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Monitoring sheep and reindeer consumption by brown bears using molecular methods

Alexander Kopatz, Marie Davey, Frode Fossøy, Kristin Forfang, Line Birkeland Eriksen, Øystein Flagstad, Oddmund Kleven

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Alexander Kopatz
Marie Davey
Frode Fossøy
Kristin Forfang
Line Birkeland Eriksen
Øystein Flagstad
Oddmund Kleven

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Female brown bear with its yearling © Alexander Kopatz

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CONTACT DETAILS

NINA head office
P.O.Box 5685 Torgarden
NO-7485 Trondheim
Norway
P: +47 73 80 14 00

NINA Oslo
Sognsveien 68
0855 Oslo
Norway
P: +47 73 80 14 00

NINA Tromsø
P.O.Box 6606 Langnes
NO-9296 Tromsø
Norway
P: +47 77 75 04 00

NINA Lillehammer
Vormstuguvegen 40
NO-2624 Lillehammer
Norway
P: +47 73 80 14 00

NINA Bergen:
Thormøhlens gate 55
NO-5006 Bergen.
Norway
P: +47 73 80 14 00

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Abstract

Kopatz, A., Davey, M., Fossøy, F., Forfang, K., Eriksen, L.B., Flagstad, Ø. & Kleven, O. 2023. Monitoring sheep and reindeer consumption by brown bears using molecular methods. NINA Report 2276. Norwegian Institute for Nature Research.

Large carnivores, such as brown bears (*Ursus arctos*), are often involved in conflict cases in areas where humans keep their livestock. Livestock depredation conflicts in Norway caused by brown bears are largely related to domestic sheep (*Ovis aries*) and semi-domestic reindeer (*Rangifer tarandus*). Data on which individual brown bears have consumed sheep or reindeer in a given area during a specific time period is useful for supporting predator management and creating a better-informed basis for decision making. For example, depredation events are unlikely to be directly witnessed, and information on brown bear diet and possible consumption of livestock could prove crucial to identifying which individual brown bears may be involved. Droplet digital PCR (ddPCR) is a DNA-based method with proven applications for detecting and quantifying diet items in faeces from a variety of animals and has the capacity to provide this type of information to the predator management. In this pilot study, we successfully developed and tested two ddPCR-based assays, one for sheep and one for reindeer, that allow us to detect and quantify their consumption by brown bears by analysing DNA from collected faecal samples.

To test the potential for integrating such an approach with existing population monitoring, we assessed the sheep and reindeer consumption from brown bear faeces collected in Trøndelag county in 2022 as part of the Norwegian Large Predator Monitoring Program. In this program biological samples, like faeces, are regularly collected across Norway and often in the context of depredation events and are subsequently genotyped to link the samples to individual brown bears. We assessed sheep and reindeer consumption by screening 124 faecal samples collected by the program in 2022 using the sheep and reindeer ddPCR assays we developed. Sheep or reindeer were detected in 34 samples (27%). Eight of these were registered to be linked to depredation events in Rovbase, indicating the ddPCR assays can provide information on consumption events that may otherwise go unnoticed and provide both spatial and temporal overview for this consumption. We stress that consumption does not universally indicate a predation event, as brown bears are omnivores that can both predate on livestock or scavenge on existing carcasses. By analysing faecal samples from the Norwegian Large Predator Monitoring Program, we were able to link diet data to at least 18 different individual brown bears (ten males, eight females), thereof at least 12 consumed sheep or reindeer at some point during the monitoring season. Nine of the 34 samples that were positive for sheep or reindeer could not be linked to a specific brown bear individual. Reindeer consumption seemed to occur primarily during May, while sheep consumption occurred in July, August and November. Female bears appeared to consume reindeer more frequently than male bears. However, these results must be interpreted with caution, due to the low numbers of positive samples, potential sampling bias, and uncertainty surrounding the relationship between defecation date and faeces collection date.

The results of this pilot project highlight the potential to use samples from the Norwegian Large Predator Monitoring Program to elucidate the extent and frequency that brown bears feed on livestock, and assess spatial-, temporal-, and sex-related patterns in consumption. This can provide a better understanding of predator ecology and depredation risks and could help to inform management decisions. We anticipate ddPCR diet analysis of faecal samples could be a valuable component in rapid analyses, particularly in ambiguous conflict cases, not only by confirming consumption by a specific individual, but by potentially providing clues to time since consumption. The ddPCR assays developed here have the potential to be applied to other sample types and to samples from other large carnivore species, such as wolf and wolverine.

Alexander Kopatz, Norwegian Institute for Nature Research (NINA), alexander.kopatz@nina.no
Marie Davey, Norwegian Institute for Nature Research (NINA), marie.davey@nina.no
Frode Fossøy, Norwegian Institute for Nature Research (NINA), frode.fossoy@nina.no

Kristin Forfang, Norwegian Institute for Nature Research (NINA), kristin.forfang@nina.no
Line Birkeland Eriksen, Norwegian Institute for Nature Research (NINA), line.eriksen@nina.no
Øystein Flagstad, Norwegian Institute for Nature Research (NINA), oystein.flagstad@nina.no
Oddmund Kleven, Norwegian Institute for Nature Research (NINA), oddmund.kleven@nina.no

Sammendrag

Kopatz, A., Davey, M., Fossøy, F., Forfang, K., Eriksen, L.B., Flagstad, Ø. & Kleven, O. 2023. Overvåking av sau og tamrein som del av dietten til brunbjørn ved hjelp av molekylære metoder. NINA Rapport 2276. Norsk institutt for naturforskning.

Store rovdyr, som for eksempel brunbjørn (*Ursus arctos*), er ofte involvert i konflikter med mennesker og beitedyr. Slike konflikter forårsaket av bjørn i Norge er som oftest relatert til sau (*Ovis aries*) og tamrein (*Rangifer tarandus*). Informasjon om hvilke bjørner som har spist sau eller tamrein i et gitt område for et gitt tidspunkt kan være nyttig for å gi et bedre grunnlag for beslutninger innen rovdyrforvaltningen. For eksempel er det sjelden slike angrep på sau bevitnes, og kunnskap om diett og eventuelle beitedyr i dietten hos enkeltindivider av bjørn kan gi viktig informasjon om hvilke dyr som har vært involvert. Digital PCR (ddPCR) er en DNA-basert metode som tidligere har vært brukt for å påvise og kvantifisere diett i skittprøver fra ulike dyrearter, og som kan være et mulig verktøy for rovdyrforvaltningen. I dette pilotstudiet har vi utviklet og testet ddPCR-assay for sau og rein, som gjør det mulig å påvise og kvantifisere nærvær av disse artene i skittprøver fra brunbjørn.

For å teste potensialet til å integrere en slik metode med eksisterende bestandsovervåking, analyserte vi forekomst av sau og rein i skittprøver fra brunbjørn innsamlet i Trøndelag som del av den nasjonale overvåkingen av store rovdyr. Dette overvåkingsprogrammet samler inn biologiske prøver, som skittprøver, fra hele Norge, og ofte i sammenheng med rovdyrkonflikter. Skittprøvene blir i utgangspunktet brukt til å identifisere hvilket individ som har stått bak angrepet. Vi analyserte forekomst av sau og tamrein ved hjelp av de nye assayene i 124 skittprøver samlet inn i 2022. Sau og/eller rein ble påvist i 34 (27%) av prøvene. Kun åtte av disse var registrerte som del av en kjent konflikt i Rovbase, noe som viser at ddPCR-analyser kan gi viktig informasjon på konsum av beitedyr i tid og rom som ellers ikke ville ha vært kjent. Vi vil understreke at konsum av beitedyr ikke nødvendigvis er koblet til et angrep på sau eller rein, siden brunbjørn er altetende og konsum kan være knyttet til åtselseting av døde dyr. Ved å analysere skittprøver innsamlet som del av det nasjonale overvåkingsprogrammet kunne vi knytte diettdata til minst 18 forskjellige brunbjørner (10 hanner og 8 hunner), hvor 12 bjørner hadde spist sau eller rein i løpet av 2022. Ni av de 34 prøvene som påviste sau eller rein kunne ikke knyttes til en individuell bjørn. Konsum av rein foregikk hovedsakelig i mai, mens konsum av sau ble påvist i juli, august og november. Hunnbjørner spiste rein oftere enn hannbjørner. Disse resultatene må tolkes forsiktig, da det er få positive prøver, mulig sampling bias og usikkerhet rundt hvor ferske skittprøvene var ved innsamlingstidspunktet.

