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The Problems with Pooling Poop: Confronting Sampling Method Biases in Wolf (*Canis lupus*) Diet Studies

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T.D. Gable, S.K. Windels, and J.G. Bruggink

Abstract: Wolf (*Canis lupus* L., 1758) diet is commonly estimated via scat analysis. Several researchers have concluded that scat collection method can bias diet estimates but none of these studies properly accounted for inter-pack, age-class, and temporal variability, all of which could bias diet estimates. We tested whether different scat collection methods yielded different wolf diet estimates after accounting for these other potential biases. We collected scats (n = 2 406) monthly from 4 packs via 3 scat collection methods (at homesites, at clusters of GPS locations, and opportunistically) in and adjacent to Voyageurs National Park, Minnesota during April 2015–October 2015. Diet estimates were not affected by scat collection method but did vary temporally, among packs, and by age-class. To more accurately estimate wolf population diets, researchers should collect 10–20 adult scats/pack/month from homesites and/or opportunistically from packs that are representative of the population of interest. Doing so will minimize the potential biases associated with temporal, inter-pack, and age-class variability.

Keywords

Biases, *Canis lupus,* diet, gray wolf, Minnesota, scat analysis, wolf diet

Introduction

"Carefully correcting for biases inherent in indirect methods of diet determination has a profound effect on the assessment of diet composition and the estimated number of prey animals killed by a carnivore population." – Wachter et al. 2012

Estimating the diet of carnivores is important for understanding predator behavior and ecology, including predator-prey relationships, disease transmission, and energetics. Carnivore diets are most commonly determined by collecting scats and identifying the prey remains present (Klare et al. 2011). The assumption when estimating diet via scat analysis is that the scats collected are representative of all the scats deposited for a particular population (Steenweg et al. 2015). When this assumption is violated, diet estimates are biased to some, often unknown, degree. Because diet estimates from scat analysis are indirect, biases will always be present to some degree but should be addressed whenever possible to reduce error and increase the accuracy of diet estimates.

Many biases in gray wolf (*Canis lupus* L., 1758) diet estimation via scat analysis have been identified (Ciucci et al. 1996, 2004; Spaulding et al. 2010), and in some cases, solutions to minimize biases have been developed (Floyd et al. 1978; Weaver and Fritts 1979; Weaver 1993). Recently, Steenweg et al. (2015) concluded that scats collected at homesites yielded a different estimated diet than scats collected on roads or trails (we refer to these as opportunistically-collected scats hereafter), which is consistent with several other studies (Theberge et al. 1978; Scott and Shackleton 1980; Fuller 1989; Trejo 2012). However, these studies pooled scats over meaningful pack (Voigt et al. 1976; Fuller and Keith 1980; Potvin et al. 1988), age-class (Theberge and Cottrell 1977; Bryan et al. 2005), and temporal (Van Ballenberghe et al. 1975; Kohira and Rexstad

1997; Tremblay et al. 2001) sampling units prior to examining the affect of scat collection methods on diet estimates. Indeed, pooling scats over these meaningful sampling units is pervasive in wolf diet studies and diet estimates from many studies could be biased (e.g. similar to ‘pooling fallacy’, Machlis et al. 1985) due to temporal, inter-pack, or age-class variability (Schooley 1994). Thus, our objectives were to 1) determine whether different scat collection methods (scats collected opportunistically, at homesites, or at GPS clusters) yield different wolf diet estimates after accounting for the 3 potential biases mentioned above (pack, age-class, and temporal) and 2) provide a practical sampling framework to collect scats for estimating wolf population diet while confronting these 3 potential biases.

Materials and Methods

Study area

Our study area was conducted in and adjacent to Voyageurs National Park (VNP; 48°30' N, 92°50' W), Minnesota, USA, an 882 km^2 protected area along the Minnesota-Ontario border. This area is in the Laurentian Mixed Forest Province, a transition zone between the southern boreal forest and northern hardwood forest (Bailey 1980). The portion of our study area south of VNP was primarily in the Kabetogama State Forest, which is actively managed for timber, resulting in a mosaic of clear cuts, young aspen (*Populus* spp.) stands, mature deciduous-coniferous stands, and wetlands. Four large lakes (Kabetogama, Rainy, Namakan and Sandpoint) cover 342 km^2 (39%) of the park and many smaller lakes are scattered throughout the landmasses in and adjacent to the park. Beaver impoundments are abundant throughout our study area, and VNP has sustained high beaver densities for over 40 yr (Johnston and Windels 2015). Lakes in

VNP freeze during late October to mid-November with ice-out occurring during late April to early May (Kallemeyn et al. 2003).

White-tailed deer (*Odocoileus virginianus* Zimmerman, 1780) are common in this area while moose (*Alces americanus* L., 1758) are relatively rare (Windels and Olson 2016; Gable et al. 2017). Wolf densities are high (4–6 wolves/100 km^2) in the park with average home ranges of 115.8 km^2 (Gable 2016). Coyotes (*Canis latrans* Say, 1823) are rare in our study area (VNP, unpubl. data). Hunting and trapping are not allowed in the park. However, harvest of white-tailed deer and American beaver (*Castor Canadensis* Kuhl, 1820) and other furbearers is legal south of the park. Wolves were federally protected throughout Minnesota during our study but were illegally killed outside VNP occasionally (VNP, unpubl. data).

