Cloacal swabbing as a tool to study diet in migrating raptors using DNA metabarcoding

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Abstract

While much research has gone into understanding the timing and patterns of migration, little has been done to understand the diet of raptors during migration. Most raptor dietary studies focus on the breeding season or winter, but migratory diet may be quite different due to differences in habitat type and prey availability along migration flyways. Here, we tested the efficacy of DNA metabarcoding to detect prey DNA on cloacal swabs. In 2019, we collected cloacal swabs from raptors during spring and fall migration in Duluth, MN. We analyzed 287 cloacal swabs from 11 species of raptors. We hypothesized that detection of dietary DNA on cloacal swabs would be influenced by the species of raptor swabbed, the size of the raptor, and migratory flight strategy (passive/soaring flight vs. active flight). Prey DNA was detected on 18.46% of cloacal swabs. Using a generalized linear model, we found that neither species, size, nor migratory flight strategy were better than the null model at explaining differences in detection of dietary DNA. To our knowledge, this is the first study to use cloacal swabbing and DNA metabarcoding to detect dietary DNA and our results indicate that this method has potential for further use.

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Introduction:

The diet of raptors during migration has not been well studied and new methodologies are necessary to study migratory diet. Raptors, or birds of prey, are a paraphyletic group of predatory birds characterized by a hooked beak, talons, and keen eyesight. Raptors are generally accepted to include Falconiformes (falcons and caracaras), Accipitriformes (eagles, hawks, kites, and Old World vultures), Cathartiformes (New World vultures), and Strigiformes (owls). A recent paper defines raptors as "*species within orders that evolved from raptorial landbirds (Telluraves) in which most species maintained raptorial lifestyles*," and this definition would also include Cariamiformes (seriemas) as raptors (McClure et al. 2019).

Of the 54 species of raptors native to North America, 35 species migrate in at least part of their range. While some species only migrate short distances in the far northern portions of their range (e.g., black vulture, *Coragyps atratus*), others migrate long distances to Central and South America (e.g., broad-winged hawk, *Buteo platypterus*). Diet during migration may differ from diet at other times of the year because of changes in habitat and prey availability compared to breeding and wintering grounds. Feeding frequency may also vary depending on how a species migrates. Species that soar on thermals, such as broad-winged hawks, may feed infrequently because their flight is not very energetically costly. In contrast, species that migrate by powered flight, such as sharp-shinned hawks (*Accipiter striatus*) may need to hunt frequently along migrations routes (Hofslund 1973). Research into the diet of raptors during migration is necessary to fully understand their ecology and to work towards their conservation.

Importance of studying diet in raptors

Diet is a key aspect of the life history of animals. In raptors, more study is needed to address differences in diet based on sex, age, habitat, and season. Because female raptors are larger than their male conspecifics, average prey size may differ between the sexes. Reduced intersexual competition for food has been suggested as one explanation for sexual size dimorphism in raptors (Krüger 2005), yet empirical evidence is needed to support such a hypothesis. Foraging may also differ based on the age of the raptor, since hunting is a skill that must be developed. On migration, juvenile American kestrels (*Falco sparverius*) may depend more heavily on dragonflies than adults do (Nicoletti 1997). Young raptors may opt for nutritionally less valuable prey to stave off starvation, while adults who have better hunting skills may pursue more difficult yet nutritionally valuable prey. Habitat type also influences diet within a species (Miller et al. 2014), so it is necessary to study diet across a species' range. Finally, season likely has a strong impact on diet, for migratory and non-migratory raptors, since prey availability differs in summer and winter due to prey migration and hibernation. Understanding variation in the diet of raptors is needed to apply their life history to their conservation.

Of the 557 species of raptors found worldwide, 52% are declining in global populations and 18% are threatened with extinction (McClure et al. 2018). Raptors are at risk from habitat loss and degradation, bioaccumulation and biomagnification of environmental contaminants, and persecution. Raptors with specialist diets may be more susceptible to changes in prey availability and dietary studies can be used to inform risk assessments, management, and conservation. Conservation efforts for raptors are important not only to help raptor populations, but also to help whole ecosystems. Raptors

have been referred to as both sentinel/indicator species and umbrella species (Sergio et al. 2006). Dietary studies for raptors can contribute to their conservation by informing land management, ecotoxicology, and public education.

Understanding trophic interactions in ecosystems is important for land management. Duluth, Minnesota is one of the most important raptor migration sites in North America, with an average of 76,000 raptors counted at Hawk Ridge each fall (hawkridge.org). Sites along migration flyways, like Duluth, could use dietary information to provide and protect habitat that is beneficial to important prey species. Additionally, sites that are dangerous for raptors, such as airports, could discourage raptors by making the land less attractive for prey species (Coghlan et al. 2013). Further study of diet and hunting habits of raptors during migration will also support the protection of existing habitat in migration flyways by demonstrating how migratory raptors use this habitat. As habitats are altered by climate change, wildlife managers will need to know what prey species to prioritize if their goal is to conserve raptors. Climate change may also bring about changes in diet and so it is necessary to have a baseline for prey species before major habitat alterations occur. Over the next 50-60 years, boreal forests are expected to decline considerably in the Northern Superior Uplands landscape surrounding Duluth, MN (Galatowitsch et al. 2009), and what effects this will have on raptors and their prey is unknown.

As top predators, raptors are susceptible to bioaccumulation and biomagnification of environmental contaminants. In the mid-20th century, DDT devastated bald eagle (*Haliaeetus leucocephalus*) and peregrine falcon (*Falco peregrinus*) populations. Current research on contaminants in raptors includes many environmental contaminants, such as

mercury, organochlorines, polychlorinated biphenyls (PCBs), per- and polyfluoroalkyl substances (PFAS), lead, and rodenticides. In a previous study at Hawk Ridge, both merlins (*Falco columbarius*) and sharp-shinned hawks were found to have mercury levels that placed them at moderate risk of adverse effects (Keyel et al. 2020). Both merlins and sharp-shinned hawks are small raptors that mainly eat small birds. Since raptors are primarily exposed to contaminants via their food, diet studies can be used to track contaminant exposure pathways in ecosystems. Dietary studies will not only show how raptors are being exposed to contaminants but will also provide a greater understanding of the whole ecosystem effects of contaminants (Baudrot et al. 2018).

Public perception of raptors, whether positive or negative, can have profound effects on raptor populations. Up until the mid-20th century, raptors in North American were heavily persecuted because they were viewed as pests to livestock, gamebirds, and songbirds (Bildstein 2001). McAttee and Stoddard (1930) wrote "There is no prejudice stronger, save that about snakes, than the universal hatred of hawks and owls." Today, raptors around the world are still killed because of the perceived effects of their diet on species of economic value. In Great Britain, hen harriers (*Circus cyaneus*) are still commonly illegally shot because their diet includes a popular gamebird, red grouse, *Lagopus lagopus scotica* (Murgatroyd et al. 2019). Persecution of raptors is by no means a rural phenomenon. In Rome, shooting was the second most common injury of raptors admitted to rehabilitation centers, and the most common cause for falcons, with the exception of kestrels (Cianchetti-Benedetti et al. 2016). Further research into the diet of raptors may help to reduce misconceptions about diet and give greater ecological context to predation.

Methods of studying diet

In the late 19th and early 20th century, stomach and crop content analysis was used to study diet in raptors with the goal of determining which species were agricultural pests (Sherrod 1978). This was a lethal method limited by whether or not the bird had eaten recently, and by the size and type of prey consumed. Small vertebrate prey items are more likely to be eaten whole and have identifiable bones in the stomach, while larger prey items are more likely to have flesh stripped away from the bones and be unidentifiable in the stomach. Modern DNA methods of stomach analysis reduces the prey size biases of morphological methods. While stomach content analysis could still be used today on raptors that are already dead, such as those killed in collisions (Coghlan et al. 2013) or by lead poisoning (Nadjafzadeh et al. 2012), it is no longer acceptable to destructively sample a healthy raptor on the chance of identifying its last meal.

Pellet dissection is a common method for studying diet in owls. A pellet is a regurgitated lump of non-digestible material, such as fur, feathers, and bones. Pellet dissection has been used to describe the diet of many species of owls including northern saw-whet owl, *Aegolius acadicus* (Grove 1985; Swengel and Swengel 1992), barn owl, *Tyto alba* (Raun 1960; Doerksen 1969), long-eared owl, *Asio otus* (Dziemian et al. 2012) and short-eared owls, *Asio flammeus* (Clark 1975). While falcons, hawks, and eagles also cast pellets, their stomachs breakdown bones more efficiently than owl stomachs do (Errington 1932) because owl gastrointestinal tracts are less acidic than those of other raptors (Duke et al. 1975). Thus, studies for diurnal raptors often combine data from pellets and prey remains (Fitch et al. 1946; Bradley and Oliphant 1991; Santillan et al. 2009). Pellet collection occurs at nests or roost sites and prey remains are identified by

morphological characteristics or by extracting DNA (Taberlet and Fumagalli 1996). DNA analysis has been shown to identity more invertebrate species in owl pellets than traditional morphological identification (Benamane et al. 2019). Using pellets to study diet during migration would only be possible if a captured bird was held until it cast a pellet, which could take several hours.

