

The problems with pooling poop: confronting sampling method biases in wolf (*Canis lupus*) diet studies

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 interpack, 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 ($n = 2406$) monthly from four packs via three scat collection methods (at home sites, at clusters of GPS locations, and opportunistically) in and adjacent to Voyageurs National Park, Minnesota, USA, during April–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 per month from home sites and (or) opportunistically from packs that are representative of the population of interest. Doing so will minimize the potential biases associated with temporal, interpack, and age-class variability.

Key words: biases, *Canis lupus*, diet, gray wolf, Minnesota, scat analysis, wolf diet.

Résumé : Des estimations du régime alimentaire du loup (*Canis lupus* L., 1758) sont couramment établies à la lumière d'analyses d'excréments. Plusieurs chercheurs ont conclu que la méthode de collecte des excréments peut biaiser les estimations du régime alimentaire, mais aucune de ces études n'a adéquatement tenu compte de la variabilité temporelle, entre meutes et selon la classe d'âge, des facteurs qui peuvent tous biaiser les estimations du régime alimentaire. Nous avons vérifié si différentes méthodes de collecte d'excréments produisent différentes estimations du régime alimentaire une fois ces autres biais potentiels pris en considération. Nous avons recueilli des excréments ($n = 2406$) mensuellement de quatre meutes en utilisant trois méthodes de collecte (à des points de rendez-vous, dans des groupes d'emplacements relevés par GPS et de manière opportuniste) à l'intérieur et à proximité du parc national Voyageur (Minnesota, États-Unis) d'avril à octobre 2015. La méthode de collecte d'excréments n'a pas d'incidence sur les estimations du régime alimentaire, mais ces dernières varient dans le temps, entre meutes et selon la classe d'âge. Afin d'estimer avec plus d'exactitude les régimes alimentaires de populations de loups, les chercheurs devraient prélever de 10 à 20 excréments d'adulte/meute par mois de points de rendez-vous ou de manière opportuniste de meutes qui sont représentatives de la population d'intérêt. Ils minimiseraient ainsi les biais potentiels associés à la variabilité temporelle, entre meutes et selon la classe d'âge. [Traduit par la Rédaction]

Mots-clés : biais, *Canis lupus*, régime alimentaire, loup gris, Minnesota, analyse d'excréments, régime alimentaire des loups.

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 wolf (*Canis lupus* L., 1758) diet estimation via scat analysis have been identified (Ciucci et al. 1996, 2004; Spaulding et al. 2000), 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 home sites 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. 2006), and temporal (Van Ballenberghe et al. 1975; Kohira and Rexstad 1997; Tremblay et al. 2001) sampling units prior to examining the effect 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 the “pooling fallacy”; Machlis et al. 1985) due to temporal, interpack, or age-class variability (Schooley 1994). Thus, our objectives were to (i) determine whether different scat collection

Received 7 December 2016. Accepted 14 June 2017.

T.D. Gable and J.G. Bruggink. Department of Biology, Northern Michigan University, 1401 Presque Isle Avenue, Marquette 49855, USA.
S.K. Windels. Voyageurs National Park, 360 Highway 11 East, International Falls, MN 56649, USA.

Corresponding author: T.D. Gable (email: thomasd.gable@gmail.com).

Copyright remains with the author(s) or their institution(s). Permission for reuse (free in most cases) can be obtained from RightsLink.

methods (scats collected opportunistically, at home sites, or at GPS clusters) yield different wolf diet estimates after accounting for the three potential biases mentioned above (pack, age class, and temporal) and (ii) provide a practical sampling framework to collect scats for estimating wolf population diet while confronting these three 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 km² 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 the 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 clearcuts, young aspen (species of the genus *Populus* L.) stands, mature deciduous-coniferous stands, and wetlands. Four large lakes (Kabetogama, Rainy, Namakan, and Sandpoint) cover 342 km² (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 years (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* (Zimmermann, 1780)) are common in this area, whereas moose (*Alces americanus* (Clinton, 1822)) are relatively rare (Windels and Olson 2016; Gable et al. 2017b). Wolf densities are high (4–6 wolves/100 km²) in the park, with mean home ranges of 115.8 km² (Gable 2016). Coyotes (*Canis latrans* Say, 1823) are rare in our study area (VNP, unpublished data). Hunting and trapping are not allowed in the park. However, harvest of white-tailed deer, 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, unpublished data).

Wolf capture and collaring

Wolves from four 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, USA). 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 were monitored through recovery. Fix intervals of GPS collars were set to 20 min, 4, 6, or 12 h, 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 version 2.0 extension for ArcGIS (Mills et al. 2006).

Scat collection

We collected wolf scats from four packs from April to October 2015. We collected scats opportunistically (roads and trails), at home sites, and at GPS clusters when possible. Clusters were defined as consecutive locations that were within 200 m of each other for ≥4 h (Latham 2009). We identified wolf home sites using

location data from GPS-collared wolves or from triangulation via howl surveys. We collected scats at home sites after wolves had left the home site or at the end of each month. We differentiated between adult and pup scats at home sites, assuming that scats with a diameter <2.5 cm were pup scats, whereas 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 1–3 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 hair from each of 12 randomly selected points). Each of these 12 hairs were then identified to species and age class, where possible, based on their micro- and macroscopic characteristics (Gable 2016). We selected 12 hairs/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 nonmammalian prey items, as well as the percent volume of prey remains other than hair (bone, hooves, claws, etc.). Scats containing only one 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 one prey item as 1% of the scat.

