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Diet of *Sistrurus catenatus* in Ontario and Ohio: Effects of Body Size and Habitat

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ABSTRACT.—Knowing what venomous snakes eat is relevant both to their conservation and to understanding the functional diversity of their venom proteins. We used fecal samples to quantify the diet of Eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*) in Ontario and Ohio. Small mammals comprised almost the entire diet of both populations, collectively comprising 13 species, five of which were common to both populations. Consistent with their habitat use, Ontario snakes ate both forest and field mammals. The unexpected occurrence of Eastern Chipmunks (*Tamias striatus*) in the Ohio samples suggested that those snakes either moved out of the fields in which they were caught to feed, or encountered chipmunks dispersing along fence rows. Large snakes did not drop small prey species from their diets and the occurrence of large prey species in diets indicates that juvenile small mammals are important prey. Limited effects of snake size on diet composition suggest that ontogenetic shifts in venom composition are unlikely to occur in the Eastern Massasauga. The similarity of diets between populations makes it unlikely that populations differ in venom composition because of local adaptation of venom proteins to different suites of prey.

What an animal eats is a fundamental aspect of its natural history (Greene, 1994). Diet is likely to affect the habitats an animal uses, and knowing what a species eats can also be informative about its evolution. For venomous snakes, diet and venom composition are expected to coevolve (Daltry et al., 1996). There is increasing evidence that a high level of functional diversity in venom proteins at both the species and population levels (Daltry et al., 1996; Sanz et al., 2006) is generated through strong positive diversifying selection acting on genes coding for venom proteins (Gibbs and Rossiter, 2008) that can have prey-specific effects (Jorge da Silva and Aird, 2001). An essential step in establishing a link between venom composition and diet is identifying what snakes eat and whether diet differs among populations. Here we report results of diet analyses for populations of Eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*) in Ontario and Ohio.

The Eastern Massasauga is distributed from western New York and southern Ontario to eastern Iowa and Missouri but occurs in a number of disjunct populations within this range. Although all of these populations are of conservation concern (Szymanski, 1998), little is known about the evolutionary distinctiveness of each population. H. L. Gibbs and J. Chiucchi (unpubl. data) have found differences in venom composition between *S. c. catenatus* populations that parallel differences in microsatellite DNA loci (Gibbs et al., 1997). Thus, local adaptation of venom composition to diet could occur at a population level, making this an appropriate taxon for relating population-level venom variation to variation in diet (Gibbs and Rossiter, 2008). Our goal of documenting diets of two populations will contribute to these broader issues.

Holycross and Mackessy (2002) reviewed available information on *Sistrurus* diets. For Eastern Massasaugas, this consisted of anecdotal information plus diet studies from Michigan (Hallock, 1991) and Wisconsin (Keenlyne and Beer, 1973). More recently, Shepard et al. (2004) studied the diet and prey preferences of neonatal Eastern Massasaugas from Illinois. From these studies, two patterns emerge. First, Eastern Massasaugas prey primarily on small mammals. Second, although adults feed almost exclusively on small mammals, neonates eat small mammals but also include snakes in their diet. Ontogenetic shifts in diet are common in snakes, involving either broadening the diet to include bigger prey as snakes grow large enough to ingest them or dropping smaller prey as bigger prey are added to the diet (Arnold, 1993). A specific objective of our study was to determine which of these patterns occurred in the populations we investigated.

In providing diet information from two massasauga populations for which diets have not been analyzed previously, we also assess the extent to which diet shifts with a change in habitat. Through much of its range, the Eastern Massasauga is described as occurring primarily in wetlands and grasslands (Wright, 1941; Reinert and Kodrich, 1982; Seigel, 1986; Johnson, 2000). However, the Ontario population we studied primarily uses forested areas, with less use of open-canopy habitats (Weatherhead and Prior, 1992; Harvey and Weatherhead, 2006a). Thus, the Ontario snakes' diet may be broader than those of other populations, including the Ohio population sampled here.