Wolf capture and collaring

Wolves from 4 packs (Ash River Pack, Moose River Pack, Sheep Ranch Pack, Shoepack Lake Pack) were captured during 2012–2015 using #7 EZ Grip foothold traps (Livestock Protection Company, Alpine, Texas). Wolves were immobilized with 10 mg/kg ketamine and 2 mg/kg xylazine using a syringe pole. Once immobilized, wolves were fitted with global positioning system (GPS) telemetry collars (Lotek IridiumTrackM 1D or 2D, Lotek Wireless Inc, Newmarket, Ontario, Canada; Vectronic Vertex Survey, Vectronic Aerospace, Berlin, Germany). Morphological measurements, tissue samples, and blood were collected. Sex and age also were recorded. Wolves were reversed with 0.15 mg/kg of yohimbine, and monitored through recovery. Fix intervals of GPS collars were set to 20 minutes, 4 hours, 6 hours or 12 hours, depending on the collar type, where the pack was located, and whether or not there was >1 collar in the pack at that time. All capture and handling of wolves was approved by the National Park Service's Institutional Animal Care and Use Committee (protocol MWR_VOYA_WINDELS_WOLF). We estimated home ranges during the ice-free season (April–October) using the 95% adaptive kernel home range method and the Home Range Tools 2.0 extension for ArcGIS (Mills et al. 2006).

Scat collection

We collected wolf scats from 4 packs from April 2015 to October 2015. We collected scats opportunistically (roads and trails), at homesites, and at GPS clusters when possible. Clusters were defined as consecutive locations that were within 200 m of each other for ≥4 hours (Latham 2009). We identified wolf homesites using location data from GPS-collared wolves or from triangulation via howl surveys. We collected scats at homesites after wolves had left the homesite or at the end of each month. We differentiated between adult and pup scats at homesites, assuming that scats with a diameter <2.5 cm were pup scats, and those ≥2.5 cm were adult scats (Ausband et al. 2010; Stenglein et al. 2010). We assumed that scats collected opportunistically or at GPS clusters were only from adult wolves. We collected scats opportunistically in known wolf home ranges on the same network of trails and roads every 1 to 3 weeks as well as at the end of each month to ensure a known month of deposition. Collected scats were placed into individual plastic sample bags labeled with date and location information.

We sterilized the scats by transferring them to nylon stockings and placing them in boiling water for >45 min (Chenaux-Ibrahim 2015). We then washed the scats in a washing machine, and allowed them to air dry for >12 h. We identified prey remains in each scat using the point-frame method (Ciucci et al. 2004). In our application of this method, we placed a grid with 12 randomly-selected points over the evenly spread-out dried scat contents and selected 12 hairs (1 from each of 12 randomly-selected points). Each of these 12 hairs were then are identified to species and age class, where possible, based on their micro- and macroscopic characteristics (Gable 2016). We selected 12 hairs per scat as sensitivity analysis has demonstrated that there is no difference in diet estimates when selecting 12 or 25 hairs/scat (Chenaux-Ibrahim 2015). When necessary, we made casts of the cuticula using all-purpose household cement. After the 12 hairs were identified, each scat was visually examined to verify all prey items had been identified. If >1 prey item was identified in the scat via the point-frame method or visual examination, we then visually estimated the relative dry volume (we refer to this as 'percent volume') of each prey item to the nearest 5% (Tremblay et al. 2001; Chavez and Gese 2005). We quantified the percent volume of each prey item using visual examination because this allowed us to estimate the percent volume of non-mammalian prey items as well as the percent volume of prey remains other than hair (e.g., bone, hooves, claws, etc.). Scats containing only 1 prey item were considered to constitute 100% of the volume of that scat. We considered trace amounts of hair detected (i.e., ≤10 individual hairs) from 1 prey item as 1% of the scat.

We used Weaver's (1993) regression equation (Eq. 1) to convert from percent volume to percent biomass.

$$\hat{Y} = 0.439 + 0.008 \times X \qquad \text{Eq. 1}$$

In Equation 1, X is the live mass of a prey species and $\hat{Y}$ is the prey mass per scat. The percent biomass is calculated by multiplying the $\hat{Y}$ by the percent volume.

We used a live mass of 4 kg for deer fawns from May and June, 14 kg for July and August, and 75 kg for adult deer from June to August (Fuller 1989; Chenaux-Ibrahim 2015). We were only able to differentiate between adult and neonate ungulate hair until the end of August. As a result, we estimated the live mass of deer consumed by wolves from September and October using the ratio of 7 adults:3 fawns found at kill sites in and around the study area in the fall to give weighted mean masses of 60.9 kg in September and 63.3 kg in October (Fuller 1989). We considered the mass of adult moose to be 444 kg and calf moose to be 20 kg from May to June (Chenaux-Ibrahim 2015). We only documented adult moose in wolf diet during May–August and calves during May–June. We used 14.4 kg and 16.7 kg for the spring (April–June) and fall (July–October) live mass of beaver, respectively, based on beaver trapping data (Windels, unpubl. data) and the average age of wolf-killed beavers in the area (Gable, unpubl. data). We used 1.5 kg for snowshoe hares (*Lepus americanus* Erxleben, 1777), 0.25 kg for small mammals, and 100 kg for black bears (*Ursus americanus* Pallas, 1780) (Chenaux-Ibrahim 2015). We converted percent volume of berries (primarily *Vaccinium* spp. and *Rubus* spp.) to biomass using a conversion factor of 0.468 kg/scat (Gable et al. 2017).

We determined how many scats/pack/month should be collected to estimate monthly pack diets using rarefaction curves (Prugh et al. 2008; Dellinger et al. 2011). To do so, we randomly subsampled without replacement from the scats collected from each pack each month, and determined diet diversity (Shannon's diversity index) as each scat was added to the monthly sample (Prugh et al. 2008). We repeated this 100 times and took the mean of the 100 simulations to yield a rarefaction curve. We used 9 categories (adult deer, fawn deer, adult moose, calf moose, beaver, berries, black bear, small mammals, snowshoe hare) to assess diet diversity. When rarefaction curves reached an asymptote we assumed that was the 'true' diet diversity (Prugh et al. 2008). For curves that had not reached an asymptote, we estimated where the curve would likely reach an asymptote based on the shape of the curve. We then estimated diet diversity at 10 and 20 scats for each month and calculated what percent of the 'true' monthly diet diversity that was. We then averaged these percentages to estimate how close diet diversity was to the 'true' diet diversity if 10 and 20 scats had been collected. We also calculated standard deviation of these means and estimated 95% confidence intervals (2 x SD).

