MARCH 2020 PREGNANCY ASSESSMENT USING SAMPLES FROM BATHURST, BLUENOSE-EAST AND BEVERLY BARREN-GROUND CARIBOU HERDS

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ABSTRACT

Pregnancy rates of caribou and other wildlife can be assessed non-invasively through assays of fecal samples in mid- to late gestation for progesterone, which is elevated in pregnant females and low in non-pregnant females. Fecal samples were collected by staff with the Government of the Northwest Territories (GNWT) Department of Environment and Natural Resources (ENR) during composition surveys of the Bathurst, Bluenose-East and Beverly barren-ground caribou herds in March 2020 as an approach to estimate late winter pregnancy rates in these herds.

Samples of fecal pellets were collected opportunistically (as encountered) at 19 sites, with a target number at each site of 15 or 20 samples (total 331). Each sample was from a different pile of fecal pellets. Sites were chosen based on the presence of mostly calf:cow caribou that had just been classified and based on the presence of abundant bedding and feeding sign in the snow. A further 46 fecal samples from caribou collected by a graduate student in February and March 2020 during field studies near the Gahcho Kue mine road were also used, for a total sample size of 377.

Fecal samples were subdivided and sent to two labs for analysis. One subset was sent to the Western College of Veterinary Medicine at the University of Saskatchewan and assayed for progesterone. Samples with progesterone higher than 100 ng/g were assessed by the lab as pregnant. A second set of samples was sent to a genetics lab at Trent University. DNA analyses enabled identification of duplicate samples (more than one sample from the same individual caribou) and determination of males and females.

Of the 331 samples collected by ENR, 32 (9.7%) were duplicates and 18 (5.4%) were unsuitable for genetic analysis. Of the remaining 281 samples, 98 (34.9%) were identified as males and 183 (65.1%) as females; 111 of the 183 female samples (60.7%) were assessed as pregnant. The 46 samples collected by the graduate student included five duplicates and two that were unsuitable for DNA analysis; of the remaining 39 samples, 34 were males and five were females (four non-pregnant). Further analyses were focused on the 331 samples collected by ENR.

A demographic model was used to assign likely proportions of female calves (nine months old; 16.4%), female yearlings (21 months old; 7.4%), and females ≥33 months old (76.3%) in the data from all three herds in March 2020. If the female calves and yearlings are assumed to be non-pregnant, then an adult pregnancy rate of 79.5% (111/139.7) resulted for females at least two years old. Other approaches, such as using the calf:cow ratio estimate from the March survey to estimate the proportion of calves, resulted in similar estimates. Similar calculations were carried out to estimate herd-specific pregnancy rates, however sample numbers were much smaller.

Results were assessed with respect to cost, field time needed, proportions of samples that were likely to be duplicates, unsuitable for DNA assays, and males, and likely proportions of female calves, yearlings and adult females in the sample data set. We assessed the sample size needed for an acceptable coefficient of variation. To obtain a sample of herd-specific pregnancy rate in about 116 females ≥33 months old, about 300 samples would need to be collected across a representative range of sites.

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INTRODUCTION

The Bathurst, Bluenose-East and Beverly barren-ground caribou herds have annual ranges spanning the Northwest Territories (NWT) and Nunavut (NU). The Bathurst herd has a calving ground west of Bathurst Inlet, and the Bluenose-East herd has a calving ground west of Kugluktuk, both in NU, with portions of the summer range in NU and the remainder of the ranges in the NWT (Figure 1). The Beverly[1] herd has a calving ground in the Queen Maud Gulf lowlands with much of its range in NU and the NWT. Historically the Bathurst and Beverly herds have ranged as far south as northern Saskatchewan.

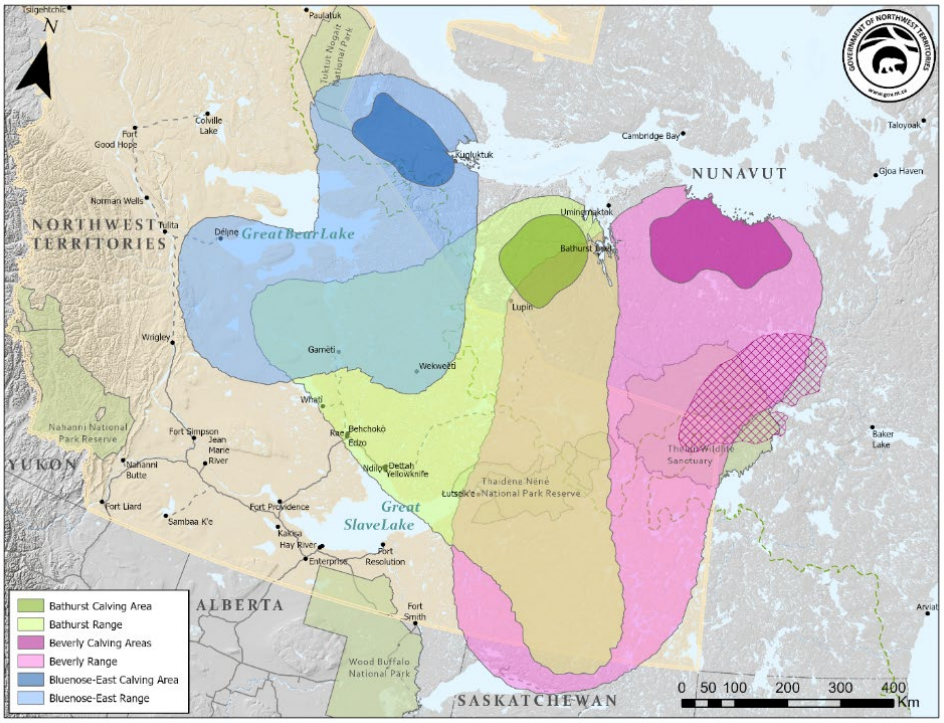

Figure 1. Annual ranges and calving grounds of the Bluenose-East, Bathurst, and Beverly herds, based on accumulated radio collar locations of cows (adapted from Nagy et al. 2011).

In 2021, the Bathurst caribou herd was estimated at about 6,200 caribou after a decline of nearly 99% from peak numbers estimated at 470,000 in 1986 (Adamczewski et al. 2022). In 2021, the Bluenose-East herd was estimated at about 23,200 caribou, suggesting a stabilizing trend between 2018 and 2021 after a steep decline between 2010 and 2018 (Boulanger et al. 2022). The Beverly herd was estimated at about 103,000 in 2018 (Campbell et al. 2019) with a slow declining trend of about 5%/year. Late-winter calf:cow ratios have been

1 The Beverly herd described in this report is the herd defined by the Government of Nunavut (GN) as calving in the central and western Queen Maud Gulf (Campbell et al. 2019). This herd may not correspond exactly to the Beverly herd defined prior to 2009 with an inland calving ground south of Garry Lakes (Adamczewski et al. 2015).

estimated in all three herds from helicopter-based composition surveys. These ratios are an index of calf survival through about the first nine and a half months of life, although these ratios are also influenced by pregnancy rate or initial productivity of calves in June.

One of the vital rates that affects population trend in caribou herds is the pregnancy rate (Bergerud 2000, Bergerud et al. 2008, Boulanger et al. 2011, Crête et al. 1996). Pregnant and non-pregnant female caribou can be distinguished non-invasively by assaying fecal samples for metabolites of progesterone in mid-late gestation (Messier et al. 1990). Progesterone in blood (serum) and feces is elevated in pregnant females and much lower in non-pregnant females (Messier et al. 1990, Morden et al. 2011a and b, Joly et al. 2015, Flasko et al. 2017). Fecal samples have been collected previously to determine pregnancy in NWT barren-ground caribou herds (ENR unpublished). In March 2020 during helicopter-based composition surveys of the Bathurst, Bluenose-East and Beverly herds (Adamczewski et al. 2020), fecal samples were collected across their winter ranges to assess pregnancy rates.

The samples were analyzed to attempt to generate an estimate of herd-wide pregnancy rate in each of the Bathurst, Bluenose-East and Beverly herds. The 2020 collection results were used to assess logistical and operational considerations in using this method of measuring late winter pregnancy rate in NWT migratory barren-ground caribou. An assessment of the sample size needed for an adequate sample of herd-wide pregnancy rate was undertaken.

METHODS

Field Sampling and Sites

Survey methods and results for March 2020 composition surveys of the Bathurst, Bluenose-East and Beverly herds were described by Adamczewski et al. (2020), and methods for fecal pregnancy assessment were based on previous work by ENR (unpublished) based on Morden et al. (2011a and b). In conjunction with the composition surveys, caribou fecal samples were collected at a total of 19 sites via helicopter (Figure 2). The target number of samples per site was initially 15, then increased to 20 to increase the overall sample size. In total 331 samples were collected. A GPS location was recorded for each site.

Figure 2. Feeding and bedding sites of caribou during March 2020 composition surveys of the Bathurst, Bluenose-East and Beverly caribou herds. A close-up of a fecal pellet group is at bottom left. Photos: GNWT/J. Adamczewski, ENR.

The approach in the field was to classify caribou from the helicopter in a particular area, then land to opportunistically collect fecal samples nearby, or as they were encountered. All sites had primarily cow:calf groups in the vicinity; no samples were gathered from areas where classified groups had a substantial representation of bulls. In forested areas, sites for fecal

collection were frozen lakes or clearings where the helicopter could land, and where there was abundant evidence of feeding and bedding of caribou. On the tundra, sites were also chosen for abundant sign of caribou feeding and bedding. Samples included pellets from piles of smaller and larger pellets.

Each sample was collected from an individual pile of fecal pellets to minimize the likelihood of duplicate samples from the same caribou. Individual samples were generally about a handful in volume and 40-50 pellets. They were labelled with the site number and then consecutive numbers from 1-20 (or fewer) up to the total number of samples taken from that site. Samples were stored in individual plastic bags, placed in a freezer at the end of the sampling day and kept frozen at -20˚C until they were sent to a lab.

A further set of fecal samples was collected by MSc student Angus Smith in February and March 2020 as part of a study on barren-ground caribou responses to winter roads (Smith 2022). The main study area for this project was the winter road to the Gahcho Kue diamond mine northwest of Yellowknife. One of the approaches to assessing responses of barren-ground caribou to the winter road was collecting fecal samples near the road and further away and assaying them for glucocorticoids as stress indicators (Smith 2022). Forty-six samples were made available by Smith for pregnancy analysis. Collar data indicated that caribou near the Gahcho Kue winter road in February and March 2020 were predominantly Beverly caribou, thus it reasoned that these samples might add to the number of samples that could be tested for pregnancy for that herd. In total 377 fecal samples were available from the ENR sampling in March 2020 (331) and Smith's sampling in February and March 2020 (46).

Samples collected in the field were kept frozen in a -20˚C freezer, brought out temporarily to subsample, then refrozen. The fecal samples were divided into three subsets, one for genetic analysis, a second for steroid hormone assays, and the third retained as a reserve in Yellowknife.

Genetic Analyses

One subset of 377 caribou fecal samples was sent to a genetics lab at Trent University in Peterborough, Ontario. These were analyzed for DNA for two purposes: (1) to identify males and females, and (2) to identify any duplicate samples. Samples were shipped and arrived at the lab on March 5, 2021 and final results were received June 22, 2022.

A description of the methods used for DNA analysis was provided by B. Redquest (pers. comm.) and is provided here (greater detail can be found in Klütsch et al. 2016). A swab was used to remove the mucous layer on the collected fecal pellets - this outside layer contains the DNA needed for downstream profiling. The swab was then placed into a small tube containing an extraction buffer where it was further broken down by an enzyme and heat. The sample was then ready for extraction using the Qiagen DNeasy Blood and Tissue kit. This

protocol uses unique filters that help remove the impurities commonly seen in samples originating from fecal samples. Once this protocol was complete, purified DNA was ready for profiling. Extracted DNA samples were then amplified at nine microsatellite loci - Bm848, Bm888, Map2C, Rt24, Rt30, Rt5, Rt6, Rt7, Rt9 and one sex specific marker. These ten markers were fluorescently labelled and amplified using PCR (Polymerase Chain Reaction). Amplified products were then loaded onto the ABI3730 and the output generated from the machine was run through the program GENEMARKER (SoftGenetics, LLC). All samples were then analyzed by two different people and final profiles were compared on an online server to detect inconsistencies and errors. The final data were then run through an R script called Allelematch that pairs duplicate profiles together so that the final number of unique individuals can be seen.

For a number of samples, DNA was found to be of insufficient quality/quantity, or the sample was considered contaminated. Fecal samples may sometimes have insufficient quantity or quality of DNA for full analysis (Ball et al. 2007).

Assays for Progesterone and Testosterone

A second subset of the 377 samples was sent to the Endocrine Services Lab at the University of Saskatchewan (Western College of Veterinary Medicine, WCVM) and assayed for progesterone. The lab identified samples from females with ≥100 ng/g progesterone in feces as being from pregnant animals. Samples low in progesterone (<100 ng/g; n=257) were also assayed for testosterone to test whether this would allow for males to be identified. Samples were sent to the WCVM on August 28, 2021 and final assay results were received November 27, 2021.