During the breeding season, nests are commonly used to study diet in raptors by collecting prey remains and pellets, expelling food from the gullet of nestlings, and by setting up cameras. Each method has its own limitations. First, prey remains at nests do not equally represent the number of prey items brought to the nest, and amphibians and other small items may be dramatically underrepresented (Tornberg and Reif 2007). While cameras may give a better quantification of the types of prey brought to the nest, identification to genus and species level is more difficult than with remains. Gullet content examination involves forcing a nestling to regurgitate food from its gullet. While it was suggested as a more reliable method for studying nestling diet than prey remains in the nest (Errington 1932), this method is not commonly used anymore. Studying diet at the nest is obviously biased to the breeding season diet which may be quite different from diet at other times of the year due to seasonal changes in prey abundance. A raptor may migrate into or out of geographic range of certain prey species and prey species may migrate or hibernate. For example, cameras at northern goshawk, *Accipiter gentilis,* nests in south-central Idaho showed Belding's ground squirrel, *Urocitellus beldingi*, made up the majority of nestling diet, but that diet shifted away from mammals near the end of the season (Miller et al 2014). Given that Belding's ground squirrels hibernate for 5 to 7

months, it follows that northern goshawk breeding season diet must differ markedly from their fall and winter diets.

Perhaps one of the more difficult methods of systematically studying diet is to directly observe hunting and feeding events. Although there are a few observational studies of diet and hunting behavior in nocturnal raptors (Abbruzzese and Ritchison 1997; Mo et al. 2016), this method lends itself better to conspicuous diurnal species that hunt in open areas, such as bald eagle (Mersmann et al. 1992), snowy owl, *Bubo scandiacus* (Boxall and Lein 1982), and peregrine falcon (Varland 2018). Direct observation of hunting is time intensive and precise identification of prey items is difficult (Rosenberg and Cooper 1990). Observers may also risk influencing the feeding behavior of the raptor.

Advances in molecular biology in the past 20 years has opened the door to novel methods of studying diet. DNA metabarcoding uses polymerase-chain reactions (PCR) to amplify unique DNA barcodes that are then sequenced to identify taxon-specific DNA present in the sample (Pompanon et al. 2012). While sanger sequencing can be used to identify a single prey species (Bourbour et al. 2018), DNA metabarcoding can sequence many different DNA molecules in a sample at the same time and thus it has quickly become a popular molecular method to study diet. In dietary studies of birds, DNA metabarcoding has been used to identify DNA from stomach contents (Coghlan et al. 2013), buccal swabs (Nota et al. 2019), pellets (Benamane et al. 2019), and feces (Jedlicka et al. 2013; Mansor et al. 2018; Trevelline et al. 2018). To collect feces, birds are often held in a cloth bag or box until they defecate. This method would be difficult to

apply to species like raptors as these extended holding times may place raptors at an unacceptable risk of stress injury.

Here, we describe the use of cloacal swabs to study the diet of migrating raptors. To our knowledge, no studies have yet used cloacal swabbing as a source of fecal material for diet analysis. Cloacal swabbing requires only a short handling time and is safe for all species of raptors. We predicted that cloacal swabbing can be used to obtain DNA of prey/dietary items and that the absence of prey DNA on cloacal swabs may have several explanations including:

1) infrequent feeding during migration
2) low concentration of fecal residue in cloaca
3) low quality of prey DNA after passing through the gut.

Additionally, **we hypothesized that the DNA return on cloacal swabs will vary between raptor species based on physical size and feeding behavior.** We predicted that larger raptors would have more prey DNA on the swabs than smaller raptors because a larger cloaca means more surface from which DNA can be swabbed. Finally, we predicted that raptors that migrate with powered flight would have more prey DNA on the swabs than raptors that migrate by soaring, because they would need to feed more frequently during migration.

Methods:

Field Collection:

In 2019 we collected cloacal swabs from 11 species of raptors during spring and fall migration (Table 1). The Spring 2019 (late April/early May) samples were collected

at Wisconsin Point, in Superior, WI. Our primary goal for these samples was to establish if there was quantifiable DNA on the cloacal swabs. We swabbed a total of 81 raptors in the spring, mostly consisting of sharp-shinned hawks (*Accipiter striatus*). The bulk of our sample collection occurred between August and November 2019 at Hawk Ridge in Duluth, MN. Raptors were trapped and banded by staff of Hawk Ridge Bird Observatory as part of a long-term study of raptor migration in Duluth (Evans et al. 2012). Raptors were swabbed after they were banded. We swabbed the cloaca by inserting the swab tip fully into the cloaca so that the cotton was not visible, and then rotating the swab for several seconds before removing it. Because the size of raptors sampled ranged from less than 100g (e.g. northern saw-whet owls) to well over 1kg, we used two different sizes of swabs in order to maximize the amount of fecal residue recovered. For raptors larger than 200g, we used a Puritan medical 6" sterile standard cotton swab with a wooden handle. For raptors smaller than 200g, we used a Puritan Medical 6" tapered mini cotton swab with a wooden handle. In order to prevent abrasion to the cloaca on especially small raptors, we lightly dampened the swab tip with deionized water prior to swabbing. After swabbing, we put the swab tip into an empty 1.7 mL microcentrifuge tube and snapped off the wooden handle using the side of the tube. Samples were kept on ice in the field and then stored at or below -20°C.

As a positive control, we analyzed high quality prey remains and fecal material collected from eleven American kestrel nest boxes located near Sax-Zim Bog (45 miles northwest of Duluth, MN). Samples were collected once from each nest box when the nestlings were banded. The samples from each nest box were subdivided into 2 to 4 subsamples for extraction, amplification and sequencing. When possible, prey remains in

each subsample were informally identified to a general taxonomic group (e.g. rodent, passerine, insect) prior to DNA extraction.

DNA extraction, amplification, and sequencing:

We used DNeasy PowerSoil Kits (Qiagen, Hilden, Germany) to extract DNA from the swabs. Before starting the manufacturer's protocol, we trimmed off any of the swab's wooden handle that did not have visible fecal material in order to reduce the amount of liquid the wood could absorb during the first extraction step. We also pipetted the buffer solution from the first step of the extraction protocol into the swab's original tube to recover any residue from the swab left in the tube. This solution was then returned to the extraction tube along with the swab tip. We then followed the rest of the extraction protocol.

We used MiBird (Ushio et al. 2018) and MiMammal (Ushio et al. 2017) primers to amplify a region of the mitochondrial 12S rRNA gene (~171bp). Each extracted DNA sample was amplified twice; once with each primer. The 20 µL PCR reaction contained 6.8 µL ddH_20, 5 µL of extracted DNA, 4 µL of 1X Bovine Serum Albumin, 2 µL 10X buffer, 0.6 µL of 50mM $MgCl_2$, 0.5 µL each of the forward and reverse primers (10 µM), 0.4 µL 10mM dNTP, and 0.2 µL Platinum™ Taq Polymerase (Invitrogen Corp., San Diego, California). We followed a touchdown PCR protocol as follows: an initial denaturation at 95°C for 3 minutes followed by **1)** 10 cycles of 95°C for 30 s, 63°C for 45 s, and 72°C for 1 min. **2)** 10 cycles of 95°C for 30 s, 60°C for 45 s, and 72°C for 1 min. **3)** 30 cycles of 95°C for 30 s, 58°C for 45 s, and 72°C for 1 min. **4)** final extension of 72°C for 10 minutes. After amplification, the DNA was sequenced using Ilumina MiSeq high-throughput sequencing (Illumina, Inc, San Diego, California).

Data analysis:

CutAdapt (v. 1.12; Martin 2011) was used to trim primer and adapter sequences from the 5' end of both the forward and reverse FASTQ reads (9.5 million pairs) and sequences were merged using USEARCH (v. 9.2; Edgar and Flyvbjerg 2015). Merged pairs were filtered to a subset of sequences with a length of 165bp (6.8 million) that had ≤1 expected errors (ee1). The ee1 set was filtered removing 83870 singletons and dereplicated using UNOISE2 (Edgar 2016), retaining 130,788 unique reads. These reads were clustered into OTUs at 97% identity, resulting in 119 OTUs (Edgar 2010; Edgar 2013) and mapped to an OTU table of all barcoded samples. The threshold for detection of an OTU was determined by the frequency of the OTU sequence in the dataset.

We identified OTUs using NCBI GenBank database. We filtered out OTUs that had no matches, had only matches with less than 100% query coverage, and those that matched bacterial or human sequences. For OTUs without a perfect match (<100% Identity), we reported the closest match that occurs in North America. If none of the top matches occurred in North America, we report the sequence with the highest percent identity.

We used generalized linear models in RStudio (R version 3.5.2, 2018) to test our hypotheses of the effect of species, body size (average body weight), and migration strategy (active vs. passive flight) on the probability of detecting prey DNA on a cloacal swab. Species with no prey detections were dropped from the analysis. We ranked fitted models using Akaike's Information Criterion corrected for small sample size (AIC_c, Burnham and Anderson 2002).

Results:

Operational Taxonomic Units (OTUs):

We initially identified 83 OTUs with 100% query coverage. From there, 16 OTUs were dropped because they were suspected to be nuclear mitochondrial DNA (NUMT), and they were redundant with other OTUs. Eleven OTUs corresponded to the 11 species of raptors we sampled, leaving 56 prey OTUs identified between the swab and nest box samples (Table 2). 38 of these prey OTUs were birds, 17 were mammals, and one was a fish. 35 prey OTUs appeared on the cloacal swab samples, 22 of which were unique to the cloaca swabs. Two of these OTUs, however, occurred only on swabs that were excluded due to signs of cross contamination of raptor DNA between swabs. These OTUs were perfect matches for pig (*Sus scrofa*) and a small fish, the central stoneroller (*Campostoma anomalus*). Twenty-nine prey OTUs occurred in the nest box samples, 15 of which were unique to the nest box samples. Finally, six OTUs were unique to a swab of a red-tailed hawk beak that we sampled opportunistically.

