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Using Noninvasive Genetics for Estimating Density and Assessing Diet of Urban and Rural Coyotes in Florida, USA

Bryan M. Kluever[1], Martin B. Main[2], Stewart W. Breck[3], Robert C. Lonsinger[4], John H. Humphrey[1], Justin W. Fischer[3], Michael P. Milleson[5], and Antoinette J. Piaggio[3]

Abstract - Coyotes *(Canis latrans)* are expanding their range and due to conflicts with the public and concerns of Coyotes affecting natural resources such as game or sensitive species, there is interest and often a demand to monitor Coyote populations. A challenge to monitoring is that traditional invasive methods involving live-capture of individual animals are costly and can be controversial. Natural resource management agencies can benefit from contemporary noninvasive genetic sampling approaches aimed at determining key aspects of Coyote ecology (e.g., population density and food habits). However, the efficacy of such approaches under different environmental conditions is poorly understood. Our objectives were to 1) examine accumulation and nuclear DNA degradation rates of Coyote scats in metropolitan and rural sites in Florida to help optimize methods to estimate population density; and 2) explore new genetic methods for determining diet of Coyotes based on vertebrate, plant, and invertebrate species DNA identified in scat. Recently developed DNA metabarcoding approaches make it possible to simultaneously identify DNA from multiple prey species in predator scat samples, but an exploration of this tool for assessing Coyote diet has not been pursued. We observed that scat accumulation rates (0.02 scats/km/day) did not vary between sites and fecal DNA amplification success decreased and genotyping errors increased over time with exposure to sun and precipitation. DNA sampling allowed us to generate a Coyote density estimate for the urban environment of eight Coyotes per 100 km2, but lack of recaptures in the rural area precluded density estimation. DNA metabarcoding showed promise for assessing diet contributions of vertebrate species to Coyote diet. Feral Swine *(Sus scrofa)* were detected as prey at higher frequencies than previously reported. We identify several considerations that can be used to optimize future noninvasive sampling efforts for Coyotes in the southeastern United States. We also discuss strengths and drawbacks of utilizing DNA metabarcoding for assessing diet of generalist carnivores such as Coyotes.

Introduction

Canis latrans Say (Coyotes) in North America are generalists that have colonized both rural (Mastro et al. 2011) and urban landscapes (Gehrt et al. 2009), including those in Florida (Grigione et al. 2011). Coyotes are usually the top predator in urban areas and can

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positively impact urban and rural ecosystems through predation and competition (Crooks and Soulé 1999). However, Coyotes are also involved in conflicts with the public, primarily through attacks on pets, livestock, and occasionally people (Poessel et al. 2016). Coyotes in urban environments have been reported to be bolder than Coyotes living in rural or wildland environments (Breck et al. 2019) and have the potential to maintain and transmit diseases (Brown et al. 2012). In rural areas, Coyotes are a source of concern among livestock producers (Boughton et al. 2016).

Despite their widespread distribution, information on Coyote population parameters (e.g., density, abundance), life history traits (e.g., survival, recruitment), diet, and habitat use in both urban and rural environments is scarce for many areas (Poessel et al. 2017, Scotten 2019).

Traditionally, invasive sampling has been employed to study mammalian carnivore space use and population dynamics, but use of noninvasive genetic sampling, specifically using scats, is becoming more common because of advantages gained (e.g., less cost and no handling of animals) and the ability to answer multiple questions (e.g., diet, population density) with the same samples. For example, using the same scat samples, researchers studying canids in the Great Basin Desert were able to determine scat accumulation rates (Lonsinger et al. 2015), estimate density (Lonsinger et al. 2018) and test theories on competition using an occupancy framework (Lonsinger et al. 2017). A comparison between morphological and genetic-based approaches for diet analyses of Coyotes revealed that for leporids, a guild commonly consumed by Coyotes, detection occurred with greater frequency with the molecular method (Gosselin et al. 2017). Recently developed DNA metabarcoding approaches make it possible to simultaneously identify the taxon of various prey DNA present in scat samples by sequencing in parallel thousands of DNA barcodes (Taberlet et al. 2012). This approach has been used for mammalian carnivore investigations and findings indicate this approach can detect prey species otherwise likely not to be detected and reduces species misidentifications (De Barba et al. 2014, Monterroso et al. 2018).

Many factors can affect accumulation of scat sample samples (e.g., latrine locations and use, animal density, home range size) and quality of the scats (i.e., the potential for DNA degradation). Pilot studies have been recommended to optimize spatial and temporal sampling efficiency before undertaking investigations at larger spatio-temporal scales (Lonsinger et al. 2015). Once pilot studies are completed, scat sampling at larger scales for genetic capture-recapture studies can be achieved (Lonsinger et al. 2018). Pilot studies are especially important in the far southeastern United States because scat sampling for DNA analysis for canids has been attempted only at small insular spatial scales (e.g., Sanibel Island, FL; Jim Beasley, University of Georgia, unpublished study, 2017). We conducted a study examining scat accumulation, DNA degradation rates, and determination of diet from DNA using Coyote scats collected from rural and urban sites in Florida. Our objectives were to: 1) determine the optimal sampling interval for collecting Coyote scats in Florida for use in genetic-based population assessment, and 2) evaluate Coyote diets from scats collected in rural and urban study sites using a DNA metabarcoding approach.

Methods

Study Area

We conducted our study in two distinct Florida environments; one urban, one rural (Fig. 1). We chose Jacksonville as our urban site as it contained an extensive network of city parks and trails and roads along power lines providing abundant transects for sampling. The rural study site was the MacArthur Agro-ecology Research Center at Buck Island Ranch

(MAERC), Highlands County. This location was selected based on access, a network of secondary dirt roads to facilitate on-foot transect surveys, and baseline information available regarding the Coyote population at this location (Boughton et al. 2016).

Coyotes in rural areas in Florida have average home range sizes of approximately 25 km2 (Thornton et al. 2004, Zhang 2017). Urban Coyotes often have smaller home ranges

