolinensis*. Ruffed Grouse and Snowshoe Hares *Lepus americanus* comprised the grouse/hare group.

We compared the proportion of occurrence of prey groups within the stomach and within the intestines using a chi-square test. We calculated kilocalories (kcal) for prey groups using mean body mass of prey (Brewer 1991, Kurta 1995, Cumberland *et al*. 2001). We initially regressed body mass of prey against known calorific values of prey species reported by Cumberland *et al.* (2001). This regression line was forced through the origin as a body mass of 0 g has no calories. We then used this linear regression model to estimate calorific values (kcal) of prey species where calorific values were unknown.

We compared kilocalories 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, we 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.

We then 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

We sampled 318 American Martens of which 151 contained food in both stomach and intestines that were used for analyses. Composition of the sample included 68 males and 83 females or 47 juveniles (<1 year old) and 104 adults (≥1 year old).

Proportions of prey groups in the stomachs and intestines of the 151 martens were similar (χ^2 = 2.13, df = 5, P = 0.83; Fig. 1), as were comparisons when half the number of the stomach content samples were removed from analyses (χ^2 = 1.56–4.80, df = 5, P = 0.44–0.91). Estimated kcal present in stomachs and intestines by prey group were also 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 (χ^2 = 7.57, df = 5, P = 0.18; Fig. 2), as were proportions of prey groups when half the number of female samples were removed from analyses (χ^2 = 2.87–10.12, df = 5, P = 0.07–0.72). Proportions of prey groups consumed by juvenile and adult martens did not differ (χ^2 = 4.45, df = 5, P = 0.487). Similarly, diet was comparable when half the number of juvenile samples were removed from analyses (χ^2 =2.06–5.06, df = 5, P = 0.41–0.84).

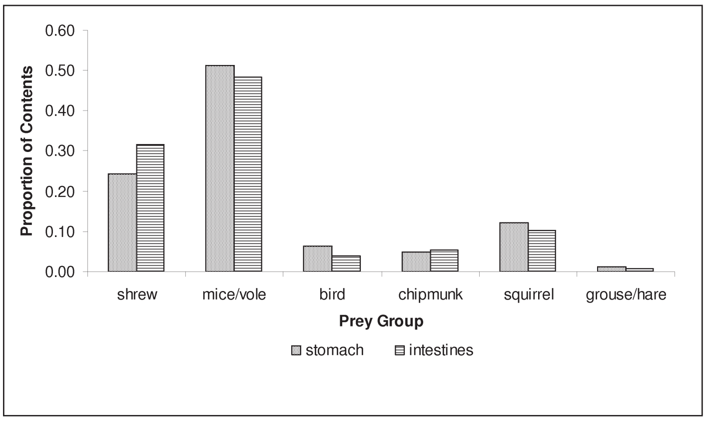

Fig. 1. Proportion of prey groups in the gastrointestinal tracts of 151 trapped American Martens, Upper Peninsula of Michigan, USA, Decembers of 2000 to 2004.

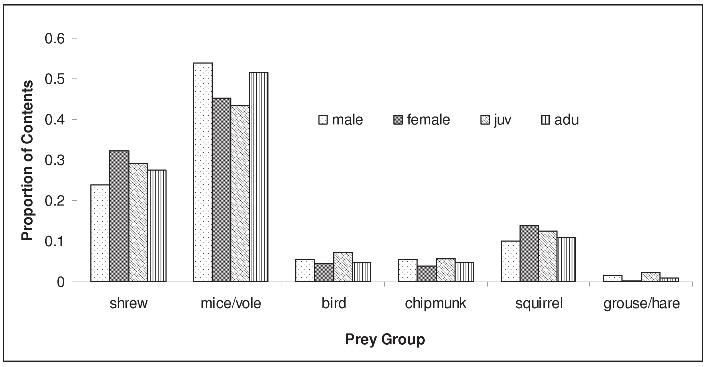

Fig. 2. Proportion of prey groups in male (n = 83), female (n = 68), juvenile (n = 47), and adult (n = 104) American Marten gastrointestinal tracts, Upper Peninsula of Michigan, USA, Decembers of 2000 to 2004.

Discussion

Often, collecting samples for research is opportunistic. However, small sample sizes increase the probability of variability due to sampling error (Trites & Joy 2005). Diversity of individuals sampled (e.g., by sex or age class) must also be considered (Trites & Joy 2005). Larger sample sizes can increase power of test results and investigator confidence in interpretation of data.

In this study, the stomach generally was the organ without prey items. Traps typically used to capture martens in Michigan are body-gripping and foot-holding traps. Body-gripping traps kill captured animals quickly, preventing further digestion and passage of contents through the GI tract. In contrast, foot-holding 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 GI tract.

Our data did not support the contention of Nagorsen *et al*. (1989) that stomach and intestinal food contents of American Martens need to be kept separate to avoid potential bias of some martens having multiple meals represented in analyses. Martens in Michigan's Upper Peninsula during early winter used a small number of prey species which may explain in part the similarity in diet between organ contents. Regardless, there was no difference in dietary content when analysing the contents of stomach or intestines. Therefore, at least in our study area, all marten specimens (e.g., stomach, intestine) may be included in a diet study which will markedly increase sample size.

This method of sampling may not be appropriate for studies conducted under different conditions. As animals consume more food, digestive transit (the speed at which prey travels through the stomach) increases (Randall *et al*. 2000), which will influence GI contents. Also, relative abundance of prey species and number of prey species available can influence diet contents. For example, if martens are sampled in a location where more types of prey are available, the probability of the number of species present in the entire GI tract being greater than the number of species present in the stomach or intestines increases. Under this scenario, the probability of organ contents differing also increases.

Acknowledgements

We thank A. Buck, J. Kustin and L. Johnson for their assistance with laboratory work. J. Bruggink provided helpful comments on an earlier draft of this manuscript. Funding was provided by Northern Michigan University, Department of Biology and the National Park Service, Pictured Rocks Science Center.

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