Raptor DNA detection – cloacal swabs:

Raptor DNA was detected on 311 out of 318 swabs. The majority of swabs (n = 280) returned a high number of sequences for the OTU that matched the individual from which the swab was collected. Seven swabs did not have any significant level of any raptor OTU, and these swabs also did not detect any prey DNA. We excluded 31 of the 318 swabs because they showed high levels of an OTU for a raptor species that did not match the individual swabbed, indicating either a mislabeling in the field or cross contamination. Sixteen of the 31 excluded samples were traced to a single round of extraction.

Prey DNA detection – cloacal swabs:

Prey DNA was detected on 53 out of 287 (18.46%) cloacal swabs (Table 3). Of the species sampled only sharp-shined hawk (n=28, 19.86%), Cooper's hawk (n= 2, 20%), northern goshawk(n=11, 30.56%), red-tailed hawk (n=5, 14.71%), and Northern Saw-whet Owl (n=7, 31.82%) had swabs that contained prey DNA (Figure 1). Of the cloacal swabs on which DNA was detected, 81.11% (n=43) had one prey OTU detected, 15.09% (n=8) had two prey OTUs detected, 1.89% (n=1) had 3 prey OTUs detected, and 1.89% (n=1) had 8 prey OTUs detected. The swab with eight prey OTUs came from an adult male sharp-shinned hawk, and there were two pairs of OTUs that matched the same species, which may indicate that two different individuals of the same species were detected.

Generalized Linear Model

We tested seven models against the null (homogenous background rate of prey detection) to evaluate the effect of species, body size (average body weight), and migration strategy (passive vs. active flight) on the probability of detecting prey species from a cloacal swab (Table 4). None of the models performed better than the null model. The differences between the models were small, indicating a lack of power. Our models using both species and migration strategy performed especially poorly because passive migration and red-tailed hawks are linearly dependent covariates. In other words, because red-tailed hawk was the only species with a prey detection that migrates via passive migration the effects of passive migration and red-tailed hawk cannot be separately estimated.

Discussion:

We have demonstrated that cloacal swabs from raptors can be used to obtain prey DNA. The majority of cloacal swabs (81.54%) did not return any prey DNA, but this was not surprising since we observed very little fecal material on the swabs. Some of these prey detections may have been contamination from domesticated species and species used to lure raptors for trapping (non-native doves and European starlings, *Sturnus vulgaris*). If all these species are excluded, then the percent of prey detection on cloacal swabs would drop from 18.46% to 12.2%. However, since DNA from lure species and domestic animals was not widespread amongst the total 318 cloacal swabs analyzed, there is no strong evidence to support their exclusion due to contamination. Furthermore, domestic animals and the species used to lure raptors are potential dietary items and discarding these sequences outright may ignore interesting trophic pathways.

We found little support for species, body size, and migration strategy (active vs. passive flight) as predictive of prey DNA detection on cloacal swabs. Our ability to detect a stronger effect of species, body size, and migration strategy may have been affected by the imbalance of samples collected between species. We collected cloacal swabs based on the availability of raptors trapped at Hawk Ridge, and some species were much more common than others. While we could have limited the number of species sampled or the number of swabs collected to have a more balanced sample between species, we prioritized collecting as many swabs as financially feasible from a variety of raptor species in order to be able to describe the overall effectiveness of cloacal swabbing at detecting prey DNA.

Overall, the prey species detected on the cloacal swabs aligned well with the general types of prey expected for each raptor species. All northern saw-whet owl swabs that detected prey DNA identified small rodent species and the majority of sharp-shinned hawk prey items were birds (Figure 2). Northern goshawk swabs detected typical prey species such as ruffed grouse (*Bonasa umbellus*), American crow (*Corvus brachyrhynchos*), and red squirrel (*Tamiasciurus hudsonicus*), but also some unexpected species such as Virginia opossum (*Didelphis virginiana*), cat (*Felis catus*), and dog (*Canis lupus familiaris*). Of the two Cooper's hawk swabs which detected prey DNA, one detected a typical prey species (ruffed grouse) and the other detected a more surprising species, cow (*Bos taurus*). Red-tailed hawk swabs had proportionally the most unusual prey DNA compared to their expected diet. Two of the red-tailed hawk swabs out of the five that detected prey DNA showed a typical prey species (red squirrel). Two of the remaining three red-tailed hawk swabs contained dog DNA and the third had dog and cedar waxwing (*Bombycilla cedrorum*).

Domestic animals: Contamination, predation or scavenging?

There were six sequences that were perfect matches for domesticated species (Table 2). While these sequences may be the result of contamination from pet dander or food, they may well represent predation or scavenging events. Of these sequences, cat (*Felis catus*) seems highly likely to be evidence of a predation event. Cat DNA was detected on a single swab from a male northern goshawk. We found one example of northern goshawk predation of cats in the literature (Kennedy 1991), and since northern goshawks are large hawks and fierce hunters this appears to be a plausible predation event. Of the domesticated species detected in our study, dog (*Canis lupus familiaris*)

seems most likely to be due to contamination. We occasionally had a dog with us in the field, and some samples were collected by a dog owner. Interestingly though, dog DNA was only detected on northern goshawk and red-tailed hawk swabs and all swabs were collected on different days. However, if dog DNA was due to contamination of six random samples, we would expect a more random distribution among the species sampled. Given that 24.39% of our samples were red-tailed hawk and northern goshawk, the hypergeometric probability of all random dog contamination occurring in just those samples is less than $2 * 10^{-4}$. While predation of dogs by northern goshawks and red-tailed hawks has not been documented in scientific literature, red-tailed hawks have been frequently documented to scavenge and northern goshawks have occasionally been documented to scavenge (Sherrod 1978). While we cannot draw any firm conclusions on the source of the dog DNA on these samples, these results provide supporting evidence of scavenging behavior in northern goshawks and red-tailed hawks and suggest further investigation is warranted.

Contamination of domesticated animal DNA from human food seems less likely than contamination from pets. We did not have food with meat in the field and no food was in the laboratory. Swabs that detected the same domesticated animal OTUs were collected on different days. Four livestock species were detected on swabs: cow (*Bos taurus*), pig (*Sus scrofa*), chicken (*Gallus gallus gallus*), and turkey (*Meleagris gallopavo*). Chicken was detected on one sharp-shinned hawk swab. Although sharp-shinned hawks have been documented to eat chickens (Sherrod 1978), they are likely to only take a young chicken or very small breed. Cow was detected for one Cooper's hawk and one sharp-shinned hawk. Pig was detected on one swab, but this swab was excluded

from analysis because it contained unexpected raptor DNA. These are clearly not predation events, but we did consider whether cow and pig DNA could be from a prey species foraging in manure, that was subsequently captured and eaten. However, none of the swabs that contained livestock DNA detected other prey DNA. Therefore, scavenging is the next best explanation. Scavenging behavior varies among species of raptors. Among the species we sampled, northern harrier (McTee et al. 2019, Peterson et al. 2001), merlin (McIntyre et al. 2009), peregrine falcon (Varland 2018), northern goshawk (Sherrod 1978), and Cooper's hawks (Peterson et al. 2001) have all been documented to scavenge.

Turkey (*Meleagris gallopavo*) may have been from either domesticated or wild turkey. Turkey was detected in three different sharp-shinned hawk samples collected in late April and early May. Sharp-shinned hawks are very small raptors. Males generally weigh around 100g and females weigh around 160g, making it impossible for a sharp-shinned hawk to depredate an adult turkey. We suggest three possible explanations: predation of a wild turkey chick, predation of a domesticated turkey chick, and scavenging of a turkey carcass. Although wild turkey chicks in southern Texas (Watts 1969) and Florida (Williams and Austin 1988) begin hatching in late April, in Wisconsin chicks hatch around early to mid-June (Wisconsin DNR 2015). If the turkey DNA we detected came from a wild turkey chick, then the predation event would have to have occurred in the southern United States. Sharp-shinned hawks fly at an average speed of 48 km/hr (Broun and Goodwin 1943), and it would take several days for a sharp-shinned hawk to fly from the southern USA to northern Wisconsin. While we do not know exactly how long prey DNA persists in the cloaca, it seems unlikely it would be detected

more than 24 hours after feeding based on gut retention times (Barton 1992). As for predation of a domesticated turkey chick, temperatures are still quite cold at the end of April and early May in Minnesota and Wisconsin and young turkey chicks on free-range farms would likely still be indoors at that time of year. This leaves scavenging as the most likely explanation. Turkey hunting season runs from mid-April to the end of May in Minnesota and Wisconsin. These dates are concurrent with our spring sampling and provide an explanation for prevalent turkey remains available for scavenging in the region. Interestingly, we could only find one report in the scientific literature of a sharp-shinned hawk scavenging (Kostechke et al. 2001). There is also one report of a sharp-shinned hawk with porcupine (*Erethizon dorsatum*) quills in its foot caught in early May in the Upper Peninsula of Michigan (Kelley and Kelley 1969). While this may have been a predation attempt on a baby porcupine, it may also have been a scavenging event. Sharp-shinned hawks are often described as secretive birds, and their inconspicuousness outside of migration season could contribute to the lack of documentation of scavenging events.

Limitations of cloacal swabbing and DNA Metabarcoding:

In general, DNA metabarcoding cannot detect cannibalism in samples obtained from an organism (i.e. cloacal swabs, feces, stomach contents, etc.), because any cannibalized prey DNA would match that of the predators' unless the genetic loci used in barcoding is polymorphic within species. Cannibalism has been reported in four different families of raptors (Accipitridae, Cathartidae, Strigidae and Tytonidae) and most documented cannibalism occurred during the breeding season as filicide or non-parental infanticide (Allen et al. 2020). Conspecific strife and scavenging have also been

documented forms of cannibalism in red-tailed hawks (Allen et al. 2020). Although cannibalism is unlikely to be common in raptors, more research is needed to determine its extent. While DNA metabarcoding is unsuited to study cannibalism because primers are selected to target loci that vary between species, a barcoding method could be designed using microsatellites, which have the ability to distinguish individuals.