Resultatene fra dette pilotprosjektet viser potensialet for å bruke skittprøvene samlet inn som del av det nasjonale overvåkingsprogrammet for store rovdyr til å belyse omfanget og frekvensen av beitedyrkonsum og vurdere effekter av tidspunkt, geografi og kjønnsfordeling. Dette kan øke vår forståelse av risikoen for angrep og bidra til den generelle kunnskapen knyttet til rovdyrøkologi, som kan være et viktig bidrag til rovdyrforvaltningen. Vi tenker at ddPCR analyser av skittprøver kan bli en verdifull del av en rask analyse, spesielt i vanskelige konfliktsaker, ikke bare for å bekrefte hvilke bjørner som har spist beitedyr, men også potensielt angi tid siden konsum. Den utviklede ddPCR-metoden vil også kunne brukes på andre prøvetyper som for eksempel mageinnhold, eller på prøver fra andre store rovdyr som ulv (*Canis lupus*) og jerv (*Gulo gulo*).

Alexander Kopatz, Norsk institutt for naturforskning (NINA), alexander.kopatz@nina.no
Marie Davey, Norsk institutt for naturforskning (NINA), marie.davey@nina.no
Frode Fossøy, Norsk institutt for naturforskning (NINA), frode.fossoy@nina.no
Kristin Forfang, Norsk institutt for naturforskning (NINA), kristin.forfang@nina.no
Line Birkeland Eriksen, Norsk institutt for naturforskning (NINA), line.eriksen@nina.no
Øystein Flagstad, Norsk institutt for naturforskning (NINA), oystein.flagstad@nina.no
Oddmund Kleven, Norsk institutt for naturforskning (NINA), oddmund.kleven@nina.no

Contents

Foreword

Animals must consume food for energy, growth and to maintain their health. Large carnivores generally feed on animal prey, although the brown bear in particular has an omnivore diet, feeding not only on animals but also plants and insects according to their seasonal availability throughout the year. In Norway, faeces samples represent a large part of the samples analysed annually in the national DNA-based monitoring program that assess the number and sex of individual predators, such as wolverine, wolves, and brown bears. This faeces material contains both the DNA of the individual, but also the DNA of the food items the animal has recently consumed before defecation. To date, the DNA of prey items has been largely left uninvestigated, despite the fact it has the potential to catalogue the diet of an individual predator across seasons, years, areas and species.

Human-wildlife conflicts often arise when predators feed on livestock. In Norway, this involves mainly predation on domestic sheep and semi-domestic reindeer. Assessing whether livestock DNA can be found within faeces samples could provide management authorities and also the wider public with additional, valuable information on the extent of livestock consumption. This could thereby allow further assessments of predation rates and comparison to livestock losses. We therefore conducted this pilot study to develop a molecular-based method to detect but also quantify the amount of sheep and reindeer in brown bear faeces collected during the Norwegian National Predator Monitoring Program for brown bears in Trøndelag. We analysed faeces collected across one whole monitoring season in order to evaluate this approach under applied, realistic, and comprehensive population monitoring conditions in Norway. If successful, such a “diet-analysis-module” would be straightforward to implement into the established monitoring routine.

We evaluated a relatively new molecular tool called droplet digital PCR (ddPCR), and successfully implemented it. However, this proof-of-concept pilot project was not without challenges. We discovered that the existing, publicly available and already published species-specific genetic markers to detect reindeer were not suitable for ddPCR analyses. We therefore designed new primer-probe sets to provide reliable detection and quantification of reindeer. Documentation of this comprehensive development process and testing and validation of our assays are presented here in the appendix. The results of our pilot study highlight the potential to use the existing DNA-based brown bear monitoring program to extend our knowledge of large predator diets and elucidate the extent of livestock and other species consumption. This approach could also be applied to other large carnivore species such as in the monitoring of wolves and wolverines.

Trondheim, April 2023

Alexander Kopatz

1 Introduction

The presence of large carnivores in areas with animal husbandry can pose a risk for conflicts with humans like livestock depredation. Responsible authorities, such as the large predator management must routinely make well informed and effective management decisions such as to compensate affected owners of killed or injured livestock, or to translocate or remove damage-causing individuals. However, identifying the individual predator responsible for a conflict may be challenging without direct observation and when multiple individuals can be present near the area and time of the incident. Therefore, detecting and quantifying livestock consumption among the routinely collected faecal samples used for Norway's DNA-based predator monitoring program, e.g., for brown bears (*Ursus arctos*), could potentially provide the management with important information (see e.g., Waits and Paetkau 2005), especially during the crucial time of spring and summer.

Brown bear livestock depredation conflicts in Norway are largely related to domestic sheep (*Ovis aries*) and semi-domestic reindeer (*Rangifer tarandus*), which are subject to attacks especially during spring and summer when alternative food resources may not be available (Dahle et al. 1998). However, the brown bear is an omnivore species feeding widely on animals, insects and plants, and as such its diet changes seasonally with the availability of different food resources (Stenset et al. 2016). Detecting and quantifying sheep and reindeer consumption by brown bears would provide important information on the effect bears have on livestock depredation and with that would extend the basis of decision-making for their management. Also, identifying specific diet items also increases our understanding of large carnivore's feeding ecology (Elfström et al. 2014, Fortin et al. 2013, Klare et al. 2011).

All living organisms contain DNA which constitutes a unique genetic code for that organism. We take advantage of it in the DNA-based monitoring to identify both the target species, individual and sex from its biological samples left behind, e.g., faeces, hairs, or tissue from dead recoveries (see e.g., Brøseth et al. 2023). When an animal consumes its diet, the DNA in these plants, insects, or animals is rarely completely broken down during the digestive process. As such, carnivore faeces contain high amounts of DNA from the animals they have consumed. Molecular methods can be used both to identify from which organisms this DNA comes from, as well as to estimate how much of that organism was recently consumed. Detecting prey in large carnivore faeces using DNA-based techniques has proven valuable for identifying food items (see e.g., Latham et al. 2013, Schwartz et al. 2014, McPherson et al. 2015, Morin et al. 2019). Over the last decade several molecular approaches and methods have been developed (see e.g., Valentini et al. 2009, De Barba et al. 2014 and previous references), with few having the capacity to absolutely quantify the amount of prey species DNA in a sample. Furthermore, these methods are rarely tested in wildlife populations and under realistic monitoring conditions as well as at larger geographical scales (Lamb et al. 2019).

Droplet digital PCR (ddPCR) is a relatively new method for detecting and quantifying specific DNA targets (**Figure 1**). The technique effectively counts individual DNA molecules of a specific type in a sample (Hindson et al. 2011). In ddPCR, DNA is first isolated from sample material (for example faeces), after which it is fractionated into approximately 20,000 droplets of one nanolitre (nL) in volume and each containing DNA fragments from the original sample. A fluorescent detector that binds to a specific DNA sequence is then used in a polymerase chain reaction (PCR) to cause droplets containing DNA from the target organism to fluoresce. The ddPCR machine then screens each droplet, recording it as positive or negative based on this fluorescence. By counting the number of positive droplets, it is then possible to estimate the concentration of target DNA (see **Figure 1**). The method has been tested for detecting rare and cryptic diet items in rodents and performs well even when diet items are present at trace amounts that cannot be detected by microscopy-based methods (Groen et al. 2022). This technique has the advantage of allowing both the calculation of detection rates and quantitative comparisons between individual samples across the landscape and sampling times.