A description of the progesterone and testosterone assays was provided by WCVM (S. Cook, pers. comm.). A known amount of dried feces was weighed into a 16x75 polypropylene tube and 5 ml methanol were added. Tubes were capped and mixed many times over several days. Samples were centrifuged and aliquoted 2 ml to a 12x75 polypropylene tube, then dried. 100 µl ethanol were added to dissolve the bile acids, then 2 ml assay steroid diluent were added and mixed. This solution went directly to the assay. The concentration of ng progesterone/g feces was calculated as: ng/ml*5 ml/g of feces.

Fecal Pellet Size and Possible Identification of Calf Samples

In an Alaskan study of sampling caribou fecal pellets for pregnancy and other attributes, Joly et al. (2015) avoided collecting fecal samples from piles with small pellets as these could be from calves, which are unlikely to be pregnant; including female calf samples would affect the estimation of overall pregnancy rate. Flasko et al. (2017) measured fecal pellet size in boreal caribou samples and found that calf pellets were usually identifiably smaller than those of older caribou.

In the field fecal samples were sampled opportunistically, or as they were encountered. Later in a lab setting, we attempted to assess the size of fecal pellets in each sample to test whether a subset of our samples might be identified as being from calves. In July 2022, reserved fecal samples collected in March 2022 were taken out of a freezer and assessed visually as either small or large. Pellet sizes were compared side-by-side across the samples from each site by two of the authors (J. Williams and J. Adamczewski). This assessment was carried out "blind", in the sense that the assay results for individual samples was not known. The samples assessed as small were then compared to the overall assay results which identified males and females and pregnant and non-pregnant females. If a high proportion of the female samples identified as small were from calves, then we expected that those samples would be identified as predominantly or entirely non-pregnant.

Pregnancy Rate Estimates

Results of the genetic analyses identified samples that were duplicates from the same individual caribou and individuals that were males and females. Of the samples that were unique individuals and females, pregnant and non-pregnant females were identified. These female samples likely included calves, nine months old in March and very unlikely to be pregnant, yearling females, 21 months old in March that could have been pregnant but likely at lower rates than older females, and females at least 33 months old in March, which would have adult pregnancy rates (Dauphiné 1976, Parker 1981, Thomas and Kiliaan 1998).

Two principal approaches were used to obtain a pregnancy rate estimate for adult caribou from the sample of fecal pellets. First, the number of fecal samples of female caribou was adjusted to only contain adult females using information from calf:cow ratios collected during the survey or using estimates from a demographic IPM. Second, the fecal data set was used as an input for the IPM, which then produced a refined pregnancy rate estimate based on the fecal data as well as other survey, collar, and composition data. We cover the first approach in this section, and the IPM analysis is detailed further in Appendix 1.

As an initial estimate, the ratio of samples of pregnant females ($S_{pregnant}$) to total individual female fecal samples (S_{female}) was used to estimate the naïve fecal pregnancy rate (PF). Bootstrap and binomial-based standard errors and confidence limits were generated based on the sites sampled, similar to the approach used with calf:cow ratios.

$$PF = \frac{S_{pregnant}}{S_{female}}$$

Because the ratio included subadult females (yearlings and calves), it was a negatively biased estimate of adult female pregnancy rate. One potential approach to reduce bias was to use the calf:cow ratio estimated during the March survey ($CCratio$) to eliminate the estimated proportion of calves from the total count of females.

$$PF_{CC} = \frac{S_{pregnant}}{S_{female} - S_{female} * CCratio/2}$$

An IPM (Boulanger et al. 2011 and 2022, Adamczewski et al. 2022) was applied to estimate adult pregnancy rate from the fecal data set. The IPM uses data sources from population surveys, collar data, and composition survey data to estimate pregnancy rate and other demographic parameters. It also estimates the number of adult females, calves and yearlings during the March surveys. The proportion of female yearlings (p_y) and calves (p_c) is then estimated as the number of female yearlings or calves divided by total females during the March survey. This information was then used to subtract the estimated numbers of yearling and calf samples from the total number of individual samples to derive a corrected fecal pregnancy rate estimate (PF_{IPM}) that only includes adult caribou. The pregnancy rates of yearling females and female calves were assumed to be 0 based primarily on Dauphiné's (1976) results (female calves 0.0% pregnant, yearling females 1.8% pregnant), thus the predicted numbers of female calves and yearling calves were assumed to account for non-pregnant female samples only.

$$PF_{IPM} = \frac{S_{pregnant}}{S_{female} - S_{female} * p_y - S_{female} * p_c}$$

We also assessed the potential pregnancy rates in females ≥33 months old with a yearling female pregnancy rate of 12% (11 of 92) recorded by Thomas and Kiliaan in the Beverly herd (1998) (PFY_{IPM}).

$$PFY_{IPM} = \frac{S_{pregnant}}{S_{female} - S_{female} * p_y * .12 - S_{female} * p_c}$$

Binomial-based standard errors and confidence limits were derived for each of the pregnancy rate estimates. These estimates mainly considered variation due to sample sizes of fecal pellets without considering uncertainty in the IPM or calf:cow ratios. Future analyses in unison with the IPM analysis will produce more robust standard error and confidence limit estimates.

Estimates were compared to the pregnancy rate estimate from the IPM for 2020 (IPM_P) given that composition surveys were not conducted in 2020 on the calving grounds; these would result in estimates of the proportion of breeding females among females at least two years old in June. We also developed a method to include the fecal pregnancy rate as an input data set to the IPM (Appendix 1).

Each pregnancy rate estimator type is summarized in Table 1.

Table 2. A summary of estimators of pregnancy rate used for the March 2020 caribou data.

Estimator	Acronym	Data source(s)	Objective/Use
Fecal pregnancy	PF	Fecal progesterone & DNA	Pregnancy rate for all females (including female calves and yearlings) from fecal field data
Fecal pregnancy adjusted using calf:cow ratios	PF_{CC}	Fecal progesterone & DNA & March survey composition data	Pregnancy rate with female calves excluded using calf:cow ratio from concurrent composition surveys; includes female yearlings and adults
Fecal pregnancy adjusted with IPM estimate	PF_{IPM}	Fecal progesterone & DNA & IPM	Fecal pregnancy with subadults excluded based on IPM estimates of proportions of each age class, assuming no yearlings are pregnant; includes adult females only
Fecal pregnancy adjusted with IPM estimate with 12% pregnant yearlings	PFY_{IPM}	Fecal progesterone & DNA & IPM	Fecal pregnancy with calves excluded based on IPM estimates of proportions of each age class, assuming 12% of yearlings are pregnant
IPM adult pregnancy	IPM_P	IPM	Model-based estimate of adult female pregnancy rate (no fecal data); excludes female calves and yearlings

The initial estimates of fecal pregnancy rate were made using all samples from genetically unique individuals identified as females; this included samples collected on all three herd ranges (see Figure 3). Model-based estimates of female calf and female yearling proportions for the Bathurst and Bluenose-East herds were averaged. Thereafter, fecal pregnancy rates were estimated by similar methods for samples from Bathurst-only, Bluenose-East-only, Bathurst/Bluenose-East mixed, and Beverly ranges.

RESULTS

Field Sampling Sites and Herd Assignments

Locations of field sites where caribou fecal samples were collected in March 2020 by ENR and in February and March 2020 by A. Smith are shown in Figure 3. Collared caribou locations for March 10, 2020 are included and areas assigned to caribou herds for the classification survey are shown as coloured polygons.

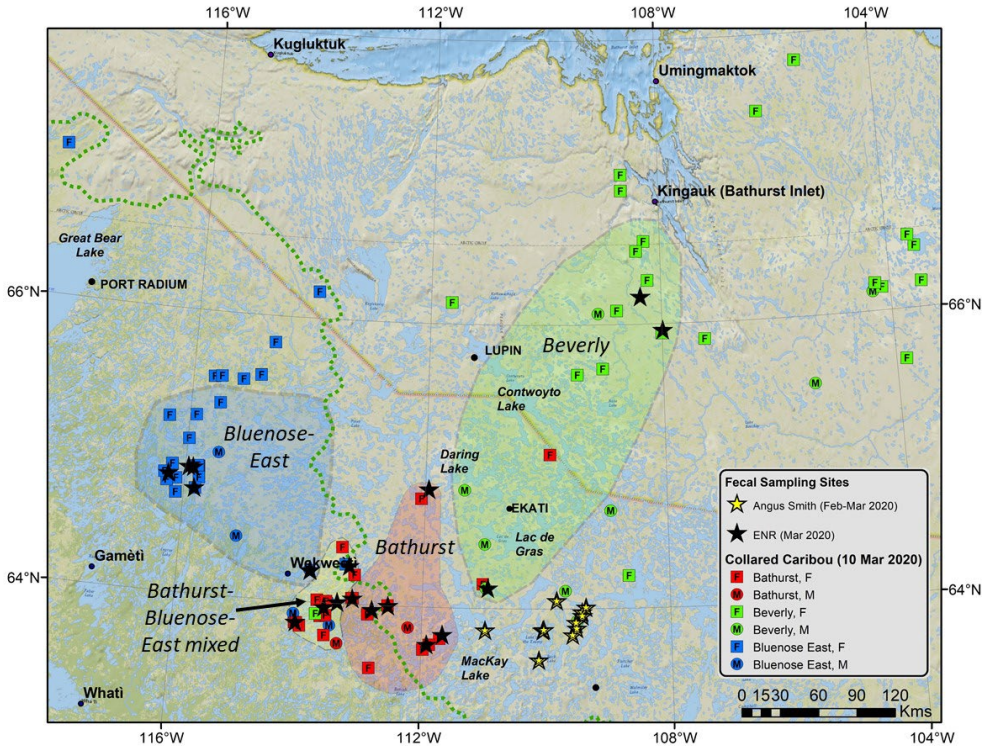

Figure 3. Sites where caribou fecal samples were collected by ENR (black stars) in March 2020 and by Smith (yellow stars) in February and March 2020, together with collared caribou locations (March 10, 2020) and areas assigned to herds for classification results.

The light green area was assigned as Beverly caribou, the light blue area as Bluenose-East caribou, the light red area as Bathurst caribou and the smaller yellow area as Bathurst/Bluenose-East caribou mixed. The light green Beverly polygon was modified slightly from the map in Adamczewski et al. (2020) to include the fecal sampling site southwest of Ekati. The coloured polygons were based on the areas flown in the March 2020 surveys; collared caribou outside the coloured polygons were not sampled during the surveys.

Locations of sites one to 20 at which caribou fecal samples were collected in March 2020 are shown in Figure 4 and the polygons assigned to herds in Figure 3 are included. Initially there was a site numbered 8 but this was approximately 300-400 m from site 7 so samples from sites 7 and 8 were combined as site 7. Based on these polygons and the collar locations in

Figure 3, sites 18, 19 and 20 were assigned as Beverly caribou; sites 10, 11, 12, 13, 14 and 15 were assigned as Bluenose-East caribou; sites 1, 2, 3, 5, 16 and 17 were assigned as Bathurst; and sites 4, 6, 7 and 9 were assigned as Bathurst/Bluenose-East mixed. This mixed area also had one Beverly collared cow mixed with Bathurst and Bluenose-East collared cows.

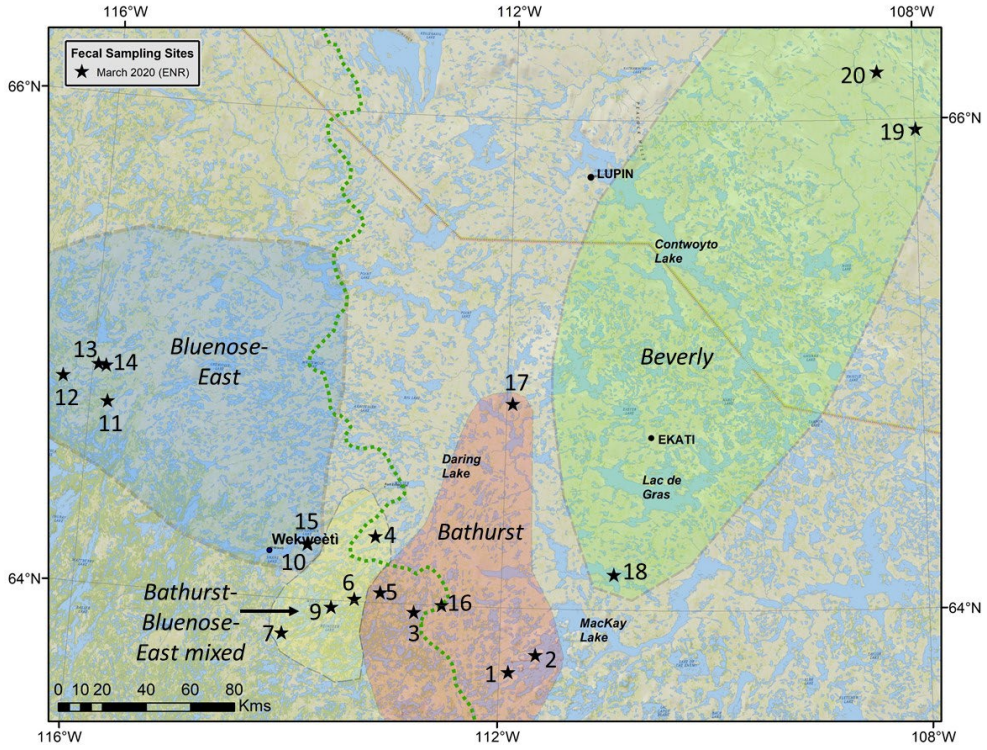

Figure 4. Sites where caribou fecal samples were collected in March 2020 by ENR during composition surveys of Bathurst, Bluenose-East and Beverly herds. Sites 10 and 15 were relatively close together and thus appear as one star.