MATERIALS AND METHODS

Data from Ontario were collected at two study sites 12.5 km apart on the Upper Bruce Peninsula between 2001 and 2004 as part of a broader study. Snakes at both locations had ready access to a variety of forested

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and nonforested habitats, including wetlands. Details of the general study methods and habitat are provided by Harvey and Weatherhead (2006a). Fecal samples for diet analysis were collected opportunistically from snakes that defecated while being held in captivity for 2–3 days for transmitter implantation or marking. Collection dates were relatively evenly distributed between 2 June and 1 October, covering most of the active season for this population. Data from Ohio were collected between 2004 and 2007 from seven study sites scattered around the state. One of the Ohio study sites was a cedar bog, whereas the other sites were all open habitats (remnant prairie, field, fen) near wetlands. Most Ohio snakes were captured using cover boards, with some captured opportunistically by researchers walking through the study sites. Fecal samples were collected while snakes were in captivity for marking. Sampling dates were relatively evenly distributed between 2 May and 6 October, again covering most of the active season for the population. All snakes in Ontario were sexed and measured (snout-vent length) with the exception of two individuals for which diet information came from direct observation of feeding in the field. Also, all snakes in Ohio were sexed and measured; although for some fecal samples, those data were not recorded. Thus, for analyses of diet relative to snake size, sample sizes are smaller than for the overall analyses.

Fecal samples were mixed with ethanol and examined under a dissecting microscope to identify the general category of prey. Mammalian prey included hair, which we subsequently used to identify prey species using hair impressions on polyvinyl acetate (Williamson, 1951). We used hair samples from museum specimens as our reference collection. We examined a minimum of three hairs per sample if the hairs appeared uniform within the sample and up to 10 hairs when hairs appeared more variable. Although it is not unusual for a single snake to contain more than one prey item (Hollycross and Mackessy, 2002), previous analyses of snake feces suggest that finding more than one prey type in a single fecal sample is uncommon (Weatherhead et al., 2003; Carfagno et al., 2006), presumably because different prey items are consumed at different times and travel through the gut separately. Snake prey in fecal samples were identified by skin, but we did not attempt to identify snake species. For analyses of prey size, we used average adult sizes of mammals from Burt and Grossenheider (1964), although we had no way to determine the size of the actual individual that had been eaten. A potential shortcoming of using fecal analysis to determine diet is that some prey species (e.g., anurans) do not leave identifiable remains in feces. However, such prey items would still result in feces being produced; thus, we documented the number of samples that contained no identifiable remains (i.e., hair or snake skin).

RESULTS

We identified prey from 44 of 48 fecal samples from Ontario and from 40 of 44 samples from Ohio. The four samples with unidentified prey from Ontario contained neither hair nor snake skin. One of the unidentified Ohio samples contained neither hair nor snake skin, and the other three were mammalian prey

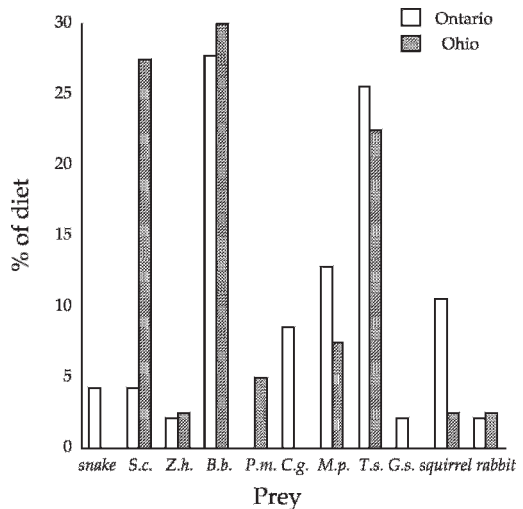


FIG. 1. Diet composition of *Sistrurus catenatus* from Ontario and Ohio based on samples with identifiable prey. Mammalian prey, ordered by increasing adult size from left to right, are Masked Shrew (*Sorex cinereus*), Meadow Jumping Mouse (*Zapus hudsonicus*), Northern Short-Tailed Shrew (*Blarina brevicauda*), Deer Mouse (*Peromyscus maniculatus*), Boreal Redback Vole (*Clethrionomys gapperi*), Meadow Vole (*Microtus pennsylvanicus*), Eastern Chipmunk (*Tamias striatus*), Northern Flying Squirrel (*Glaucomys sabrinus*), Red Squirrel (*Tamiasciurus hudsonicus*)—ON/Eastern Fox Squirrel (*Sciurus niger*)—OH and Snowshoe Hare (*Lepus americanus*)—ON/Eastern Cottontail (*Sylvilagus floridanus*)—OH.