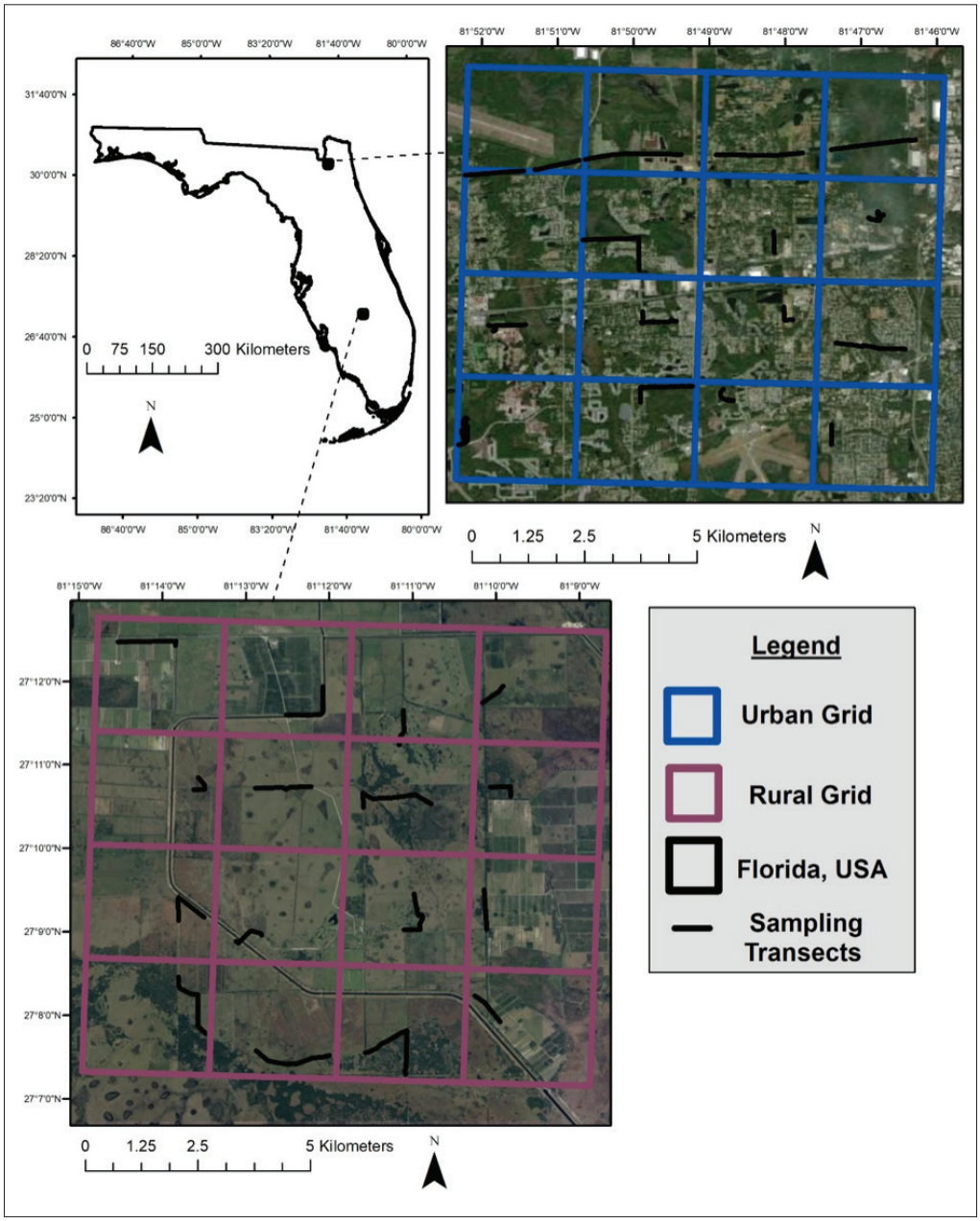

Figure 1. Urban and rural study areas located in Jacksonville and Highlands County, FL used to collect coyote scat samples during January-February 2020. Study area size was 100 km2 divided into 16 cells of 6.25-km2 each. Transects range from 500-m to 2-km in length.

than their rural counterparts, but home range sizes can be highly variable (e.g., 1.1–22.3 km2; Grubbs and Krausman 2009, Jantz 2011). Following previous research by Lonsinger et al. (2018), we used a 6.25 km2 cell size based on capture-recapture density estimates undertaken on Coyotes with a similar home range of 19.0–35.2 km2. In both urban and rural locations, we established study areas of 100 km2, each of which was divided into 16 cells (2.5 x 2.5 km). In each cell we identified 500-m to 2-km long transects for scat sampling areas. In the urban study area, transect locations were limited to public domain areas including hiking trails, sidewalks, and roads along power lines. In the rural area, transects were limited to pasture fence lines and dirt roads.

Field Scat Sampling

Because we anticipated DNA degradation being most problematic in summer due to higher levels of moisture and UV radiation (Brinkman et al. 2010, Murphy et al. 2007), our field scat sampling efforts were conducted during Florida's cool and dry winter months of January and February. We conducted surveys in each 16-cell grid in both the rural and urban study sites. All transects were initially cleared of all scats (hereafter "clear survey") and then repeatedly surveyed to collect freshly deposited scats of known age (hereafter "collection survey") (Lonsinger et al. 2018). Surveyors were trained to search for scats within 2m of the centerline of each transect. Transects were clearly visible/displayed at all times on data collection tables and/or smart phones using ARCGIS Collector (ESRI, West Redlands, CA, USA). Repeated sampling on transects is an established methodology for answering important questions pertaining to canid biology and ecology (Dempsey et al 2014, Kluever and Gese 2016, Kluever et al. 2017). To provide information regarding scat accumulation and DNA degradation rates needed for development of a sampling protocol for estimating Coyote abundance, we employed a staggered interval survey where subsets of transects were sampled at different intervals, ranging from one to six days between surveys. Including the clear survey, all transects were surveyed at least five times.

For each scat encountered during double-observer collection surveys (Dempsey et al. 2015), we recorded UTM coordinates and collected a 1–2 cm long piece of scat using a razor blade and placed the segment of fecal sample in 15-ml tubes containing 10 ml of DETs buffer (Seutin et al. 1991). These samples were sent to the Wildlife Genetics Lab (WGL) at the USDA Wildlife Services National Wildlife Research Center (NWRC) Headquarters, Fort Collins, CO, USA, for species identification and, if identified as Coyote, individual identification. We placed an additional 1-2 cm long piece of scat in 20-ml tubes and sent these samples frozen to Jonah Ventures (Boulder, CO, USA) for Coyote diet analysis with DNA metabarcoding. For scats that were collected within 24 hours of the clear survey or the previous collection survey, an additional 0.5 g of scat was placed in a 2-ml tube containing 1 ml of DETs buffer and used for time zero of the degradation study (see below). The remaining scat, which usually comprised a large proportion of the original scat was then collected.

Species and Individual Coyote Identification and Abundance Estimation

We extracted DNA from fecal samples using Qiagen's QIAamp® Fast DNA Stool Mini Kit. Extracted DNA samples were then amplified and cycle-sequenced using general mitochondrial (mtDNA) control region primers L15926 5' – CAATTCCCCGGTCTTGTAAACC and H16340 5' – CCTGAAGTAGGAACCAGATG (Vilà et al. 1999). Sequences were compared to NCBI GenBank using BLAST, and top matches were selected to identify species. Low quality sequences were amplified and sequenced a second time. When fecal samples were identified as Coyote, we amplified 10 nuclear microsatellite loci with a multiple tubes

approach (Taberlet et al. 1996) to identify individuals. We performed four independent PCR replicates on each sample for each microsatellite panel. We determined a consensus allele call at each locus from the four PCR replicates by following common scoring protocols for noninvasive studies (Frantz et al. 2003, Lonsinger et al. 2015). As a general rule, matching heterozygotes must be observed ≥2 times, and single alleles ≥3 times for homozygote confirmation. Multiple detections of individual Coyotes were considered recaptures and allowed for estimation of abundance using a capture-recapture framework. Due to data scarcity, we employed the capture with replacement (Capwire) population model (Miller et al. 2005) using the package CAPWIRE in R (R Core Team 2020).

Coyote Scat Accumulation

Identification of Coyote scats allowed us to calculate daily scat accumulation rates, a metric employed where scat accumulation rate is standardized across transects to generate a daily accumulation rate reported as scats/km/day (Lonsinger et al. 2015). We generated daily scat accumulation rates for the urban site, rural site, and both sites combined. Daily accumulation rates in concert with DNA degradation rates can allow natural resource managers and biologists to understand and plan the appropriate sampling effort needed for a noninvasive genetic approach capable of yielding informative results (Lonsinger et al. 2015).

DNA Degradation

To determine factors that influence DNA degradation in Coyote scats we designed an experiment in which 24 scats were randomly assigned to one of three treatments: 1) full exposure to sun and precipitation events (FE), 2) exposure to sun but no precipitation (NP), and 3) ambient air only (NPS). Following methods of Kierepka et al. (2016), we tested for nuclear DNA degradation of scats at "time zero" when the sample was collected in the field and after 12, 24, 72, 120, and 168 hours of exposure. After the 12 hour sampling period, we observed ants consuming scats. Because we were more interested in Coyote DNA degradation than biodegradation rates of scat contents (i.e., consumption by insects or other organisms), we treated a 2 meter area buffer around the experimental array with insecticide. We observed minimal ant presence during the 24 hour sampling period and zero ants during remaining sampling events.