Numbered sites where Smith collected caribou fecal samples in February and March 2020 are shown in Figure 5. Based on the presence of five collared Beverly caribou and one collared Bathurst caribou in the study area sampled by Smith and the large disparity in herd sizes (Bathurst 6,200 estimated in 2021 and Beverly 103,000 estimated in 2018), it was assumed that the fecal samples collected at these sites were almost entirely from Beverly caribou.

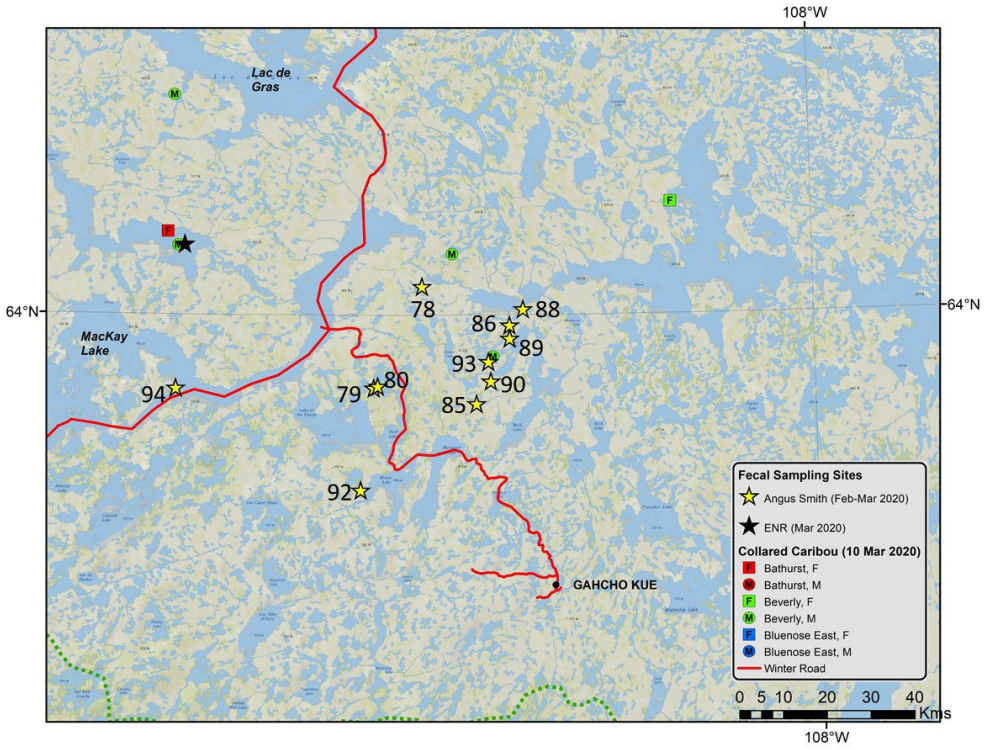

Figure 5. Sites at which caribou fecal samples were collected by MSc student Smith in February and March 2020. Collar locations are from March 10, 2020.

Genetic Analysis Results

Of the 331 samples collected during caribou surveys, 32 (9.7%) were identified as duplicates and 18 (5.4%) were identified as unsuitable for genetic analysis. Of the remaining 281 samples, 98 (34.9%) were identified as males and 183 (65.1%) as females; 111 of the 183 female samples (60.7%) were assessed as pregnant. The 46 samples from Smith included five duplicates (10.9%) and two samples with unsuitable DNA (4.3%); of the remaining 39 samples, 34 were males (87.1%) and five were females (12.8%) thus this subset of samples contributed little to analysis of pregnancy rates. More detailed results are provided below in combination with the results of the progesterone assays.

Progesterone and Testosterone Assay Results

There were, in total, 320 individual progesterone values, after removing the duplicates and unsuitable-DNA samples. Of these 320 samples, 132 were from males, 77 from non-pregnant females and 111 from pregnant females. Mean values in ng/g with 95% confidence intervals (CI) were: males 13.54±2.17, pregnant females 266.59±28.09, and non-pregnant females 47.62±6.95 (Figure 6). The 95% CI were non-overlapping thus the means were significantly different. The WCVM lab labeled progesterone values in females of <100 ng/g as non-pregnant.

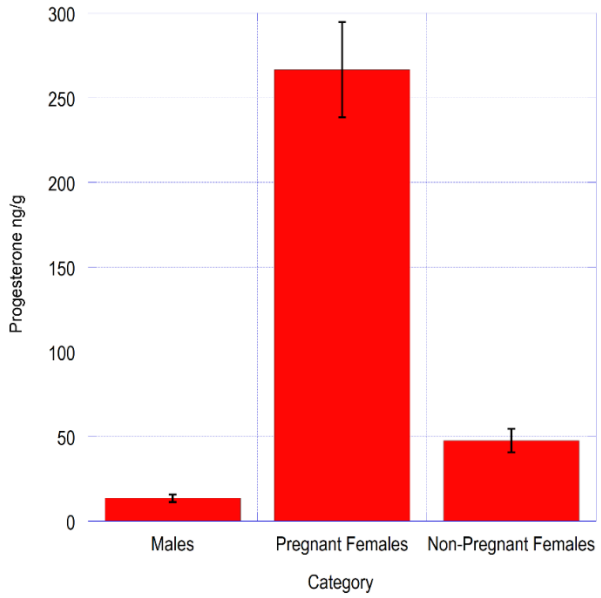

Figure 6. Mean concentrations of progesterone in fecal samples from male, pregnant female and non-pregnant female caribou collected in March 2020 by ENR and in February and March 2020 by Smith from the Bathurst, Bluenose-East and Beverly herds. Error bars are 95% CI.

Assays for testosterone were carried out for non-pregnant females and males to assess whether higher testosterone levels in males might allow them to be separated from non-pregnant females; by mistake three samples from pregnant females were also assayed for testosterone. The total samples of males, pregnant females, and non-pregnant females tested for testosterone were 132, three and 77 respectively. Mean values and 95% CIs for testosterone in ng/g were: males 5.34±0.53, pregnant females 4.83±2.31, and non-pregnant females 5.19±0.80 (Figure 7). The large CI for the pregnant females likely reflects the much smaller sample size of this category. The mean values for all three categories were very similar and the 95% CIs showed considerable overlap, thus fecal testosterone levels were not significantly different and did not allow for individual samples to be assigned to any of the three categories.

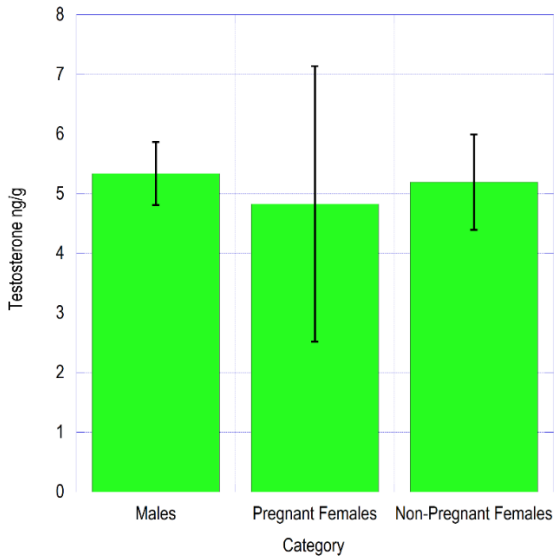

Figure 7. Mean concentrations of testosterone in fecal samples from male, pregnant female and non-pregnant female caribou collected in March 2020 by ENR and in February and March 2020 by Smith from the Bathurst, Bluenose-East and Beverly herds. Error bars are 95% CI.

Results Including Genetic and Progesterone/Testosterone Assays

Results for caribou sex and pregnancy status are summarized by site in Table 2. Numbers of duplicate occurrences are included, as are numbers of samples where no DNA result was obtained. Results from all of Smith's sites were combined as the numbers per site were limited and a high percentage of these were males. A listing of all individual samples, including duplicates and samples where DNA was not successfully obtained, sex and pregnancy status in females, and progesterone and testosterone levels recorded, is in Appendix 2. Further estimation of pregnancy rates included only the 281 samples collected by ENR in March 2020 that were not duplicates, had valid DNA results, and where progesterone and testosterone levels were recorded. The samples collected by Smith were nearly all males, thus added little to information about pregnancy rate, and likely were not gathered from cow:calf groups, as in ENR's March 2020 sites.

Table 2. Summary by sampling site of caribou fecal pellet analyses for sex and pregnancy status from samples collected in March 2020 by ENR and in February and March 2020 by Smith.

Site#	Initial Sample #	Duplicates	Inadequate DNA (Trent U)	Males Total	Females Total	Pregnant Females	Non-Pregnant Females
ENR1-2020	15	2	1	9	3	2	1
ENR2-2020	15	0	1	5	9	4	5
ENR3-2020	14	1	1	7	5	2	3
ENR4-2020	15	5	0	6	4	2	2
ENR5-2020	13	2	0	3	8	5	3
ENR6-2020	14	2	0	4	8	6	2
ENR7-2020	15	0	1	4	10	5	5
ENR9-2020	13	1	1	4	7	4	3
ENR10-2020	20	0	5	6	9	8	1
ENR11-2020	20	0	1	4	15	9	6
ENR12-2020	20	2	0	5	13	7	6
ENR13-2020	20	0	0	4	16	11	5
ENR14-2020	20	3	2	11	4	2	2
ENR15-2020	18	1	2	6	9	5	4
ENR16-2020	19	4	0	3	12	2	10
ENR17-2020	20	3	0	7	10	8	2
ENR18-2020	20	2	0	3	15	11	4
ENR19-2020	20	4	2	4	10	9	1
ENR20-2020	20	0	1	3	16	9	7
Totals ENR	**331**	**32**	**18**	**98**	**183**	**111**	**72**
Percentages ENR		9.7% of samples	5.4% of samples	34.9% of 281 M+F	65.1% of 281 M+F	60.7% of 183 F total	39.3% of 183F total
Totals Smith	**46**	**5**	**2**	**34**	**5**	**1**	**4**
Percentages Smith		10.9% of samples	4.3% of samples	87.1% of 39 M+F	12.8% of 39 M+F	20.0% of 5 F total	80.0% of 5 F total
Overall Totals	**377**	**37**	**20**	**132**	**188**	**112**	**76**
Percentages Overall		9.8% of samples	5.3% of samples	41.2% of 320 M+F	58.8% of 320 M+F	59.6% of 188 F total	40.4% of 188 F total

From the ENR samples, there were 281 samples where sex was identified along with pregnancy status. This included 98 males (34.9%) and 183 females (65.1%). Of the 183 females, 111 (60.7%) were pregnant and 72 (39.3%) were not.

Fecal Pellet Size Assessment

Samples were visually assessed as small and presumably from smaller caribou, or large and presumably from larger animals. Total numbers of males, pregnant females and non-pregnant females by site are shown in Table 3 from all 281 caribou where sex was identified. The results for pellets considered small were a sub-set of 65 of the 281 samples.

Table 3. Site summary of numbers of caribou (males and females and pregnant and non-pregnant females) from ENR sampling in March 2020, including all 281 samples (left) and only a subset of 65 pellet samples identified in July 2022 as small and potentially from calf or yearling caribou (right).

All Pellet Samples					Pellet Samples Considered Small Only			
Site#	**Males Total**	**Females Total**	**Pregnant Females**	**Non-Pregnant Females**	**Males Total**	**Females Total**	**Pregnant Females**	**Non-Pregnant Females**
ENR1-2020	9	3	2	1	2	1	0	1
ENR2-2020	5	9	4	5	1	2	1	1
ENR3-2020	7	5	2	3	2	0	0	0
ENR4-2020	6	4	2	2	2	2	2	0
ENR5-2020	3	8	5	3	2	5	4	1
ENR6-2020	4	8	6	2	0	4	3	1
ENR7-2020	4	10	5	5	2	4	2	2
ENR9-2020	4	7	4	3	0	3	2	1
ENR10-2020	6	9	8	1	0	0	0	0
ENR11-2020	4	15	9	6	2	1	0	1
ENR12-2020	5	13	7	6	1	3	1	2
ENR13-2020	4	16	11	5	0	3	1	2
ENR14-2020	11	4	2	2	3	3	1	2
ENR15-2020	6	9	5	4	1	3	2	1
ENR16-2020	3	12	2	10	0	3	0	3
ENR17-2020	7	10	8	2	1	2	2	0
ENR18-2020	3	15	11	4	0	2	1	1
ENR19-2020	4	10	9	1	1	2	2	0
ENR20-2020	3	16	9	7	1	1	1	0
Totals	**98**	**183**	**111**	**72**	**21**	**44**	**25**	**19**
	34.9% of 281 M+F	65.1% of 281 M+F	60.7% of 183 F total	39.3% of 183F total	32.3% of 65 M+F	67.7% of 65 M+F	56.8% of 44F total	43.2% of 44 F total

Overall, the proportions of males and females for the larger sample of 281 caribou were very similar to those in the small pellet subset: 34.9% of the 281 samples were males and 65.1% were females, compared to 32.3% males and 67.7% females among the small-pellet subgroup. In addition, of the 183 females in the larger sample, 60.7% were pregnant and 39.3% were not; in the small-pellet subset there were 44 females, of which 56.8% were pregnant while 43.2% were not.