Our study could not identify predation on other species of raptors included in the sampling because of bleed over between samples during high-throughput sequencing. This bleed over between samples is only likely to occur for DNA present at high concentrations, such as the birds' own DNA. Prey DNA from a cloacal swab is unlikely to bleed over during sequencing. While by no means a major part of their diet, predation and scavenging of raptors by other raptors has been documented in many species. Sherrod (1978) noted raptors as dietary items for golden eagle (*Aquila chrysaetos*), northern goshawk, Cooper's hawk, northern harrier, ferruginous hawk (*Buteo regalis*), red-tailed hawk, red-shouldered hawk (*Buteo lineatus*), Harris's hawk (*Parabuteo unicinctus),* gyrfalcon (*Falco rusticolus*), and peregrine falcon. The raptors that were found in their diets were generally small raptors, such as screech owls (*Megascops sp.*) and American kestrels, although large raptor species were documented multiple times in golden eagle diets. Although it is quite possible that some of the species included in our study could have eaten another raptor, bleed over signal of raptor DNA between samples during high-throughput sequencing was as strong and even stronger than some prey detections. The one exception was an opportunistic beak swab from a red-tailed hawk which contained bald eagle DNA. Red-tailed hawks are known facultative scavengers and road-killed bald eagles in northern Minnesota are known to occur. Since bald eagle

was not a species included in our study, we are considering this sample as documentation of a scavenging event. This example raises the question of raptors scavenging other raptors during migration. To get around the issue of cross-contamination of raptor DNA between samples, close attention should be paid to sterile technique in the field when multiple species of raptors are being sampled.

Improving the method:

To our knowledge, this is the first study to attempt to use cloacal swabbing as a method for describing diet. While our overall percentage of cloacal swabs with prey DNA was low (18.46%) we believe further experimentation could improve the technique. A controlled experiment on a captive population of raptors would help to establish the length of time DNA from a prey item remains in the cloaca, the success rate of cloacal swabbing at detecting all dietary items, the effect of time of day, and whether cloacal swabbing is more successful on some species of raptors than on others. Taking more than one cloacal swab per bird may increase the chance of recovering prey DNA from the cloaca, although eventually consecutive swabs will have less fecal material on them. Furthermore, finding ways to reduce the bird's stress levels may increase the amount of fecal material in the cloaca. We swabbed birds after the banding process, but it may be better to swab them immediately after capture or after a resting period.

We further recommend including the time of day into experimental design. Mean gut retention of time of food in raptors ranges from 6 to 8 hours (Barton 1992). We sampled diurnal raptors in both the morning and afternoon, but owls were only swabbed during the first half of the night. In hindsight, it may have been better to swab owls later in the night towards morning after they had a chance to hunt and digest a meal. A

nocturnal hunter like the northern saw-whet owl caught soon after sunset is unlikely to have eaten since the previous night and thus have already cleared its cloaca. Interestingly though, northern saw-whet owl had highest percentage of swabs that detected prey DNA. This higher detection rate may have been due to the less acidic nature of gastrointestinal tract of Strigiformes compared to Accipitriformes and Falconiformes (Duke et al. 1975). Further dietary cloacal swab work should include multiple species of owls to determine if the method is more effective for Strigiformes than other raptors. For diurnal raptors, more research is needed on feeding behavior during migration to understand the timing of hunting. If a species generally hunts in the morning, then it would be better to do a cloacal swab in the afternoon. A species that hunts in the late afternoon would be trickier to time cloacal swabbing because if caught in the afternoon digestion will not have reached the cloaca and if caught in the morning, digestion may already be complete. A better understanding of feeding patterns will help target the best time of day to collect cloacal swabs.

Finally, cloacal swabbing may be improved by refining the molecular methods. We used two types of primers, MiBird and MiMammal, which target an approximately 171bp section of the mitochondrial 12S rRNA gene. We chose these primers because we anticipated that the majority of prey items would be avian or mammalian. Our choice in primers missed arthropod, amphibian, and reptile prey, however we were financially limited in the number of sequencing runs we could perform. Experimenting with the number and types of primers used may improve the return of prey DNA. When selecting a barcode region, there is a tradeoff off between taxonomic coverage (the diversity of species that can be captured) and taxonomic resolution (the taxonomic level to which a

species can be identified) (Pompanon et al. 2012). Using multiple primers can improve both taxonomic coverage and resolution, but it will add to the cost of analysis.

Depending on the goals of the study, pooling swabs for DNA extraction from different individuals of the same species, sex, and age class may improve the amount of prey DNA obtained. DNA molecules from a sample can be lost at each step of the DNA extraction process, left behind in a pipette tip or microcentrifuge tube. Pooling multiple samples together may reduce the probability that rare DNA will be lost. Additionally, it would reduce the cost of analysis per sample. However, compositing samples requires a large sample size and can reduce analytical power. We did a small test of compositing sharp-shinned hawk swabs. We pooled together 10 juvenile female swabs and 10 juvenile male swabs. Both the male and female composited samples yielded one prey OTU each (Table 2). Given that the individual sharp-shinned hawk swabs had an 19.86% success rate at detecting prey DNA, compositing samples may not improve the detection rate. However, a more rigorous study of composited swabs is necessary to determine whether or not it is a viable method.

Further research:

While the number of species that have had their genomes fully or partially sequenced has increased dramatically over the past twenty years and continues to grow, more North American species need to have the mitochondrial 12S rRNA gene sequenced. As a locus for DNA metabarcoding 12S shows great potential because of its high specificity to target taxa, but it lacks a reference database as robust as the more commonly used COI locus, cytochrome c oxidase subunit I (Collins et al. 2019). We did an ad hoc search of the NCBI Nucleotide database for possible avian and mammalian

prey and recorded whether 12S was fully, partially, or not sequenced (Table 5). For birds, only 34% had 12S fully sequenced while 54% did not have any portion of 12S sequenced. For mammals, the story was slightly better: 12S was fully sequenced for 48% of species and not sequenced for 30% of species. Imperfect matches for OTUs leaves a level of uncertainty that limits a study's ability to answer deeper questions. While we were able to conclude a Family identification for some of our OTUs with imperfect matches, others could only be identified to Order. Sequencing 12S for missing species would increase the power of analysis for DNA metabarcoding studies and would be applicable to a variety of predation and biomonitoring studies.

More research is also needed on the frequency and timing of feeding of raptors during migration. Due to the various complexities and challenges of obtaining data on feeding during migration, research in this area remains limited and will be more qualitative than quantitative until new techniques are developed. A simple first step to collecting more data on migratory feeding habits would be to record whether a captured raptor has signs of a recent meal, such as a full crop or prey remains on its beak/talons. Food in a crop can be easily detected by touching the outside of a bird's neck. Since the crop is a temporary food storage location, food in the crop is a sign the bird fed recently, and this information can be used determine the timing of feeding during migration. As described above, understanding the timing of feeding is important in determining the ideal time of day to take a cloacal swab to maximize the chance of obtaining fecal material. Recording signs of recent feeding would likely underestimate feeding frequency because diurnal raptors are generally caught with bait, and a raptor that fed recently may be less likely to be caught than a hungry individual. However, this type of

study would still be useful because it would give a relative comparison of feeding frequency between species, ages, and sexes. Understanding the relative feeding frequency of raptors would help researchers know which species to prioritize for research since the diet of infrequent feeders will be more difficult to study.

Other applications of cloacal swabbing:

Previously, cloacal swabbing has been used to detect diseases, such as West Nile virus (Komar et al. 2002) and avian influenza (Wang et al. 2008), to study microbiomes (Xenoulis et al. 2010), and collect genetic material (Mucci et al. 2014). Here, we propose that cloacal swabbing can be used as a tool to study diet. While we only investigated its use in raptors, cloacal swabbing could be used to study diet in a wide variety of other species for which obtaining feces is difficult. Cloacal swabbing may be most effective at studying diet in medium to large birds, and on species which feed regularly throughout the day. Future studies could use cloacal swabbing to take repeated diet samples of the same individual throughout the breeding season. Cloacal swabbing could also be used to verify whether a predator is targeting a threatened or endangered species. The effectiveness of cloacal swabbing should be compared to buccal, beak, and talon swabs, and multiple types of swabs could be used on one individual to increase the chances of detecting prey DNA.

Conclusion:

We have demonstrated that cloacal swabbing can be used to obtain prey DNA for the purpose of diet analysis. Given the low percent of swabs with prey DNA, this method will need further refinement to be an effective and efficient method for studying diet in raptors. In the past decade DNA metabarcoding has become a common method for studying diet in a variety of taxa and it has potential for further innovation. This study presents a new application of DNA metabarcoding to study diet in raptors during migration. Migratory diet is an understudied part of the life history of raptors. Our findings suggest that scavenging may be a more important feeding strategy during migration than previously thought. More research is needed to understand how the diet of raptors differs between migration, breeding and nonbreeding season.

Tables and Figures

Table 1: Cloacal swabs collected from raptors during spring and fall migration in 2019. 31 additional swabs were collected, however these swabs were excluded from analysis due to possible mislabeling the field and/or contamination.