We used PCR amplification and genotyping error rates to assess DNA degradation rates of scats. To qualify for inclusion in the degradation component of our study, scat had to be collected within 24 hours of deposition. Our initial efforts in January and February 2020 did not result in a sufficient sample size that met this criterion, thus we supplemented our sample size with four additional Coyote scats collected from the Jacksonville study area during May 2020 and 16 Coyote scats collected from captive Coyotes housed at the NWRC Field Station in Logan, UT, during June 2020. Captive Coyotes were fed 650 g of commercial mink food (Fur Breeders Agricultural Cooperative, Logan, UT, USA) daily, using a technique referred to as scatter-feeding, where the food is distributed broadly within each enclosure. Water was provided *ad libitum*. All scats were frozen within 12 hours of being collected.

For the degradation experiment, scats were placed in a secure netted aviary and one of three treatment boxes (Fig. 2), which protected scats from removal by vertebrates. The treatment boxes were constructed using 1.5 x 3.8 x 8.9 cm dimensional lumber framing to form a rectangular frame with internal dimensions of 61.0 x 34.3 x 8.9 cm). The top of the box was covered by metal window screening secured by staples to minimize invertebrate access to

samples from above. The boxes were divided into cells by securing string to the bottom of the box evenly dividing the box into 8 cells. The treatment boxes were placed outdoors and within the aviary, and placed on an elevated and leveled sandy soil mound topped with metal window screening to minimize invertebrate access to the samples from below ground. Individual treatments except for FE were created through the use of clear (NP) or silver painted (NPS) Plexiglas panels (80.0 cm x 53.3 cm x 0.64 cm) fitted with aluminum framing secured to the underside of the panels for rigidity. Panels were centered and elevated above the box 5.08 cm using 1.2 cm diameter aluminum rods inserted into holes drilled at each corner of the box to allow airflow and reduce condensation on the underside of the panel while protecting the samples from the desired elements per the treatment. Average daily temperature during the duration of the DNA degradation experiment was 29.3°C (SD = 1.9) and ranged from 22.1 to 42.8°C.

Genotypes were obtained for each sample using 10 microsatellite markers (Multiplex A, B, C from Table 1 of Hopken et al. 2016). We used a multiple tubes approach (Taberlet et al. 1996) with four replicates of each PCR. To analyze error rates across genotypes—false alleles (FA) and allelic dropout (ADO)—we used gimlet v1.3.3 (Valière 2002). As there were no reference genotypes from the animals to compare to the fecal genotypes, we generated consensus genotypes both across all time points for each sample (n = 10 genotypes), and using the threshold method in gimlet set to 2 repeats as a minimum. Resulting consensus genotypes (n = 24) were reviewed and, in cases where two alleles showed up more than twice and with equivalent frequency, the call was changed to a null (000) unless one of those alleles showed up more often in the time 0 replicates, then that allele was called. These consensus genotypes were used as the reference for comparison with replicate genotypes from each time point and to assess the frequency of null alleles and false alleles across the study and within each treatment.

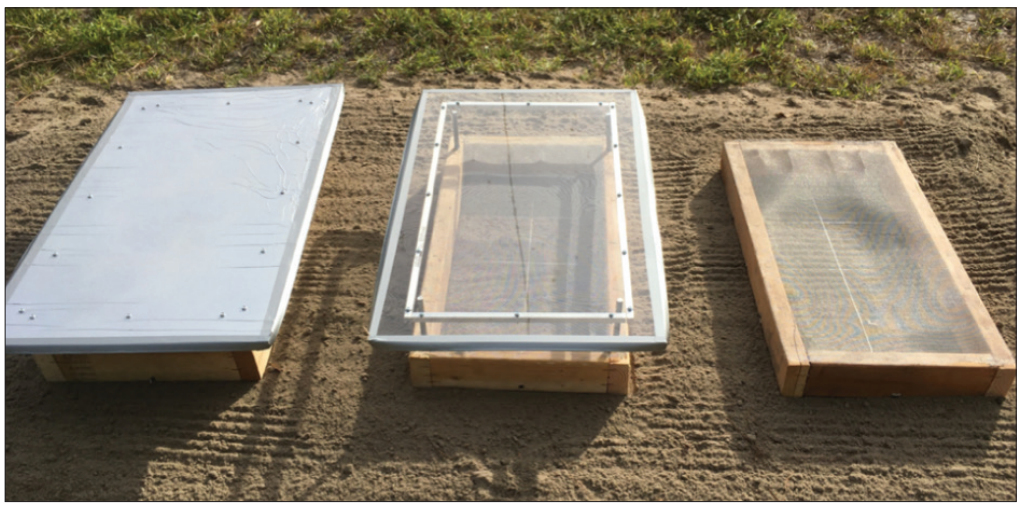

Figure 2. Experimental array for coyote scat DNA degradation study conducted at the U.S. Department of Agriculture (USDA) Wildlife Services National Wildlife Research Center, Gainesville, FL, in July 2020. Twenty four scats (8 scats per treatment box) were randomly assigned a treatment and sampled at 1, 3, 5, and 7 days post placement. Treatments (from left to right) are 1) ambient air only (no sun or precipitation (NPS), 2) exposure to sun but not precipitation (NP), and 3) exposure to sun and precipitation (FE).

To assess degradation rates across all samples and treatments and per treatment, we distilled genotypes for 24 fecal samples across 6 time periods (0, 12, 24, 72, 120, 168 hours) into general amplification rates, averaging the four PCR replicates. We then evaluated amplification rates as a 2-factor, repeated-measures design in which time was a continuous variable considered as a within-subject factor, and treatment (FE = full exposure, NP = no precipitation, and NPS = no sun or precipitation exposure) was considered a between-subject factor. Further, individual scats were nested within a treatment given that each was only subjected to one of the three treatments. We evaluated the relationship of time and treatment on amplification rates using a linear mixed model with a continuous autoregressive [AR(1)] correlation structure (amplification rate = individual random intercept + treatment + time + treatment × time (and reduced subsets of factors as appropriate)) in program R (package = nlme; r v3.3.3; R Core Team 2017). Factors were retained as significant based on an alpha value of 0.1 given the limited sample size (Kluever et al. 2013).

To further assess DNA degradation rates in scats, we evaluated PCR amplification success (PCR success), FA, and ADO as binary response variables with mixed-effects logistic regression models. We included a random effect for sample to resolve pseudoreplication effects due to multiple observations per sample. We included fixed effects for the scat age and treatment type (i.e., FE, NP, or NPS), and an interaction between age and treatment. For PCR success, a successful amplification was coded as a one and failure to amplify was coded as a zero. For models of FA and ADO, the presence of an error was coded as a one, whereas the lack of an error (for a sample with a successful amplification) was coded as zero. Based on the model results for each of the three response variables, we estimated the probability of PCR success, FA, and ADO as a function of sample age and treatment. All mixed-effects logistic regression analyses were conducted using program R (R Core Team 2020).

Coyote Diet

We used DNA metabarcoding techniques to identify the composition and occurrence of plant and animal (vertebrate and invertebrate) material in Coyote scats collected during field scat sampling. DNA in fecal samples were extracted and primers used to amplify the number of copies of short segments of DNA that are universal for taxonomic groups of interest. In this case, BatR01 and Ac12s were used to amplify vertebrate DNA, ArthCOI for insects, and trnL primers were used to amplify higher plant DNA. BatR01 is geared toward mammal and avian species detection while Ac12s is known to be more robust for detection of herpetofauna, though there is some overlap in species detectability across the two primers (Joseph Craine, Jonah Ventures, personal communication). After amplification, DNA was tagged with a unique index to identify different samples and then samples were pooled before sequencing on an Illumina Miseq. After sequencing, data were processed to provide a list of sequences and their abundances for each sample and primer set. These sequences were then compared to reference databases to identify the species from which the DNA sequences originated. DNA isolation and quantification, as well as sequence processing was performed as in Robeson et al. (2018) to produce representative OTU (Operational Taxonomic Unit) sequences in the form of Exact Sequence Variants (ESVs; Callahan et al. 2017).