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

$$(1) \quad \hat{Y} = 0.439 + 0.008X$$

where 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 to October using the ratio of seven adults to three 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 and 16.7 kg for the spring (April–June) and fall (July–October) live masses of beaver, respectively, based on beaver trapping data (S.K. Windels, unpublished data) and the mean age of wolf-killed beavers in the area (T.D. Gable, unpublished 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

Table 1. Statistical comparisons of diet estimates used to identify the potential biases in scat-based wolf (*Canis lupus*) diet estimates from four wolf packs in and adjacent to Voyageurs National Park, Minnesota, USA, 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
Interpack 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	April vs. May		POP	6	0.008	Yes
	May vs. June		POP	6	0.008	Yes
	June vs. July		POP	6	0.008	Yes
	July vs. August		POP	6	0.008	Yes
	August vs. September		POP	6	0.008	Yes
	September vs. October		POP	6	0.008	
Age-class variability	AR adult vs. pup	May–August	AR	2	0.025	Yes
	MR adult vs. pup	May–August	MR	2	0.025	No

^aOpp: opportunistic; Home: home sites.

^bDenning season: April–August; Ice-free season: April–October.

^cAR: Ash River pack; MR: Moose River pack; SR: Sheep Ranch pack; SHOE: Shoepack Lake pack; POP: anytime ≥ 2 pack diet estimates were combined.

^dNumber of Fisher's exact tests used to test a particular hypothesis.

^eCritical value determined via Bonferroni correction ($\alpha = 0.05/\text{number of statistical tests}$).

^fAll four pack diets averaged to yield diet of population.

(*Ursus americanus* Pallas, 1780) (Chenau-Ibrahim 2015). We converted percent volume of berries (primarily species of the genera *Vaccinium* L. (blueberries) and *Rubus* L. (blackberries)) to biomass using a conversion factor of 0.468 kg/scat (Gable et al. 2017a).

We determined how many scats/pack per 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 sampled 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 smooth rarefaction curve. We used nine 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 percentage 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 ($1.96 \times \text{SD}$).

We used five categories (adult deer, fawn deer, adult moose, beaver, other) for comparison of diet estimates among packs, months, scat collection methods, and age classes (Table 1). We used percent biomass to assess wolf diets because 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 two 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 two 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 nine 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 three 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 five prey items in one diet estimate were statistically different from the distribution of the percent biomass of the same five prey items in another diet estimate (i.e., 2×5 contingency table). Pairwise comparisons of pack diets (Ash River vs. Moose River, Ash River vs. Sheep Ranch, etc.) during the ice-free season were used to assess interpack variability in diet estimates. Similarly, we used pairwise comparisons of the population's diet in consecutive months (April vs. May, May vs. June, etc.) during the ice-free season to assess monthly variability in diet estimates. We used an $\alpha = 0.05$ for

Table 2. Number of adult wolf (*Canis lupus*) and pup scats from three different collection methods (GPS clusters, home sites, and opportunistic) from four wolf packs in and adjacent to Voyageurs National Park, Minnesota, USA, during April–October 2015.

Pack	Age	Method	Month							Total
			April	May	June	July	August	September	October	
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 Lake ^a	Adult	Total	51	54	29	32	108	60	134	468
Total			262	265	512	551	393	176	247	2406

^aScats pooled from opportunistic collections (April–July) and from home sites and clusters (September–October).

statistical tests. When >1 Fisher's exact test was used to test a single hypothesis, we used the Bonferroni correction ($\alpha/\text{number of statistical tests}$) to reduce the probability of making a type I 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 two tests (one for the Moose River pack and one for the Ash River pack) to test the hypothesis.

We used a percentile bootstrap approach to determine the 95% confidence intervals (95% CI) of diet estimates by using 1000 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 2406 scats (1895 adult scats, 511 pup scats) from April 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 and 20 scats/month, monthly diet diversity was 86% (95% CI = 70%–100.0%) and 94% (95% CI = 85%–100.0%), respectively, of the “true” monthly diet diversity; both confidence intervals overlap 100%.

Diet estimates during the denning season did not differ (Figs. 2A–2D) based on (i) scats collected opportunistically versus those collected at home sites 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 ($p = 0.820$, $\alpha = 0.05/4$); (ii) scats collected at home sites versus 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 ($p = 0.224$, $\alpha = 0.05/3$); (iii) scats collected opportunistically versus 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 (Figs. 3A–3C) during the ice-free season did not differ based on scats collected opportunistically versus 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.