that we could not match to our reference collection. Among identified prey, all Ontario samples included a single prey type, whereas one of the Ohio samples included two prey species. Two Ontario samples were snakes and the rest (88%) were mammals (including the two direct feeding observations). In the Ohio samples, all the identified prey were mammals; thus, overall, mammals accounted for 97.6% of Ohio prey. We identified nine different mammal species from Ontario samples and eight mammal species from Ohio (Fig. 1). Five mammal species were common to the diets of both populations. For both populations, the most common prey species was *Blarina brevicauda*. Chipmunks (*Tamias striatus*) were the second most common prey in Ontario and the third most common in Ohio. The occurrence of chipmunks and squirrels in the diets of both populations suggests that the snakes were foraging in forest, although the occurrence of species such as *Microtus pennsylvanicus* in samples from both populations indicates that the snakes were also hunting in more open habitats.

The range of prey sizes was extensive, from *Sorex cinereus* (4 gm) to rabbits that would be much larger than the snakes as adults. However, we found little evidence of ontogenetic shifts in diet (Fig. 2). The two samples that contained snake remains came from small individuals, but for samples with mammalian prey, individual prey species occurred across a range of snake sizes. If prey are categorized into small

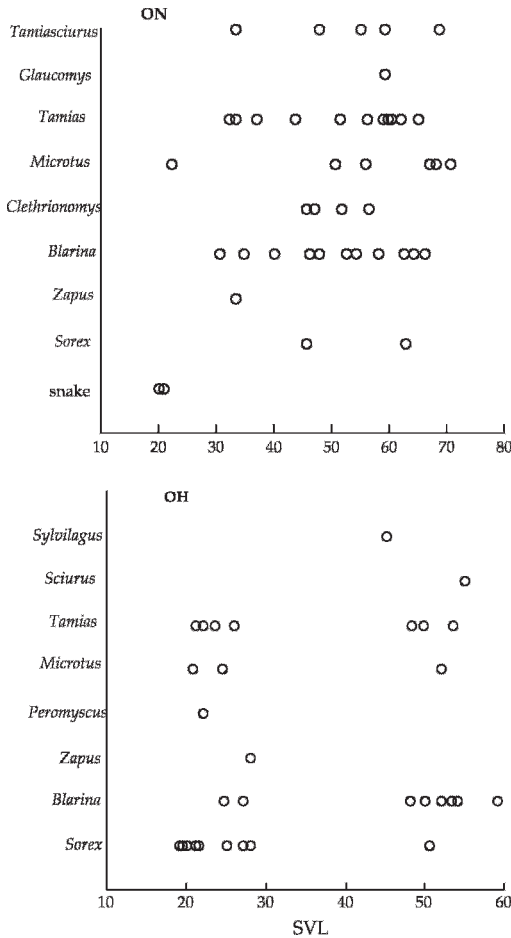


FIG. 2. Prey of *Sistrurus catenatus* relative to size (snout-vent length) of the snake from which the sample was obtained for Ontario and Ohio samples with identifiable prey. Mammals are arranged in ascending order of adult size (for full names, see Fig. 1). Note that not all samples are represented here because of missing SVL measurements.

(<50 g mean adult size: *Microtus* and smaller) and large (>90 g mean adult size: *Tamias* and bigger), the mean SVL \pm SD of snakes eating small and large prey did not differ for Ontario (50.7 ± 12.7 cm vs. 52.8 ± 11.9 , $t = -0.54$, $P = 0.59$, $N = 26, 18$) or Ohio (34.2 ± 14.4 vs. 38.2 ± 14.7 , $t = -0.70$, $P = 0.48$, $N = 24, 9$). These results suggest that the absence of intermediate-sized snakes in the Ohio samples (Fig. 2) is unlikely to have affected the analysis of prey size versus snake size.