For the vertebrate and invertebrate metabarcoding data, we followed De Barba et al. (2014) and considered only sequences with match identity >95% in order to increase the accuracy of the automatic taxonomic assignation and exclude chimeric species. We also removed vertebrate species we felt were clearly linked to the DNA of either the host species or human based on the criteria that the species did not occur in the study area and

were highly genetically related to Coyotes or human samplers. ESVs that yielded the same vertebrate species were combined and the read counts and relative contribution of diet item were summed across the species. Additional ESVs that identified species not known to occur in the study area were considered chimeras and were also removed (Taberlet et al. 2018). For the invertebrate data, we followed the same general approach, but because > 50% of the ESVs did not contain either species, genus, or family identification, we aggregated and interpreted data at the level of order. Though we report on both the frequency of occurrence and the relative contribution of items associated with Coyote scats based on the absolute number of times a given species (vertebrates) or order (invertebrates) was read by the sequence, we elected to focus on frequency of occurrence due to interpretation issues associated with sequence reads (Sullins et al. 2018).

We used the same approach for evaluating the genetic metabarcoding of plant material in Coyote scats as for vertebrate and invertebrates, considering only those ESVs that had match identity >95%. However, we did not identify plants in Coyote scats to the species level because many of the ESV records did not include information to the level of either species or genus and many that did were determined to be unreliable because they represented taxa that do not occur in Florida as determined by comparison of suspect records against herbarium accounts in the Atlas of Florida Plants (https://florida.plantatlas.usf.edu/). Taxonomic designations were based on comparisons of DNA metabarcoding from samples to plant DNA available in the National Center for Biotechnology Information (NCBI) library and that ESV taxonomic designations represented the closest match available, but were not necessarily accurate representations of plant material at the species or genus level. For this reason, we summarized ESV records by family. From a dietary perspective it was most useful to review families based on the types of diet resources they provide (e.g., woody species that produce soft mast, etc.), so we further grouped families into nine plant dietary groups. We separated legumes from non-mast producing forbs because legumes (e.g., Peanuts, *Arachis hypogaea* Linnaeus) have been reported to be important diet components for Coyotes during winter (Cherry et al. 2016). Data are reported as frequency of occurrence (number of records) and percent relative contribution (i.e., percentage of the absolute number of times a given taxonomic sequence was read by the sequencer) for scats collected from each study area plus a combined sites category. Percent relative contribution data were relativized to 100% for comparison purposes (McCune and Grace 2002).

Results

Coyote Scat Accumulation Rates and Abundance Estimates

Daily Coyote scat accumulation rates averaged 0.02 scats/km/day (SD = 0.02, range 0–0.07, n = 17). This accumulation rate means that, on average, 50 km per day or 10 km every five days needs to be searched to find one Coyote scat. Average daily accumulation rates of Coyote scats did not differ between rural and urban sites (rural: average = 0.02, SE = 0.01, range = 0-0.07, n = 8; urban: average = 0.02, SE = 0.01, range = 0-0.04, n = 9).

DNA analysis of the 88 scat samples (n = 50 urban, n = 38 rural) resulted in species identification for 69 samples (Table 1). Nine samples failed to provide useful information due to failed DNA amplification, contamination, or other reasons. Of the 69 scats that were identified to species, 36 were identified as Coyote with 26 and 10 of those samples collected from urban and rural study areas, respectively. Of the 36 scats identified as Coyote, nuclear DNA amplification resulted in identification of 13 individuals across both sites, with seven and six individual Coyotes identified in the urban and rural study sites, respectively. Three

Coyotes in the urban study site were detected (or recaptured) on three separate occasions, which yielded an abundance estimate of eight (95% Confidence Interval: 7–11) Coyotes per 100 km2, or a density estimate of one Coyote per 12.5 km2. No individual Coyotes were detected on multiple occasions (i.e., no recaptures) in the rural environment. Consequently, we were unable to calculate a Coyote density estimate, but we can state that a minimum of six Coyotes used the rural study area during our sampling period.

DNA Degradation

The treatment with the lowest error rates and best PCR success was NP (Fig. 3), whereas NPS had similar error rates to FE, but with a higher PCR success rate overall. One sample (Sample O) was dropped, as it only had the two earliest time points represented as nothing was left after insects consumed it. We identified a marginally significant interaction effect between time and treatment (F-value = 2.61, P = 0.07), suggesting changes in amplification

Table 1. Species ID for scats collected in an urban and rural site in Florida in 2020. A total of 69 scats analyzed by the Wildlife Genetics Lab were identified to species. We calculated percent for each species by dividing the number of scats by the total number found at both sites.

	Sites Combined		Rural Sites		Urban Sites	
Species	n	%	n	%	n	%
Coyote	36	52	10	14	26	38
Bobcat	15	22	12	17	3	4
Domestic Dog	6	9	3	4	3	4
White-tailed deer	2	3	2	3	0	0
Feral Swine	3	4	3	4	0	0
Opossum	2	3	0	0	2	3
Cow	1	1	1	1	0	0
Gray Fox	1	1	0	0	1	1
Raccoon	1	1	1	1	0	0
Red Fox	2	3	0	0	2	3
Total	69	100	32	46	37	54

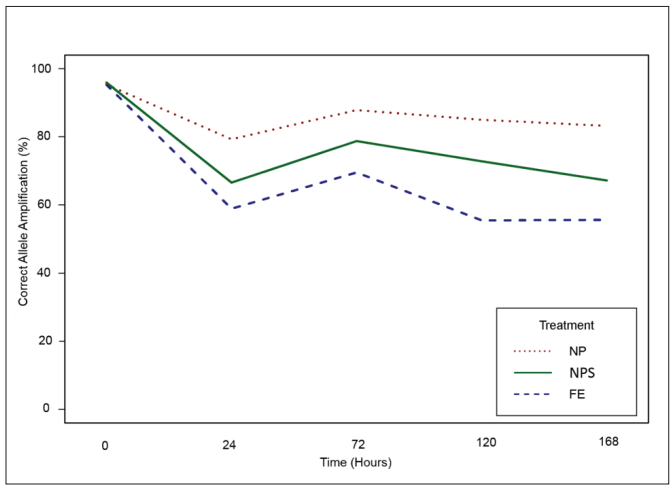

Figure 3. Percent amplification of nuclear DNA from coyote scat exposed to environmental treatments for 1, 3, 5, and 7 days. FE = Full exposure of scat to environment (sun + precipitation), NPS = No exposure to sun or precipitation, NP = no exposure to precipitation.

rate differed among treatments over time. Across all three treatments, we observed the biggest rate of decrease in amplification rate from time 0 to 12 hours. From 12 to 168 hours, amplification rate was generally stable (Fig. 4). The FE treatment exhibited the highest number of null alleles, the highest number of false alleles across loci, and the lowest percent positive PCR amplification across loci (Table 2).

Based on the results of the mixed-effects logistic regression, the probabilities of PCR success, FA, and ADO were significantly influenced by sample age (Table 3), with older samples typically having a lower probability of PCR success and higher probabilities of FA and ADO (Fig. 5). Although the probability of PCR success for the NP treatment appeared to increase, 95% confidence intervals (not presented) suggested the pattern was relatively stable over the 7-day sampling window. Still, we detected a significant interaction between sample age and the NP treatment for the probabilities of both PCR success and FA, but not for ADO.