The objective of this pilot study was to develop a ddPCR method to detect and quantify domestic sheep *Ovis aries* and semi-domestic reindeer *Rangifer tarandus* DNA from faeces samples collected during the national monitoring of brown bears in Trøndelag, Norway, in 2022. After being genotyped for individual identification (Brøseth et al. 2023), we used the same sample material to conduct ddPCR analyses assessing the content for the two target species: sheep and reindeer.

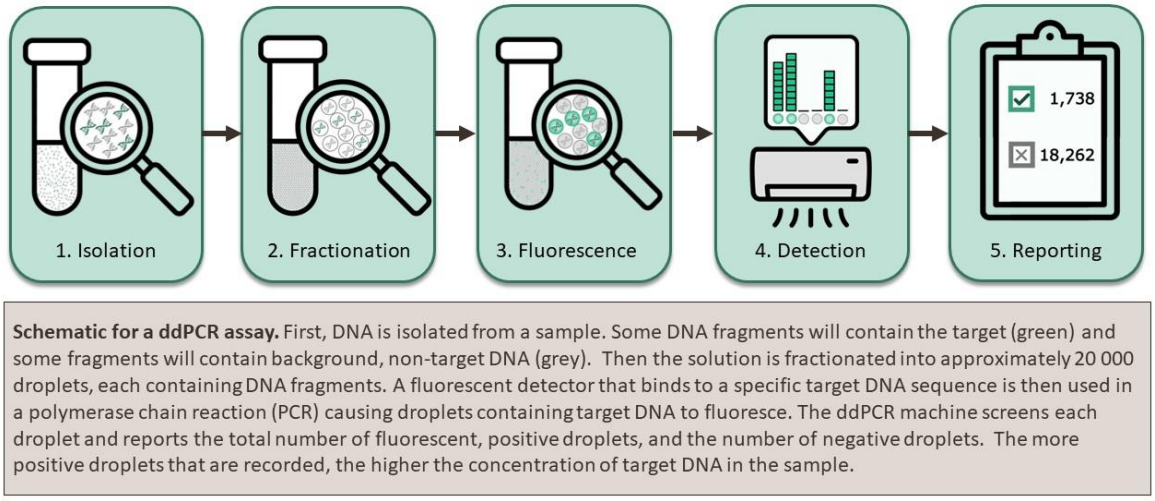
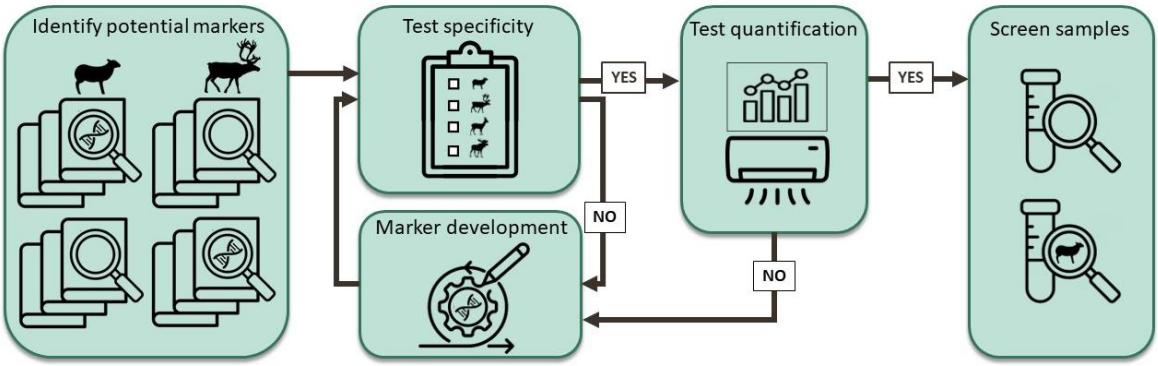


***Figure 1**. A schematic diagram showing the different steps in the DNA-based droplet digital PCR (ddPCR) assay we used to analyse faeces for sheep and reindeer consumption in Trøndelag in 2022. The assay detects and quantifies the amount of DNA from a specific target organism in a sample. The ddPCR assay can be run simultaneously on 48 or 96 samples, making it scalable for the analyses of single to large amounts of samples.*

2 Material and methods

2.1 Marker selection, development and validation

In order to detect and quantify sheep and reindeer DNA in brown bear faeces, we developed ddPCR based assays for each of these prey items according to the workflow described in **Figure 2**. A full description of the assay development process and results can be found in the appendix (Appendix, section 7.1 Marker selection, development and validation) and the primer combinations investigated are listed in **Table 1**. Briefly, we initially screened existing scientific literature for potential markers for these prey items and then tested two of these markers (**Table 1**): ovisPDE for sheep (Laube et al. 2007) and reinRC for reindeer (Shim et al. 2011) against a panel of tissue samples from potential brown bear prey items to ensure the marker would not yield false positives through non-specific detection of other prey items. When the first reindeer marker tested was found to be specific, but not quantitative, we developed two new markers and then tested their specificity against the same prey-item panel. Analysis with ddPCR was then conducted and tested using the sheep-specific and reindeer-specific markers against control-samples (faeces) with added known amounts of DNA from these prey items. This allowed us to confirm whether the markers could measure successfully how much sheep or reindeer DNA was present in a sample. The sheep primer ovisPDE and reindeer primer reinCOI were found to be species-specific and quantitative (**Appendix Figure A2**) and were further used to screen the faeces samples collected in Trøndelag in 2022 (see **Table 2** and **Appendix Table A1**). The reinCOI marker for reindeer was very sensitive, detecting reindeer when it was present in as little amounts as 0.001 ng/uL, while the ovisPDE marker for sheep was somewhat less sensitive, detecting as little as 0.01 ng/uL sheep DNA. These amounts are more than sufficient to detect the target species in a sample.



Workflow for developing prey assays. Potential markers for specific prey items (sheep, reindeer) were identified by scanning existing scientific literature. These markers were then tested against a panel of potential brown bear prey items*. When existing markers failed to discriminate between potential prey items in the brown bear diet, new markers were developed and again tested against the prey item panel. Markers that succesfully distinguished specific prey items were then used for screening and quantifying their presence among 124 brown bear faecal samples from Trøndelag county.

* Prey test panel comprised of: badger, brown bear, capercaillie, moose, red deer, reindeer, roe deer, sheep, wild boar, willow ptarmigan

***Figure 2**. Workflow diagram showing the testing and validation process used to develop droplet digital PCR (ddPCR) assays for detecting and quantifying sheep (Ovis aries) and reindeer (Rangifer tarandus) consumption from faecal samples collected during the national brown bear monitoring in Trøndelag in 2022.*

***Table 1**. Primer information for the sheep and reindeer markers tested and developed for diet assays to quantify sheep and reindeer consumption from faeces.*

Marker Name	Target	F Primer	R Primer	Probe	Reference
ovisPDE	Sheep	ACCCGTCAAGCAGACTCTAACG	TAAATATTTCAGCTAAGGAAAAAAAAGAAG	CAGGATTTTTGCCGCATTCGCTT	Laube et al. 2007
reinRC	Reindeer	CGTACATATATGGTCCTGTAC	GTACTATGTACTGTAAATAATGTC	CCCCATGCTTATAAGCAAGT	Shim et al. 2011
reinCytB187	Reindeer	TCACATCTGTCGAGACGTCAATT	TGCTCCGTTGGCATGTATGTA	TGGCTGAATCATCCG	*This study*
reinCytB189	Reindeer	ACTCACATCTGTCGAGACGTCAA	TGCTCCGTTGGCATGTATGTA	TGGCTGAATCATCCG	*This study*
reinCOI	Reindeer	CTGGAGCAGGAACAGGTTGAA	GCTCCTGCGTGAGCTAGGTT	TGTTTACCCTCCTTTAGCTGG	*This study*