DISCUSSION

Eastern Massasaugas in Ontario and Ohio had similar diets. Mammals comprised 88% of the diet in Ontario and over 97% of the diet in Ohio. Diets were diverse in both populations, including eight and nine mammal species, respectively, that ranged from small shrews to rabbits and included both forest and grassland species. We found little evidence of an

ontogenetic shift in diet. Although the only two samples that contained snake remains came from small individuals, small snakes also consumed a wide variety of mammal species. Given that adults of some of the mammal species we identified would be much too large for many of our snakes to consume, juvenile mammals appear to be common prey. Based on the four samples from Ontario and one from Ohio that contained neither hair nor snake skin, anurans could be a minor component of the diets of both populations. However, because other potential prey (e.g., neonatal rodents) could also account for these unknown samples, they should be interpreted cautiously.

Our results are consistent with evidence from other populations of Eastern Massasaugas in several ways. All Eastern Massasaugas appear to be dietary generalists; anurans are rare prey at best; and among the variety of mammal species eaten, shrews are especially important prey (Holycross and Mackessy, 2002; Shepard et al., 2004). Based on documented patterns of habitat selection (Weatherhead and Prior, 1992; Harvey and Weatherhead, 2006a), we had predicted that Ontario snakes would have a broader diet that included prey found in forests. In fact, we found this pattern in Ontario and Ohio. Chipmunks were common prey in both locations, and squirrels were common in Ontario and one was found in an Ohio sample.

The occurrence of forest prey in the Ohio samples was not a result of the snakes specializing on forest mammals at one study location because chipmunks occurred in samples from five sites. There are two possible explanations for the Ohio results. First, it is possible that snakes foraged in forests and then moved to fields to use cover boards for thermoregulation following prey ingestion (e.g., Blouin-Demers and Weatherhead, 2001). Because the nearest forests were at least several hundred meters distance, this would have required considerable commuting by the snakes. Alternatively, the forest mammals may have moved out of the forest. Chipmunks use fencerows as dispersal corridors (e.g., Bennet et al., 1994; Bowman and Fahrig, 2002); hence, the Ohio snakes may have been ambushing chipmunks moving along nearby fencerows.

We infer from the collective dietary data that the Eastern Massasauga is an opportunistic predator. Large snakes continue to include small prey in their diet, suggesting that they take prey as they are encountered. Also, changes in habitat appear to result in changes in diet. For example, in Wisconsin more than 85% of the diet was *Microtus pennsylvanicus*, which likely reflected prey availability where the snakes were studied (Keenlyne and Beer, 1973), whereas in our study *Microtus* was a relatively minor prey type. From a conservation perspective, this dietary flexibility should be a positive attribute. As long as there are small mammals available, the snakes should be able to eat; therefore, other aspects of habitat quality (e.g., availability of suitable hibernacula; Harvey and Weatherhead, 2006b) are likely to be more important to the snakes than the abundance of one particular prey species.

These results have implications for understanding venom evolution in *Sistrurus*. First, confirmation of the

result from previous studies that mammals dominate the diets of *Sistrurus catenatus* implies that, if a link exists between venom toxicity and prey consumed (e.g., Daltry et al., 1996), then venom of *S. catenatus* should be especially toxic toward mammals relative to other vertebrate prey (e.g., anurans) that are rarely consumed. This appears to be the case (H. L. Gibbs and S. P. Mackessy, unpubl. data). Second, the lack of strong age-related effects on diet composition suggests that the ontogenetic shifts in venom composition documented in other species (e.g., Mackessy, 1988) may occur to only a limited extent in *S. c. catenatus*, as has been found in *Sistrurus miliarius barbouri* (Deyrup et al., 2000). Finally, the overall similarity of diets between these two populations of *S. catenatus* implies that any population level differences in venom composition are not likely caused by local adaptation of venom proteins to different suites of prey (e.g., Daltry et al., 1996). Therefore, other explanations for differences in venom composition among populations must be sought (e.g., Gibbs et al., 2009).

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