We used 5 categories (adult deer, fawn deer, adult moose, beaver, other) for comparison of diet estimates between packs, months, scat collection methods, and age classes (Table 1). We used percent biomass to assess wolf diets as this is more accurate than using percent volume (Weaver 1993; Klare et al. 2011). Scats in the other category consisted of snowshoe hare, berries, black bear, small mammals, and in 2 instances, calf moose. To determine the diet during a particular period of interest >1 month (*e.g.*, denning season), we averaged the monthly diet estimates to yield an estimate for the larger period. We considered the denning season to be 5 months (April–August), and the ice-free season to be 7 months (April–October). We never pooled scats from different months, packs or age-classes when estimating diets, and we omitted pup diets when comparing pack diet estimates or monthly population diet estimates. For example, to estimate the diet of a pack during the ice-free season we averaged the monthly adult diet estimates from April to October to yield the ice-free season diet of that pack.

We use the term population to denote any time 2 or more pack diet estimates were combined. We did this to determine if, and how biases would change when several pack diets were combined into a single diet estimate. We estimated the diet of the population as the mean of the estimated pack diets of interest. To minimize any temporal bias when comparing diet estimates, we omitted monthly diet estimates from the denning or ice-free season diet estimates if a sufficient number of scats could not be collected from both packs, methods, or age-classes during that month (e.g., we omitted May when comparing differences in collection methods from the Sheep Ranch Pack).

We did not compare adult and pup scats from the Sheep Ranch Pack because we only collected 9 pup scats over the course of the denning season. Similarly, we did not examine differences in sampling method from the Shoepack Lake Pack because we were not able to collect a sufficient sample over several months to accurately compare whether there were differences among the 3 sampling methods.

We determined whether diet estimates differed using pairwise Fisher's exact tests (Trites and Joy 2005). Specifically, we compared whether the distribution of the percent biomass of the 5 prey items in one diet estimate were statistically different to the distribution of the percent biomass of the same 5 prey items in another diet estimate (i.e., 2 x 5 contingency table). Pairwise comparisons of pack diets (i.e., Ash River vs. Moose River, Ash River vs. Sheep Ranch, etc.) during the ice-free season were used to assess inter-pack variability in diet estimates. Similarly, we used pairwise comparisons of the population's diet in consecutive months (e.g., Apr. vs May, May vs. Jun, etc.) during the ice-free season to assess monthly variability in diet estimates. We used an $\alpha = 0.05$ for statistical tests. When >1 Fisher's exact test was used to test a single hypothesis, we used the Bonferroni correction (α/number of statistical tests) to reduce the probability of making a type 1 error. For example, we used an α of 0.025 (0.05/2) to determine whether adult and pup diets were different because we ran 2 tests (1 for the Moose River pack and 1 for the Ash River pack) to test the hypothesis.

We used a percentile bootstrap approach to determine the 95% confidence intervals of diet estimates by using 1 000 bootstrap simulations and then selecting the 25th and 975th highest values for each food item in a particular diet estimate (Andheria et al. 2007). All analyses were completed using program R (version 3.1.3, R Core Team 2015).

Results

We collected 2 406 scats (1 985 adult scats, 511 pup scats) from April 2015 to October 2015 (Table 2). Most rarefaction curves (96%; $n = 28$) appeared to reach an asymptote once 10–20 scats were included in the sample based on visual examination, (Fig. 1). Similarly, at 10 scats/month and 20 scats/month, monthly diet diversity was 86% (95% CI = 70-100.0%) and 94% (95% CI = 85-100.0%) of the 'true' monthly diet diversity; both confidence intervals overlap 100%.

Diet estimates during the denning season did not differ (Fig. 2) based on: 1) scats collected opportunistically vs those collected at homesites in the Ash River Pack ($p = 0.752$, $\alpha = 0.05/4$), Moose River Pack ($p = 0.400$; $\alpha = 0.05/4$), Sheep Ranch Pack ($p = 0.536$; $\alpha = 0.05/4$), or the population ($p = 0.820$, $\alpha = 0.05/4$); 2) scats collected at homesites vs those collected at clusters of GPS locations in the Ash River Pack ($p = 0.625$; $\alpha = 0.05/3$), Moose River Pack ($p = 0.031$; $\alpha = 0.05/3$), and the population ($p = 0.224$, $\alpha = 0.05/3$); 3) scats collected opportunistically vs those collected at clusters of GPS locations in the Ash River Pack ($p = 0.441$; $\alpha=0.05/3$), Moose River Pack ($p = 0.065$, $\alpha=0.05/3$), and the population ($p = 0.363$, $\alpha = 0.05/3$). Diet estimates (Fig. 3) during the ice-free season did not differ based on scats collected opportunistically vs those collected at clusters in the Ash River Pack ($p = 0.273$; $\alpha = 0.05/3$), Moose River Pack ($p = 0.114$; $\alpha = 0.05/3$), and the population ($p = 0.540$; $\alpha = 0.05/3$).

Adult and pup diets of the Ash River Pack were different ($p < 0.025$; $\alpha = 0.05/2$) but adult and pup diets of the Moose River Pack were not ($p = 0.273$; $\alpha = 0.05/2$; Fig. 4). Although we only collected 10 Ash River pup scats during May, the rarefaction curve appeared to reach an asymptote at 10 scats, which suggested our sample size was adequate.

Because sampling method did not affect diet estimates, we pooled scats collected via different sampling methods for each pack, and estimated pack diet from April through October for each of the 4 packs by averaging the monthly diet estimates for each pack during this period. There was a difference ($p < 0.008$ for all pairwise pack diet comparisons; $\alpha = 0.05/6$; Fig. 5A) in diet between every pack except the Moose River Pack and Shoepack Lake Pack ($p = 0.010$ for pairwise diet comparison between Moose River and Shoepack Lake Pack). Population diet estimates differed between consecutive months ($p < 0.008$ for pairwise comparisons of consecutive month's diets; $\alpha = 0.05/6$; Fig. 5B) except between September and October ($p = 0.029$ for pairwise diet comparison between September and October).