Pregnancy Rate Estimates

An initial estimate of pregnancy rate was made using all 281 samples collected across the Bathurst, Bluenose-East and Beverly herds in March 2020 (Table 4).

Table 4. Estimates of pregnancy rates across all three herds and for individual herds based on fecal samples assayed for DNA and progesterone collected in March 2020 during composition surveys of the Bathurst, Bluenose-East and Beverly herds. The female calf age class is assumed non-pregnant in all cases, and female yearling age class is assumed to be either 0% or 12% pregnant. Adult females are ≥33 months old in March. Percentages of female calves, yearlings and adults for Bathurst and Bluenose-East herds are from the IPM.

	Herd/Herds				
Measurement	**All Herds**	**Bathurst**	**Bluenose-East**	**Bathurst/BNE**	**Beverly**
Site #s	1-7, 9-20 (all)	1,2,3,5,16,17	10,11,12,13,14,15	4,6,7,9	18,19,20
Total Samples #	281	81	102	47	51
Males #	98	34	36	18	10
Females Total #	183	47	66	29	41
Females Pregnant #	111	23	42	17	29
Females Non-Pregnant #	72	24	24	12	12
Calves as % of Females	16.4	13.1	17.3	16.4	
Yearlings as % of Females	7.4	11	6.4	7.4	
Adults as % of Females	76.3	76	76.4	76.3	
Calves Female #	30.0	6.1	11.4	4.7	
Yearlings Female #	13.6	5.1	4.2	2.2	
Adults Female #	139.7	35.7	50.4	22.1	
Yearling Female Pregnancy 0%					
Female Calves % Pregnant	0	0	0	0	
Female Yearlings % Pregnant	0	0	0	0	
Female Adults % Pregnant	79.5% (111/139.7)	63.3 (23/35.7)	83.3 (42/50.4)	76.9% (17/22.1)	
Confidence Limits (Female Adult % pregnant)	73.0-84.7	48.8-75.7	72.3-90.5	58.3-88.7	
Yearling Female Pregnancy 12%					
Female Calves % Pregnant	0	0	0	0	
Female Yearlings % Pregnant	12 (1.6/13.6)	12 (0.6/5.1)	12 (0.5/4.2)	12 (0.3/2.2)	
Females Adult % Pregnant	78.9 (109/140.8)	63.2 (23/36.2)	82.5 (42/50.9)	76.9 (17/22.1)	

Calculations assume that the fecal sampling was random and included female calves, yearlings and older caribou in proportion to their relative numbers in the areas where fecal pellets were collected. The results of our attempt to separate small pellet samples from larger pellet samples, described above, suggested that the small pellet samples included pregnant and non-pregnant females and males in about the same proportions as the overall sample set. Thus, there was no rationale for excluding the small-pellet sub-set of results from analyses.

Results for all the samples combined from all three herds used combined Bathurst and Bluenose-East IPM values of 16.4% female calves, 7.4% female yearlings and 76.3% adult females, which translate to 30.0 female calves, 13.6 female yearlings and 139.7 adult females. Estimates from the IPM were weighted by the estimated total number of females for each herd in March therefore accounting for differences in relative abundance of the herds. If all female calves and yearlings are assumed to be non-pregnant, then the adult female pregnancy rate was 79.5% across all three herds. If the yearling female pregnancy rate is assumed to be 12% (Thomas and Kiliaan 1998), then a similar adult pregnancy rate estimate of 78.9% results. Parker (1981) found a yearling pregnancy rate of 43% in the George River herd in an increasing phase, however population trends in the Bathurst, Bluenose-East and Beverly herds in 2020 was likely either stable or declining, thus this yearling pregnancy rate is unlikely to be applicable to these three herds.

Using similar calculations, estimates of adult female pregnancy rates of 63.3% in the Bathurst herd, 83.3% in the Bluenose-East herd and 76.8% in the mixed Bathurst/Bluenose-East herds were generated in Table 4. It should be noted that the total numbers of female samples (including all age classes) for these three categories were limited (Bathurst 47, Bluenose-East 66 and Bathurst/Bluenose-East mixed 29). For the Beverly herd, modeling using the IPM has not been developed to date, thus the model-based estimates of proportions of females of different age-classes and model-based estimates of pregnancy rate were not possible. An estimate was developed, however, based on the March 2020 calf:cow ratio estimated for the Beverly herd (91.5%); this estimate includes female yearlings and adult females and excludes calves. The Beverly sample had the highest simple proportion of pregnant female samples (29/41 or 70.7%) of the three herds, but the total female sample for this herd was a limited 41 individuals.

For greater clarity, the estimators of pregnancy rates described in Table 1 are shown for the Bathurst and Bluenose-East herds in Table 5 and in Figure 8.

Table 5. Pregnancy rate estimates for the Bathurst, Bluenose-East, and Beverly herds in March 2020. IPM-based estimates were not available for the Beverly herd given that an IPM model has not been developed for this herd.

Pregnancy Estimator	Description	Estimated Pregnancy Rate	95% Confidence Limits	
Bathurst Herd				
PF	Fecal Raw Estimate March (includes all females)	48.9%	35.1%	62.9%
PF_{cc}	Fecal Estimate March; female calves excluded based on calf:cow adjustment (CC ratio=0.304); includes yearling and adult females	57.7%	43.3%	70.9%
PF_{IPM}	Fecal Estimate with IPM adjustment; female calves and yearlings excluded	64.4%	49.9%	76.7%
PFY_{IPM}	Fecal Estimate with IPM adjustment and yearling females with 12% pregnancy	63.3%	48.8%	75.7%
IPM_P	IPM Pregnancy (model only; no fecal results included)	74.0%	46.0%	92.4%
Bluenose-East Herd				
PF	Fecal Raw Estimate March (includes all females)	63.6%	51.5%	74.3%
PF_{cc}	Fecal Estimate March; female calves excluded based on calf:cow adjustment (CC ratio=0.418; includes yearling and adult females	80.5%	69.1%	88.3%
PF_{IPM}	Fecal Estimate with IPM adjustment; female calves and yearlings excluded	83.3%	72.3%	90.5%
PFY_{IPM}	Fecal Estimate with IPM adjustment and yearling females with 12% pregnancy	82.5%	71.4%	89.9%
IPM_P	IPM Pregnancy (model only; no fecal results included)	82.0%	68.0%	94.0%
Beverly Herd				
PF	Fecal Raw Estimate March (includes all females)	70.7%	55.2%	82.6%
PF_{cc}	Fecal Estimate March; female calves excluded based on calf:cow adjustment (CC ratio=0.45) ; includes yearling and adult females	91.5%	78.2%	97.0%

The lowest estimates of pregnancy rate for both herds were the raw estimates of fecal pregnancy (red in Figure 8), which included calves and yearlings. The pregnancy estimates based only on the model (purple) can be most directly compared to the values based on fecal samples adjusted for the IPM, with yearlings assumed to be either all non-pregnant (green) or 12% pregnant (blue). These comparisons indicate reasonable similarity and overlapping CIs.

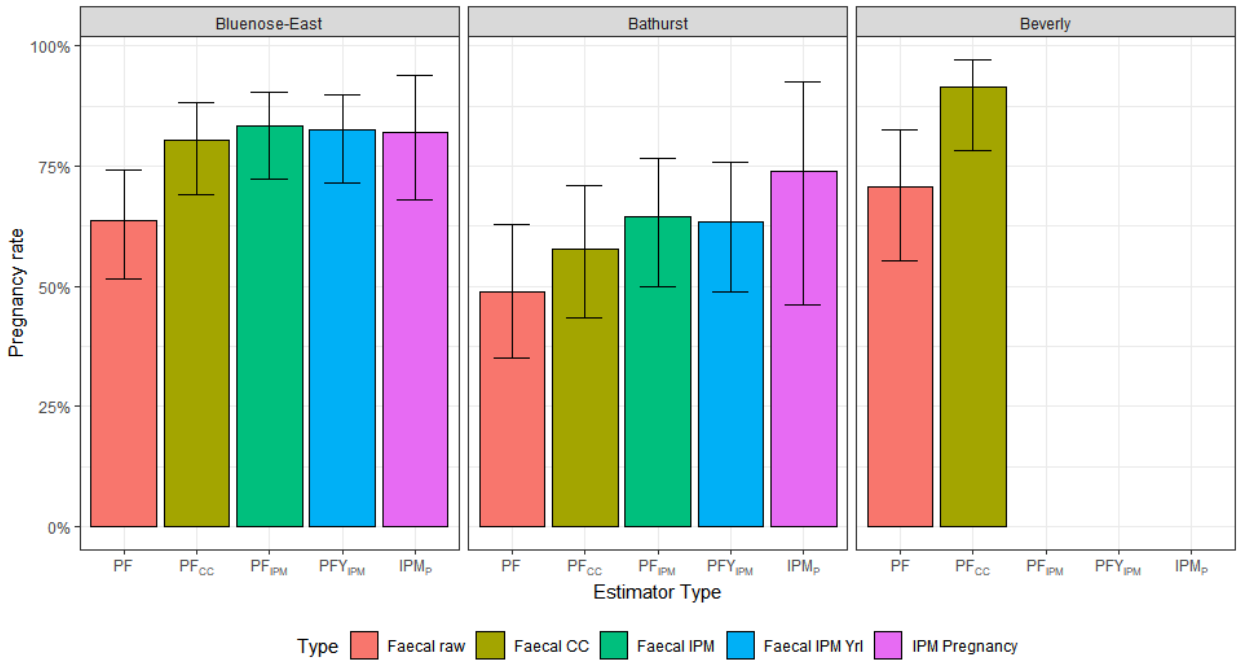

Figure 8. Comparison of estimates of pregnancy rate with estimators defined in Tables 1 and 4, for Bluenose-East, Bathurst and Beverly herds in March 2020.

The main factor influencing differences between the fecal calf:cow based estimator (PF_{CC}) and fecal IPM-based estimators (PF_{IPM} and PFY_{IPM}) was the relative proportion of female yearlings in each herd during the March survey. If this proportion was lower in 2020, such as with the Bluenose-East herd (Table 4: 6.4%), the difference in estimates was minimal. At higher yearling proportions, such as the Bathurst (11%), differences were greater. The covariance between productivity and estimates is explored further in a later section on IPM modeling.

June calving ground surveys produce an estimate of the proportion of breeding females on the calving grounds, which would be the closest other measure to assess pregnancy rate of females in late gestation. Because a June 2020 calving ground survey was not conducted, a comparison of estimates from March 2020 and June 2020 was not possible.

Binomial confidence limits on estimates were likely optimistic given that they did not fully consider variation in calf:cow ratios or IPM estimates. Future analyses will develop more comprehensive methods to estimate confidence limits on these ratios.

Cost of Assays and Field Time

Costs of progesterone assays were 393 samples at $12.50/sample or $4,912.50. Costs of testosterone assays were 257 samples at $18.50/sample or $4,754.50, with the total for these steroid assays of $9,667.00. A total of 377 samples were sent for genetic analysis to Trent University at $60.00/sample and the total cost was $23,751.00. Total costs of lab

assays at Trent University and University of Saskatchewan were $33,418.00. The costs of the helicopter charter, fuel, fuel caching, accommodations and other expenses in March 2020 are not included here; those costs are likely of comparable overall scale as June composition surveys (described further on).

In March 2020, 19 sites were sampled. Two of the authors (J. Williams and J. Adamczewski) collected fecal samples at each site. On average each stop to pick up fecal samples was 20-25 minutes. The stops added up to about seven hours in the field, with the effort spread over the five days of the helicopter survey.

Sample Size Needed for Fecal Pregnancy Assessment

An evaluation of the numbers of samples needed to assess pregnancy rate in a caribou herd was made based on the following approach. As a first step, a statistical evaluation was made of the numbers of samples of females needed to estimate pregnancy rate for a given Coefficient of Variation (CV; Figure 9). If it is assumed that pregnancy rate estimation is described as a set of binomial trials then it is possible to estimate precision of the ratio estimate of pregnancy rate (Cochran 1977). This estimate assumes that individuals are identified (no duplicates) so that the resulting fecal samples are independent. In addition, this approach also assumes that samples are spatially representative, i.e. sampling needs to be dispersed across a herd's range and random, in proportion to fecal samples encountered. The results observed in this study suggested that small pellets did not reliably identify samples from younger age classes of caribou.