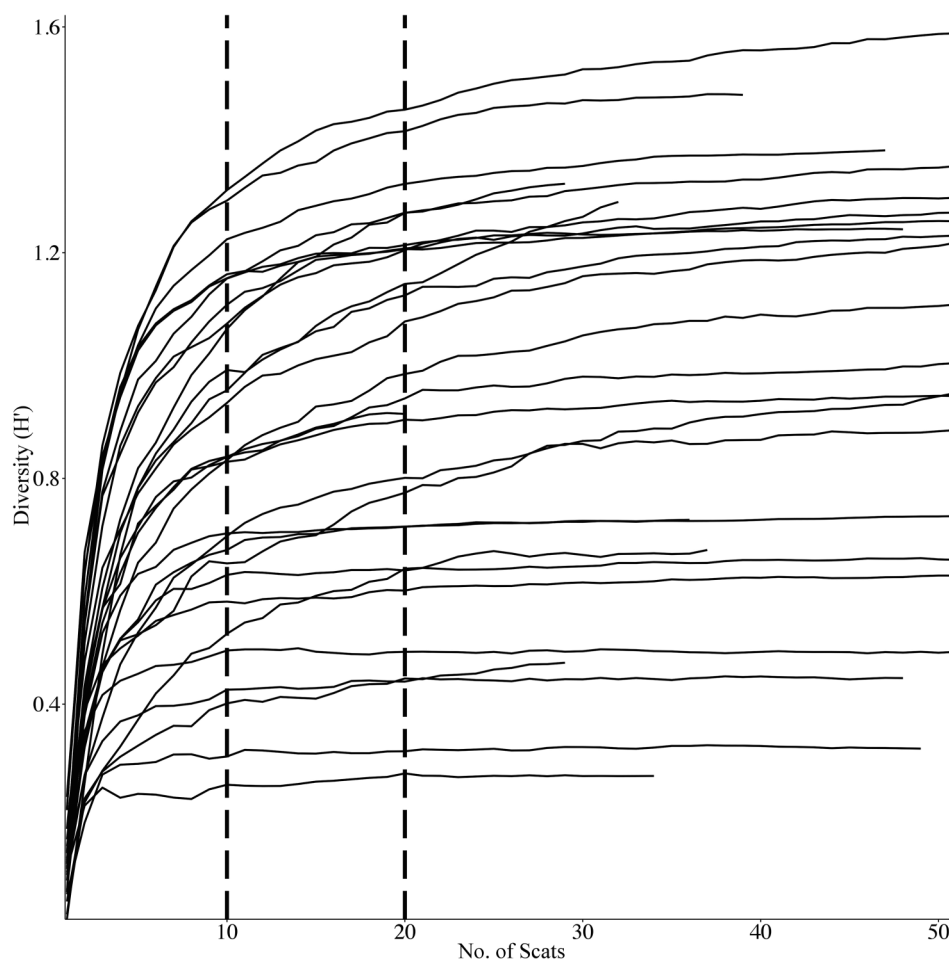
Because sampling method did not affect diet estimates, we pooled scats collected via different sampling methods for each pack, and we estimated pack diet from April through October for each of the four packs by averaging the monthly diet estimates for each pack during this period. There was a difference ($p < 0.008$ for all pairwise pack diet comparisons, $\alpha = 0.05/6$; Fig. 5A) in diet between every pack except the Moose River pack and the Shoepack Lake pack ($p = 0.010$ for pairwise diet comparison between Moose River pack 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, interpack, 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 data set. Theberge et al. (1978), Scott and Shackleton (1980), Fuller (1989), Marquard-Petersen (1998), Trejo (2012), and Steenweg et al. (2015) all concluded that scats collected at home sites yielded different diet estimates than those collected opportunistically (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 home sites, as well as local prey (e.g., beavers) availability around home sites. However, none of these studies accounted for temporal, interpack, 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 1994; Ciucci et al. 2007). Furthermore, Theberge et al. (1978), Marquard-Petersen (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

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



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 (opportunisticly or at home sites), we are uncertain of the generality of our results regarding clusters. Collecting scats at GPS clusters is problematic because 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), as well as how many clusters are actually visited. Clusters that span longer time frames 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 autocorrelated 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.