Discussion

Scat collection methods

Scat collection method had no effect on wolf diet estimation at the pack or population level after we controlled for temporal, inter-pack, and age-class variability. Our study is unique in that we obtained a robust sample of scats that allowed us to test assumptions related to each of these factors within the same dataset. Theberge et al.

(1978), Scott and Shackleton (1980), Fuller (1989), Marquard-Peterson (1998), Trejo (2012), and Steenweg et al. (2015) all concluded that scats collected at homesites yielded different diet estimates than those collected opportunistically (e.g., roads, trails, etc.). Theberge et al. (1978) and Steenweg et al. (2015) posited that these differences were due to the proximity of kill sites to homesites, and local prey (e.g., beavers) availability around homesites. However, none of these studies accounted for temporal, inter-pack, and/or age-class variability but instead pooled scats across these meaningful sampling units, which makes their conclusions regarding sampling method and the mechanisms that cause these supposed differences suspect (Schooley et al. 1994; Ciucci et al. 2007). Further, Theberge et al. (1978), Marquard-Peterson (1998), and Steenweg et al. (2015) used frequency of occurrence of food items to estimate wolf diets rather than percent biomass, which is the most accurate method available to estimate carnivore diets from scats (Klare et al. 2011), and this could have led these researchers to incorrectly conclude that scat collection method affects diet estimates.

Although diet estimates from scats collected at clusters were the same as diet estimates from scats collected using other methods (opportunistically or at homesites), we are uncertain of the generality of our results regarding clusters. Collecting scats at GPS clusters is problematic as the quantity and content of the scats collected can depend on how a cluster is defined (e.g., length of interval and how close locations must be), and how many clusters are actually visited. Clusters that span longer timeframes could be biased toward kill sites of larger ungulate prey, thus biasing overall diet estimation (Webb et al. 2008). As the variation among prey sizes in wolf diet increases (e.g., from snowshoe hare to adult moose in our study), this bias would increase. Similarly, scats at clusters during the ice-free season are more likely to be from a single individual instead of the entire pack because pack cohesion is weakest during this time (Demma et al. 2007; Barber-Meyer and Mech 2015). Thus, individual characteristics such as the age or breeding status of the collared wolf could bias diet estimates. Moreover, scats collected at kill site clusters could represent the same prey meal and be highly auto-correlated in space and time, which could potentially bias diet estimates (Marucco et al. 2008). Therefore, we do not recommend basing wolf diet estimates solely on scats collected at GPS clusters.

Inter-pack variability

We documented several potential biases other than scat collection method that could have affected diet estimates if they were not taken into account. Most notably, there was inter-pack variability among every pack except the Shoepack and Moose River packs (Fig. 5A). Inter-pack variability in diet probably results from the differing abundance of available prey in each territory (Fuller and Keith 1980), or packs specializing on particular prey. Further, it seems likely that there is less variability in diet among individuals within a pack than between packs. Therefore, we suggest that packs should be the sample unit when estimating the diet of a population, i.e., scats from different packs should not be pooled. Rather, the diet of each pack should be estimated, and then the pack diets averaged to yield the diet of the population of interest. Pooling scats from several packs, which is common in wolf diet studies (Van Ballenberghe et al. 1975; Theberge et al. 1978; Fritts and Mech 1981; Fuller 1989; Forbes and Theberge 1996; Latham et al. 2011; Steenweg et al. 2015; Chenaux-Ibrahim 2015), should be avoided unless each pack is adequately and uniformly sampled. Otherwise, the packs that are most easily sampled will be over-represented.

Age-class variability

Most scat-based studies of wolf diet have pooled adult and pup scats collected at homesites with the assumption that pup and adult diet is the same (Van Ballenberghe et al. 1975; Theberge et al. 1978; Fritts and Mech 1981; Steenweg et al. 2015). In our study, this assumption was valid for the Moose River Pack, but not for the Ash River Pack. Differences between adult and pup diet estimates suggests certain pack members (e.g., breeding males and females) bring disproportionally greater amounts of food to the pups than other members, or that pups are consuming food items that are abundant around homesites (Van Ballenberghe et al. 1975; Theberge and Cottrell 1977; Fuller 1989; Bryan et al. 2005). There was no difference in pup and adult diets at homesites in Grand Teton National Park (Trejo 2012) whereas pup scats in Kluane National Park contained more small mammals than adult scats due to a colony of ground squirrels near the homesite (Theberge and Cottrell 1977). Further research is needed to determine the factors that affect differences in pup and adult diets (e.g., prey densities, prey base composition, pack composition, geography; Bryan et al. 2005).

The best way to reduce bias associated with age class is to differentiate between pup and adult scats collected at homesites using an appropriate size cutoff while acknowledging such cutoffs are imperfect. Many studies have considered scats <2.5 cm in diameter at homesites to be pup scats (Latham 2009; Ausband et al. 2010; Stenglein et al. 2010, 2011) although others have used more conservative cutoffs of <1.5–2.0 cm (Theberge and Cottrell 1977; Trejo 2012; Derbridge et al. 2012) We used <2.5 cm as the cutoff to differentiate between adult and pup scats at homesites. We acknowledge that we almost certainly classified some adult wolf scats as pup scats using this cutoff (*see* Weaver and Fritts 1979) but believe there was little misclassification of pup scats as adult scats because pups were substantially smaller than adults (Van Ballenberghe and Mech 1975) during this period (May–August). In other words, it is very unlikely pups <6 mo old can produce large (≥2.5 cm), adult-sized scats but adult wolves can, at times, produce pup sized scats (<2.5 cm) (Weaver and Fritts 1979).