2.2 Determining sheep and reindeer consumption in Trøndelag brown bears

In 2022, a total of 225 biological samples were collected during the national monitoring of brown bears in Trøndelag. Of these, 141 were registered as faeces samples, collected between April and November and 128 were successfully genotyped for individual identification for the national monitoring (see Rovbase and Brøseth et al. 2023). We used 124 of these faecal samples to screen for sheep and reindeer consumption. DNA was isolated from 0.037 to 1.662 g of faecal material from each sample using a FastDNA Spin Kit for Soil (MP Biomedicals, USA). Faecal material was weighed and placed in a 50 mL tube containing Lysing Matrix E. Sodium phosphate buffer (9.8 mL) and MT buffer (1.2 mL) were then added to the sample, which was subsequently homogenized on a FastPrep 96 instrument (MP Biomedicals) for 60 s at 1600 rpm. Samples were then centrifuged at 11,500 rpm for 10 minutes and 500 µL of supernatant was transferred to a 2 mL tube. DNA was then isolated according to the manufacturer's specifications using the FastDNA Soil Kit for 2 mL, and DNA was eluted in 200 µL AE-buffer (Qiagen, Germany). Extracted DNA was then used in ddPCR assays for sheep and reindeer using the ovisPDE and reinCOI marker-probe systems, respectively (**Table 1**). Assays were run on a Biorad QX600™ ddPCR system using ddPCR Supermix for probes (no dUTPs) from Biorad with final primer concentrations of 0.9 µM and final probe concentrations of 0.25 µM. A minimum of three positive droplets were required to consider a sample as testing positive for sheep or reindeer. The number of positive droplets was used to determine the number of DNA copies per volume of DNA extract, which was then converted into DNA copies per gram dried faeces.

2.3 Determining individual composition and temporal trends

To examine spatial, temporal, and sex related trends in sheep and reindeer consumption, prey consumption results from the Trøndelag brown bears were then merged with individual location, identity and sex data generated prior to this study from the same fecal samples as part of the DNA-based monitoring program for brown bears in Norway. Genotyping was conducted using PCR and STR analysis on a 3500xL Genetic Analyzer instrument (Applied Biosystems) and a detailed description of the genotyping and individual identification process is available in Brøseth et al. (2023). Among the 124 faecal samples that underwent diet screening, a total of 18 individual brown bears were identified: eight females and ten males (Brøseth et al. 2023, **Tables 2** and

A1). Two of the samples analysed were collected near active den sites. While these results are reported in the summary tables and figures, the denning locations are protected and are therefore not included on any of the maps.

***Table 2**. Overview of the analysed faeces samples in this study, collected during the national brown bear monitoring in Trøndelag in 2022. Individual identification* and sex* is based on the results of the Norwegian national brown bear monitoring, published in the report by Brøseth et al. (2023).*

Sex*	**Number of samples analysed**	**Number of individuals identified***	**Number of samples positive for sheep** (ovisPDE)	**Number of samples positive for reindeer** (reinCOI)
Male	25	10	3	6
Female	29	8	1	15
Unknown ID	70	-	2	7
Total	124	18	6	28

3 Results

3.1 Sheep and reindeer consumption in Trøndelag brown bears

Reindeer and sheep consumption occurred at low frequencies in the analysed faeces and the quantity of DNA detected varied up to 10-fold in sheep and up to 10,000-fold in reindeer. Of the 124 faecal samples from the national brown bear monitoring analysed from Trøndelag in 2022, 28 (22.5%) contained reindeer DNA and six (4.8%) contained sheep DNA. Neither sheep nor reindeer were detected in the remaining 90 (72.7%) faecal samples. Faecal samples containing reindeer were mainly collected in the north-eastern part of Trøndelag during May, while faeces samples containing sheep were collected mostly near the European Route E6 and across the brown bear active season (**Tables 2** and **3**, **Figure 3**, **Appendix Table A1**).

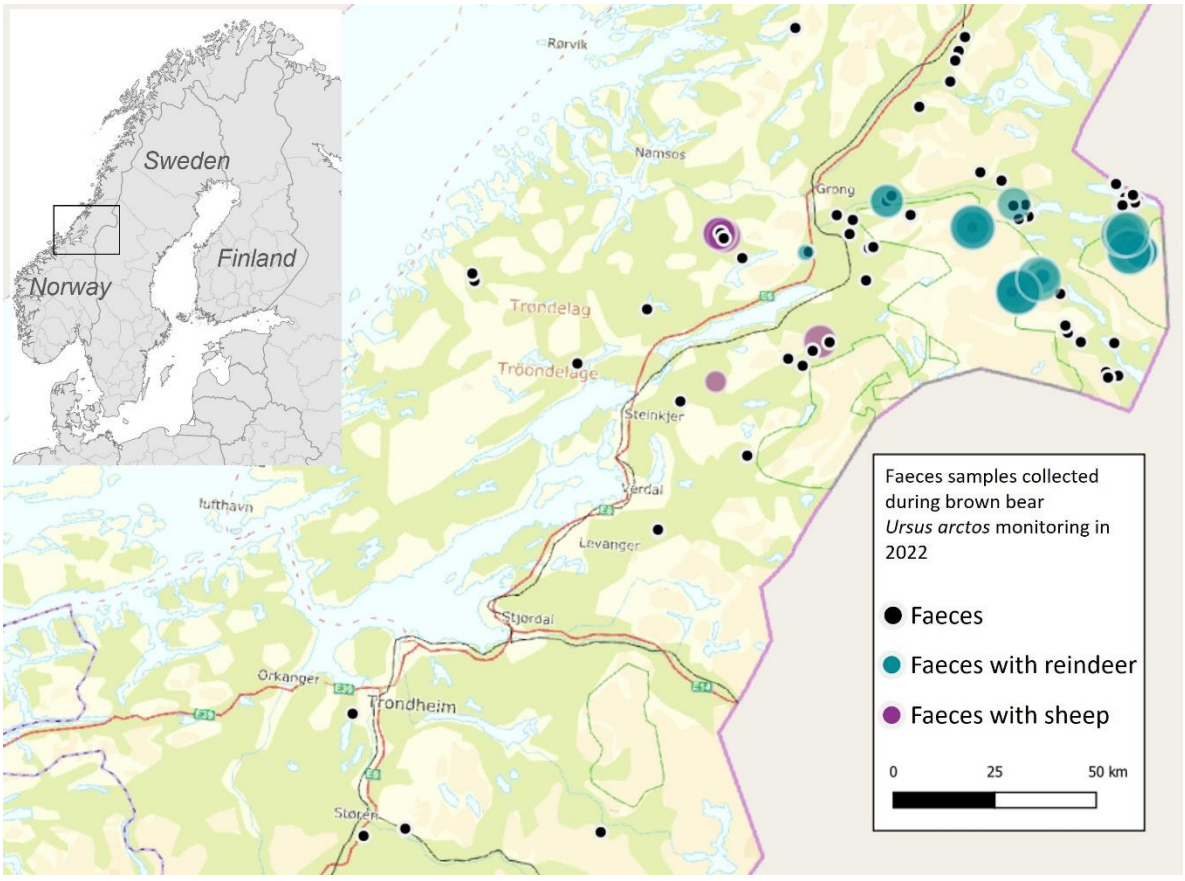


***Figure 3**. Locations of the faeces samples collected in 2022 in Trøndelag, Norway, during brown bear monitoring and analysed (N=124) for sheep and reindeer consumption in this pilot study (N=122 locations are show as two samples collected near an active den site are not shown, as denning locations are protected). Faecal samples containing reindeer (blue) and sheep (purple) are sized in accordance with the concentration of the consumed species found. Faeces samples containing neither of the two species are shown in black.*

3.2 Temporal-, sex-, and individual-related trends

A total of ten brown bear individuals, five females and five males, were confirmed to have consumed reindeer (**Table 3**). An additional seven samples tested positive for reindeer but were not successfully genotyped for individual identification (Brøseth et al. 2023). Faecal samples testing positive for reindeer were collected primarily in May (86% of positive samples, **Figure 4A**). Faeces from one female and one male tested positive for sheep DNA (**Table 3**) as well as an additional two samples that were not successfully genotyped for individual identification (see **Tables 2** and **A1**). Faeces samples containing sheep were collected in July, August, and November (**Figure 4B**). We detected more frequent consumption of reindeer in the faeces from female brown bears, but it was also detected in males at lower concentrations (**Tables 3** and **A1**, **Figure A5**). By contrast, only one male and one female bear each consumed sheep. However, we emphasize that these sample sizes are too small to statistically test sex-based differences in diet and draw robust conclusions. Multiple faecal samples (from two to nine samples, see **Table 3**) were analysed for fourteen of the eighteen individuals encountered in this study. Unsurprisingly, reindeer and sheep consumption varied among individuals, with some brown bears testing positive for a specific prey item at multiple time points, and other brown bears testing consistently negative for both prey items throughout the season (**Figure 4**, **Figure A5**). Our prey consumption assays also captured diet variability over time in specific individuals. For example, individual BI060033 NT97 scats tested positive for reindeer in May, but not in August or October (**Figure 5A**).