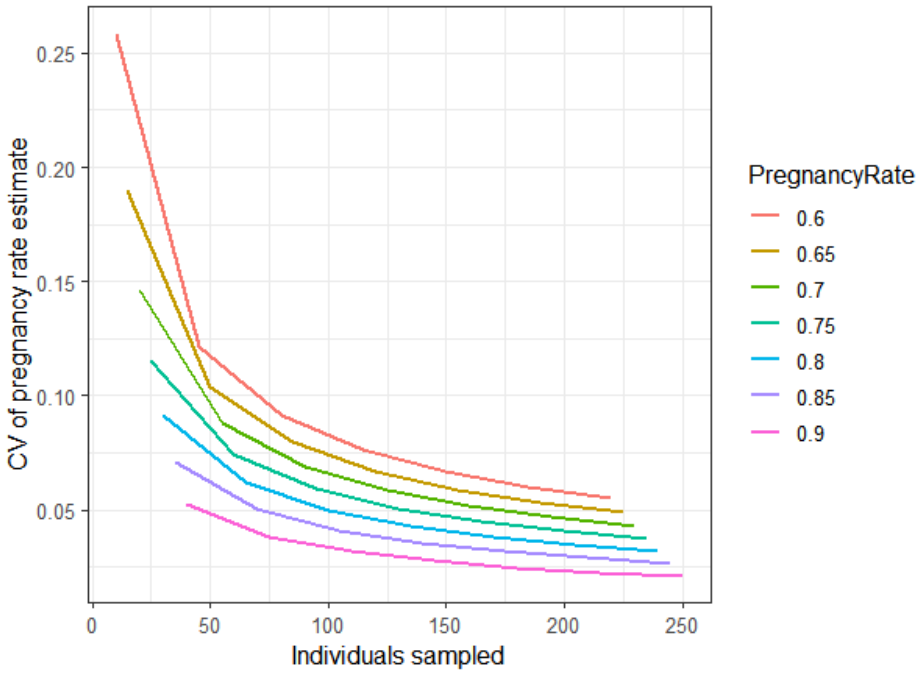

Figure 9. Variation in CV of pregnancy rate estimate from fecal samples with number of individuals sampled and the pregnancy rate.

If the assumptions of independent and random samples are met, then the curves in Figure 9 describe estimated precision. As pregnancy rate increases, the number of samples required for a precise estimate decreases. If the target CV is 0.1 then at least 100 samples are required with a pregnancy rate estimate of 0.6 (i.e. 60%). It is likely that the binomial estimate of variance is optimistic and therefore 100 samples of female caribou would be a minimum requirement.

Secondly, the results of the March 2020 fecal sampling carried out by ENR demonstrated that an overall total number of samples collected would result in sequential reductions due to duplicate samples, samples unsuitable for DNA analysis, male samples, and likely inclusion of samples from female calves and female yearlings. One potential example sequence which begins with 300 total fecal samples collected from one herd's range is presented in Table 6.

Table 6. Likely numerical outcomes from collecting 300 fecal samples to estimate pregnancy rate in a barren-ground caribou herd, based on results of ENR March 2020 sampling during composition surveys.

Step Number	Outcome
1	300 individual samples collected at 20 sites
2	30 samples less (10%), identified as DNA duplicates; 15 samples less (5%), identified as poor DNA
3	255 unique samples with suitable DNA remaining
4	89 samples identified as males (35%)
5	166 samples identified as females (65%)
6	20% of female samples identified as calves (9 months old)
7	10% of female samples identified as yearlings (21 months old)
8	116 samples identified as adult females (≥33 months old)
9	Pregnancy rate assessed from either females ≥33 months old or from females ≥21 months old

Achieving a target of about 116 samples/herd from adult females ≥33 months old would require collection of about 300 samples in the field. This approach assumes that sampling would be random across smaller and larger fecal pellets, likely proportions of female calves and yearlings can be identified, and proportions of duplicates, unsuitable samples, and males/females would be similar to the results from the ENR March 2020 sampling described in this report.

DISCUSSION

Overall, results from fecal samples collected in March 2020 during ENR's helicopter-based composition surveys suggest that this approach has value under some conditions, particularly in years when June composition surveys are not flown or if precise estimates of late winter pregnancy would help inform a detailed understanding of herd status and evaluation of management actions. Any method used to assess reproductive status of caribou in late pregnancy needs to include an adequate sample size that is representative of the reproductively active segment of a herd. Fecal sampling during composition surveys will include some duplicate samples, poor-DNA samples, and some proportion of samples from males, and sampling effort would have to accommodate for this to achieve required sample sizes.

Fecal Pellet Sampling and Pellet Size

Flasko et al. (2017) weighed and measured boreal caribou fecal pellet size and concluded that calf pellets were identifiably smaller than those of caribou at least 21 months old, and Joly et al. (2015) did not pick up the smallest caribou fecal samples on the assumption these were from calves. However, results of this study suggested that caribou pellets assessed as small had male/female and pregnant/non-pregnant proportions that were very similar to those in the larger sample. In our case, excluding the samples with smaller pellets might have excluded some calves and yearlings, but it would also have excluded a substantial proportion of pregnant females. There appeared to be a gradient in pellet size rather than a categorical separation of pellets from calves and older caribou. The proportion of male samples (about 35%) among our samples was surprisingly high given that fecal samples were collected only in areas where predominantly calf:cow groups were classified. A substantial proportion of the male samples could have been from male calves and male yearlings, based on the proportions of female calves and female yearlings in Table 4.

If pellet samples from calves nine months old can be reliably and categorically identified as distinct from pellets from caribou yearlings 21 months old and adults ≥33 months old, then avoiding those pellet groups may be a viable approach, as suggested by Flasko et al. (2017). However, these results left considerable uncertainty as to the age class that small-pellet samples were from. Further, in the field during the helicopter-based composition surveys in March, sampling on the ground is time-constrained as the main purpose of the surveys is the classification.

An alternative approach is to sample piles of fecal pellets opportunistically found in the field, then use demographic information or a model to estimate likely proportions of female calves, female yearlings and adult females to retroactively assign a percentage of the female fecal samples to each of these categories. Female calves are very unlikely to be pregnant and the proportion of yearling females that are pregnant is likely to be low (Dauphiné 1976, Thomas and Kiliaan 1998); thus an estimate of likely pregnancy rate in females ≥33 months old can

then be made (see Tables 4 and 5). Sampling fecal pellet piles opportunistically is a simple approach to use in the field and does not require a judgement call as to which samples are small. A random sampling approach may be preferable to an approach of discarding small pellets where there is uncertainty as to the age classes of pellet samples kept or discarded.

Costs of Assays and Field Time for Fecal Pellet Sampling During Helicopter Surveys

At the end of the results section, one scenario (Table 6) was proposed that would begin with 300 samples collected in the field for a caribou herd and result in about 116 samples from females ≥33 months old, assuming that portions of the 300 samples would be duplicates, unsuitable DNA samples, and males as found in this study. The results obtained for testosterone suggest that the range of levels of this hormone in caribou feces in males, pregnant females and non-pregnant females did not allow an individual sample to be assigned to one of these classes. Further assays for testosterone would not likely serve any purpose in this type of study. At a cost of $12.50/sample, 300 samples assayed for progesterone would be $3,750.00. At $60.00/sample for DNA analysis as carried out in this study, 300 samples would cost $18,700.00. The total cost for 300 samples would be $22,450. These costs are just those associated with laboratory costs and do not include costs associated with the composition surveys themselves and would increase if a larger sample size was required.

Costs of assessing pregnancy rate from caribou fecal samples would be substantially reduced if the genetic analyses were not carried out and only the progesterone assays were completed. Mean levels of progesterone in males, non-pregnant females and pregnant females were well separated (Figure 6) and the 95% CI were also well separated, which suggests that fecal progesterone alone could separate the three categories of caribou with good confidence. However, avoiding the DNA analyses would likely mean that about 10% of the samples could be duplicate samples, which would add to the overall uncertainty of estimating herd-specific pregnancy rates.

The stops made at 19 sites in the field in March 2020 added up to about seven hours in the field, with the effort spread over the five days of the helicopter survey. This effort did not greatly hinder the survey flying. If collection of fecal samples was to involve sample collection of 300 samples (15 samples from each of 20 sites) from each of two or three herds, the time needed for stops on the ground would increase accordingly and add significantly to field time to complete the helicopter-based surveys.

Comparison of % Breeding Females from June Composition Surveys and March Fecal Pregnancy Rate Estimation

A helicopter and ground-based composition survey is part of June calving ground photo surveys (e.g. Boulanger et al. 2022). The June composition survey is flown across the distribution of calving female caribou at or just after the peak of calving. Proportions of

newborn calves, yearlings, breeding females, non-breeding females, young bulls and prime bulls are estimated. Numbers of caribou classified usually number thousands. For example, 3,977 caribou, including newborn calves, were classified in June 2021 on the Bathurst calving ground (Adamczewski et al. 2022) and 8,166 caribou were classified on the Bluenose-East calving ground in June 2021 (Boulanger et al. 2022). In June 2019, composition surveys were flown on the Bathurst and Bluenose-East calving grounds as stand-alone surveys to estimate the proportion of breeding females; the total numbers of caribou classified were 1,161 for the Bathurst herd and 5,347 for the Bluenose-East herd (Adamczewski et al. 2021).

One of the key statistics estimated from the June composition survey is the proportion of breeding females, which is a proxy for a direct measure of pregnancy rate. Females potentially of breeding age are two years old or older in early June. Yearlings are classified separately from breeding and non-breeding cows, thus the estimate of % breeding females does not include yearling females, which would have been nine months old in March. Breeding females include cows that have any of the following features: (1) a newborn calf; (2) a distended udder; or (3) hard antlers, because pregnant cows retain these until a few days after giving birth while non-pregnant cows will have shed the antlers well before June. Cows lacking all of these features are considered non-breeders, i.e. they were non-pregnant that winter.

A second valuable statistic from the June composition surveys is a calf:cow ratio in all females older than yearlings and in breeding females. As the composition survey is often flown about a week after the peak of calving, the calf:cow ratio in breeding females can provide an index of early calf mortality.

June composition surveys on the calving grounds estimate the proportion of breeding females in a caribou herd, and analyzing fecal samples collected during composition surveys in March gives an estimate of pregnancy rate, each of these methods offer advantages and disadvantages.

Advantages of June composition surveys to estimate the proportion of breeding females include:

(1) An overall sample size of females at least an order of magnitude larger in June than in March, making it logistically easier to achieve representative sample sizes.

(2) Uncertainty of identifying individual female classes on the June survey is limited to including females two years old vs females 3+ years old in June. By comparison, fecal samples collected in March will likely include calves, yearlings, and adult females, a substantial proportion of males, as well as duplicate samples and samples with poor-quality DNA. The exact proportions of female calves, yearlings and adult females among

fecal samples is difficult to identify with confidence. Higher numbers of fecal samples could be collected to reduce uncertainties, which would have an increased cost.

(3) The June surveys can provide an estimate of early calf mortality through the calf:cow ratio in breeding cows, while fecal sampling in March could provide insights into potential late gestational or early post-calving losses if data from June are available.

(4) Results of helicopter-based composition surveys are usually available immediately, while results of progesterone and testosterone assays require a period of months before data are available.

Fecal sampling in March for pregnancy estimation also offers the following advantages:

(1) Fecal sampling is non-invasive to the caribou. Helicopter-based composition surveys can result in some degree of short-term stress to the caribou, and June surveys create some short-term stress to caribou cows with very young calves. Fecal sampling from caribou can also be carried out using ground-based methods (e.g. Polfus et al. 2017) and larger sample sizes may be possible with this approach. Polfus et al. (2017) had samples from 655 individual caribou for genetic studies, which were collected over an extended period.

(2) Costs of progesterone assays and DNA analyses, estimated at about $22,450 for 300 fecal samples, were substantially less than the $71,500 cost of a June composition survey. However, this fecal sampling was only possible as an add-on to helicopter-based surveys with overall costs on a similar scale as the June helicopter-based surveys.

(3) Fecal samples can also be assayed for other purposes, such as estimating caribou diet (Newmaster et al. 2013, Joly et al. 2015) stress levels (Joly et al. 2015, Smith 2022) and presence and prevalence of parasites (e.g. Johnson et al. 2010). These applications are not possible from composition surveys.

Both methods should be able to create a sample set that is reasonably representative of the herd's distribution, in large part because the June composition surveys and the March composition surveys used to collect fecal samples are both designed to sample in proportion to regional numbers of caribou found and are guided by the satellite collar distribution of the herd. Ground-based sampling of fecal samples by skidoo would be feasible and non-invasive to caribou (Polfus et al. 2017). Sampling widely across a herd's range in remote areas (see Figure 3) could be challenging, particularly if results are required from a limited time period.

Considerations for Potential Future Assessment of Caribou Pregnancy Rate from Fecal Samples

Potential future applications of fecal sampling for pregnancy rate in barren-ground caribou herds should consider the following:

1. To obtain a sample of at minimum 100-120 fecal samples from females of ≥33 months old in March in a herd, sample collection should include at least 300 total fecal samples from 15-20 sites with good spatial representation.
2. Although Flasko et al. (2017) and Morden et al. (2011a) found that size of fecal pellets allowed separation of samples from calves and older caribou/reindeer, our test of small vs large fecal pellets suggested that pellets identified as small included a substantial proportion of pregnant females. Unless there is a categorical separation of pellet sizes, it may be preferable to sample fecal pellets piles at random in the field and then use demographic information or modeling to identify likely proportions of female calves, yearlings and adults. In a field situation where time is a constraint (e.g. during helicopter-based surveys), sampling opportunistically may be more practical than attempting to classify pellet sample sizes.
3. Sampling across a herd's distribution of females in winter is essential to obtaining a representative sample. Distribution of collared females can be used to distribute sampling sites, in the same way that collared caribou assist in planning aerial surveys.
4. Assaying fecal samples for testosterone was not useful in identifying males or pregnant/non-pregnant females.
5. Progesterone was useful in identifying males, pregnant females and non-pregnant females, and DNA analysis was useful in identifying duplicate samples (approximately 10% of samples) and eliminating them from the analysis.
6. Ground-based fecal sampling in winter, as for example, carried out by Polfus et al (2017) could be used to increase sample sizes, however sampling widely across a herd's range, over a short timeframe, in remote areas may be challenging.

