Revealing Seasonal Dietary Niche Overlap Among Sympatric Large Carnivores using DNA Metabarcoding

Biodiversity and Conservation

Jessica R. Patterson[1], Stéphanie Périquet-Pearce[2,3,4], Madeline Melton[1], Brennan PetersonWood[1], Rubén Portas[5], Ortwin Aschenborn[5], Claudine Cloete[6], Laura E. Peirson[7], Diana J.R. Lafferty[7], James C. Beasley[1]

[1]University of Georgia, Warnell School of Forestry and Natural Resources, Savannah River Ecology Lab, P.O. Box Drawer E, Aiken, SC 29802, USA

[2]Ongava Research Centre, Private Bag 13 419, Southern Industrial, Windhoek, Namibia

[3]Department of Conservation Management, Faculty of Science, George Campus, Nelson Mandela University, Madiba Drive, George, 6530, South Africa

[4]Panthera, New York, NY, USA

[5]Department of Evolutionary Ecology Leibniz Institute for Zoo and Wildlife Research of Berlin, Alfred-Kowalke St. 17, 10315 Berlin

[6]Etosha Ecological Institute, Etosha National Park, Namibia.

[7]Northern Michigan University, Department of Biology, Wildlife Ecology and Conservation Science Laboratory, Marquette, MI 49855, USA.

Corresponding author:

Jessica R. Patterson

University of Georgia, Warnell School of Forestry and Natural Resources, Savannah River Ecology Lab, P.O. Box Drawer E, Aiken, SC 29802, USA

jessypatterson311@gmail.com

Text S1. Methods: Carnivore Chemical Immobilization Protocol

The chemical immobilization protocol included vehicular darting with baits (zebras [*Equus quagga*], springbok [*Antidorcas marsupialis*]) and calls to lure animals in (Smuts et al., 1977), using a combination of dart-administered Zoletil and Medetomidine for immobilization, and Atipamezole and Yohimbine as reversal agents, with dosages determined by the veterinarian based on species and individual body size. Typically, this included 40 mg Zoletil and 5 mg Medetomidine reversed with 20-30 mg Atipamezole and 12 mg Yohimbine for hyenas, 80-110 mg Zoletil and 10 mg Medetomidine reversed with 40-50 mg Atipamezole and 20-50 mg Yohimbine for male lions, 60-80 mg Zoletil and 8-10 mg Medetomidine reversed with 40-50 mg Atipamezole and 20-30 mg Yohimbine for female lions. All immobilizations and collaring procedures were performed by veterinarians registered with the Namibian Veterinary Council and the Ministry of Environment, Forestry, and Tourism and were approved by the Namibian Ministry of Environment, Forestry, and Tourism (permit #AN202101004) as well as the University of Georgia Institutional Animal Care And Use Committee under protocols A2024 05-009-06 and A2021 04-013-Y3-A11.

Text S2. Methods: Sequencing and Bioinformatics

Each 25 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 12.5ul Master Mix, 0.5 µl of each primer, 1.0 µl of gDNA, and 10.5 µl DNase/RNase-free H2O. DNA was PCR amplified using the following conditions: initial denaturation at 94 C for 3 minutes, followed by 45 cycles of 30 seconds at 94 C, 30 seconds at 52 C, and 1 minute at 72 C, and a final elongation at 72 C for 10 minutes. Sample library pools were prepped for sequencing on an Oxford Nanopore Technologies MinION (Oxford, England) using the Ligation Sequencing Kit V14 (cat# SQK-LSK114). Final basecalling was completed using AWS Batch and EC2 g5.xlarge instances running ONT's dorado software (v0.7.1) in super high accuracy mode using the dna_r10.4.1_e8.2_400bps_sup@v5.0.0 basecalling model.

Raw Nanopore sequencing output was converted from pod5 to fastq format using dorado v0.7.1 (Oxford Nanopore Technologies, 2024) and the super-high accuracy basecalling model. Cutadapt v3.4 (Martin, 2011) was then used to trim outer adapters and reorient reads in a consistent 5’ to 3’ direction. The resulting reads were sorted into individual samples by demultiplexing with pheniqs v2.1.0 (Galanti et al., 2021), allowing no more than one mismatch in each of the paired 10 bp molecular indices. Cutadapt was then used again to extract the target amplicon by removing the gene primers, discarding any reads where one or both primers were not found or where the resulting amplicon sequences were < 300 bp or > 450 bp. Exact sequence variants (ESVs) were then identified for each sample using the unoise3 denoising algorithm (Edgar, 2016a) as implemented in vsearch (Torbjørn et al., 2016). Only reads that were observed 4 or more times and with a maximum expected error rate (Edgar and Flyvbjerg, 2015) > 1 bp were considered as candidate ESVs and putative chimeras were filtered using the uchime3

algorithm (Edgar, 2016b). Final read counts were determined for each sample by mapping unfiltered raw reads to the identified ESVs using the vsearch function usearch_global with a minimum identity of 95%. For each final ESV, a consensus taxonomy was assigned using a custom best-hits algorithm and a reference database consisting of publicly available sequences on NCBI GenBank (Benson et al., 2005), as well as Jonah Ventures voucher sequences records. Reference database searching used vsearch to conduct exhaustive semi-global pairwise alignments, and final match quality was quantified using a custom, query-centric approach, where the % match ignores terminal gaps in the target sequence, but not the query sequence. A consensus taxonomic assignment was then generated using either all 100% matching reference sequences or all reference sequences within 1% of the top match, accepting the reference taxonomy for any taxonomic level with > 90% agreement across the top hits.