Interpack variability

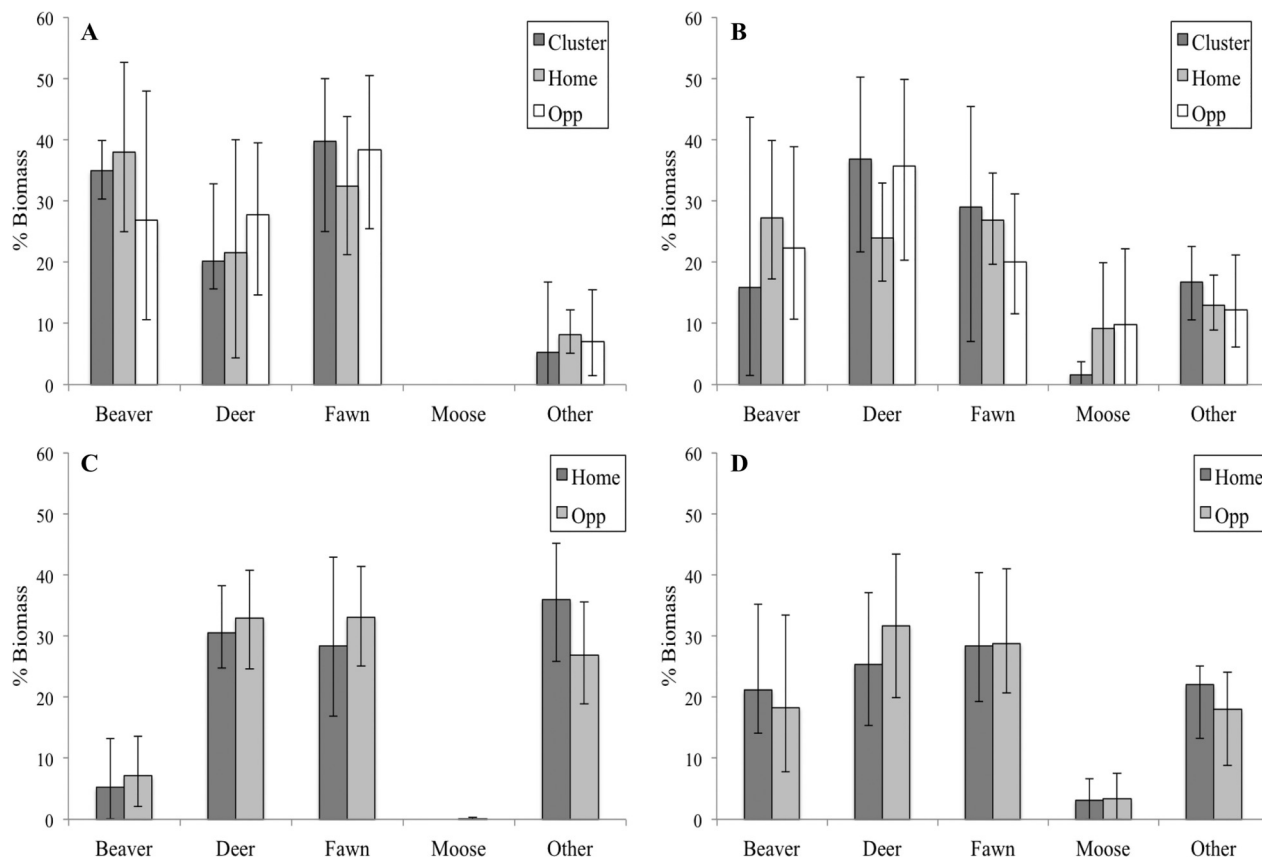
We documented several potential biases other than scat collection method that could have affected diet estimates if they were

not taken into account. Most notably, there was interpack variability among every pack except the Shoepack and Moose River packs (Fig. 5A). Interpack 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. Furthermore, 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; Chenaux-Ibrahim 2015; Steenweg et al. 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 home sites 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 suggest that certain pack members (e.g., breeding males and females) bring disproportionately greater amounts of food to the

Fig. 2. Estimated diet of three 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, Minnesota, USA, based on three scat collection methods (clusters, home sites, and opportunistic (Opp)) during the 2015 denning season (April–August). Error bars represent the 95% confidence intervals.



pups than other members, or that pups are consuming food items that are abundant around home sites (Van Ballenberghe et al. 1975; Theberge and Cottrell 1977; Fuller 1989; Bryan et al. 2006). There was no difference in pup and adult diets at home sites in Grand Teton National Park (Trejo 2012), whereas pup scats in Klutane National Park contained more small mammals than adult scats due to a colony of arctic ground squirrels (*Spermophilus parryi* (Richardson, 1825)) near the home site (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. 2006).

The best way to reduce bias associated with age class is to differentiate between pup and adult scats collected at home sites using an appropriate size cutoff while acknowledging such cutoffs are imperfect. Many studies have considered scats <2.5 cm in diameter at home sites 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; Derbridge et al. 2012; Trejo 2012). We used <2.5 cm as the cutoff to differentiate between adult and pup scats at home sites. We acknowledge that we almost certainly classified some adult wolf scats as pup scats using this cutoff (see Weaver and Fritts 1979), but we believe that 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 months old can produce large (≥2.5 cm), adult-sized scats; however, adult wolves can, at times, produce pup-sized scats (<2.5 cm) (Weaver and Fritts 1979).

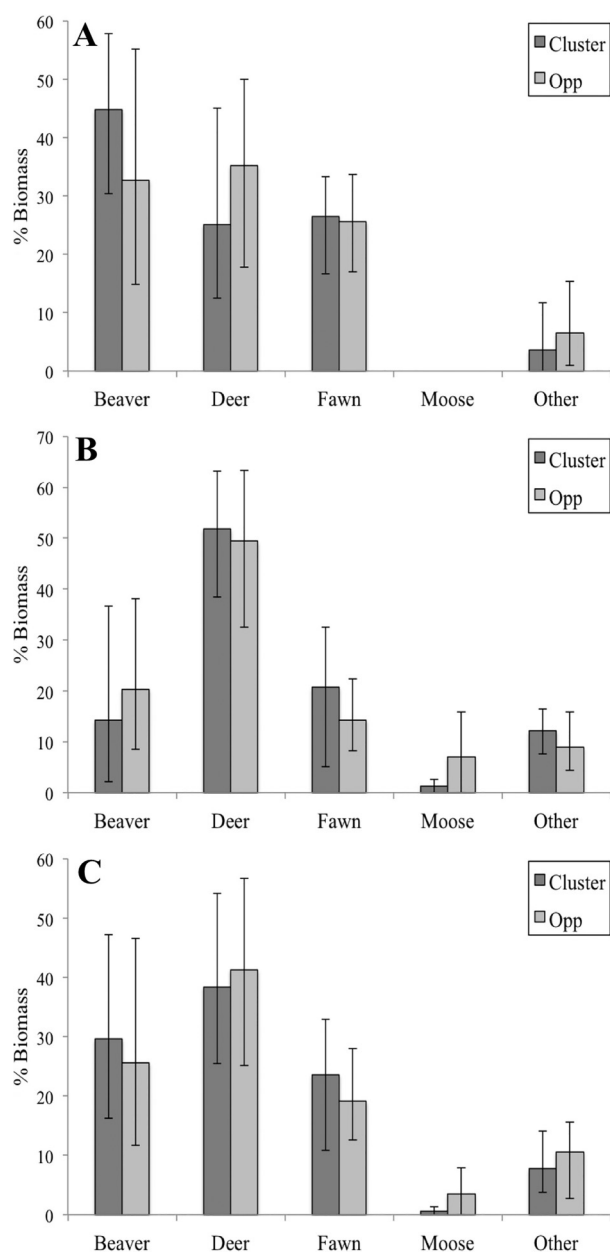
As pups approach adult size, bias from age-class variability cannot be minimized (unless genetic techniques are used to identify