***Table 3**. Overview of reindeer and sheep consumption among brown bear individuals analysed in this study. Individual identification* and sex* is based on the results of the Norwegian national brown bear monitoring, published in the report by Brøseth et al. (2023).*

Individual brown bear ID*	**Sex***	**Number of samples per individual**	**Number of samples positive for sheep** (ovisPDE)	**Number of samples positive for reindeer** (reinCOI)
BI060033 NT97	Female	6	0	4
BI060035 NT99	Male	1	0	1
BI403866 NT102	Female	5	0	5
BI406278 NT125	Female	2	1	0
BI406284 NT131	Female	9	0	0
BI408808 NT142 +	Female	2	0	2
BI417049 NT179	Male	4	3	0
BI418303 NT182 +	Male	2	0	2
BI418304 NT183 +	Female	3	0	3
BI418824 NT185	Male	4	0	1
BI418825 NT186	Male	3	0	0
BI418826 NT187	Male	3	0	0
BI418827 NT189	Male	3	0	0
BI418828 NT188	Male	2	0	1
BI418829 NT190	Female	1	0	1
BI418830 NT191	Male	2	0	0
BI418911 NT192	Female	1	0	0
BI418925 NT193	Male	1	0	1
Unknown ID	Unknown	70	2	7

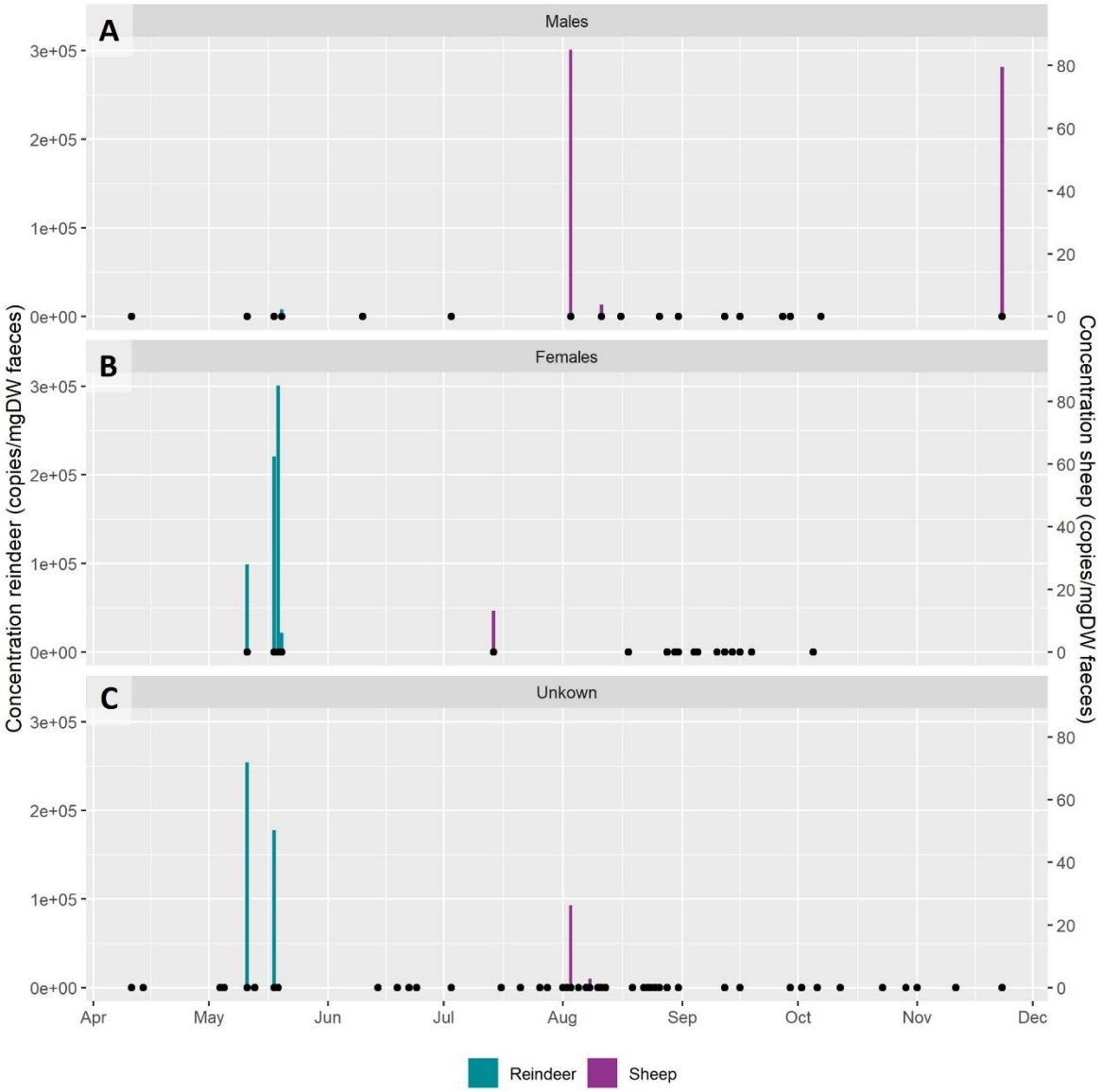


***Figure 4**. Sheep and reindeer consumption among brown bears in Trøndelag in 2022 based on ddPCR assays of faecal samples collected in the Norwegian Large Predator Monitoring Program. Each black point represents an analysed faecal sample and the date it was collected. The presence and quantity of sheep (blue) or reindeer (purple) DNA are indicated by coloured bars. The results are presented for A: male and B: female brown bears, as well as C: unknown individuals, i.e., samples for which genotyping, and sex assignment was unsuccessful (Brøseth et al. 2023).*

4 Discussion

4.1 Using molecular-based diet assays to monitor predation

We have successfully developed two ddPCR based assays that allow us to not only detect consumption of sheep and reindeer, but also to quantify how much DNA of each species is present in a faecal sample. By linking this diet information to already existing genotyping data from the Norwegian Large Predator Monitoring program, we can provide snapshots of an individual bear's diet through space and time. In a management context, this information is valuable, and of particular interest when associating individuals to a conflict or depredation event. However, when relying on faecal samples to infer diet, predation, and recurrent behavioural patterns in an individual, there are a few limitations to bear in mind. Conclusions must be drawn with caution and results must be evaluated in light of the following considerations:

1. **Sampling bias is possible and occurs.**
   It is highly unlikely to find and collect every faeces from every individual brown bear in an area. Studies that rely on opportunistic sampling, such as this one, usually exhibit biases, for example towards easily accessed areas, conflict locations, and areas like dens where high traffic by specific individuals is expected (see e.g., Nellemann et al. 2007). Also, female and male bears may exhibit different detection probabilities (see e.g., Bellemain et al. 2005). Although the Norwegian brown bear monitoring program benefits from a large network of wildlife professionals that collect and submit samples, it is unlikely that all brown bear faeces dropped by the animals in an area are collected throughout a year. This can contribute to, for example, missing predation events for specific individuals that occur in remote areas, or an over representation of predation events in the data because of preferential collection of faeces from around carcasses.