As pups approach adult size, bias from age-class variability cannot be minimized (unless genetic techniques are used to identify parentage of individuals) as adult and pup scats will be indistinguishable based on morphology. When pup diet is different from adult diet, pooling scats could bias overall summer adult wolf diet estimates. The impact of this bias would increase as the proportion of pup scats relative to adult scats at homesites increases. Thus, we suggest providing pup diet estimates alongside adult diet estimates as adult diet is a better metric for summer wolf pack diet as pups are incapable of hunting large prey.

Temporal variation

Wolf diet changes quickly in response to the availability and abundance of vulnerable prey (Van Ballenberghe et al. 1975; Fuller 1989; Theberge and Theberge 2004; Wiebe et al. 2009). Indeed, wolf diet in our study differed between consecutive months except September and October (Fig. 5B). Despite this, scats from several months are commonly pooled together with the implicit assumption that wolf diet is similar in every month of the larger sampling period (e.g., season or year). Our results indicate that such pooling introduces potentially significant bias into diet estimates. For example, beavers composed a substantial proportion (0.42) of wolf diet in the VNP area during April–May, and fawns composed a substantial proportion (0.40) during June–August. If we had collected more scats during April–May than June–August and pooled all scats we would have overestimated beaver in wolf diet during this period. The extent to which particular prey items would be over or underestimated would only increase as the disparity in sample size among months increases. Although scats could be pooled for a season as long as there is equal sampling in each month, equal sampling rarely occurs in scat-based diet studies.

We recommend estimating monthly diet in order to minimize potential bias from temporal variability in diet estimates regardless of the sample size collected in each month. We acknowledge that a monthly sampling period is somewhat arbitrary (i.e., versus a 15, 25, or 40-day period, for example) but it provides a convenient period that should capture intra-seasonal variability in wolf diet while still being logistically feasible. Further, this period is widely used in diet studies and should allow for broader comparisons within and among different study areas.

Determining an adequate sample size

Given the temporal and inter-pack variability in wolf diets, adequate numbers of scats from each pack each month are needed to correctly estimate the diet of the larger population. Although 10 scats/pack/month appears sufficient to estimate monthly pack diet, we suggest collecting 20 scats/pack/month when possible as this will increase the accuracy of the diet estimate (Fig. 1). Because wolf diet diversity has little affect on the sample size needed (Dellinger et al. 2011; Chenaux-Ibrahim 2015; Fig. 1), it is not surprising that multiple studies have determined that between 10–30 scats were sufficient to estimate wolf diets regardless of the time interval (monthly, seasonal, annual) over which scats were collected, or whether scats were collected from individual packs or populations. For example, 20 scats were deemed sufficient to estimate the annual diet of red wolf (*Canis rufus* Audobon and Bachman, 1851) packs (Dellinger et al. 2011) and 15–30 scats appeared sufficient to estimate the seasonal diet of wolf populations in Minnesota (Chenaux-Ibrahim 2015). Although rarefaction curves estimate how many scats would be needed to adequately represent the pool of scats collected they cannot account for the biases that could be present in the pool of scats collected (Trites and Joy 2005). Therefore, diet estimates can be inaccurate even when adequate sample sizes have been collected. Many researchers simply pool scats among months, seasons or years to increase sample sizes, but doing so often introduces a new source of bias in an attempt to remove another.

Setting a higher standard for scat-based wolf diet studies

We have demonstrated that inter-pack, age-class, and temporal variability can bias scat-based wolf diet estimates which is consistent with several studies across wolf range (*see* Introduction). However, most wolf diet studies have not confronted all of these potential biases. Therefore, a higher standard is necessary. To accurately estimate wolf diets, we recommend future studies strive to account for 1) monthly variability in diet, 2) inter-pack variability in diet, 3) age-class variability in diet, and 4) differences in wolf diet estimates due to scat collection methods. We suggest all 4 of these potential biases can be minimized by collecting 10–20 adult scats/pack/month from homesites and/or opportunistically on roads and trails. Addressing the potential biases we have identified can be done in a practical and reasonable manner, but is contingent on a well-developed study design that identifies the packs that are both representative of the larger population, and that can be realistically sampled (Trites and Joy 2005; Steenweg et al. 2015). We are confident that using our approach will increase the quality and accuracy of wolf diet estimates, which could ultimately influence management decisions.

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Fig. 1. Rarefaction curves examining the impact of scat sample size on 2015 monthly (April–October) wolf (*Canis lupus*) pack diet diversity in Voyageurs National Park, Minnesota. The dotted vertical lines represent when most curves are approaching an asymptote.

Fig. 2. Estimated diet of 3 wolf (*Canis lupus*) packs–Ash River Pack (A), Moose River Pack (B), Sheep Ranch Pack (C)–and the population (D) in and adjacent to Voyageurs National Park based on 3 scat collection methods (clusters, homesites, and opportunistic) during the 2015 denning season (April–August). Error bars represent the 95% confidence intervals.

Fig. 3. Estimated diet of 2 wolf (*Canis lupus*) packs–Ash River Pack (A), Moose River Pack (B)–and the population (C) in and adjacent to Voyageurs National Park based on 2 scat collection methods (at clusters and opportunistically) during the 2015 ice-free season (April–October). Error bars represent the 95% confidence intervals.

Fig. 4. Comparison between adult and pup wolf (*Canis lupus*) diet for the Ash River and Moose River packs from May–August 2015. Error bars represent the 95% confidence intervals.

Fig. 5. Inter-pack (A) and monthly (B) variability in wolf (*Canis lupus*) diet in and adjacent to Voyageurs National Park from April 2015–October 2015. Error bars represent the 95% confidence intervals.

Table 1. Statistical comparisons of diet estimates used to identify the potential biases in scat-based wolf (*Canis lupus*) diet estimates from 4 wolf packs in and adjacent to Voyageurs National Park, MN during April–October 2015.