parentage of individuals) because 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 home sites increases. Thus, we suggest providing pup diet estimates alongside adult diet estimates because adult diet is a better metric for summer wolf pack diet since 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, then 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.

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



We recommend estimating monthly diet 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 (versus a 15, 25, or 40 day period, for example), but it provides a convenient period that should capture intraseasonal variability in wolf diet while still being logistically feasible. Furthermore, 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 interpack variability in wolf diets, adequate numbers of scats from each pack each month are needed

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

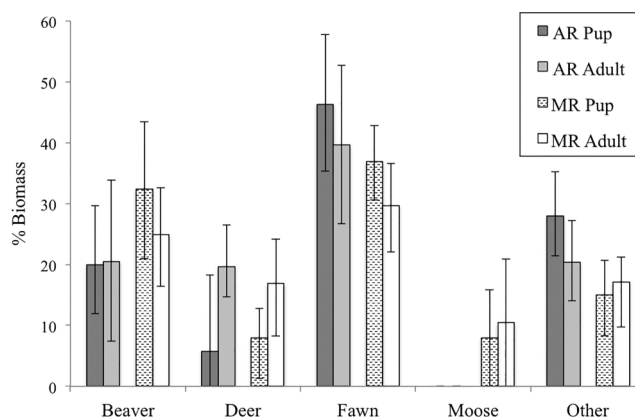
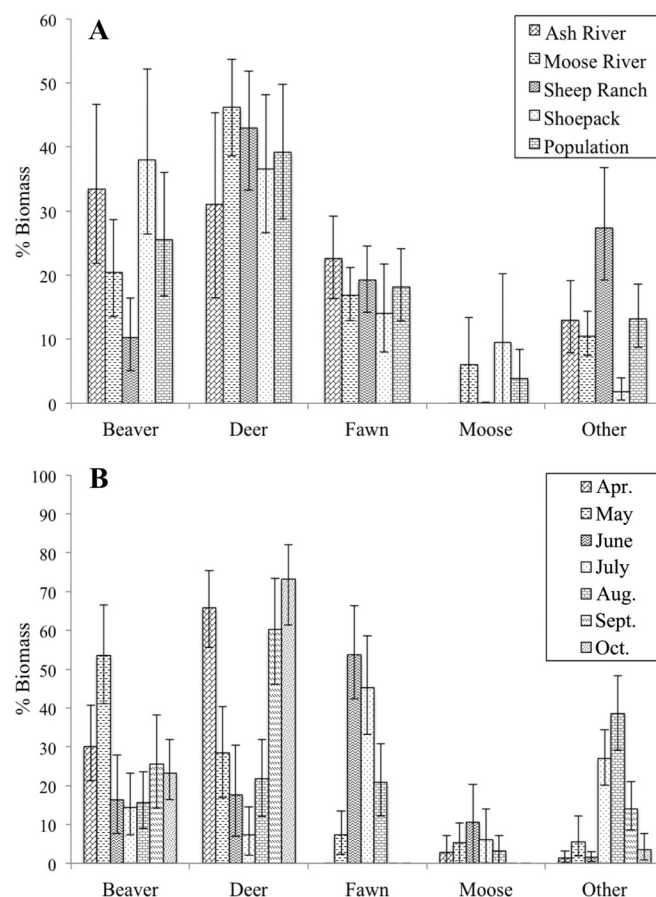


Fig. 5. Interpack (A) and monthly (B) variabilities in wolf (*Canis lupus*) diet in and adjacent to Voyageurs National Park, Minnesota, USA, from April to October 2015. Error bars represent the 95% confidence intervals.



to correctly estimate the diet of the larger population. Although 10 scats/pack per month appears sufficient to estimate monthly pack diet, we suggest collecting 20 scats/pack per month when possible because this will increase the accuracy of the diet estimate (Fig. 1). Because wolf diet diversity has little effect 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 and 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* Audubon 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 interpack, age class, and temporal variability can bias scat-based wolf diet estimates, which is consistent with several studies across the range of wolves (see the 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 (i) monthly variability in diet, (ii) interpack variability in diet, (iii) age-class variability in diet, and (iv) differences in wolf diet estimates due to scat collection methods. We suggest all four of these potential biases can be minimized by collecting 10–20 adult scats/pack per month from home sites and (or) opportunistically on roads and trails. Addressing the potential biases that 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.