Species	Total Samples	Females	Males	Juvenile	Adult	Juvenile Females	Adult Females	Juvenile Males	Adult Males
American kestrel (*Falco sparverius*)	9	4	5	8	1	3	1	5	
Merlin (*Falco columbarius*)	13	7	6	10	3	6	1	4	2
Peregrine falcon (*Falco peregrinus*)	2	1	1	1	1		1	1	
Sharp-shinned hawk (*Accipiter striatus*)	141	108	33	52	89	43	65	9	24
Cooper's hawk (*Accipiter cooperii*)	10	7	3	5	5	3	4	2	1
Northern goshawk (*Accipiter gentilis*)	36	13	23	28	8	8	5	20	3
Northern harrier (*Circus hudsonius*)	16	8	8	8	8	3	5	5	3
Red-tailed hawk (*Buteo jaimacensis*)	34			25	8				
Broad-winged hawk (*Buteo platypterus*)	3			3					
Rough-legged hawk (*Buteo lagopus*)	1	1			1		1		
Northern saw-whet owl (*Aegolius acadicus*)	22	13	3	12	9	7	7	3	
Total	287	162	92	152	133	73	90	49	33

Table 2: Summary of OTU matches in GenBank BLAST search. OTUs are listed in order of the most sequences detected in the dataset to the least number of detected sequences. "Closest Match?" denotes whether the species listed for the OTU had the highest percent identity of the matching sequences in GenBank. When "N", the species listed is the species within geographic range of Duluth, MN, U.S.A. "Multiple Matches?" denotes if there were multiple sequences in GenBank with the same percent identity as the species listed for the OTU. Subsequent columns of sample types list the number of samples where the OTU was detected. Because many samples detected multiple different OTUs, the sample columns do not add up to the total number of samples. "Nest Samples" counts the total number of nest box subsamples in which an OTU was detected and "Nest Box" is the total number of unique nest boxes where the OTU was detected. "Beak Swab" is a single swab from the exterior of a Red-tailed Hawk (*Buteo jamaicensis*) beak. "Composited Samples" are two samples that composited 10 cloacal swabs together from Sharp-shinned Hawk (*Accipiter striatus*) for extraction. "Excluded samples" are the cloacal swabs that were excluded from analysis due to evidence of cross contamination of raptor DNA.

GenBank BLAST results	Voucher Sequence	% Identity	E Value	Closest Match?	Multiple Matches?	Cloacal Swabs	Nest Samples	Nest Boxes	Beak Swab	Composited Samples	Excluded Samples
Eurasian sparrow hawk (*Accipiter nisus*)	MN122826.1	95.15	8E-66	Y	N	142				1	5
Red-tailed hawk (*Buteo jamaicensis*)	GQ264619.1	100	4E-79	Y	N	34					10
Vole spp. (*Microtus multiplex)*	AJ972918.1	95.15	3E-65	Y	N	1	32	11	1		1
Norther goshawk (*Accipiter gentilis*)	MN122867.1	97.59	2E-72	Y	N	32					7

GenBank BLAST results	Voucher Sequence	% Identity	E Value	Closest Match?	Multiple Matches?	Cloacal Swabs	Nest Samples	Nest Boxes	Beak Swab	Composited Samples	Excluded Samples
Northern saw-whet owl (*Aegolius acadicus*)	U83759.1	100	4E-79	Y	N	22					3
Merlin (*Falco columbarius*)	KM264304.1	96.36	4E-69	Y	N	12					18
American kestrel (*Falco sparverius*)	DQ780880.1	100	4E-79	Y	N	9	31	11			2
Hen harrier (*Circus cyaneus*)	KU237286.1	98.79	8E-76	Y	N	15					3
Northern goshawk (*Accipiter gentilis*)	MN122867.1	90.91	4E-54	Y	N	9					4
Broad-winged hawk (*Buteo platypterus*)	GQ264632.1	100	4E-79	Y	N	3					1
Song sparrow (*Melospiza melodia*)	FJ236290.1	98.18	4E-74	N	N		10	6			1
Peregrine falcon (*Falco peregrinus*)	JX029991.1	100	4E-79	Y	N	2					
Dog (*Canis lupus familaris*)	MN181403.1	100	4E-79	Y	N	5					
Rough-legged hawk (*Buteo lagopus*)	KP337337.1	100	4E-79	Y	N	1					
White-tailed deer (*Odocoileus virginianus*)	KM612273.1	100	4E-79	Y	N				1		
Bobolink (*Dolichonyx oryzivorus*)	AF447226.1	100	4E-79	Y	N		2	1			
Dog (*Canis lupus familaris*)	CP050601.1	100	4E-79	Y	N	1					

GenBank BLAST results	Voucher Sequence	% Identity	E Value	Closest Match?	Multiple Matches?	Cloacal Swabs	Nest Samples	Nest Boxes	Beak Swab	Composited Samples	Excluded Samples
Cow (*Bos taurus*)	MN714195.1	100	4E-79	Y	N	2					4
Zonotrichia leucophrys	FJ236292.1	95.15	8E-66	Y	N	2	7	4			
Common yellowthroat (*Geothlypis trichas*)	AF447233.1	100	4E-79	Y	N		5	3			1
Vole spp. (*Eothenomys smithii*)	LC424768.1	92.77	4E-59	Y	Y	1					1
Orange-crowned warbler (*Vermivora celata*)	FJ236284.1	97.58	2E-72	Y	Y		1	1			
Dove spp. (*Streptopelia orientalis*)	KT182929.1	92.12	2E-57	Y	N	5					
Pig (*Sus scrofa*)	MH603005.1	100	4E-79	Y	N						1
Star-nose mole (*Condylura cristata*)	KX754488.1	100	4E-79	Y	N				1		
Turkey (*Meleagris gallopavo*)	KM224338.1	100	4E-79	Y	N	3					
Song sparrow (*Melospiza melodia*)	FJ236290.1	95.76	2E-67	Y	N		3	2			
Virginia opossum (*Didelphis virginiana*)	AY012091.1	100	4E-79	Y	N	1					
White-crowned sparrow (*Zonotrichia leucophrys*)	FJ236292.1	94.55	4E-64	N	Y		3	3			
White-crowned sparrow (*Zonotrichia leucophrys*)	FJ236292.1	92.12	2E-57	N	Y	2	3	2			

GenBank BLAST results	Voucher Sequence	% Identity	E Value	Closest Match?	Multiple Matches?	Cloacal Swabs	Nest Samples	Nest Boxes	Beak Swab	Composited Samples	Excluded Samples
Pine grosbeak (*Pinicola enucleator*)	KM078781.1	93.33	6E-62	N	Y	2	4	3			
Cedar waxwing (*Bombycilla cedrorum*)	KJ909187.1	100	4E-79	Y	N	1					
Chicken (*Gallus gallus gallus*)	MN013407.1	100	4E-79	Y	N	1	1	1			1
Dove (*Streptopelia decaocto*)	NC_037513.1	93.33	8E-61	Y	N	2					
Cat (*Felis catus*)	AP023162.1	100	4E-79	Y	N	1					
Dark-eyed junco (*Junco hyemalis*)	FJ236293.1	92.77	4E-59	Y	Y		1	1			
Snow bunting (*Plectrophenax nivalis*)	AF447251.1	93.94	2E-62	N	N	1	1	1			
Ruffed grouse (*Bonasa umbellus*)	KC785605.1	100	4E-79	Y	N	4					
Dark-eyed junco (*Junco hyemalis*)	FJ236293.1	93.33	2E-62	N	Y		1	1			
Mallard (*Anas platyrhynchos*)	MK770342.1	100	4E-79	Y	N		1	1			
Vole spp. (*Eothenomys miletus*)	KX014874.1	96.97	8E-71	Y	N	1	1	1			1
Woodpecker (*Dendropicos griseocephalus*)	AY940749.1	90.96	5E-53	Y	N	1					
Northern short-tailed shrew (*Blarina brevicauda*)	NC_042734.1	99.39	2E-77	Y	N	1	1	1	1		
Common shrew (*Sorex araneus*)	MN122909.1	94.58	4E-64	Y	N		6	4			

GenBank BLAST results	Voucher Sequence	% Identity	E Value	Closest Match?	Multiple Matches?	Cloacal Swabs	Nest Samples	Nest Boxes	Beak Swab	Composited Samples	Excluded Samples
White-throated sparrow (*Zonotrichia albicollis*)	MN356386.1	100	4E-79	Y	N	1	6	4		1	
Red-winged blackbird (*Ageliaus phoeniceus*)	FJ236289.1	97.58	2E-72	Y	Y	4	1	1			
Starling spp. (*Sturnus tristis*)	HQ915864.1	98.18	4E-74	Y	N	1	2	2			
Eastern phoebe (*Sayornis phoebe*)	U83765.2	92.12	2E-57	Y	N		2	1		1	
Wren spp. (*Campylorhynchus zonatus*)	KF509924.1	95.15	8E-66	Y	N		3	3			
Northern cardinal (*Cardinalis cardinalis*)	MH700631.1	100	4E-79	Y	N	5	1	1			
Yellow-bellied sapsucker (*Sphyrapicus varius*)	AY940770.1	100	4E-79	Y	N	1					
Deer mouse (*Peromyscus maniculatus*)	NC_039921.1	98.79	8E-76	N	N	4					
White-crowned sparrow (*Zonotrichia leucophrys*)	FJ236292.1	92.12	2E-57	N	Y		4	4			
American crow (*Corvus brachyrhynchos*)	KP403809.1	100	2E-81	Y	N	2					
Tree swallow (*Tachycineta bicolor*)	JQ071614.1	100	4E-79	Y	N		2	1			