Potential Bias	Comparisons[a]	Time Period[b]	Packs Used[c]	No. of Tests[d]	α [e]	$p < \alpha$?
Scat collection method						
	Opp vs. Home	Denning	AR,MR,SR,POP	4	0.013	No
	Opp vs. Clusters	Denning	AR,MR,POP	3	0.017	No
	Home vs. Clusters	Denning	AR,MR,POP	3	0.017	No
	Opp vs. Clusters	Ice-Free	AR,MR,POP	3	0.017	No
Inter-pack variability						
	AR vs. MR	Ice-Free	AR,MR	6	0.008	Yes
	AR vs. SR	Ice-Free	AR,SR	6	0.008	Yes
	AR vs. SHOE	Ice-Free	AR,SHOE	6	0.008	Yes
	MR vs. SHOE	Ice-Free	MR,SHOE	6	0.008	No
	MR vs. SR	Ice-Free	MR,SR	6	0.008	Yes
	SR vs. SHOE	Ice-Free	SR,SHOE	6	0.008	Yes
Temporal variability[f]						
	Apr vs. May		POP	6	0.008	Yes
	May vs. Jun		POP	6	0.008	Yes
	Jun vs. Jul		POP	6	0.008	Yes
	Jul vs. Aug		POP	6	0.008	Yes
	Aug vs. Sep		POP	6	0.008	Yes
	Sep vs. Oct		POP	6	0.008	
Age-class variability						
	AR adult vs. pup	May-Aug	AR	2	0.025	Yes
	MR adult vs. pup	May-Aug	MR	2	0.025	No

[a] Opp = opportunistic, Home = homesites.
[b] Denning season = Apr–Aug, Ice-free season = Apr–Oct.
[c] AR = Ash River Pack, MR = Moose River Pack, SR = Sheep Ranch Pack, SHOE = Shoepack Lake Pack, and POP denotes anytime ≥2 pack diet estimates were combined.
[d] Number of Fisher's Exact Tests used to test a particular hypothesis.
[e] Critical Value determined via Bonferroni correction ($\alpha = 0.05$/no. of statistical tests).
[f] All 4 pack diets averaged to yield diet of population.

Table 2. Number of adult wolf (*Canis lupus*) and pup scats from 3 different collection methods (GPS-clusters, homesites, and opportunistic) from 4 wolf packs in and adjacent to Voyageurs National Park, MN during April–October 2015.

			Month							
Pack	Age	Method	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Total
Ash River	Adult	Clusters	23	6	3	4	-	4	19	59
		Home	16	34	19	55	28	-	-	152
		Opp.	21	19	15	17	11	16	17	116
		Total	60	59	37	76	39	20	36	327
	Pup	Home	-	10	27	57	28	-	-	122
Moose River	Adult	Clusters	8	16	8	36	3	39	42	152
		Home	99	36	75	121	34	-	-	365
		Opp.	10	16	31	38	36	10	6	147
		Total	117	68	114	195	73	49	48	664
	Pup	Home	-	26	201	118	44	-	-	389
Sheep Ranch	Adult	Clusters	-	1	-	-	-	-	19	20
		Home	11	-	21	30	17	-	-	79
		Opp.	23	47	83	43	84	47	10	337
		Total	34	48	104	73	101	47	29	436
Shoepack[a]	Adult	Total	51	54	29	32	108	60	134	468
Total			**262**	**265**	**512**	**551**	**393**	**176**	**247**	**2406**

[a] Scats pooled from opportunistic collections (April–July) and from homesites and clusters (Sept–Oct).

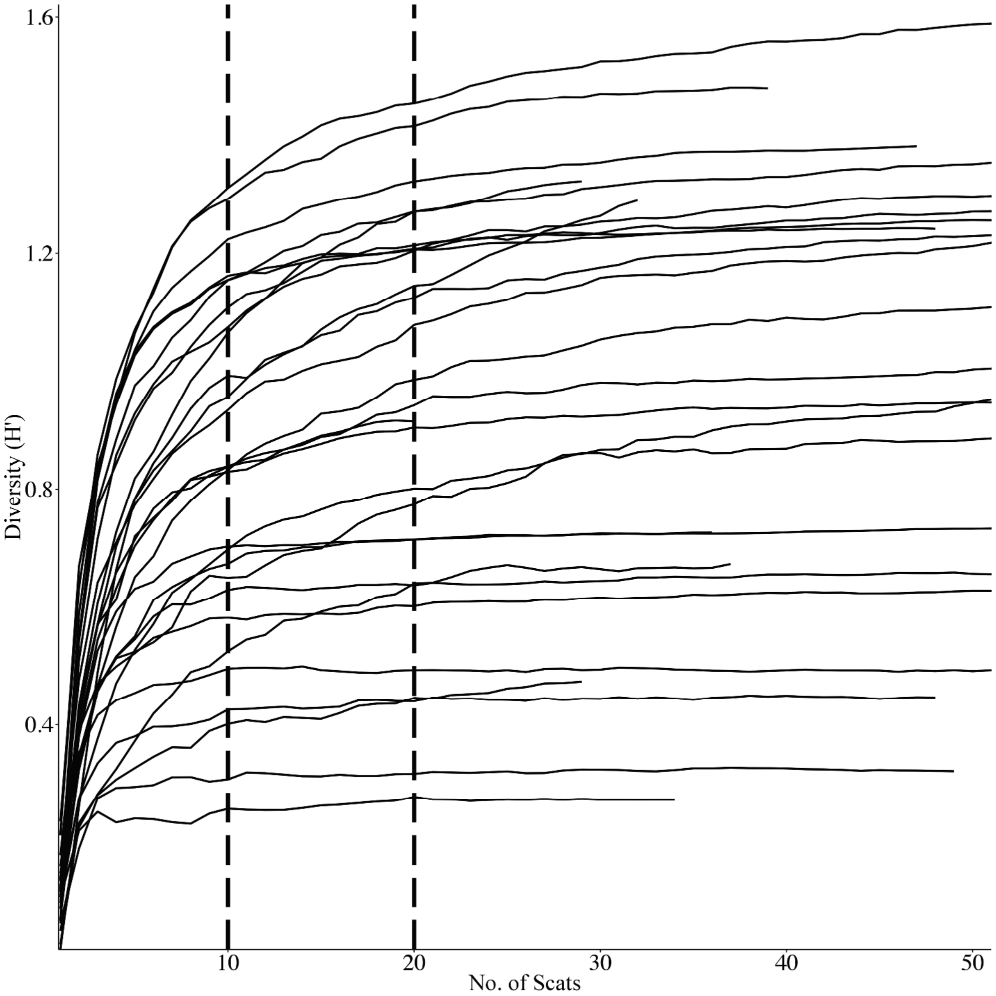

Fig. 1. Rarefaction curves examining the impact of scat sample size on 2015 monthly (April–October) wolf (*Canis lupus*) pack diet diversity in Voyageurs National Park, Minnesota. The dotted vertical lines represent when most curves are approaching an asymptote.