2. **Collection date is not necessary the defecation date.**
   The date a faecal sample is collected is not necessarily the date of defecation. The period between the individual brown bear being at the sample location, defecation and that sample being found and collected can be hours to weeks or even months. Here, experienced personnel can subjectively estimate the "age" of a faeces sample. However, such estimates can be challenging, because, depending on habitat and environmental conditions, faecal samples may decompose differently (Kopatz et al. 2021). As such, collection dates must be interpreted as the latest possible date of defecation.

3. **Gut retention time combined with brown bear movement creates a delay.**
   Each faecal sample is a snapshot of the brown bear's diet consumed roughly over the last 24 hours, prior to defecation. But small traces of diet may be still present for a longer period, especially if high volumes were consumed (Elfström et al. 2013). The time food items need to pass through the digestive tract and be deposited as faeces is called gut retention time. One can assume a time delay of approximately eight to 18 hours between the consumption of meat or carcass item and when it is excreted as faeces while the diet item passes through a brown bear's digestive tract: with a median gut retention time of about 14 hours and 30 minutes (Elfström et al. 2013). As such, a faecal sample does not provide a picture of an individual bear's current foraging activity and the faeces may be deposited in a different area than from where the diet items present in it were consumed. Roaming brown bears are capable of long-distance movement, especially during mating season and when dispersing out for their natal area. Such distances can, depending on season and availability of food etc., range from a few kilometres up to 10 kilometres within 24 hours (Ordiz et al. 2014, Bartoń et al. 2019, Hertel et al. 2019). Individuals testing positive for sheep or reindeer should only fall under suspicion for a given depredation event if positive scats are within these plausible distances. Also, various signs and information should be combined with other evidence before concluding involvement of an individual in specific conflicts.

4. **Small quantities of diet items can occur for multiple reasons.**
   The detection of high and significant quantities of a particular prey species' DNA in a faeces indicate recent consumption of considerable quantities of that species. By contrast, interpreting small quantities is more challenging as this can indicate recent consumption of a small amount of the diet item or, alternatively, consumption of larger amounts several days to a week prior to defecation (Elfström et al. 2013).

5. **Consumption does not always indicate predation.**
   The detection of a particular prey species' DNA in a faecal sample confirms that the individual ate that species but does not provide any conclusive information on how this diet item was obtained. While bears can actively hunt and predate on livestock, they are also opportunistic scavengers and will feed on carcasses of animals that have died for other reasons or were killed by other individuals (Dahle et al. 1998, Swenson et al. 2007). Again, different sources of evidence and information should be combined to deliver conclusive proof.

4.2 Sheep and reindeer consumption by brown bears in Trøndelag

Of the 141 faecal samples collected in Trøndelag for the brown bear monitoring program in 2022, we successfully used the developed ddPCR assays to screen 124 of them for sheep and reindeer consumption (**Table 2**). Although more samples tested positive for reindeer than for sheep, we cannot conclude that one or the other is a more frequent diet component of brown bears in Trøndelag, because the reindeer assay is ten times more sensitive than the sheep assay (see section **4.4.** below and **Appendix**) and we have insufficient statistical power to resolve a difference between the two. However, by linking this data with individual genotyping data (Brøseth et al. 2023), we were able to both examine the diet of individual brown bears and map the consumption of sheep and reindeer over both space (**Figure 3**) and time (**Figure 4**).

Faeces with sheep were mainly collected around the European Route E6 with corresponds to areas in Trøndelag with variable levels of sheep herding. Four positive samples originated in Overhalla with 4,342 grazing sheep in 2021, while a single positive sample was collected in Snåsa and Steinkjer, where 4,000 and 9,991 sheep, respectively, were released to graze in 2021 (grazing statistics from https://beitestatistikk.nibio.no/ accessed 24. April 2023). These statistics have been comparably stable over the last five years in the regions of interest. Reindeer consumption was also detected primarily in the area of Trøndelag with active reindeer husbandry. Reindeer appeared to be a more frequent part of the brown bear's diet in the early season, with 86% of all scats that tested positive for reindeer being collected in May (**Figure 4**). Indeed, the Scandinavian brown bear shows seasonal preferences of food items (Stenset et al. 2016). Such selection can be expected, due to seasonal availability of certain food as well as the tendency of brown bears to use meat as protein resources during springtime and summer, I.e., during the calving season (Stenset et al. 2016). Consumption of sheep occurred later, in July, August and November (**Figure 4**), which may seem unexpected as wild berries is by far the most important food resource during this period, which implies that a reduction of depredation events could be expected. However, as brown bears can tend towards sheep as food, when available (Dahle et al. 1998), the observed result could be due to one factor or a combination thereof, for example local variation in berry availability and a product of situational, opportunistic foraging. Alternatively, the result may also reflect a sampling bias or stochastic variation as only six samples were positive for sheep consumption, originating from two to four different individuals (**Table 3**).

4.3 Tracking the diet of individual brown bears

Multiple samples were collected from the majority of individuals detected in this study (**Table 3**), allowing us to track and map livestock consumption throughout the season for specific individual brown bears. Data for each individual bear is found in **Appendix Table A1** and we highlight the value of these data by presenting here three case studies from the eighteen individuals detected in this study:

1. **Female brown bear BI060033NT97**
   Six faecal samples were collected from this bear. Three samples from May were collected in an area with reindeer husbandry and our analysis detected significant amounts of reindeer DNA in each of these faecal samples (**Figures 5A** and **6A**). Faeces collected later in the season from the same individual did not contain reindeer DNA. Combining these results from single feces highlight how ddPCR assays can be used to track variation in diet over the season.

2. **Female brown bear BI406284NT131**
   Nine scats were collected from this bear in an 18-day period in late August to early September, but there was no evidence of sheep or reindeer consumption during this time period (**Figures 5B** and **6B**). This illustrates the potential for faecal samples to provide diet data with high temporal resolution, although the results must be interpreted with caution, as the collection date is not necessarily reflective of the defecation date, as discussed above, in section 4.1.

3. **Male brown bear BI417049NT179**
   Faeces of this bear were collected twice in August and twice in November. In August, a sample collected beside a sheep carcass (Rovbase) contained high amounts of sheep DNA, while eight days later, only traces of sheep DNA were present in a second sample nearby (**Figures 5C** and **6C**). As discussed above, this may reflect recent consumption of a small amount of sheep, or simply reflect the large amount of sheep that had been consumed the previous week. In November, two faecal samples were taken in close proximity to two sheep carcasses and while both belonged to the male brown bear BI417049NT179, only one tested positive for sheep DNA. This may illustrate the confounding influence of gut retention time that is discussed above on our ability to interpret faecal-based diet assessments. While the absence of sheep DNA in one of these scats could reflect a false negative in the ddPCR assay it could also reflect a scat containing diet items consumed before bear BI417049NT179 arrived in the area and began consuming sheep, as discussed in section 4.1.