GenBank BLAST results	Voucher Sequence	% Identity	E Value	Closest Match?	Multiple Matches?	Cloacal Swabs	Nest Samples	Nest Boxes	Beak Swab	Composited Samples	Excluded Samples
White-crowned sparrow (*Zonotrichia leucophrys*)	FJ236292.1	92.12	2E-57	N	Y	1	2	2			
Downy woodpecker (*Picoides pubescens*)	KT119343.1	100	4E-79	Y	N	2					
Central wtoneroller (*Campostoma anomalus*)	KP013113.1	100	4E-79	Y	N						1
Dove (*Streptopelia decaocto*)	NC_037513.1	92.73	4E-59	Y	N	2					
White-headed woodpecker (*Picoides albolarvatus*)	AY940760.1	98.79	8E-76	Y	N	2					
Moose (*Alces alces*)	MK644928.1	100	4E-79	Y	N				1		
Florida scrub jay (*Aphelocoma coerulescens*)	MN356421.1	99.39	2E-77	Y	N	4					
Red squirrel (*Tamiasciurus hudsonicus*)	AY227555.1	100	4E-79	Y	N	3					
Bald eagle (*Haliaeetus leucocephalus*)	GQ264658.1	100	2E-81	Y	N				1		
Coyote (*Canis latrans*)	KT448277.1	100	2E-81	Y	N				1		
Rose-breasted grosbeak (*Pheucticus ludovicianus*)	AF447248.1	100	4E-79	Y	N		1	1			
Wilson's snipe (*Gallinago gallinago*)	DQ674576.1	100	2E-81	Y	N		2	1			

Table 3: GenBank results for cloacal swabs with Prey DNA. Fifty-three cloacal swabs detected prey DNA. "Species" is the raptor species from which the cloacal swab was collected. "Age" is the age of the swabbed raptor (HY= hatch year, AHY= after-hatch year, SY=second year, ASY=after-second year, TY=third year, ATY= after-third year, and U=unknown age). "Sex" is the sex of the swabbed raptor (F=female, M=male, U=Unknown). "Date" is the day the swab was collected. "Prey" is the non-raptor OTU(s) detected on the swab. When no exact match was found in GenBank, prey species is recorded as the closest species within geographic range of Duluth, MN. If none of the closest matches were within geographic range, prey is reported at a higher taxonomic level. In two cases where there was a very close match to a species outside geographic range (>98% Identity, the closest match is reported. * denotes a match closely related to a species used to lure raptors during trapping. "% Identity" is the percent similarity of the detected OTU sequence to the prey species listed. "E Value" is the probability the OTU sequence and GenBank sequence would be that similar due to chance. "Accession #" is the unique identifier of the matching sequence in GenBank.

Species	Age	Sex	Date	Prey	% Identity	E Value	Accession #
Sharp-shinned hawk	ATY	F	4/27/2019	Turkey (*Meleagris gallopavo*)	100	4.00E-79	KM224338.1
Sharp-shinned hawk	SY	F	4/27/2019	Northern cardinal (*Cardinalis cardinalis*)	100	4.00E-79	MH700631.1
Sharp-shinned hawk	SY	M	5/4/2019	Turkey (*Meleagris gallopavo*)	100	4.00E-79	KM224338.1
Sharp-shinned hawk	AHY	M	5/4/2019	Northern cardinal (*Cardinalis cardinalis*)	100	4.00E-79	MH700631.1

Species	Age	Sex	Date	Prey	% Identity	E Value	Accession #
Sharp-shinned hawk	SY	M	5/4/2019	*Dove spp.	92.12	2.00E-57	KT182929.1
				*Dove spp.	92.73	4.00E-59	NC_037513.1
Sharp-shinned hawk	SY	M	5/4/2019	White-crowned sparrow (*Zonotrichia leucophrys*)	95.15	8.00E-66	FJ236292.1
				*Dove spp.	92.12	2.00E-57	KT182929.1
				White-crowned sparrow (*Zonotrichia leucophrys*)	92.12	2.00E-57	FJ236292.1
				Pine grosbeak (*Pinicola enucleator*)	93.33	6.00E-62	KM078781.1
				Red-winged blackbird (*Agelaius phoeniceus*)	97.58	2.00E-72	FJ236289.1
				Northern cardinal (*Cardinalis cardinalis*)	100	4.00E-79	MH700631.1
				White-crowned sparrow (*Zonotrichia leucophrys*)	92.12	2.00E-57	FJ236292.1
				*Dove spp.	92.73	4.00E-59	NC_037513.1
Sharp-shinned hawk	ASY	M	5/4/2019	*Dove spp.	93.33	8.00E-61	NC_037513.1
Sharp-shinned hawk	SY	M	5/4/2019	White-crowned sparrow (*Zonotrichia leucophrys*)	95.15	8.00E-66	FJ236292.1
				White-crowned sparrow (*Zonotrichia leucophrys*)	92.12	2.00E-57	FJ236292.1
				Pine grosbeak (*Pinicola enucleator*)	93.33	6.00E-62	KM078781.1
Sharp-shinned hawk	SY	F	5/4/2019	Red-winged blackbird (*Agelaius phoeniceus*)	97.58	2.00E-72	FJ236289.1
Sharp-shinned hawk	TY	F	5/4/2019	Red-winged blackbird (*Agelaius phoeniceus*)	97.58	2.00E-72	FJ236289.1

Species	Age	Sex	Date	Prey	% Identity	E Value	Accession #
Sharp-shinned hawk	SY	F	5/4/2019	Snow bunting (*Plectrophenax nivalis*)	93.94	2.00E-62	AF447251.1
Sharp-shinned hawk	SY	F	5/4/2019	Northern cardinal (*Cardinalis cardinalis*)	100	4.00E-79	MH700631.1
Sharp-shinned hawk	TY	F	5/4/2019	Cow (*Bos taurus*)	100	4.00E-79	MN714195.1
Sharp-shinned hawk	TY	F	5/5/2019	*Dove spp.	92.12	2.00E-57	KT182929.1
Sharp-shinned hawk	SY	F	5/5/2019	Red-winged blackbird (*Agelaius phoeniceus*)	97.58	2.00E-72	FJ236289.1
				Northern cardinal (*Cardinalis cardinalis*)	100	4.00E-79	MH700631.1
Sharp-shinned hawk	SY	F	5/5/2019	White-headed woodpecker (*Picoides albolarvatus*)	98.79	8.00E-76	AY940760.1
Sharp-shinned hawk	ATY	F	5/5/2019	Turkey (*Meleagris gallopavo*)	100	4.00E-79	KM224338.1
Sharp-shinned hawk	HY	F	9/5/2019	Downy woodpecker (*Picoides pubescens*)	100	4.00E-79	KT119343.1
				White-headed woodpecker (*Picoides albolarvatus*)	98.79	8.00E-76	AY940760.1
Sharp-shinned hawk	HY	F	9/5/2019	Florida scrub jay (*Aphelocoma coerulescens*)	99.39	2.00E-77	MN356421.1
Sharp-shinned hawk	HY	F	9/6/2019	Florida scrub jay (*Aphelocoma coerulescens*)	99.39	2.00E-77	MN356421.1
Sharp-shinned hawk	HY	F	9/7/2019	Ruffed grouse (*Bonasa umbellus*)	100	4.00E-79	KC785605.1
Sharp-shinned hawk	HY	F	9/14/2019	Yellow-bellied sapsucker (*Sphyrapicus varius*)	100	4.00E-79	AY940770.1

Species	Age	Sex	Date	Prey	% Identity	E Value	Accession #
Sharp-shinned hawk	HY	F	9/21/2019	*Dove spp.	92.12	2.00E-57	KT182929.1
				Florida scrub jay (*Aphelocoma coerulescens*)	99.39	2.00E-77	MN356421.1
Sharp-shinned hawk	ASY	F	9/21/2019	*Sturnus spp.	98.18	4.00E-74	HQ915864.1
				Florida scrub jay (*Aphelocoma coerulescens*)	99.39	2.00E-77	MN356421.1
Sharp-shinned hawk	U	F	9/21/2019	Downy woodpecker (*Picoides pubescens*)	100	4.00E-79	KT119343.1
Sharp-shinned hawk	HY	F	9/22/2019	Woodpecker spp.	90.96	5.00E-53	AY940749.1
Sharp-shinned hawk	HY	F	9/23/2019	Chicken (*Gallus gallus*)	100	4.00E-79	MN013407.1
Sharp-shinned hawk	HY	M	9/24/2019	White-throated sparrow (*Zonotrichia albicollis*)	100	4.00E-79	MN356386.1
Cooper's hawk	HY	F	9/15/2019	Cow (*Bos taurus*)	100	4.00E-79	MN714195.1
Cooper's hawk	ASY	F	10/6/2019	Ruffed grouse (*Bonasa umbellus*)	100	4.00E-79	KC785605.1
Northern goshawk	SY	M	5/5/2019	*Dove spp.	92.12	2.00E-57	KT182929.1
Northern goshawk	HY	M	9/26/2019	American crow (*Corvus brachyrhynchos*)	100	2.00E-81	KP403809.1
Northern goshawk	HY	M	10/8/2019	Ruffed grouse (*Bonasa umbellus*)	100	4.00E-79	KC785605.1
				American crow (*Corvus brachyrhynchos*)	100	2.00E-81	KP403809.1
Northern goshawk	HY	F	10/12/2019	Dog (*Canis lupus familiaris*)	100	4.00E-79	MN181403.1
Northern goshawk	HY	M	10/17/2019	Cat (*Felis catus*)	100	4.00E-79	AP023162.1