355x355mm (300 x 300 DPI)

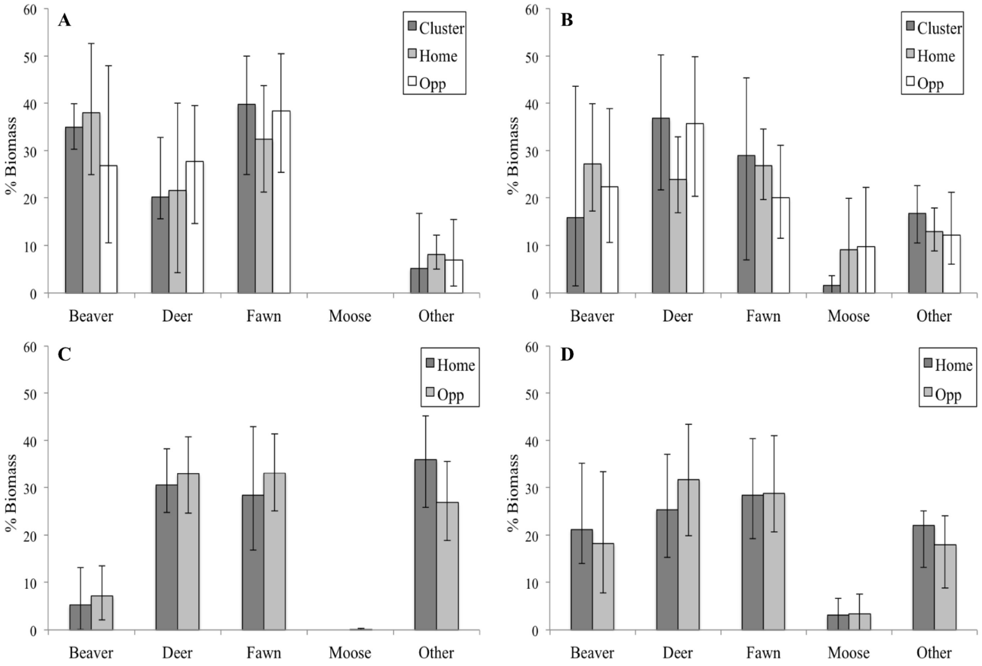

Fig. 2. Estimated diet of 3 wolf (*Canis lupus*) packs–Ash River Pack (A), Moose River Pack (B), Sheep Ranch Pack (C)–and the population (D) in and adjacent to Voyageurs National Park based on 3 scat collection methods (clusters, homesites, and opportunistic) during the 2015 denning season (April–August). Error bars represent the 95% confidence intervals.

253x171mm (300 x 300 DPI)

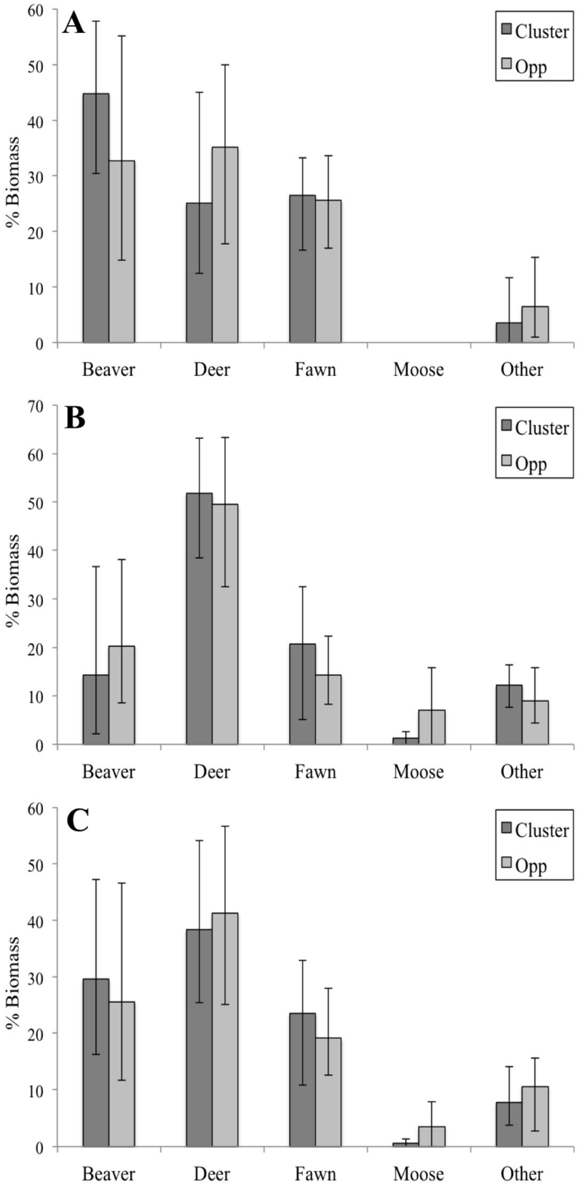

Fig. 3. Estimated diet of 2 wolf (*Canis lupus*) packs–Ash River Pack (A), Moose River Pack (B)–and the population (C) in and adjacent to Voyageurs National Park based on 2 scat collection methods (at clusters and opportunistically) during the 2015 ice-free season (April–October). Error bars represent the 95% confidence intervals.