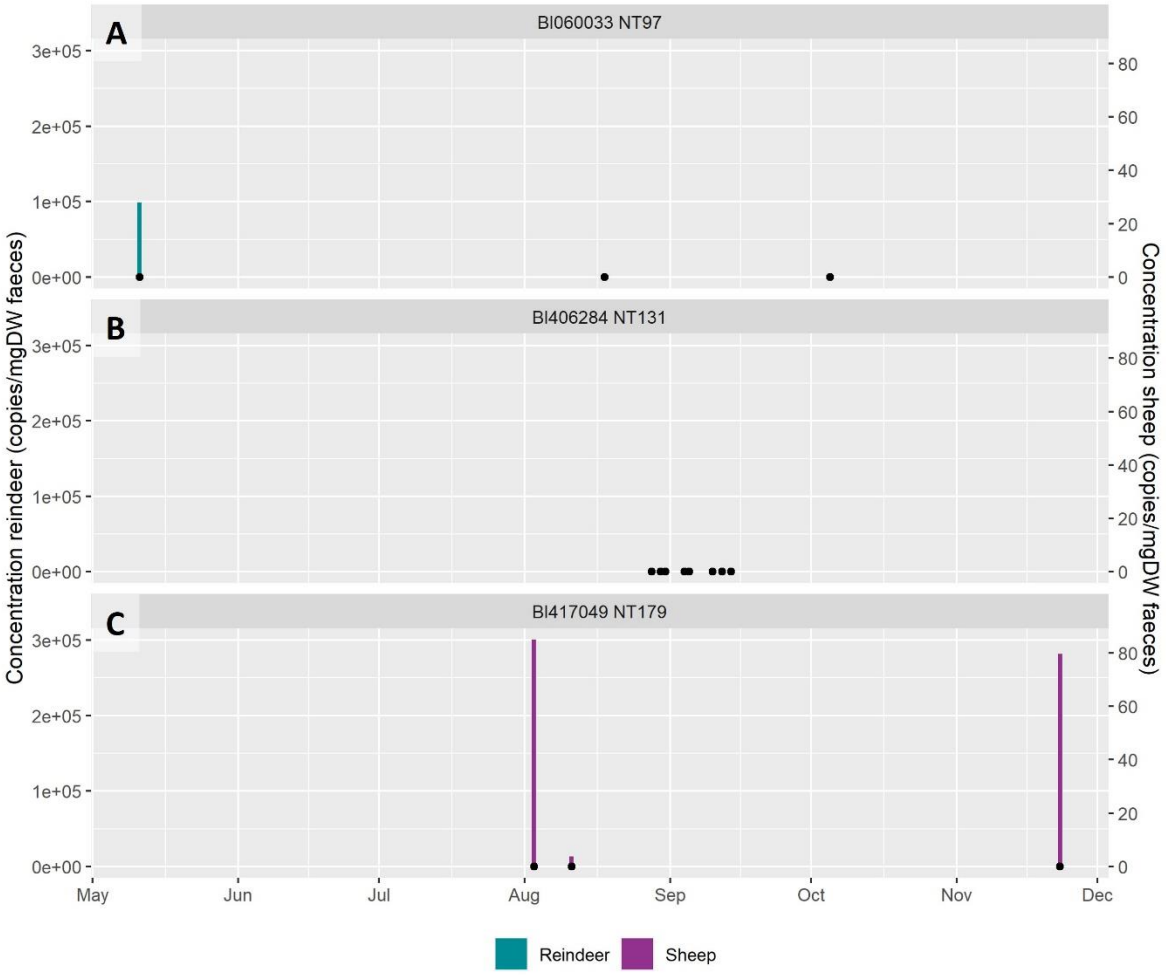


***Figure 5**. Sheep and reindeer diet components for three different bear individuals in 2022. A: BI060033NT97 (N=6 faeces), female, with reindeer detected in three faeces (blue), B: BI406284NT131 (N=9), female, with neither reindeer nor sheep detected, and C: BI417049NT179 (N=4), male, with sheep detected in three faeces (purple). Dates on which a fecal sample was collected and analysed are indicated by black points. The presence and quantity of sheep (blue) or reindeer (purple) DNA are indicated by coloured bars.*

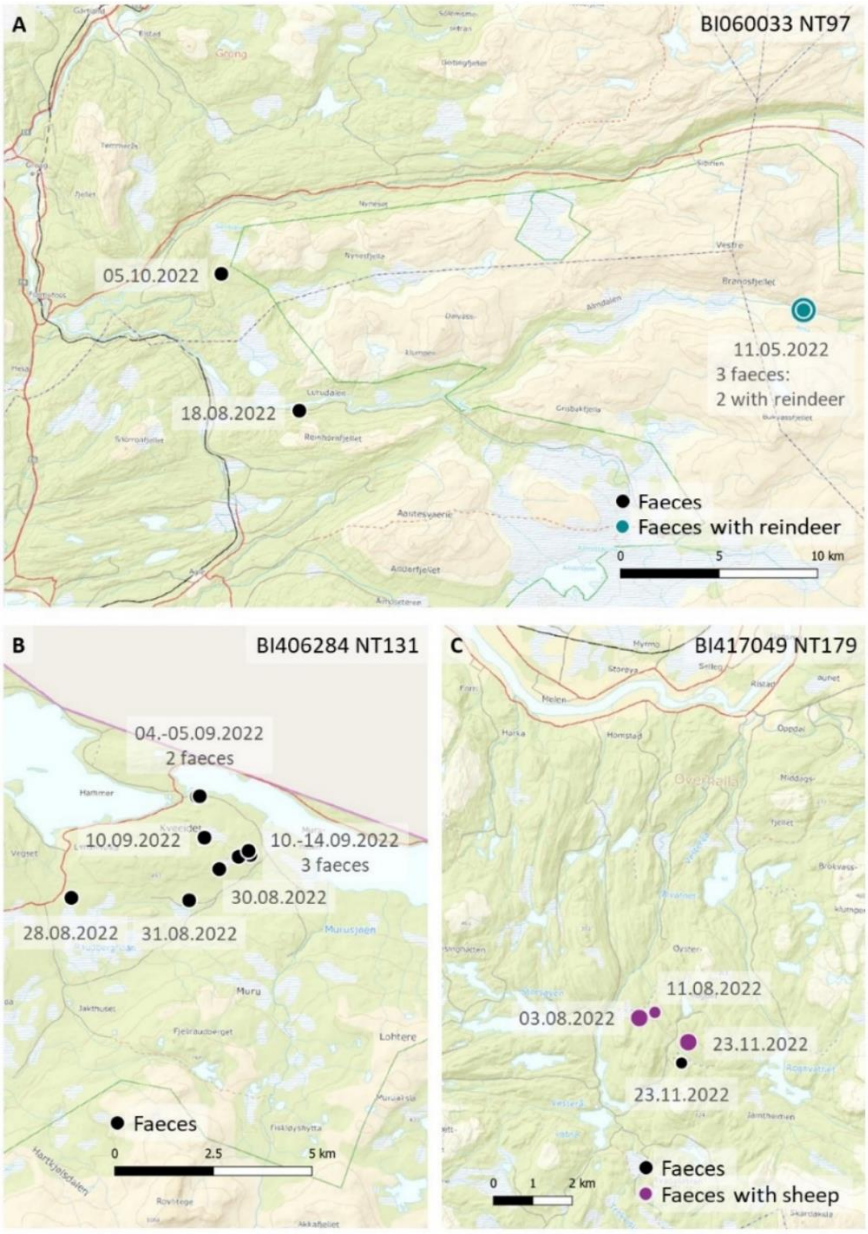


Figure 6. *Faeces sampling locations and dates of three brown bear individuals identified during the national monitoring in season 2022 in Trøndelag. A: BI060033NT97 (N=6 faeces; but one location has been shielded for publication, see Results), female, with reindeer detected in three faeces (blue), B: BI406284NT131 (N=9), female, with neither reindeer nor sheep detected, and C: BI417049NT179 (N=4), male, with sheep detected in three faeces (purple).*

4.4 The application of droplet digital PCR (ddPCR) in wildlife monitoring

We successfully developed ddPCR-based assays for both sheep and reindeer that allow us to both detect the species and quantify how much of their DNA is present in an individual faecal sample. By linking the screening of faecal samples to the existing DNA-based brown bear monitoring program in Norway (Brøseth et al. 2023), we were able to examine sheep and reindeer consumption in individual brown bears over space and time as well as assess sex-related patterns in sheep and reindeer consumption. Sheep or reindeer consumption was detected in 27% of the samples screened (34 samples total). Some samples were taken in close proximity to sheep or reindeer carcasses where predation was assumed to have occurred, but 76% of these (26 samples) were not directly linked to other evidence of sheep or reindeer consumption (https://rovbase.no/, assessed 8. March 2023), suggesting that the ddPCR method has the capacity to detect consumption of sheep and reindeer that would otherwise go unnoticed. Compared to micro- and macroscopic methods to identify prey species in predator's faeces, DNA-based techniques like ddPCR have clear-cut advantages like higher detection rates, particularly in samples containing multiple prey items, better detection of trace amounts, higher precision in identification of the target species, higher reliability, and lower observer bias (Ciucci et al. 2014, Groen et al. 2022, Mumma et al. 2016, Nørgaard et al. 2021, Shores et al. 2015). Furthermore, these methods are relatively low cost and high throughput, allowing screening of large numbers of samples. It must be noted that while the concentrations of DNA measured for a given prey species can be used to compare quantities of that species between faecal samples, these concentrations cannot be compared between sheep and reindeer. The ovisPDE marker for sheep targets cyclic GMP phosphodiesterase which is a single-copy nuclear gene and would occur once in every cell of the prey animal. By contrast the reinCOI marker for reindeer targets the mitochondrial cytochrome c oxidase subunit 1 gene, which can occur thousands of times in every cell of the prey animal. As such, the concentration of sheep or reindeer DNA measured per gram consumed will be different and these concentrations cannot be used to directly compare the amount of sheep consumed to the amount of reindeer consumed.