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APPENDIX 1. INTEGRATED POPULATION MODEL ESTIMATION OF PREGNANCY RATE AND FACTORS INFLUENCING FECAL-BASED PREGANANCY RATE

A demographic model, the Integrated Population Model (IPM) has been used for a number of years (Boulanger et al. 2011) to integrate demographic indicators and assess components of population trend in the Bathurst and Bluenose-East herds. Recent updates for the two herds are in Adamczewski et al. (2022) for the Bathurst herd and Boulanger et al. (2022) for the Bluenose-East herd. Among the outputs generated by the models is fecundity over time. The model-based fecundity values take into consideration all field-based estimates such as calf:cow ratios, bull:cow ratios, collar-based cow survival rates and the variances associated with them. In this section we explore how the fecal pregnancy data could be integrated into the modeling; in addition, the model was used to generate predicted fecal pregnancy rates for the two herds over time and these are compared to the March 2020 pilot study results.

One potential use of the fecal-based pregnancy data is as an additional dataset used to inform the IPM. These datasets could be used to further complement calving ground-based estimates of the proportion of breeding females as well as to provide pregnancy rate estimates in years when calving ground surveys are not conducted. Formulas were derived to estimate the raw March fecal-based pregnancy rates (that include calves and yearlings). Comparison of field-based and model-based estimates can then be used in future IPM runs.

A second use of the fecal dataset was to refine the IPM-based estimate of pregnancy rate. Currently, the IPM uses estimates of proportion breeding females from calving ground surveys as well as calf:cow ratios to estimate pregnancy rate. A formula that used the IPM parameter to estimate the March fecal estimate (FP listed in Table 1) using the IPM demographic parameters was developed. The IPM based estimate of fecal pregnancy rate (IPM_{Ft}) is simply the ratio of estimated pregnant females divided by the sum of adult, yearling, and calf females during the survey in March.

$$IPM_{FP,t} = \frac{\mathrm{F}_t \, N_{f,t-1} \, S_{f,t-1}}{N_{f,t-1} \, S_{f,t-1} + \, 0.5 N_{y,t-1} \, S_{y,t-1} + \, 0.5 N_{c,t-1} \, S_{c,t-1}}$$

In this equation F_t is the pregnancy rate (termed fecundity in the IPM) for the upcoming calving ground (year *t*). The number of adult females, yearling and calves is symbolized as N_f, N_y, and N_c based on IPM estimates of the previous caribou year (symbolized by *t-1*). A sex ratio of 50% was assumed for calves and yearlings. Survival rates for each cohort from the IPM were symbolized by *S.* A scaling term was applied to survival ($S_{scale}=S^{(interval/365)}$) where the interval was the number of days from assumed calving (June 11 the previous year) to when the survey occurred (271 days in 2021). Using this scaling term for survival allowed an estimate of the numbers of each cohort from the previous year that were surviving up to the time the March pellet collection survey occurred (as the product of the number on the previous calving ground and scaled survival).

IPM fecal based estimates of pregnancy were estimated for 2020 as well as previous years to explore how variation in productivity and other factors influences the deviation of fecal and June-based pregnancy rate estimates. Future IPM model runs could include the fecal data as an input data set.

The dataset for the Bathurst herd dates to the 1980s (Figure 10), while the dataset for the Bluenose-East herd (Figure 11) is of shorter duration. Fecundity tends to show a saw-tooth pattern with higher and lower values alternating.

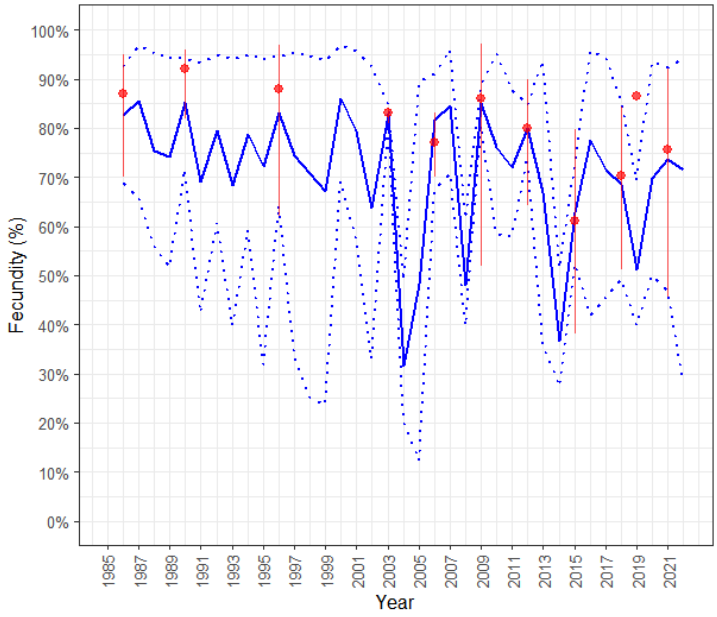

Figure 10. Fecundity in the Bathurst caribou herd 1985-2021 from an IPM in blue with 95% CI as dotted blue lines. Field-based estimates of the proportion of breeding females in June are shown as red dots with associated 95% CIs.

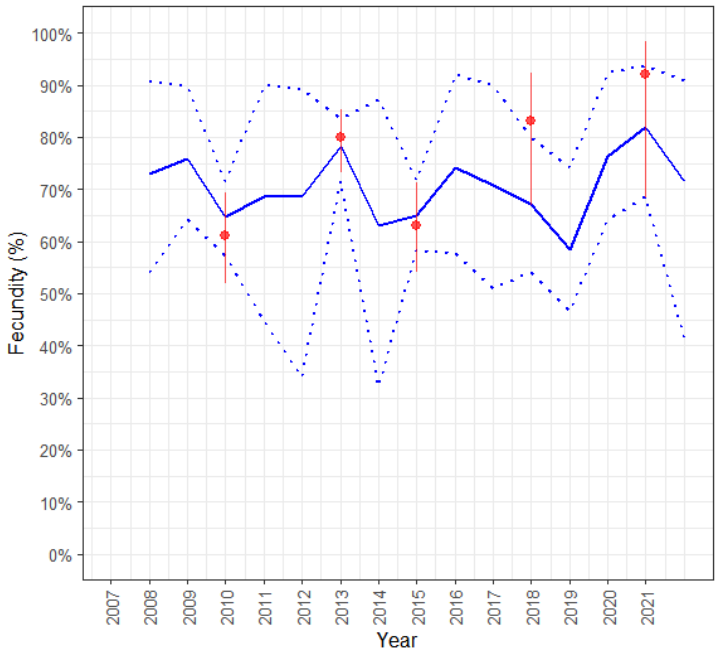

Figure 11. Fecundity in the Bluenose-East caribou herd 2007-2021 from an IPM in blue with 95% CI shown as dotted blue lines. Field-based estimates of the proportion of breeding females in June are shown as red dots with associated 95% CIs.

The models were adjusted to generate likely values for fecal-based pregnancy estimates for both herds. Figures 12 and 13 show the IPM estimates of June proportion of females pregnant and the estimates of fecal sample-based pregnancy rate for the Bathurst and Bluenose-East herds. For the Bathurst herd, the field-based estimate from March 2020 based on 23 of 47 fecal samples pregnant was 0.49 (SE=0.042, CV=0.10, CI=0.30-0.70). The binomial distribution-based CV (0.06) and confidence limits (CI=0.35-0.62) were relatively close to the bootstrap estimate. For the Bluenose-East herd, 42 of 66 samples were of pregnant females resulting in an estimate of 0.66 (SE=0.045, CI=0.55-0.73). The binomial based CI estimate was 0.51-0.74. For both herds the IPM and field estimates were relatively close with overlap of confidence limits of field estimates and model predictions.

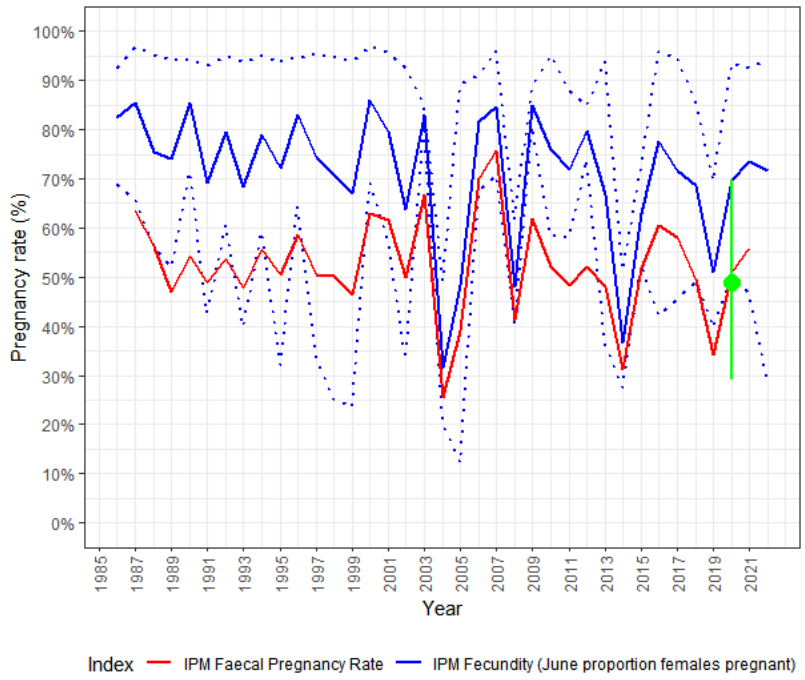

Figure 12. IPM estimates of June pregnancy rate (fecundity) and March fecal based pregnancy rate for Bathurst herd. Estimate from fecal-based pilot study in March 2020 is included in green.

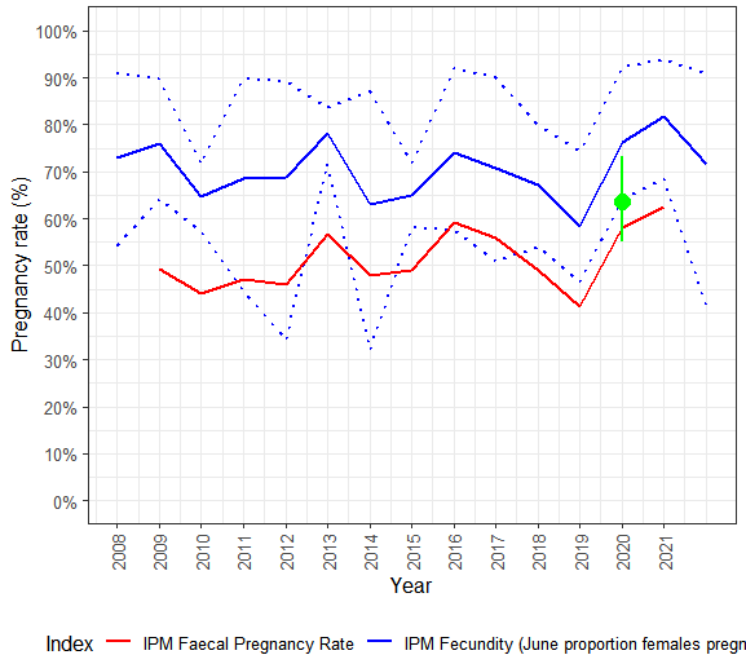

Figure 13. IPM estimates of June pregnancy rate (fecundity) and March fecal based pregnancy rate for Bluenose-East herd. Estimate from fecal samples in March 2020 is included in green.

The June estimates of proportion of breeding females and the model-based estimates of March fecal-based pregnancy parallel each other closely for both herds. The main difference between them is that the March-estimated fecal pregnancy rate is lower because this value includes all females including calves, while the June-estimated percent breeding females includes females at least two years old and females ≥3 years old. The two field-based estimates of fecal-based pregnancy rates for the Bathurst and Bluenose-East herds fit well with the model-based fecal pregnancy rates (with overlapping CIs), which suggests that these values were a reasonable fit.

One interesting trend in the pregnancy and proportion of breeding female estimates is that the difference between the June calving ground and March fecal estimates varies with the difference being minimal in some years. The general reason for this is that if productivity for a given year is low then the relative number of calves in March will also be low and therefore the difference in estimates will be less. This difference will also be affected by productivity in the preceding year (which would then also affect the number of yearlings). Figure 14 below shows how productivity in the year of the survey is related to difference in the two estimates for the Bathurst herd. If productivity is low then the difference is also lower.

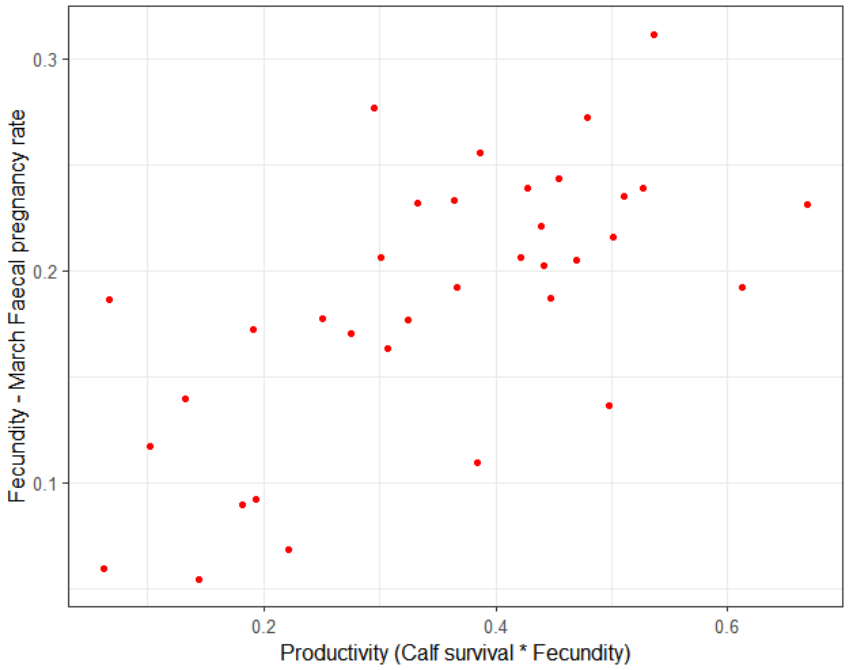

Figure 14. The difference in IPM based estimates of June and March (fecal sample) pregnancy rate as a function of productivity for the Bathurst herd (which will index the relative number of calves during the March survey).