95x190mm (300 x 300 DPI)

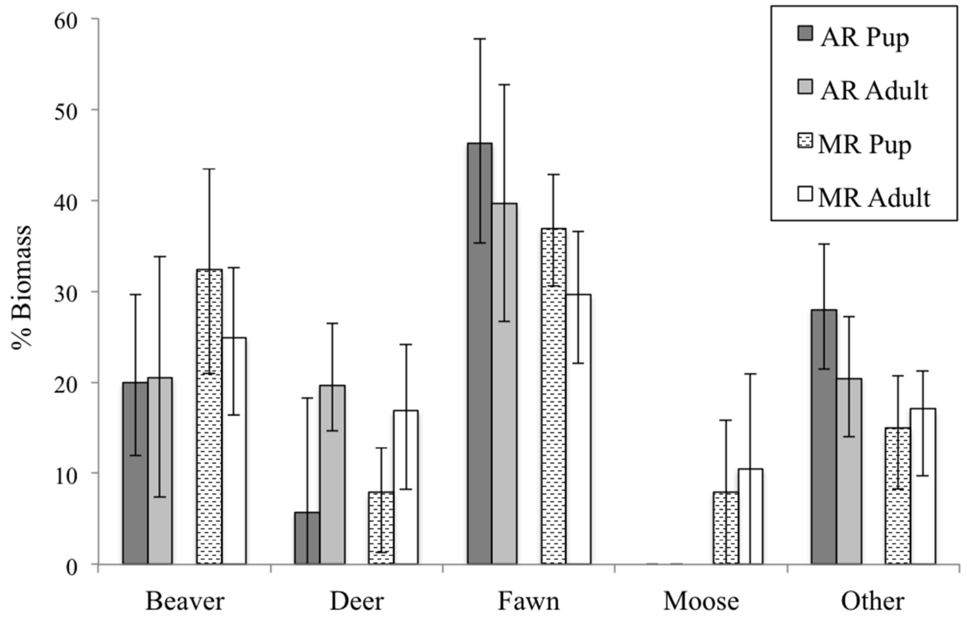

Fig. 4. Comparison between adult and pup wolf (*Canis lupus*) diet for the Ash River and Moose River packs from May–August 2015. Error bars represent the 95% confidence intervals.

88x57mm (300 x 300 DPI)

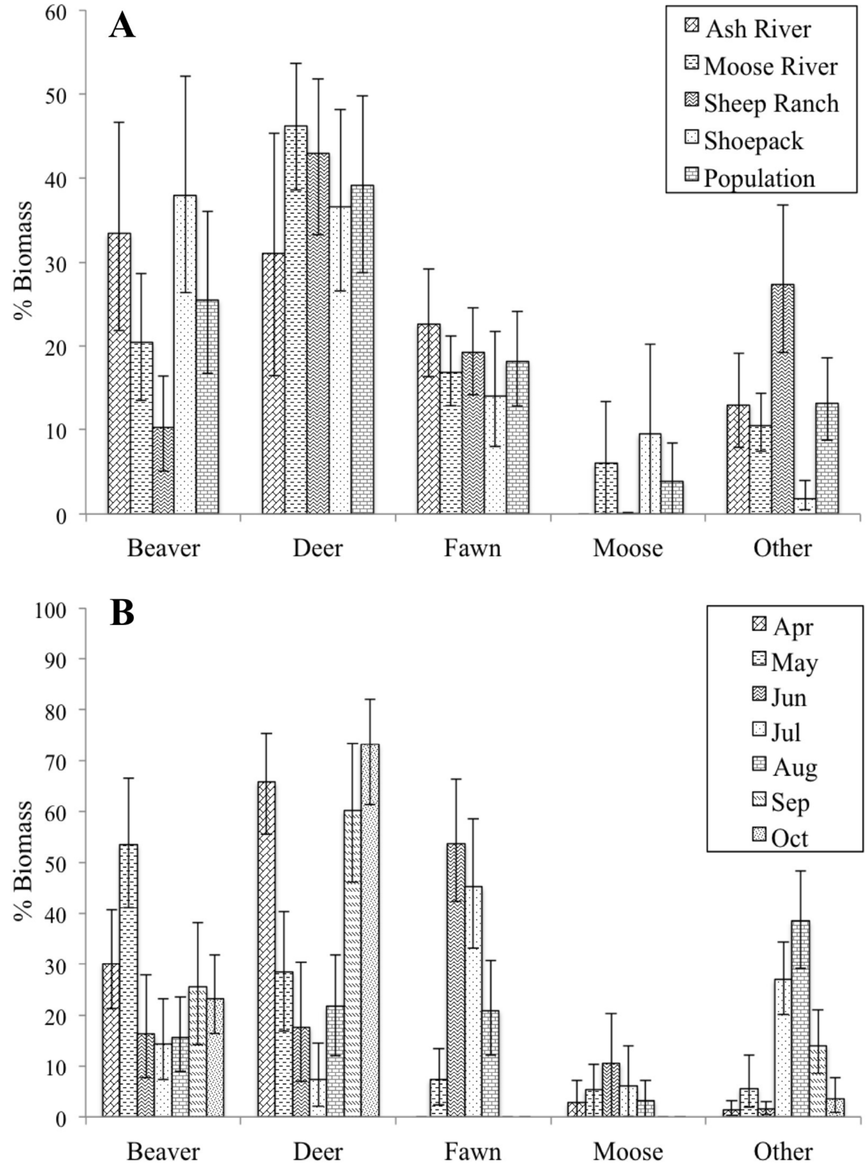

Fig. 5. Inter-pack (A) and monthly (B) variability in wolf (*Canis lupus*) diet in and adjacent to Voyageurs National Park from April 2015–October 2015. Error bars represent the 95% confidence intervals.

142x190mm (300 x 300 DPI)