The predictions based on the model of PCR success suggested that PCR success decreased rapidly for the FE and NPS treatments, with the FE treatment having the overall lowest predicted PCR success (Fig. 5A). The predictions based on the FA model suggested that FA were relatively low but increased with increasing sample age (Fig. 5B). Similarly, predictions based on the ADO model suggested that ADO increased with increasing sample age (Fig. 3).

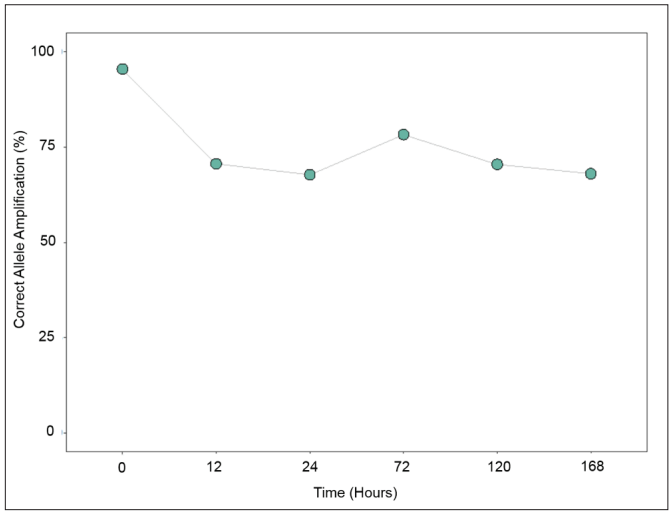

Figure 4. Percent of nuclear DNA from coyote scat that was successfully amplified following 1,3,5 and 7 days of scat exposure to three environmental treatments. See Figure 3 for a description of the treatment types.

Table 2. Summary findings of Coyote scat DNA degradation experiment conducted at the U.S. Department of Agriculture National Wildlife Research Center Florida Field Station, July 2020.

Treatment	Proportion (number) Null alleles across loci	Proportion (number) False alleles across loci	Proportion positive PCR Mean across loci
All Treatments	0.23 (594)	0.03 (22)	0.76
Full Exposure (FE)	0.27 (230)	0.02 (7)	0.68
No Precipitation with Sun (NP)	0.18 (143)	0.01 (7)	0.82
No Precipitation or Sun (NPS)	0.23 (221)	0.04 (8)	0.77

Table 3. Regression coefficients, standard errors (SE), and p-values for mixed-effects logistic regression model results for PCR success, false alleles, and allelic dropout in coyote fecal DNA samples collected over 7 days (Age) from the DNA degradation experiment conducted in July 2020 at the USDA NWRC Florida Field Station. Coyote scat were exposed for 1,3,5 or 7 days to full exposure (exposure to sun and precipitation), NP (no precipitation; exposure to sun), and NPS (no sun or precipitation; ambient air only); full exposure is the intercept.

	PCR success			False alleles (FA)			Allelic dropout (ADO)		
	Coefficient	SE	P-value	Coefficient	SE	P-value	Coefficient	SE	P-value
Intercept	1.669	0.534	0.002	-3.889	0.515	<0.001	-1.811	0.461	<0.001
Age	-0.197	0.023	<0.001	0.135	0.055	0.0136	0.213	0.029	<0.001
NP	0.189	0.784	0.809	-1.343	0.792	0.0902	-0.727	0.684	0.2
NPS	0.878	0.760	0.248	-1.109	0.768	0.1490	-0.493	0.656	0.452
Age*NP	0.251	0.037	<0.001	0.312	0.082	<0.001	-0.005	0.045	0.911
Age*NPS	-0.012	0.035	0.739	0.145	0.088	0.1007	-0.008	0.043	0.853

Coyote Diet

For the BatR01 primers, we detected 16 vertebrate species in Coyote scats (Table 4). Preliminary inspection revealed that for one urban scat, the ESV reads for Bobcat and Coyote were > 10,000 and similar in a sample identified as Coyote with mtDNA. For all other diet samples, the species identified through mtDNA typically had > 10,000 reads assigned to the same species. It was atypical to have a sample with equal reads to two species. Although this sample was identified as Coyote with mtDNA, the microsatellite markers did not amplify well. Given that the sample was not easily assigned to a species from metabarcoding analysis, we elected to censor that scat from our vertebrate diet results. All remaining rural scats (n = 9) contained at least one vertebrate species whereas six urban scats (23%) contained zero. On average, scats from urban and rural Coyotes contained 1.53 (SE = 0.23) and 3.1 (SE = 0.49) vertebrate species, respectively. In total, seven and 14 species were detected in the diets of rural and urban Coyotes, respectively. *Sus scrofa* Linnaeus (Feral Swine) was encountered most often (n = 12) and across both sites (n = 5 rural, n = 7 urban). Species detected once included *Equus caballus* Linnaeus (Domestic Horse), *Hypostomus plecostomus* Linnaeus (Suckermouth Catfish), *Salmo salar* Linnaeus (Atlantic Salmon), and *Branta canadensis* Linnaeus (Canada Goose). All species detected in Coyote scats from the rural sites were also detected in scats from the urban sites except for *Mycteria americana* Linnaeus (Wood Stork), which was detected in two scats from the rural site. Dominant diet items beside Feral Swine were *Felis catus* Linnaeus (Domestic Cat) and *Gallus gallus* Linnaeus (Domestic Chicken) in urban areas,

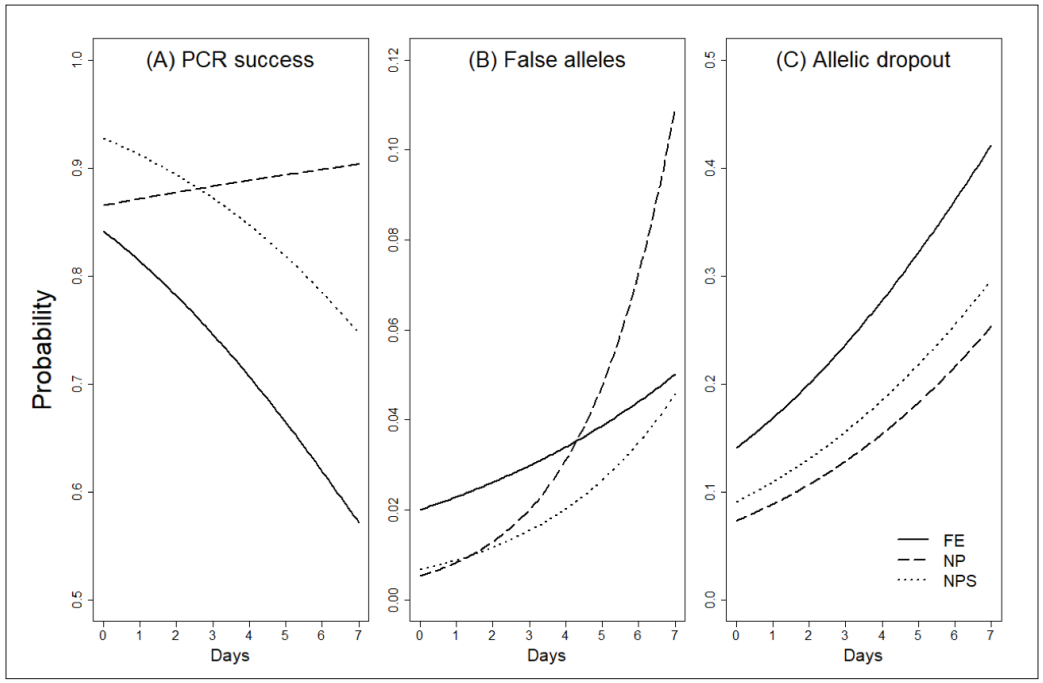

Figure 5. Mixed-effects logistic regression model results for the probabilities of (A) PCR success, (B) false alleles, and (C) allelic dropout as a function of sample age (days) of coyote fecal DNA samples exposed for 1,3,5 or 7 days to one of three treatments—full exposure (FE; exposure to sun and precipitation), no precipitation (NP; exposure to sun), and no sun or precipitation (NPS; exposure to ambient air only)— in July 2020 at the U.S Department of Agriculture National Wildlife Research Center, Florida Field Station.