Species	Age	Sex	Date	Prey	% Identity	E Value	Accession #
Northern goshawk	HY	M	10/23/2019	Virginia opossum (*Didelphis virginiana*)	100	4.00E-79	AY012091.1
Northern goshawk	SY	M	10/23/2019	Ruffed grouse (*Bonasa umbellus*)	100	4.00E-79	KC785605.1
Northern goshawk	HY	F	10/23/2019	Red squirrel (*Tamiasciurus hudsonicus*)	100	4.00E-79	AY227555.1
Northern goshawk	HY	F	10/30/2019	*Dove spp.	93.33	8.00E-61	NC_037513.1
Northern goshawk	ASY	F	11/10/2019	Dog (*Canis lupus familiaris*)	100	4.00E-79	MN181403.1
Northern goshawk	ASY	F	11/12/2019	Dog (*Canis lupus familiaris*)	100	4.00E-79	CP050601.1
Red-tailed hawk	HY	U	9/4/2019	Dog (*Canis lupus familiaris*)	100	4.00E-79	MN181403.1
Red-tailed hawk	U	U	9/23/2019	Dog (*Canis lupus familiaris*)	100	4.00E-79	MN181403.1
Red-tailed hawk	HY	U	11/1/2019	Red squirrel (*Tamiasciurus hudsonicus*)	100	4.00E-79	AY227555.1
Red-tailed hawk	HY	U	11/2/2019	Red squirrel (*Tamiasciurus hudsonicus*)	100	4.00E-79	AY227555.1
Red-tailed hawk	HY	U	11/11/2019	Dog (*Canis lupus familiaris*)	100	4.00E-79	MN181403.1
				Cedar waxwing (*Bombycilla cedrorum*)	100	4.00E-79	KJ909187.1
Northern saw-whet owl	SY	F	10/4/2010	Deer mouse (*Peromyscus maniculatus*)	98.79	8.00E-76	NC_039921.1

Species	Age	Sex	Date	Prey	% Identity	E Value	Accession #
Northern saw-whet owl	ASY	F	10/1/2019	Vole spp.	96.97	8.00E-71	KX014874.1
				Deer mouse (*Peromyscus maniculatus*)	98.79	8.00E-76	NC_039921.1
Northern saw-whet owl	HY	F	10/4/2019	Vole spp.	95.15	3.00E-65	AJ972918.1
Northern saw-whet owl	HY	F	10/4/2019	Vole spp.	92.77	4.00E-59	LC424768.1
Northern saw-whet owl	SY	U	10/4/2019	Northern short-tailed shrew	99.39	2.00E-77	NC_042734.1
Northern saw-whet owl	SY	F	10/14/2019	Deer mouse (*Peromyscus maniculatus*)	98.79	8.00E-76	NC_039921.1
Northern saw-whet owl	HY	M	10/14/2019	Deer mouse (*Peromyscus maniculatus*)	98.79	8.00E-76	NC_039921.1

Table 4: Generalized linear models of prey detection on cloacal swabs. We tested 7 models against the null to evaluate the effect of species, body size (average body size), and migration strategy (passive vs. active flight) on the probability of detecting prey species from a cloacal swab.

Model Description	AIC_c	ΔAIC_c	Number of Parameters	Model Weight
Null	256.92	0	1	0.34
Migration	257.48	0.56	2	0.25
Migration + Size	258.06	1.13	3	0.19
Size	258.95	2.03	2	0.12
Species	261.05	4.13	5	0.04
Size + species	262.21	5.28	6	0.02
Migration + species	262.71	5.78	6	0.02
Migration + Species + Size	263.87	6.94	7	0.01

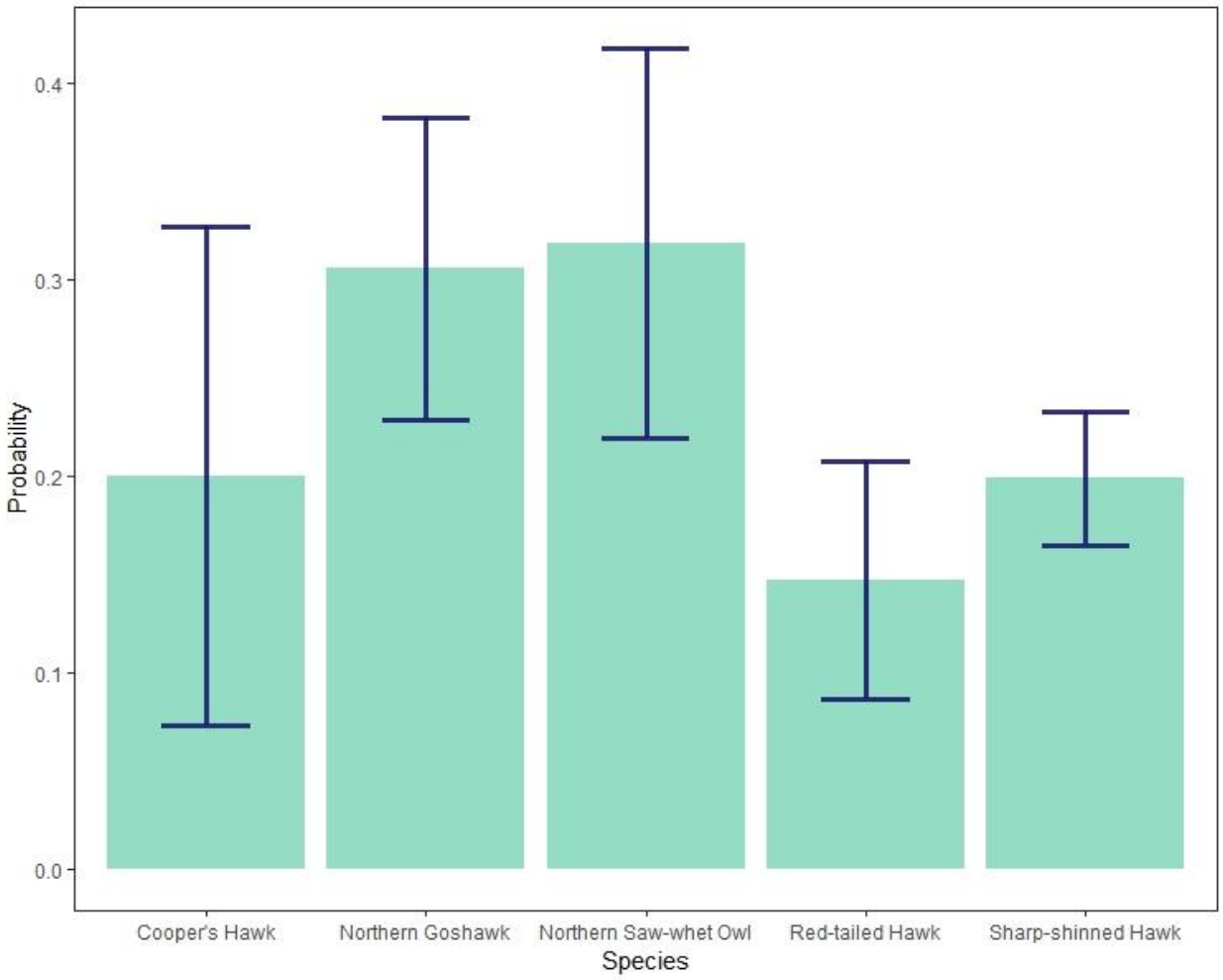

Figure 1: Probability of detecting ≥1 prey species on cloacal swabs by species. This figure was generated from the "Species" model. Species of raptors with no prey detections were omitted. The error bars are +/- one standard deviation of the predicted probability of detection for each species.

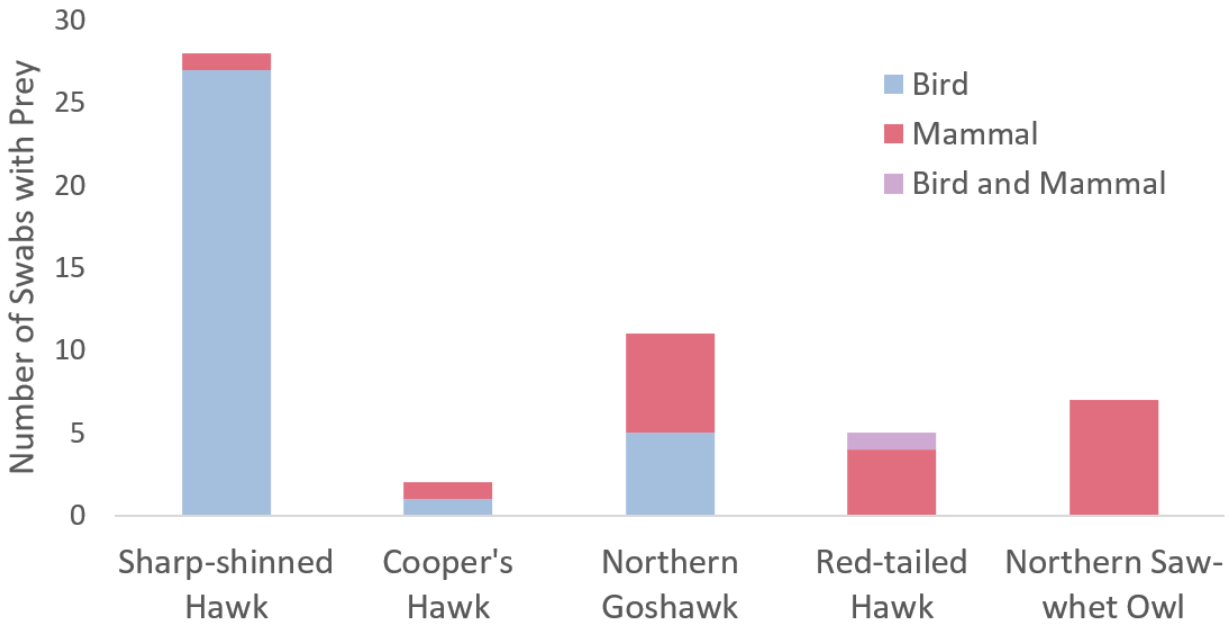

Figure 2: Type of prey on cloacal swabs by species. The number of cloacal swabs with prey DNA detection are subdivided by the taxonomic group of the prey OTU(s) on the cloacal swab. Cloacal swabs that with only avian prey are in blue, swabs with only mammalian prey are in red, and swabs with both mammalian and avian prey are in purple.