APPENDIX 2. INDIVIDUAL RESULTS FOR FECAL SAMPLES COLLECTED MARCH 2020 BY ENR OR FEBRUARY AND MARCH 2020 BY MSc STUDENT SMITH IN THE NORTH SLAVE REGION OF NWT.

Progest = progesterone; Testo = testosterone; Preg = pregnant P; NP = Not Pregnant. Grey = males; yellow = duplicate, not used; blue = no result from Trent lab (sample contaminated/insufficient); S = small pellets (others considered large); NS = no sample left.

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
ENR1-2020-1	47110	90.7		5.0	F	NP			S
ENR1-2020-2	47111	7.5	1	5.2	M	n/a			
ENR1-2020-3	47112	9.4	1	5.2	M	n/a			S
ENR1-2020-4	47113	13.5	1	5.9	M	n/a	X		
ENR1-2020-5	47114	8.8	1	7.5				X	
ENR1-2020-6	47115	17.3	1	6.6	M	n/a			
ENR1-2020-7	47116	20.5	1	6.9	M	n/a			
ENR1-2020-8	47117	231.5			F	P			
ENR1-2020-9	47118	204.8			F	P			
ENR1-2020-10	47119	279.2			F	P	X		S
ENR1-2020-11	47120	10.1	1	3.6	M	n/a			
ENR1-2020-12	47121	24.0	1	2.7	M	n/a			
ENR1-2020-13	47122	12.6	1	5.9	M	n/a			S
ENR1-2020-14	47123	11.7	1	8.6	M	n/a			
ENR1-2020-15	47124	27.6	1	11.3	M	n/a			
ENR2-2020-1	47125	67.8	1	7.0	F	NP			
ENR2-2020-2	47126	6.0	1	5.2	M	n/a			S
ENR2-2020-3	47127	59.1	1	4.6	F	NP			
ENR2-2020-4	47128	3.1	1	5.9	M	n/a			
ENR2-2020-5	47129	88.2	1	3.0	F	NP			S
ENR2-2020-6	47130	8.7	1	2.6	M	n/a			
ENR2-2020-7	47131	67.8	1	2.5	F	NP			
ENR2-2020-8	47132	133.8			F	P			S
ENR2-2020-9	47133	216.9			F	P			
ENR2-2020-10	47134	263.6						X	
ENR2-2020-11	47135	8.9	1	3.5	M	n/a			
ENR2-2020-12	47136	4.1	1	3.0	M	n/a			
ENR2-2020-13	47137	175.4			F	P			
ENR2-2020-14	47138	113.5			F	P			
ENR2-2020-15	47139	8.2	1	3.3	F	NP			
ENR3-2020-1	47140	6.3	1	4.6	F	NP			

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
ENR3-2020-2	47141	7.6	1	3.9	M	n/a			S
ENR3-2020-3	47142	7.4	1	5.7	M	n/a			
ENR3-2020-4	47143	153.3		5.4				X	S
ENR3-2020-5	47144	5.8	1	3.8	F	NP			
ENR3-2020-6	47145	27.1	1	3.4	M	n/a			NS
ENR3-2020-7	47146	16.1	1	0.9	M	n/a			
ENR3-2020-8	47147	5.9	1	1.7	M	n/a	X		S
ENR3-2020-9	47148	10.9	1	3.3	F	NP			
ENR3-2020-10	47149	8.0	1	4.1	M	n/a			S
ENR3-2020-11	47150	13.8	1	1.9	M	n/a			
ENR3-2020-12	47151	133.1			F	P			
ENR3-2020-13	47152	23.0	1	1.8	M	n/a			
ENR3-2020-14	47153	233.6			F	P			
ENR4-2020-1	47154	7.0	1	5.7	M	n/a			
ENR4-2020-2	47155	89.2	1	6.1	F	NP			
ENR4-2020-3	47156	8.8	1	9.1	M	n/a			S
ENR4-2020-4	47157	6.5	1	6.1	M	n/a			S
ENR4-2020-5	47158	6.8	1	2.7	M	n/a			
ENR4-2020-6	47159	12.4	1	1.3	M	n/a	X		
ENR4-2020-7	47160	16.4	1	3.2	M	n/a			
ENR4-2020-8	47161	326.7			F	P			S
ENR4-2020-9	47162	11.0	1	6.3	M	n/a	X		
ENR4-2020-10	47163	158.3			F	P			S
ENR4-2020-11	47164	14.0	1	5.1	M	n/a	X		
ENR4-2020-12	47165	379.3			F		X		
ENR4-2020-13	47166	9.3	1	2.0	M	n/a			
ENR4-2020-14	47167	39.7	1	3.7	F	NP			
ENR4-2020-15	47168	8.3	1	4.5	M	n/a	X		
ENR5-2020-1	47169	81.0	1	13.0	F	NP			
ENR5-2020-2	47170	50.9	1	4.3	F	NP			
ENR5-2020-3	47171	101.3		3.3	F	P			S
ENR5-2020-4	47172	148.6		4.1	F	P			S
ENR5-2020-7	47173	363.4			F	P			
ENR5-2020-8	47174	91.6	1	5.4	M	n/a			S
ENR5-2020-9	47175	21.0	1	7.5	F	NP			S
ENR5-2020-10	47176	190.5			F	P			S
ENR5-2020-11	47177	306.5			F	P			S
ENR5-2020-12	47178	2.8	1	6.7	M	n/a			
ENR5-2020-13	47179	13.4	1	4.2	M	n/a			S

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
ENR5-2020-14	47180	307.9			F		X		
ENR5-2020-15	47181	94.7	1	5.2	F		X		
ENR6-2020-1	47182	8.9	1	4.3	M	n/a			
ENR6-2020-2	47183	9.6	1	5.4	M	n/a	X		
ENR6-2020-3	47184	5.6	1	4.0	M	n/a			
ENR6-2020-4	47185	89.0	1	5.7	F	NP			
ENR6-2020-5	47186	165.0			F	P			
ENR6-2020-6	47187	204.8			F	P			
ENR6-2020-7	47188	256.2			F	P			S
ENR6-2020-8	47189	7.1	1	5.6	F	NP			S
ENR6-2020-9	47190	20.8	1	7.8	M	n/a			
ENR6-2020-10	47191	7.3	1	3.7	M	n/a			
ENR6-2020-11	47192	250.7			F	P			
ENR6-2020-12	47193	16.5	1	8.0	M	n/a	X		
ENR6-2020-13	47194	736.0			F	P			S
ENR6-2020-14	47195	276.1			F	P			S
ENR7-2020-1	47196	20.7	1	4.6	M	n/a			
ENR7-2020-2	47197	291.4			F	P			
ENR7-2020-3	47198	23.0	1	6.5	F	NP			
ENR7-2020-4	47199	62.4	1	1.8	F	NP			
ENR7-2020-5	47200	80.2	1	4.7	F	NP			
ENR7-2020-6	47201	31.6	1	7.4	M	n/a			
ENR7-2020-7	47202	31.6	1	3.6	F	NP			S
ENR7-2020-8	47203	220.5			F	P			S
ENR7-2020-9	47204	12.0	1	5.4	M	n/a			S
ENR7-2020-10	47205	12.8	1	3.7	F	NP			S
ENR7-2020-11	47206	249.7			F	P			S
ENR7-2020-12	47207	23.2	1	4.0	M	n/a			S
ENR7-2020-13	47208	584.5			F	P			
ENR7-2020-14	47209	331.0			F	P			
ENR7-2020-15	47210	20.9	1	4.5				X	NS
ENR9-2020-1	47211	651.7			F	P			
ENR9-2020-2	47212	175.2						X	
ENR9-2020-3	47213	86.7	1	3.0	F	NP			
ENR9-2020-4	47214	5.2	1	3.2	M	n/a			
ENR9-2020-5	47215	82.2	1	4.0	F	NP			
ENR9-2020-6	47216	246.1			F	P			
ENR9-2020-7	47217	15.7	1	4.5	F	NP			S

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
ENR9-2020-8	47218	557.9			F	P			S
ENR9-2020-9	47219	176.9			F	P			S
ENR9-2020-10	47220	12.5	1	4.2	M	n/a			
ENR9-2020-11	47221	12.2	1	3.3	M	n/a			
ENR9-2020-12	47222	24.3	1	4.7	M	n/a	x		
ENR9-2020-13	47223	10.4	1	4.2	M	n/a			
ENR10-2020-1	47225	122.3			F	P			
ENR10-2020-2	47226	8.2	1	4.5	M	n/a			
ENR10-2020-3	47227	6.8	1	3.3	M	n/a			
ENR10-2020-4	47228	4.8	1	2.4	M	n/a			
ENR10-2020-5	47229	4.9	1	2.8	M	n/a			
ENR10-2020-6	47230	23.6	1	5.2	M	n/a			
ENR10-2020-7	47231	27.6	1	5.0	F	NP			
ENR10-2020-8	47232	174.7			F	P			
ENR10-2020-9	47233	262.4						X	
ENR10-2020-10	47234	126.9			F	P			
ENR10-2020-11	47235	106.9			F	P			
ENR10-2020-12	47236	21.9	1	6.7				X	
ENR10-2020-13	47237	16.7	1	2.7				X	S
ENR10-2020-14	47238	8.0	1	2.8	M	n/a			
ENR10-2020-15	47239	14.7	1	3.2				X	NS
ENR10-2020-16	47240	238.4			F	P			
ENR10-2020-17	47241	286.7			F	P			
ENR10-2020-18	47242	21.6	1	8.0				X	S
ENR10-2020-19	47243	226.0			F	P			
ENR10-2020-20	47244	139.6			F	P			NS
ENR11-2020-1	47245	12.6	1	5.3	M	n/a			S
ENR11-2020-2	47246	206.0			F	P			
ENR11-2020-3	47247	3.1	1	3.5	M	n/a			
ENR11-2020-4	47248	100.9			F	P			
ENR11-2020-5	47249	82.2	1	3.6	F	NP			
ENR11-2020-6	47250	20.8	1	2.8	F	NP			
ENR11-2020-7	47251	5.4	1	1.3	F	NP			
ENR11-2020-8	47252	222.7			F	P			

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
ENR11-2020-9	47253	181.9			F	P			
ENR11-2020-10	47254	25.4	1	6.2	F	NP			S
ENR11-2020-11	47255	222.9			F	P			
ENR11-2020-12	47256	26.2	1	3.2	F	NP			
ENR11-2020-13	47257	133.8			F	P			
ENR11-2020-14	47258	17.5	1	2.8	F	NP			
ENR11-2020-15	47259	17.2	1	3.1				X	S
ENR11-2020-16	47260	132.8			F	P			
ENR11-2020-17	47261	10.7	1	3.3	M	n/a			S
ENR11-2020-18	47262	118.4			F	P			
ENR11-2020-19	47263	29.4	1	4.7	M	n/a			
ENR11-2020-20	47264	212.1			F	P			
ENR12-2020-1	47265	12.5	1	4.2	M	n/a			
ENR12-2020-2	47266	91.2	1	2.1	F	NP			
ENR12-2020-3	47267	51.5	1	2.7	F	NP			
ENR12-2020-4	47268	139.4			F	P			
ENR12-2020-5	47269	181.6			F	P			
ENR12-2020-6	47270	391.2			F		X		
ENR12-2020-7	47271	14.2	1	1.9	F	NP			S
ENR12-2020-8	47272	199.3			F	P			S
ENR12-2020-9	47273	9.1	1	2.8	M	n/a			
ENR12-2020-10	47274	260.0			F	P			
ENR12-2020-11	47275	8.7	1	1.3	F	NP			S
ENR12-2020-12	47276	87.2	1	1.9	F	NP			
ENR12-2020-13	47277	21.3	1	3.8	M	n/a			
ENR12-2020-14	47278	19.2	1	2.1	M	n/a			S
ENR12-2020-15	47279	20.6	1	1.8	F	NP			
ENR12-2020-16	47280	12.0	1	1.8	M	n/a			
ENR12-2020-17	47281	150.5			F	P			
ENR12-2020-18	47282	212.3			F	P			
ENR12-2020-19	47283	273.4			F	P			