Table 4. Species of vertebrates detected in or on Coyote scats using a DNA metabarcoding approach in Florida, 2020, using Bat01 primers. Thirty of 35 scats (85%) contained at least one species; scats yielding zero vertebrate DNA are not included in the summary below. Percent relative contribution to diet derived by dividing the Exact Sequence Variant (ESV) read count, the absolute number of times a given ESV was read by the DNA barcode sequencer, by the total number of reads.

	Sites Combined (n = 35 scats)		Rural Sites (n = 9 scats)		Urban sites (n = 26 scats)	
Species	Frequency of Occurrence	% Relative Contribution	Frequency of Occurrence	% Relative Contribution	Frequency of Occurrence	% Relative Contribution
Domestic Cat *(Felis catus)*	4	18.1	0	0	4	30.4
Feral Swine *(Sus scrofa)*	12	17.9	5	8.8	7	24.3
Eastern Cottontail *(Sylvilagus floridanus)*	8	16.1	8	39.6	2	< 1.0
Domestic Chicken *(Gallus gallus)*	8	15.5	0	0	8	26.0
Domestic Cow *(Bos taurus)*	8	10.6	6	25.5	2	<1.0
Hispid Cotton Rat *(Sigmodon hispidus)*	6	8	3	13.7	3	4.2
Turkey *(Meleagris gallopavo)*	2	4.4	0	0	2	7.5
Bobcat *(Lynx rufus)*	9	3.4	5	7.6	4	<1.0
Wood Stork *(Mycteria americana)*	2	1.8	2	4.6	0	0
White-tailed Deer *(Odocoileus virginanus)*	3	1.7	0	0	3	3.0
Domestic Sheep *(Ovis canadensis)*	3	<1.0	2	<1.0	1	<1.0
Domestic Horse *(Equus calabus)*	1	<1.0	0	0	1	3.0
Suckermouth Catfish *(Hypostomus sp)*	1	<1.0	0	0	1	1.6
Canada Goose *(Branta canadensis)*	1	<1.0	0	0	1	<1.0
Atlantic Salmon *(Salmo salar)*	1	<1.0	0	0	1	<1.0
Totals	69	100	33	100	40	s100

and *Sylvilagus floridanus* Allen (Eastern Cottontail Rabbit) and *Bos taurus* Linnaeus (Domestic Cow) in rural areas.

For the Ac12s primers, we detected at least one vertebrate species in only 15 scats (43%). Species detected in the rural environment included Feral Swine, Domestic Cow, *Ovis aries* Linnaeus (Domestic Sheep), and *Sigmodon hispidus* Howell (Hispid Cotton Rat), with Domestic Cow comprising the majority of ESV reads (82%). Species detected in the urban environment included the four species detected in the rural environment minus Domestic Cow, in addition to Domestic Chicken and Eastern Cottontail Rabbit. All species detected with Ac12s primers were also detected with BatR01, but eight additional species (53%) were only detected with the BatR01 primers (Table 4). For the Ac12s, Feral Swine and Eastern Cottontail comprised the most ESV reads at 48 and 39%, respectively.

For invertebrates, we detected 11 orders across six phyla (Table 5). Although orders from Arthropoda (insects, myriapods, arachnids, crustaceans) were most commonly encountered, this grouping only constituted 45% of (5 of 11) of all orders detected. The order Diptera (true flies, mosquitoes, gnats, midges) represented 80% relative contribution of invertebrate DNA in Coyote scats across all sites and was overwhelmingly the most often observed invertebrate group. Detections of insects from the order Orthoptera (grasshoppers, katydids, crickets) were only associated with the rural environment and detections of the order Lepidoptera (butterflies and moths) appeared to be more common in the urban environment (Table 5).

DNA metabarcoding of plant material identified 643 unique ESVs representing 50 plant families (Table S1, available online at https://eaglehill.us/URNAonline2/suppl-files/urna-191-Kluever-s1.pdf). Families that had the greatest frequency of ESV records in scats for all sites combined included Pinaceae (pines; n = 191), Poaceae (grasses; n = 140), Fabaceae (legumes; n = 55), Cupressaceae (cypress; n = 43) and Asteraceae (flowering composites; n = 31) (Table S1). All other families had <20 records. Plant dietary groups were strongly influenced by these families, with the greatest frequencies and percent relative contributions contributed by non-mast producing woody species, graminoids, non-mast producing forbs, and legumes, respectively (Table 6). Soft mast from woody species was the next most represented group, but collectively represented only 1.5% of the total relative contribution to plant material detected in scats. Frequency and percent relative contribution of plant material to Coyote scats collected from rural and urban study sites mirrored these patterns, except scats collected from rural sites had greater contributions from graminoids and non-mast producing forbs, and scats collected from urban areas had greater contributions from non-mast producing woody species, legumes and woody species with soft mast (Table 6).

Discussion

Coyote Scat Accumulation Rates and Abundance Estimates

We documented a minimum of six Coyotes using the rural study area and calculated an abundance estimate of eight Coyotes using the urban study area. Although we lacked sufficient recapture data from scats to estimate Coyote abundance at our rural study site, we were able to estimate population abundance at the urban site, which was equivalent to a population density estimate of one Coyote per 12.5 km2. It is unclear whether these Coyotes were established adults with territories or included transients and juveniles that had not yet dispersed. Juvenile dispersal typically begins during September-October but may extend well into spring (Harrison 1992, Sumner et al. 1984). Consequently, estimated Coyote densities may vary seasonally and do not necessarily reflect actual home range size or territories, which have been reported to have a large degree of overlap among territorial

Table 5. Orders of invertebrates detected in or on Coyote scats using a DNA metabarcoding approach in Florida, 2020. Twenty-four of 36 scats (66%) contained at least one order of invertebrates; scats yielding zero invertebrate DNA in are not included in the summary below. Percent relative contribution to diet derived by dividing the Exact Sequence Variant (ESV) read count, the absolute number of times a given ESV was read by the DNA barcode sequencer, by the total number of reads.

		Sites Combined (n = 36 scats)		Rural Sites (n = 10 scats)		Urban sites (n = 26 scats)	
Phylum	Order	Frequency of Occurrence	% Relative Contribution	Frequency of Occurrence	% Relative Contribution	Frequency of Occurrence	% Relative Contribution
Arthropoda	Diptera	16	80.0	6	43.6	10	91.0
Arthropoda	Lepidoptera	7	5.6	1	5.6	6	5.5
Amoebozoa	Longamoebia	6	3.2	5	10.3	1	1.0
Arthropoda	Orthoptera	2	9.2	2	40.0	0	0
Streptophyta	Poales	2	< 1.0	0	0	2	<1.0
Oomycota	Pythiales	2	1.3	0	0	2	1.6
Rotifera	Adinetida	1	< 1.0	1	<1.0	0	0
Arthropoda	Anostraca	1	< 1.0	0	0	1	<1.0
Arthropoda	Coleoptera	1	< 1.0	0	0	1	<1.0
Ochrophyta	Eustigmatales	1	< 1.0	0	0	1	<1.0
Rotifera	Philodinida	1	< 1.0	0	0	1	<1.0
Totals		40	100	15	100	25	100

Coyotes except for core use areas (Chamberlain et al. 2000). Gehrt et al. (2009), on the other hand, found that home ranges for Coyotes in a metropolitan area had little overlap except among mated pairs and did not vary among seasons or between age and sex classes. Coyotes have been reported to be more abundant in urban versus rural environments (e.g., Atwood et al. 2010). That we found a greater number of Coyotes in the urban study area and also collected greater numbers of Coyote scats suggests Coyote density may be higher in Jacksonville than at MAERC, but more robust data are needed to reliably state whether Coyote densities differed between rural and urban study areas.