Acknowledgements

Funding and logistical support was provided by Voyageurs National Park, the National Park Service Great Lakes Research and Education Center, Northern Michigan University, Rainy Lake Conservancy, The Bruggink Wildlife Research Fund, Wolf Park, and the contributions of 58 individuals via a crowd-funding campaign. A. Homkes, R. Ryan, and S. Johnson-Bice contributed significant time and effort conducting field and laboratory work for this study.

References

- Andheria, A.P., Karanth, K.U., and Kumar, N.S. 2007. Diet and prey profiles of three sympatric large carnivores in Bandipur Tiger Reserve, Indian J. Zool. 273(2): 169–175. doi:10.1111/j.1469-7998.2007.00310.x.
- Ausband, D.E., Mitchell, M.S., Doherty, K., Zager, P., Mack, C.M., and Holyan, J. 2010. Surveying predicted rendezvous sites to monitor gray wolf populations. J. Wildl. Manage. 74(5): 1043–1049. doi:10.2193/2009-303.
- Bailey, R.G. 1980. Description of the ecoregions of the United States. U.S. Dep. Agric. Misc. Publ. No. 1391.
- Barber-Meyer, S., and Mech, L.D. 2015. Gray wolf (*Canis lupus*) dyad monthly association rates by demographic group. Can. Wildl. Biol. Manage. 4(2): 163–168. Available from <http://cwbm.name/wp-content/uploads/2016/04/7-Vol-4-Issue-2-Barber-Meyer-and-Mech.pdf> [accessed 22 July 2016].
- Bryan, H.M., Darimont, C.T., Reimchen, T.E., and Paquet, P.C. 2006. Early ontogenetic diet in gray wolves, *Canis lupus*, of coastal British Columbia. Can. Field-Nat. 120(1): 61–66. doi:10.22621/cfn.v120i1.247.
- Chavez, A.S., and Gese, E.M. 2005. Food habits of wolves in relation to livestock depredations in northwestern Minnesota. Am. Midl. Nat. 154(1): 253–263. doi:10.1674/0003-0031(2005)154[0253:FHOWIR]2.0.CO;2.
- Chenaux-Ibrahim, Y. 2015. Seasonal diet composition of gray wolves (*Canis lupus*) in northeastern Minnesota determined by scat analysis. M.Sc. thesis, Department of Biology, University of Minnesota–Duluth, Duluth.
- Ciucci, P., Boitani, L., Pelliccioni, E.R., Roco, M., and Guy, I. 1996. A comparison of scat-analysis methods to assess the diet of the wolf *Canis lupus*. Wildl. Biol. 2(1): 37–48.