Table 5: 12S sequencing status of bird and mammal species. We did a select search in the NCBI Nucleotide database of 119 bird and 56 mammal species that are possible prey items and noted whether the 12S gene had been fully sequenced (Y), partially sequenced (P), or not sequenced (N) for the species.

Scientific Name	Common Name	12S sequenced
Porzana carolina	Sora	Y
Gallinago delicata	Wilson's snipe	P
Scolopax minor	American woodcock	Y
Zenaida macroura	Mourning dove	Y
Coccyzus erythropthalmus	Black-billed cuckoo	Y
Coccyzus americanus	Yellow-billed cuckoo	Y
Caprimulgus vociferus	Eastern whip-poor-will	P
Chordeiles minor	Common nighthawk	Y
Sphyrapicus varius	Yellow-bellied sapsucker	Y
Picoides pubescens	Downy woodpecker	Y
Leuconotopicus villosus	Hairy woodpecker	N
Colaptes auratus	Northern flicker	Y
Picoides dorsalis	American three-toed woodpecker	N
Picoides arcticus	Black-backed woodpecker	N
Dryocopus pileatus	Pileated woodpecker	Y
Contopus virens	Eastern wood pewee	N
Empidonax flaviventris	Yellow-bellied flycatcher	N
Empidonax virescens	Acadian flycatcher	N
Empidonax alnorum	Alder flycatcher	N
Empidonax minimus	Least flycatcher	N
Contopus cooperi	Olive-sided flycatcher	N
Sayornis phoebe	Eastern phoebe	Y
Myiarchus crinitus	Great crested flycatcher	N
Tyrannus tyrannus	Eastern kingbird	P
Lanius exubitor	Northern shrike	N
Vireo griseus	White-eyed vireo	N
Vireo flavifrons	Yellow-throated vireo	N
Vireo solitarius	Blue-headed vireo	N
Vireo gilvus	Warbling vireo	N
Vireo philadelphicus	Philadelphia vireo	N
Vireo olivaceus	Red-eyed vireo	Y
Cyanocitta cristata	Blue jay	N
Perisoreus canadensis	Gray jay	N
Poecile atricapillus	Black-capped chickadee	Y
Poecile hudsonicus	Boreal chickadee	N

Scientific Name	Common Name	12S sequenced
Sitta canadensis	Red-breasted nuthatch	N
Sitta carolinensis	White-breasted nuthatch	Y
Certhia americana	Brown creeper	N
Troglodytes aedon	House wren	N
Troglodytes hiemalis	Winter wren	N
Cistothorus palustris	Marsh wren	N
Cistothorus stellaris	Sedge wren	N
Regulus satrapa	Golden-crowned kinglet	P
Regulus calendula	Ruby-crowned kinglet	Y
Sialia sialis	Eastern bluebird	P
Catharus fuscescens	Veery	N
Catharus minimus	Gray-cheeked thrush	N
Catharus ustulatus	Swainson's thrush	Y
Catharus guttatus	Hermit thrush	P
Hylocichla mustelina	Wood thrush	P
Turdus migratorius	American robin	Y
Dumetella carolinensis	Gray catbird	P
Mimus polyglottos	Northern mockingbird	P
Toxostoma rufum	Brown thrasher	N
Bombycilla cedrorum	Cedar waxwing	Y
Bombycilla garrulus	Bohemian waxwing	Y
Vermivora cyanoptera	Blue-winged warbler	N
Vermivora chrysoptera	Golden-winged warbler	N
Vermivora celata	Orange-crowned warbler	Y
Leiothlypis peregrina	Tennessee warbler	N
Leiothlypis ruficapilla	Nashville warbler	N
Setophaga ruticilla	American redstart	N
Setophaga americana	Northern parula	N
Setophaga petechia	Yellow warbler	N
Setophaga pensylvanica	Chestnut-sided warbler	N
Setophaga magnolia	Magnolia warbler	N
Setophaga tigrina	Cape May warbler	N
Setophaga caerulescens	Black-throated blue warbler	N
Setophaga coronata	Yellow-rumped warbler	Y
Setophaga virens	Black-thoated green warbler	N
Setophaga fusca	Blackburnian warbler	N
Setophaga pinus	Pine warbler	N
Setophaga palmarum	Palm warbler	N
Setophaga castanea	Bay-breasted warbler	N
Setophaga striata	Blackpoll warbler	N
Setophaga citrina	Hooded warbler	N
Mniotilta varia	Black-and-white warbler	N
Seiurus aurocapilla	Ovenbird	P

Scientific Name	Common Name	12S sequenced
Parkesia noveboracensis	Northern waterthrush	N
Oporornis agilis	Connecticut warbler	N
Geothlypis philadelphia	Mourning warbler	N
Geothlypis trichas	Common yellowthroat	Y
Cardellina pusilla	Wilson's warbler	N
Cardellina canadensis	Canada warbler	Y
Pipilo erythrophthalmus	Eastern towhee	P
Spizella arborea	American tree sparrow	N
Spizella passerina	Chipping sparrow	N
Spizella pallida	Clay-colored sparrow	N
Spizella pusilla	Field sparrow	N
Passerculus sandwichensis	Savannah sparrow	N
Ammodramus savannarum	Grasshopper sparrow	N
Ammodramus leconteii	Le Conte's sparrow	N
Ammodramus nelsoni	Nelson's sharp-tailed sparrow	N
Passerella iliaca	Fox sparrow	N
Melospiza melodia	Song sparrow	Y
Melospiza lincolnii	Lincoln's sparrow	N
Melospiza georgiana	Swamp sparrow	N
Zonotrichia albicollis	White-throated sparrow	Y
Zonotrichia leucophrys	White-crowned sparrow	Y
Junco hyemalis	Dark-eyed junco	Y
Piranga olivacea	Scarlet tanager	Y
Cardinalis cardinalis	Northern cardinal	Y
Pheucticus ludovicianus	Rose-breasted grosbeak	Y
Passerina cyanea	Indigo bunting	P
Agelaius phoeniceus	Red-winged blackbird	Y
Sturnella magna	Eastern meadowlark	Y
Euphagus carolinus	Rusty blackbird	N
Quiscalus quiscula	Common grackle	Y
Molothrus ater	Brown-headed cowbird	P
Icterus galbula	Baltimore oriole	P
Pinicola enucleator	Pine grosbeak	Y
Haemorhous purpureus	Purple finch	Y
Haemorhous mexicanus	House finch	Y
Spinus pinus	Pine siskin	N
Loxia leucoptera	White-winged crossbill	Y
Loxia curvirostra	Red crossbill	Y
Acanthis flammea	Common redpoll	Y
Spinus tristis	American goldfinch	Y
Passer domesticus	House sparrow	Y

Scientific Name	Common Name	12S sequenced
Sorex arcticus	Arctic shrew	N
Sorex cinereus	Common shrew	P
Sorex fumeus	Smoky shrew	N
Sorex haydeni	Prairie shrew	N
Sorex hoyi	American pygmy shrew	N
Sorex palustris	American water shrew	Y
Blarina brevicauda	Northern short-tailed shrew	P
Cryptotis parva	Least shrew	P
Parascalops breweri	Hairy-tailed mole	P
Condylura cristata	Star-nose mole	Y
Myotis lucifugus	Little brown bat	Y
Myotis septentrionalis	Northern long-eared myotis	Y
Lasiurus borealis	Eastern red bat	Y
Lasiurus cinereus	Hoary bat	Y
Lasionycteris noctivagans	Silver-haired bat	Y
Pipistrellus subflavus	Tricolored bat	Y
Eptesicus fuscus	Big Brown bat	Y
Tamias minimus	Least chipmunk	N
Tamias striatus	Eastern chipmunk	Y
Marmota monax	Woodchuck	P
Spermophilus franklinii	Franklin's ground squirrel	N
Spermophilus parryii	Arctic ground squirrel	Y
Spermophilus richardsonii	Richardson's ground squirrel	Y
Ictidomys tridecemlineatus	Thirteen-lined ground squirrel	Y
Sciurus carolinensis	Eastern gray squirrel	Y
Sciurus niger	Easter fox squirrel	P
Tamiasciurus hudsonicus	Red squirrel	P
Glaucomys sabrinus	Northern flying squirrel	N
Glaucomys volans	Southern flying squirrel	Y
Geomys bursarius	Plains pocket gopher	P
Reithrodontomys megalotis	Western harvest mouse	N
Peromyscus leucopus	White-footed mouse	Y
Peromyscus maniculatus	Deer mouse	Y
Onychomys leucogaster	Northern grasshopper mouse	Y
Myodes gapperi	Southern red-backed vole	N
Myodes rutilus	Northern red-backed vole	Y
Phenacomys intermedius	Western heather vole	N
Phenacomys ungava	Eastern feather vole	N
Microtus ochrogaster	Prairie vole	Y
Microtus pennsylvanicus	Meadow vole	P
Microtus pinetorum	Woodland vole	N
Lemmus sibiricus	Brown lemming	N
Synaptomys borealis	Northern bog lemming	N

Scientific Name	Common Name	12S sequenced
Synaptomys cooperi	Southern bog lemming	N
Dicrostonyx groenlandicus	Collared lemming	Y
Rattus norvegicus	Norway rat	Y
Mus musculus	House mouse	Y
Zapus hudsonius	Meadow jumping mouse	N
Zapus princeps	Western jumping mouse	N
Perognathus flavescens	Plains pocket mouse	P
Castor Canadensis	American beaver	Y
Erethizon dorsatum	Porcupine	P
Ondatra zibethicus	Muskrat	Y
Sylvilagus floridanus	Eastern cottontail	P
Lepus americanus	Snowshoe hare	Y
Lepus europaeus	European hare	Y

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