The estimated Coyote scat accumulation rate (0.02 scats/km/day) was lower than reported elsewhere (0.076 scats/km/day; Lonsinger et al. 2015). These results may be due to lower detection probability than experienced by Lonsinger et al. (2015), who conducted their study in xeric, resource poor environments where scat detection probability may have been higher due to less vegetation on transects. An important finding to consider with regards to our reported scat accumulation rates is that the number of scats observed was highly disproportionate (non-uniform) across transects. For example, we detected zero Coyote scats during collection surveys along nine (56.3%) transects in the urban sites and eight (50%) transects in the rural sampling sites. There may be multiple explanations for the majority of Coyote scats being collected on a limited number of transects. Kluever et al. (2015) found that as vegetation on transects increased, scat detection probability decreased. Lonsinger et al. (2016) found that vehicular traffic negatively influenced scat accumulation rates, because scats run over by vehicles were more readily broken down and therefore dissipated into the environment quicker. It is also possible that some transects had low levels of Coyote use due to disturbance, poor habitat quality, seasonal variance in food resources, or Coyote management (in rural areas) that depressed the local population. It is also important to note that seasonality has been shown to influence scat accumulation (Lonsinger et al. 2015), and sampling during other times of the year in Florida may be met with greater or lesser success due to climatic and other factors.

Table 6. Plant material identified in coyote scats organized by 10 plant dietary groups representing 65 families plus an unknown category. Data are from ESV (Exact Sequence Variant) records, which represent unique identifiers of DNA of plant origin in the scats. Frequency of occurrence represents the total number of ESV records and percent relative contribution represents the percentage of the absolute number of times a given taxonomic sequence was read by the sequencer, relativized to 100% against all other ESV records for comparison purposes. Plant categories with % ESV = 0 represent values of <0.01%.

	Combined Sites (n = 36 scats)		Rural Coyotes (n = 10 scats)		Urban Coyotes (n = 26 scats)	
Plant Dietary Group	Frequency of Occurrence	% Relative Contribution	Frequency of Occurrence	% Relative Contribution	Frequency of Occurrence	% Relative Contribution
Woody Soft Mast	20	1.5	5	0.7	15	1.7
Woody Hard Mast	9	0.3	6	1.0	3	0.0
Woody (no mast)	258	68.0	42	54.0	216	73.4
Forb Soft Mast	1	0.3	0	0.0	1	0.5
Legumes	55	3.3	19	2.4	36	3.6
Forbs (no mast)	140	10.6	62	20.6	78	6.8
Graminoids	151	14.0	59	21.8	92	10.9
Vines	7	0.1	3	0.3	4	0.0
Moss	6	2.1	0	0.0	6	2.9
Totals	643	100	195	100	448	100

For noninvasive genetic sampling to be more useful for carnivores in our study areas, and likely other urban and rural environments, scat detection probability should be improved to increase capture and recapture rates. Urban areas pose challenges because the use of urban space by Coyotes has been found to be non-uniform, with animals spending a disproportionate amount of time in green spaces to meet their requirements (Ellington and Gehrt 2019, Wurth et al. 2020). Intensive cluster sampling (Humm et al. 2017, Rehman et al. 2016) in and around green spaces may increase capture and recapture rates if scat detection challenges can be addressed. Based on our findings, transects placed on residential roads and/or distribution powerline maintenance roads resulted in poor detection rates with human observers.

Our derived scat accumulation rate of 0.02 scats/km/day indicates that on average, a person searching transects for Coyote scats would need to walk 50 km to collect a single Coyote scat (e.g., 50 km/day or 10 km/day for 5 days). Establishing teams can make scat collection more efficient and productive. For example, two, 2-person teams could survey a total of 25 km of transects per day (12.5 km/day/team), which is a logistically feasible effort based on our study. This would result in an estimated 12.5 Coyote scats to be collected in one 5-day work week. Such a sampling interval should obtain an adequate sample size for use in a robust noninvasive sampling framework capable of generating density estimates for two study areas if at least four discreet sampling periods/occasions were incorporated. A methodology that could markedly increase detection probability rates for scats in areas with low detection probability would be to employ scat detection dogs as either a replacement or supplement to human observers (de Oliveira et al. 2012, Orkin et al. 2016); this approach would also presumably increase scat detection rates in rural areas.

DNA Degradation

During the first 12-hour time period, scats exposed to sun but protected from precipitation (i.e., NP) had higher amplification rates than scats protected from sun and precipitation (i.e., NPS) or those exposed to both sun and precipitation (i.e., FE). Amplification of DNA for samples in the NPS treatment declined steadily for the first 24 hours and then appeared to roughly stabilize at 70-75% amplification during subsequent 24-168 hours. DNA in scats under the NPS and FE treatments degraded more rapidly than NP during the first 12 hours. Surprisingly, DNA degradation of scats under the NP treatment was lower than NPS, but this is suspected to be an artifact of the loss of one sample entirely consumed by insects after 12 hours (Sample O) and the influence of 2 samples where no DNA amplification occurred at 12 hours (presumably due to stochastic pipetting error), but did occur at later times. These results may also reflect low daily precipitation during the experiment, which averaged only 0.13 cm/day (range 0-0.14, SD = 0.05). As a result, we speculate that had we experienced more natural precipitation or emulated precipitation events, we would have observed a greater decrease in amplification rate for this treatment. The samples associated with the NPS treatment may have retained moisture the longest, and this additional moisture may have affected DNA degradation.

As expected, samples under the FE treatment had the greatest level of DNA degradation, but this was not fully apparent until later sample time periods. Environmental conditions, including the effects of insects and decomposing organisms, are known to affect DNA degradation rates in scats. Precipitation and exposure time are of particular concern to DNA integrity (Santini et al. 2007). Based on our scat DNA degradation experiment, sampling daily and before scat deposited the previous night is exposed to the sun offers the best probability of amplification. However, if such a sampling scheme is too costly, then our results indicate a fairly stable period when amplification of scat remains stable for up to 7 days.

Future research on Coyote DNA degradation that covers a larger temporal window and incorporates either seasons with variation in precipitation or artificial precipitation would further improve our understanding of the DNA degradation process in Florida.

As found in other studies, allelic dropout rates were predicted to be higher than false allele rates, and both tended to increase with sample age and full exposure to the environment. Patterns observed for the NP treatment were inconsistent with expectations (i.e., stable or increasing for PCR success over time, increasing more precipitously than the FE treatment for FA). Due to the relatively small sample size (i.e., only 7–8 replicates per treatment), these patterns could be the result of stochastic processes associated with sample collection or laboratory procedures. For example, Gosselin et al. (2017) found differences in DNA amplification rates based on where the scat sample was collected from.

By removing small portions of each scat upon initial detection/collection to submit to our laboratory for DNA analyses, we reduced the overall scat size, thus likely creating an increased surface area to volume ratio and exposure to the environment. This may have impacted the results of the DNA degradation experiment. As such, our DNA degradation findings should be considered conservative, but still highly informative for future Coyote noninvasive sampling efforts.