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
ENR12-2020-20	47284	9.8	1	2.4	F		X		S
ENR13-2020-1	47285	27.0	1	8.7	F	NP			S
ENR13-2020-2	47286	43.2	1	2.9	F	NP			
ENR13-2020-3	47287	6.0	1	4.1	M	n/a			
ENR13-2020-4	47288	7.4	1	3.1	M	n/a			
ENR13-2020-5	47289	72.5	1	2.5	F	NP			
ENR13-2020-6	47290	85.7	1	3.8	F	NP			
ENR13-2020-7	47291	224.6			F	P			
ENR13-2020-8	47292	253.5			F	P			
ENR13-2020-9	47293	18.2	1	4.1	M	n/a			
ENR13-2020-10	47294	14.8	1	2.6	F	NP			S
ENR13-2020-11	47295	12.9	1	2.6	M	n/a			
ENR13-2020-12	47296	185.4			F	P			
ENR13-2020-13	47297	259.9			F	P			
ENR13-2020-14	47298	236.1			F	P			
ENR13-2020-15	47299	21.6	1	3.3	F	NP			
ENR13-2020-16	47300	192.1			F	P			
ENR13-2020-17	47301	377.4			F	P			
ENR13-2020-18	47302	235.7			F	P			S
ENR13-2020-19	47303	263.0			F	P			
ENR13-2020-20	47304	741.1			F	P			
ENR14-2020-1	47305	105.3			F	P			
ENR14-2020-2	47306	8.6	1	3.0	M	n/a			
ENR14-2020-3	47307	17.5	1	6.4	M	n/a			
ENR14-2020-4	47308	3.6	1	2.2	M	n/a			
ENR14-2020-5	47309	4.6	1	2.1	F	NP			S
ENR14-2020-6	47310	11.5	1	4.6	M	n/a			S
ENR14-2020-7	47311	34.2	1	11.5	M	n/a			
ENR14-2020-8	47312	24.8	1	9.1	M	n/a			
ENR14-2020-9	47313	13.5	1	6.2	M	n/a			
ENR14-2020-10	47314	23.9	1	9.4				X	
ENR14-2020-11	47315	18.6	1	6.7	M	n/a	X		
ENR14-2020-12	47316	16.3	1	5.9	F	NP			S

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
ENR14-2020-13	47317	18.4	1	7.0				X	
ENR14-2020-14	47318	6.1	1	3.3	M	n/a			S
ENR14-2020-15	47319	14.5	1	4.8	M	n/a			
ENR14-2020-16	47320	9.9	1	4.3	M	n/a			
ENR14-2020-17	47321	31.0	1	4.9	M	n/a			S
ENR14-2020-18	47322	220.6			F	P			S
ENR14-2020-19	47323	21.4	1	4.9	F		X		
ENR14-2020-20	47324	297.6			F		X		
ENR15-2020-1	47325	4.0	1	2.5	M	n/a			
ENR15-2020-2	47326	60.6	1	1.4	F	NP			
ENR15-2020-3	47327	151.4						X	
ENR15-2020-4	47328	69.5	1	3.4	F	NP			
ENR15-2020-5	47329	5.4	1	2.8	M	n/a			
ENR15-2020-6	47330	158.5						X	S
ENR15-2020-7	47331	163.1			F	P			S
ENR15-2020-8	47332	314.9			F		X		
ENR15-2020-9	47333	13.3	1	5.8	M	n/a			
ENR15-2020-10	47334	20.4	1	5.3	F	NP			
ENR15-2020-11	47335	10.9	1	3.3	F	NP			S
ENR15-2020-12	47336	12.3	1	2.4	M	n/a			
ENR15-2020-13	47337	370.3			F	P			
ENR15-2020-14	47338	671.8			F	P			S
ENR15-2020-15	47339	257.3			F	P			
ENR15-2020-16	47340	9.4	1	2.3	M	n/a			S
ENR15-2020-17	47341	188.7			F	P			
ENR15-2020-18	47342	15.0	1	5.7	M	n/a			
ENR16-2020-1	47343	58.2	1	3.6	F	NP			
ENR16-2020-2	47344	84.1	1	3.9	F	NP			S
ENR16-2020-3	47345	8.9	1	4.9	F	NP			
ENR16-2020-4	47346	93.8	1	5.3	F	NP			
ENR16-2020-5	47347	30.1	1	2.7	F	NP			
ENR16-2020-6	47348	7.9	1	3.3	F	NP			S

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
ENR16-2020-7	47349	16.4	1	7.3	M	n/a			
ENR16-2020-8	47350	157.3			F	P			
ENR16-2020-9	47351	39.2	1	2.3	M	n/a			
ENR16-2020-10	47352	7.0	1	3.5	F		X		S
ENR16-2020-11	47353	117.4			F	P			
ENR16-2020-12	47354	135.4			F		X		
ENR16-2020-13	47355	6.9	1	3.6	F	NP			S
ENR16-2020-14	47356	64.2	1	4.3	F	NP			
ENR16-2020-15	47357	80.4	1	3.8	F	NP			
ENR16-2020-16	47358	87.1	1	2.8	F		X		S
ENR16-2020-17	47359	85.8	1	2.6	F	NP			
ENR16-2020-18	47360	70.3	1	4.0	F		X		NS
ENR16-2020-19	47361	7.6	1	3.7	M	n/a			
ENR17-2020-1	47362	5.5	1	2.5	M	n/a			
ENR17-2020-2	47363	61.1	1	3.6	F	NP			
ENR17-2020-3	47364	10.6	1	6.6	M	n/a			
ENR17-2020-4	47365	107.0			F	P			
ENR17-2020-5	47366	53.1	1	5.0	F	NP			
ENR17-2020-6	47367	18.9	1	7.1	M	n/a			
ENR17-2020-7	47368	624.4			F	P			
ENR17-2020-8	47369	17.9	1	16.0	M	n/a	X		
ENR17-2020-9	47370	303.4			F	P			
ENR17-2020-10	47371	368.3			F		X		
ENR17-2020-11	47372	213.0			F	P			
ENR17-2020-12	47373	366.4			F	P			S
ENR17-2020-13	47374	408.9			F	P			S
ENR17-2020-14	47375	23.5	1	8.1	M	n/a			
ENR17-2020-15	47376	297.4			F	P			
ENR17-2020-16	47377	731.2			F	P			
ENR17-2020-17	47378	48.7	1	13.0	M	n/a			
ENR17-2020-18	47379	27.5	1	10.9	M	n/a			S
ENR17-2020-19	47380	23.0	1	12.0	M	n/a			

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
ENR17-2020-20	47381	579.2			F		X		
ENR18-2020-1	47382	10.7	1	4.2	F		X		
ENR18-2020-2	47383	137.6			F	P			S
ENR18-2020-3	47384	136.7			F	P			
ENR18-2020-4	47385	255.6			F	P			NS
ENR18-2020-5	47386	96.3	1	5.0	F	NP			
ENR18-2020-6	47387	320.0			F	P			
ENR18-2020-7	47388	271.1			F	P			
ENR18-2020-8	47389	22.9	1	7.4	F	NP			S
ENR18-2020-9	47390	31.8	1	10.0	M	n/a			
ENR18-2020-10	47391	567.0			F	P			
ENR18-2020-11	47392	312.2			F	P			
ENR18-2020-12	47393	35.3	1	10.9	F	NP			
ENR18-2020-13	47394	15.5	1	6.6	M	n/a			
ENR18-2020-14	47395	27.6	1	10.8	M	n/a			
ENR18-2020-15	47396	408.0			F	P			
ENR18-2020-16	47397	32.1	1	13.7	F	NP			
ENR18-2020-17	47398	431.7			F	P			
ENR18-2020-18	47399	18.5	1	8.3	M	n/a	X		
ENR18-2020-19	47400	197.5			F	P			
ENR18-2020-20	47401	331.7			F	P			
ENR19-2020-1	47402	233.4			F	P			
ENR19-2020-2	47403	40.7	1	9.0	M	n/a			
ENR19-2020-3	47404	10.4	1	4.2	M	n/a			
ENR19-2020-4	47405	469.8			F	P			
ENR19-2020-5	47406	335.6			F		X		
ENR19-2020-6	47407	120.3			F	P			
ENR19-2020-7	47408	367.5			F	P			
ENR19-2020-8	47409	536.6			F		X		
ENR19-2020-9	47410	516.4			F	P			S
ENR19-2020-10	47411	105.4						X	
ENR19-2020-11	47412	372.5			F	P			
ENR19-2020-12	47413	245.1						X	

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
ENR19-2020-13	47414	21.7	1	12.3	M	n/a			S
ENR19-2020-14	47415	454.3			F	P			
ENR19-2020-15	47416	611.0			F	P			S
ENR19-2020-16	47417	296.8			F	P			
ENR19-2020-17	47418	347.7			F		X		S
ENR19-2020-18	47419	18.0	1	8.8	M	n/a			
ENR19-2020-19	47420	32.9	1	14.0	F	NP			
ENR19-2020-20	47421	423.7			F		X		
ENR20-2020-1	47422	96.5	1	8.3	F	NP			
ENR20-2020-2	47423	188.1			F	P			
ENR20-2020-3	47424	97.0	1	8.4	F	NP			
ENR20-2020-4	47425	162.6			F	P			
ENR20-2020-5	47426	111.3			F	P			
ENR20-2020-6	47427	423.9			F	P			
ENR20-2020-7	47428	233.4			F	P			S
ENR20-2020-8	47429	136.6			F	P			
ENR20-2020-9	47430	204.1			F	P			
ENR20-2020-10	47431	177.9			F	P			NS
ENR20-2020-11	47432	19.3	1	10.9	F	NP			
ENR20-2020-12	47433	68.4	1	23.7	M	n/a			
ENR20-2020-13	47434	199.5						X	NS
ENR20-2020-14	47435	49.0	1	22.5	F	NP			
ENR20-2020-15	47436	18.1	1	11.8	M	n/a			
ENR20-2020-16	47437	37.9	1	18.0	M	n/a			S
ENR20-2020-17	47438	67.1	1	7.7	F	NP			
ENR20-2020-18	47439	159.2			F	P			
ENR20-2020-19	47440	73.1	1	8.3	F	NP			
ENR20-2020-20	47441	26.7	1	14.3	F	NP			
78-1-1	47442	10.0	1	5.9	M	n/a			
78-1-2	47443	30.5	1	6.5	M	n/a			
78-1-3	47444	22.0	1	5.8	M	n/a			

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
78-1-4	47445	6.7	1	8.6	M	n/a	X		
78-1-5	47446	1.3	1	5.0	M	n/a			
79-1-1		2.5	1	8.0					
79-1-2		2.4	1	9.7					
79-1-3	47447	0.9	1	4.2	M	n/a			
79-1-4	47448	1.6	1	4.0	M	n/a			
79-1-5	47449	4.7	1	4.6	M	n/a			
80-1-1	47450	5.0	1	5.4	M	n/a			
80-1-2	47451	0.0	1	5.9	M	n/a	X		
80-1-3	47452	0.0	1	5.9	M	n/a			
80-1-4	47453	87.3	1	9.3	F	NP			
80-1-5	47454	1.6	1	5.2	M	n/a			
85-1-1	47455	1.6	1	5.6	M	n/a			
85-1-2	47456	4.0	1	4.5	M	n/a			
85-1-3	47457	5.5	1	3.7	M	n/a			
85-1-4	47458	0.0	1	5.2	M	n/a			
85-1-5	47459	17.2	1	5.7	M	n/a			
86-1-1	47460	5.1	1	5.2	M	n/a			
86-1-2	47461	4.4	1	6.2	M	n/a			
86-1-3	47462	0.6	1	4.8	M	n/a			
86-1-4	47463	10.8	1	5.6	M	n/a			
86-1-5	47464	5.5	1	4.1	M	n/a			
88-1-1		3.5	1	6.0					
88-1-2	47465	1.7	1	3.4	M	n/a			
88-1-3		1.4	1	5.0					
88-1-4	47466	95.2	1	6.9	F	NP			
88-1-5	47467	54.1	1	4.7	F	NP			
89-1-1	47468	45.1	1	4.5	F	NP			
89-1-2		5.2	1	3.8					
89-1-3	47469	5.1	1	4.1	M	n/a			
89-1-4	47470	7.4	1	4.6	M	n/a	X		
89-1-5	47471	105.2	0	7.1	F	P			
90-1-1	47472	5.3	1	4.2	M	n/a			
90-1-2	47473	2.3	1	4.1	M	n/a			
90-1-3	47474	0.4	1	4.3	M	n/a	X		
90-1-4		0.0	1	2.6					
90-1-5	47475	1.5	1	4.6	M	n/a			
92-1-1	47476	5.8	1	6.3	M	n/a			
92-1-2	47477	11.7	1	3.9				X	
92-1-3		6.3	1	4.9					
92-1-4		7.1	1	4.9					

ENR Sample Number	Trent lab ID Number	Progest ng/g feces	Progest <100 ng/g	Testo ng/g feces	Sex	Preg P or NP	Duplicate not used	Trent No Result	Small/Large Pellets
92-1-5		5.5	1	4.7					
93-1-1	47478	4.2	1	3.5	M	n/a			
93-1-2	47479	7.3	1	5.5	M	n/a			
93-1-3	47480	10.1	1	5.3	M	n/a			
93-1-4	47481	5.1	1	3.8	M	n/a			
93-1-5	47482	9.4	1	7.2	M	n/a			
94-1-1	47483	0.5	1	4.6	M	n/a	X		
94-1-2	47484	5.2	1	5.0	M	n/a			
94-1-3	47485	3.0	1	5.5				X	
94-1-4	47486	3.1	1	3.8	M	n/a			
94-1-5	47487	5.1	1	5.5	M	n/a			