- Ciucci, P., Tosoni, E., and Boitani, L. 2004. Assessment of the point-frame method to quantify wolf *Canis lupus* diet by scat analysis. Wildl. Biol. 10(2): 149–153. Available from https://www.researchgate.net/publication/242145735_Assessment_of_the_point-frame_method_to_quantify_wolf_Canis_lupus_diet_by_scat_analysis [accessed 22 July 2016].
- Ciucci, P., Chapron, G., Guberti, V., and Boitani, L. 2007. Estimation of mortality parameters from (biased) samples at death: are we getting the basics right in wildlife field studies? A response to Lovari et al. (2007). J. Zool. (Lond.), 273(2007): 125–127. doi:10.1111/j.1469-7998.2007.00379.x.
- Dellinger, J.A., Ortman, B.L., Steury, T.D., Bohling, J., and Waits, L.P. 2011. Food habits of red wolves during pup-rearing season. Southeast. Nat. 10(4): 731–740. doi:10.1656/058.010.0412.
- Demma, D.J., Barber-Meyer, S., and Mech, L.D. 2007. Testing global positioning system telemetry to study wolf predation on deer fawns. J. Wildl. Manage. 71(8): 2767–2775. doi:10.2193/2006-382.
- Derbridge, J.J., Krausman, P.R., and Darimont, C.T. 2012. Using Bayesian stable isotope mixing models to estimate wolf diet in a multi-prey ecosystem. J. Wildl. Manage. 76(6): 1277–1289. doi:10.1002/jwmg.359.
- Floyd, T.J., Mech, L.D., and Jordan, P.A. 1978. Relating wolf scat content to prey consumed. J. Wildl. Manage. 42(3): 528–532. doi:10.2307/3800814.
- Forbes, G.J., and Theberge, J.B. 1996. Response by wolves to prey variation in central Ontario. Can. J. Zool. 74(8): 1511–1520. doi:10.1139/z96-165.
- Fritts, S.H., and Mech, L.D. 1981. Dynamics, movements, and feeding ecology of a newly protected wolf population in northwestern Minnesota. Wildl. Monogr. 80: 3–79.
- Fuller, T.K. 1989. Population dynamics of wolves in north-central Minnesota. Wildl. Monogr. 105: 3–41. Available from <http://www.jstor.org/stable/3830614> [accessed 22 July 2016].
- Fuller, T.K., and Keith, L.B. 1980. Wolf population dynamics and prey relationships in northeastern Alberta. J. Wildl. Manage. 44(3): 583–602. doi:10.2307/3808006.
- Gable, T.D. 2016. Wolf predation: where and how wolves hunt beavers, and confronting the biases in scat-based diet studies. M.Sc. thesis, Department of Biology, Northern Michigan University, Marquette.
- Gable, T.D., Windels, S.K., and Bruggink, J.G. 2017a. Estimating biomass of berries consumed by gray wolves. Wildl. Soc. Bull. 41(1): 129–131. doi:10.1002/wsb.730.
- Gable, T.D., Windels, S.K., and Olson, B.T. 2017b. Estimates of white-tailed deer density in Voyageurs National Park: 1989–2016. Natural Resource Report NPS/VOYA/NRR—2017/1427. National Park Service, Fort Collins, Colo.
- Johnston, C.A., and Windels, S.K. 2015. Using beaver works to estimate colony activity in boreal landscapes. J. Wildl. Manage. 79: 1072–1080. doi:10.1002/jwmg.927.
- Kallemeyn, L.W., Holmberg, K.L., Perry, J.A., and Odde, B.Y. 2003. Aquatic synthesis for Voyageurs National Park. U.S. Geological Survey, Information and Technology Report 2003-0001.
- Klare, U., Kamler, J.F., and MacDonald, D.W. 2011. A comparison and critique of different scat-analysis methods for determining carnivore diet. Mammal Rev. 41(4): 294–312. doi:10.1111/j.1365-2907.2011.00183.x.
- Kohira, M., and Rexstad, E.A. 1997. Diets of wolves, *Canis lupus*, in logged and unlogged forests of southeastern Alaska. Can. Field-Nat. 111(3): 429–435. Available from <http://biodiversitylibrary.org/page/35599330> [accessed 22 July 2016].
- Latham, A.D.M. 2009. Wolf ecology and caribou–primary prey–wolf spatial relationships in low productivity peatland complexes in northeastern Alberta. Ph.D. dissertation, Department of Biological Sciences, University of Alberta, Edmonton.
- Latham, A.D.M., Latham, M.C., McCutchen, N.A., and Boutin, S. 2011. Invading white-tailed deer change wolf–caribou dynamics in northeastern Alberta. J. Wildl. Manage. 75(1): 204–212. doi:10.1002/jwmg.28.
- Machlis, L., Dodd, P.W.D., and Fentress, J.C. 1985. The pooling fallacy: problems arising when individuals contribute more than one observation to the data set. Z. Tierpsychol. 68(3): 201–214. doi:10.1111/j.1439-0310.1985.tb00124.x.
- Marquard-Petersen, U. 1998. Food habits of arctic wolves in Greenland. J. Mammal. 79(1): 236–244. doi:10.2307/1382859.
- Marucco, F., Pletscher, D.H., and Boitani, L. 2008. Accuracy of scat sampling for carnivore diet analysis: wolves in the Alps as a case study. J. Mammal. 89(3): 665–673. doi:10.1644/07-MAMM-A-005R3.1.
- Mills, K.J., Patterson, B.R., and Murray, D.L. 2006. Effects of variable sampling frequencies on GPS transmitter efficiency and estimated wolf home range size and movement distance. Wildl. Soc. Bull. 34(5): 1463–1469. doi:10.2193/0091-7648(2006)34[1463:EOVSFO]2.0.CO;2.
- Potvin, F., Jolicœur, H., and Huot, J. 1988. Wolf diet and prey selectivity during two periods for deer in Quebec: decline versus expansion. Can. J. Zool. 66(6): 1274–1279. doi:10.1139/z88-186.
- Prugh, L.R., Arthur, S.M., and Ritland, C.E. 2008. Use of faecal genotyping to determine individual diet. Wildl. Biol. 14(3): 318–330. doi:10.2981/0909-6396(2008)14[318:UOFGTD]2.0.CO;2.
- R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from <https://www.r-project.org/>.
- Schooley, R.L. 1994. Annual variation in habitat selection: patterns concealed by pooled data. J. Wildl. Manage. 58(2): 367–374. doi:10.2307/3809404.