Coyote Diet

We documented a greater number of vertebrate species in Coyote scats collected in urban areas than rural study areas, which differs from rural-urban diet comparisons of Coyotes in Florida reported by Grigione et al. (2011). We found that Feral Swine was the most frequently detected vertebrate species in Coyote scats from both rural and urban areas. This item has been reported in Coyote scats or stomachs by other studies, but at a much lesser frequency (Cherry et al. 2016). Because Feral Swine reproduce year-round in the Southeastern United States and have large litters (VerCauteren et al. 2020), it is possible Coyotes in both urban and rural areas are actively preying on piglets and sub-adults, scavenging carrion, or engaging in coprophagia (Steinmann et al. 2011). Similarly, the frequent occurrence of Feral Swine in our analyses of Coyote diets presents opportunities to evaluate whether Coyotes exert predatory pressure on Feral Swine, which are arguably a greater threat to Florida's agricultural interests than are Coyotes (Bevins et al. 2014, Anderson et al. 2016). Besides Feral Swine, Coyote diet in urban areas largely consisted of Domestic Cats and Domestic Chickens. To our knowledge, the extent to which we detected chickens in the urban environment has not been previously recorded and may be attributed to an increase in backyard farming practices. In rural areas, dominant diet items for Coyotes besides Feral Swine were Eastern Cottontail Rabbits and Domestic Cow. Cow may be a result of fecal contamination or due to the same reasons we identified for high Feral Swine signal in the diet (see above). The dependance on Eastern Cottontail Rabbits aligns with expectations in rural areas (Grigone et al. 2011).

Lynx rufus Schreber (Bobcat) occurred in Coyote diets at high frequencies at both sites, but the species represented a minute percentage of overall ESV reads. Bobcats have been observed in Coyote scats and stomachs, but at a lower frequency than we observed (Cherry et al. 2016). Other studies examining Coyote-Bobcat interactions report that the two species co-occur by fine-scale spatial and temporal segregation that limits interaction (Lombardi et al. 2020, Thornton et al. 2004), but Coyotes have been reported to kill Bobcats (Gipson et al. 2002). Although Thornton et al. (2004) and other studies have observed agonistic interactions between Coyotes and Bobcats to be minimal, increasing densities of Coyotes could conceivably change how Coyotes and Bobcats interact. Our finding of Bobcat DNA being

present in Coyote scats at a high frequency may be at least partially driven by coprophagy of Bobcat scats or scent marking by Bobcats on Coyote scats. Unfortunately, disentangling these potential drivers was not possible with the DNA metabarcoding approach.

Some of the diet items found in scats from our urban study area were unusual, including the Suckermouth Catfish and Atlantic Salmon. We suspect the single Atlantic Salmon observation to be evidence of Coyotes consuming anthropogenic waste, a behavior reported in urban Coyotes (Grigione et al. 2011). The Suckermouth Catfish, which is an invasive species (Nico et al. 2009, Gestring et al. 2010) and considered a "trash fish" by anglers, may be due to Coyotes scavenging carrion or from opportunistic predation on fish trapped in drying ponds and drainage areas during Florida's dry winter months. Predation on Canada geese in urban environments has been reported from investigations in other regions (Brown 2007). Because the Ac12s primers detected less than half of the species detected by Bat01, we do not recommend using this primer for assessing vertebrate diet of Coyotes in Florida. However, it is important to note that this primer could be more informative for Coyote diet investigations when field sampling occurs in summer, a season when Coyotes are more likely to be consuming herpetofauna.

We speculate that dipteran species being the invertebrate taxa most frequently associated with Coyote scat was likely an artifact of Coyotes consuming fly larvae and eggs associated with carrion or as the result of scat contamination after deposition, as many dipteran species utilize dung for consumption, egg laying, or both (Brown 2013). Similarly, many lepidopteran species are attracted to dung (Krenn 2010), and this likely influenced our finding of this group being associated with scats from both urban and rural areas.

Studies of Coyote diets in Florida and the southeastern United States have reported various mixed plant material, but plant material in Coyote diets is typically dominated by soft mast (Cherry et al. 2016, Grigione et al. 2011, Santana and Armstrong 2017, Schrecengost et al. 2008, Swingen et al. 2015, Thornton et al. 2004). Coyote scats analyzed in this study also included soft mast from multiple families of woody plants and a single family of forbs (Solanaceae; Table S1). In this study, the frequency of occurrence of soft mast in Coyote scat was low and was dominated by the Rosaceae, which includes many soft mast producing trees such as *Prunus* species. The relatively low representation of soft mast in the diet analysis may be explained in part by the fact that the availability of soft mast declines for many species during winter months (Cherry et al. 2016).

DNA metabarcoding revealed an incredible diversity of plant material in scat of Coyotes, which undoubtedly was a result of plant material intentionally consumed, indirectly consumed as a component of prey items (i.e., plant material within the digestive system of a prey item), ingested unintentionally (e.g., pollen), or as the contamination of scats after deposition by seeds, pollen, or spores. Deciphering the significance of different plant materials in Coyote scat was beyond the scope of our work but offers intriguing new challenges in understanding the breadth of diets for Coyotes and other animals. For example, the single most represented plant family in ESV records was Pinaceae (*Pinus* spp.), constituting 63.1% relative contribution of total ESV records. But the significance of this result is unknown because of the various possible mechanisms for this plant species to end up in the scat.

There are several takeaway messages regarding the use of DNA metabarcoding for analysis of Coyote diets. First, as anticipated, the data provided by DNA metabarcoding indicate Coyotes are generalist feeders that consume a variety of items based on opportunity and availability. This highly generalist dietary behavior by Coyotes has been well documented in the literature. Second, DNA metabarcoding appears to be a highly effective means of documenting unexpected dietary items, such as the Suckermouth Catfish and Atlantic Salmon

that might not otherwise be identified. This specificity would make DNA metabarcoding a potentially valuable approach for documenting predation on vertebrate species of interest, such as domestic or endangered species, but scavenging and coprophagia pose potential complications for interpretation of results. Last, DNA metabarcoding may be useful for evaluating invertebrate and plant material in scats given the ability to determine how such material ended up in the scat. However, several drawbacks and potential sources of bias related to the employment of this technique for generalist carnivores, including the potential for coprophagy, scent marking, and insect and pollen/seeds/spores interacting with scats post-deposition need to be carefully considered when interpreting results. The potential source of bias of pollen/seeds/spores interacting with scats post-deposition could be potentially controlled/accounted for by placing scats of known diet (e.g., from captive Coyotes) within the study area across two treatments types, one fully exposed two the environment, the other where pollen/seeds/spores cannot interact with scats (i.e., enclosed treatment box with a sealed plexiglass cover). In addition, future investigations would benefit by including a comparison of traditional/mechanical sorting diet determination with a DNA metabarcoding approach.

Conclusion

Results from our study could aid wildlife managers in several ways. In Florida, our results suggest sampling during winter, when rainfall is minimal, and spacing scat collection periods by seven days (168 hours) would be optimal for balancing DNA degradation and scat accumulation. Such a collection timeframe should maximize the amount of time available for scat deposition without substantially reducing the ability to amplify DNA from samples. Sampling more frequently may be necessary in the event of unusually heavy rainfall or during the summer rainy season because precipitation is reported to increase the rate of DNA degradation (Santini et al. 2007). Limited scat accumulation rates appear to be the most limiting factor to implementing a noninvasive genetic-based monitoring program. Developing more robust methods for sampling in areas where scat deposition is more likely and utilizing scat detection dogs to enhance detection may increase scat collection rates.

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