- Scott, B.M.V., and Shackleton, D.M. 1980. Food habits of two Vancouver Island wolf packs: a preliminary study. *Can. J. Zool.* **58**(6): 1203–1207. doi:[10.1139/z80-166](https://doi.org/10.1139/z80-166). PMID:[7427816](https://pubmed.ncbi.nlm.nih.gov/7427816/).
- Spaulding, R., Krausman, P.R., and Ballard, W.B. 2000. Observer bias and analysis of gray wolf diets from scats. *Wildl. Soc. Bull.* **28**(4): 947–950.
- Steenweg, R., Gillingham, M.P., Parker, K.L., and Heard, D.C. 2015. Considering sampling approaches when determining carnivore diets: the importance of where, how, and when scats are collected. *Mammal Res.* **60**(3): 207–216. doi:[10.1007/s13364-015-0222-4](https://doi.org/10.1007/s13364-015-0222-4).
- Stenglein, J.L., Waits, L.P., Ausband, D.E., Zager, P., and Mack, C.M. 2010. Efficient, noninvasive genetic sampling for monitoring reintroduced wolves. *J. Wildl. Manage.* **74**(5): 1050–1058. doi:[10.2193/2009-305](https://doi.org/10.2193/2009-305).
- Stenglein, J.L., Waits, L.P., Ausband, D.E., Zager, P., and Mack, C.M. 2011. Estimating gray wolf pack size and family relationships using noninvasive genetic sampling at rendezvous sites. *J. Mammal.* **92**(4): 784–795. doi:[10.1644/10-MAMM-A-200.1](https://doi.org/10.1644/10-MAMM-A-200.1).
- Theberge, J.B., and Cottrell, T.J. 1977. Food habits of wolves in Kluane National Park. *Arctic*, **30**(3): 189–191. doi:[10.14430/arctic2699](https://doi.org/10.14430/arctic2699).
- Theberge, J.B., and Theberge, M.T. 2004. The wolves of Algonquin Park, a 12 year ecological study. Publ. Ser. No. 56, Department of Geography, University of Waterloo, Waterloo, Ont.
- Theberge, J.B., Oosenbrug, S.M., and Pimlott, D.H. 1978. Site and seasonal variations in food of wolves, Algonquin Park, Ontario. *Can. Field-Nat.* **92**(1): 91–94. Available from <http://biodiversitylibrary.org/page/28062334> [accessed 22 July 2016].
- Trejo, B.S. 2012. Comparison of two methods used to characterize the summer diet of gray wolves (*Canis lupus*). M.Sc. thesis, College of Natural Resources and Sciences, Humboldt State University, Arcata, Calif.
- Tremblay, J.P., Jolicœur, H., and Lemieux, R. 2001. Summer food habits of gray wolves in the boreal forest of the Lac Jacques-Cartier Highlands, Québec. *Alces*, **37**(1): 1–12. Available from https://www.researchgate.net/publication/236736451_Summer_food_habits_of_gray_wolves_in_the_boreal_forest_of_the_Lac_Jacques-Cartier_highlands_Quebec [accessed 22 July 2016].
- Trites, A.W., and Joy, R. 2005. Dietary analysis from fecal samples: how many scats are enough? *J. Mammal.* **86**(4): 704–712. doi:[10.1644/1545-1542\(2005\)086\[0704:DAFFSH\]2.0.CO;2](https://doi.org/10.1644/1545-1542(2005)086[0704:DAFFSH]2.0.CO;2).
- Van Ballenberghe, V., and Mech, L.D. 1975. Weights, growth, and survival of timber wolf pups in Minnesota. *J. Mammal.* **56**(1): 44–63. doi:[10.2307/1379605](https://doi.org/10.2307/1379605). PMID:[1113051](https://pubmed.ncbi.nlm.nih.gov/1113051/).
- Van Ballenberghe, V., Erickson, A.W., and Byman, D. 1975. Ecology of the timber wolf in northeastern Minnesota. *Wildl. Monogr.* **43**: 3–43. Available from <http://www.jstor.org/stable/3830388> [accessed 22 July 2016].
- Voigt, D.R., Kolenosky, G.B., and Pimlott, D.H. 1976. Changes in summer foods of wolves in central Ontario. *J. Wildl. Manage.* **40**(4): 663–668. doi:[10.2307/3800561](https://doi.org/10.2307/3800561).
- Wachter, B., Blanc, A., Melzheimer, J., Höner, O.P., Jago, M., and Hofer, H. 2012. An advanced method to assess the diet of free-ranging large carnivores based on scats. *PLoS ONE*, **7**: e38066. doi:[10.1371/journal.pone.0038066](https://doi.org/10.1371/journal.pone.0038066). PMID:[22715373](https://pubmed.ncbi.nlm.nih.gov/22715373/).
- Weaver, J.L. 1993. Refining the equation for interpreting prey occurrence in gray wolf scats. *J. Wildl. Manage.* **57**(3): 534–538. doi:[10.2307/3809278](https://doi.org/10.2307/3809278).
- Weaver, J.L., and Fritts, S.H. 1979. Comparison of coyote and wolf scat diameters. *J. Wildl. Manage.* **43**(3): 786–788. doi:[10.2307/3808765](https://doi.org/10.2307/3808765).
- Webb, N.F., Hebblewhite, M., and Merrill, E.H. 2008. Statistical methods for identifying wolf kill sites using global positioning system locations. *J. Wildl. Manage.* **72**(3): 798–807. doi:[10.2193/2006-566](https://doi.org/10.2193/2006-566).
- Wiebe, N., Samelius, G., Alisauskas, R.T., Bantle, J.L., Bergman, C., de Carle, R., Hendrickson, C.J., Lusignan, A., Phipps, K.J., and Pitt, J. 2009. Foraging behaviours and diets of wolves in the Queen Maud Gulf Bird Sanctuary, Nunavut, Canada. *Arctic*, **62**(4): 399–404. doi:[10.14430/arctic171](https://doi.org/10.14430/arctic171).
- Windels, S.K., and Olson, B.T. 2016. Moose population survey at Voyageurs National Park: 2016. Natural Resource Data Series NPS/VOYA/NRDS—2016/1031. National Park Service, Fort Collins, Colo.