DNA-based analysis of faeces has become the preferred method for diet analysis in predators, especially when, like here, a large number of faeces samples is assessed (De Barba et al. 2014, Elfström et al. 2014, Pompanon et al. 2012, Valentini et al. 2009). A technique called DNA-metabarcoding is often used to simultaneously identify all diet components and their relative abundance within a single faecal sample (see e.g., De Barba et al. 2014, Elfström et al. 2014). However, this approach does not allow us to specifically conclude that one sample contains more of a given target species than another sample. ddPCR assays for specific prey items have the advantage of providing absolute quantifications that can be compared across samples, providing high resolution data for diet items that are of specific relevance (Floren et al. 2015, Groen et al. 2022).

4.5 Can diet assays provide useful information to the predator management?

Assessing and quantifying diet items including sheep and reindeer, but also other livestock, is of particular relevance when predators come in conflict with animal husbandry. In this pilot project, we highlight the potential to use the existing DNA-based brown bear monitoring program to build a data set that elucidates the extent and frequency at which these species feed on livestock. The ddPCR assays we implement here have the ability to detect sheep and reindeer consumption events that would otherwise go unnoticed and provide both spatial and temporal context for this consumption. Further, by combining genotyping data with prey consumption, we can better understand predator ecology and assess risk of depredation of livestock on an individual level by bears or other large predators (Latham et al. 2013). In addition to genotyping for individual and sex, diet analyses (including food items other than sheep and reindeer) could be a valuable

component in rapid analyses to help illuminate a scenario, particularly in ambiguous conflict cases. The quantity of livestock DNA detected in a ddPCR analysis of a faecal sample can give clues to the time since consumption, which has practical relevance for large predator management and conflict mitigation measures. Also, the method has potential to be applied to other sample types, such as stomach contents and to samples from other large carnivore species, such as wolf (*Canis lupus*) and wolverine (*Gulo gulo*).

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7 Appendices

7.1 Marker selection, development and validation

7.1.1 Sheep and reindeer marker selection

In order to develop prey assays for each of the target species (sheep *Ovis aries*, reindeer *Rangifer tarandus),* we first searched the scientific literature for pre-existing species-specific primers that target these species. For sheep, the marker-probe system selected was ovisPDE which targets the cyclic GMP phosphodiesterase gene and was originally developed for detecting and quantifying lamb in food products (Laube et al. 2007). For reindeer, the R-FAM marker targeting the mitochondrial D-loop noncoding region was selected. This marker was originally developed to discriminate between reindeer and deer (*Cervus elaphus*, *Cervus nippon*) antler material used in eastern traditional medicine (Shim et al. 2011). The specificity of each of these markers was then tested against a panel of tissue samples that represent potential vertebrate prey items of the European brown bear including both domestic livestock, as well as wild mammal and bird prey items.

7.1.2 Sheep Marker Validation

The ovisPDE sheep marker successfully detected sheep and no other vertebrate prey items (**Figure A1**). Although goat, another potential brown bear prey item, was not included in the specificity testing panel, earlier testing of ovisPDE by Laube et al. (2007) found it to be specific to lamb without cross amplification of goat. The only other potential caprinid prey item for Norwegian brown bears is the muskox (*Ovibos moschatus*). Although the ovisPDE marker was not tested for specificity against muskox, none of the faecal samples from Trøndelag originate from areas with muskox populations, so any positive detections can be assumed to be for domestic sheep. The ovisPDE marker was then tested to confirm it not only detects the target species but provides a quantitative estimate of the amount of target DNA present. DNA from eight brown bear fecal samples originating from localities without active sheep grazing were selected for these quantification tests. DNA from these samples was combined with known amounts of sheep DNA in a process known as “spiking” and then analysed by ddPCR. The ovisPDE marker was clearly quantitative, with more positive droplets detected when more sheep DNA was present (**Figure A2**). A standard curve using known concentrations of sheep DNA was run for the ovisPDE marker and shows that the limit of its detection of sheep DNA is 0.01 ng/µL.

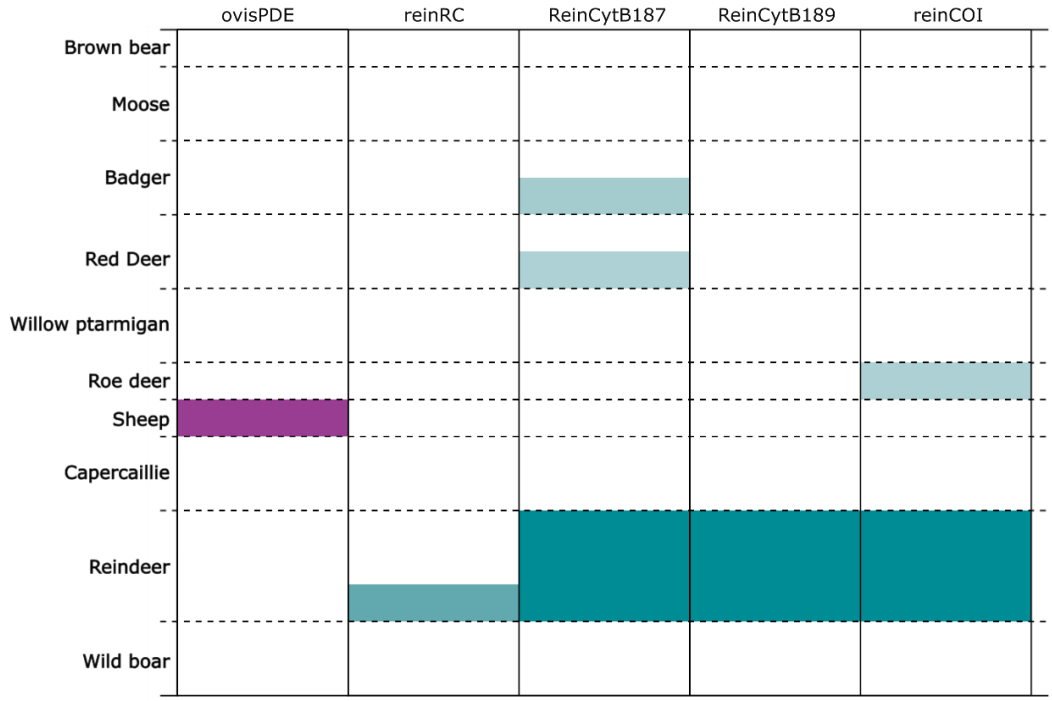

***Figure A1**. Results of specificity testing for all sheep (purple) and reindeer markers(blue). Coloured tiles indicate a tissue sample from the animal tested positive with the specified primer. The colour intensity correlates with the number of positive droplets in the ddPCR reaction, with dark colours indicating many droplets and light colours indicating few. The marker ovisPDE was designed by Laube et al. (2007), the marker reinRC was designed by Shim et al. (2011), and the remaining three markers were developed in this study.*

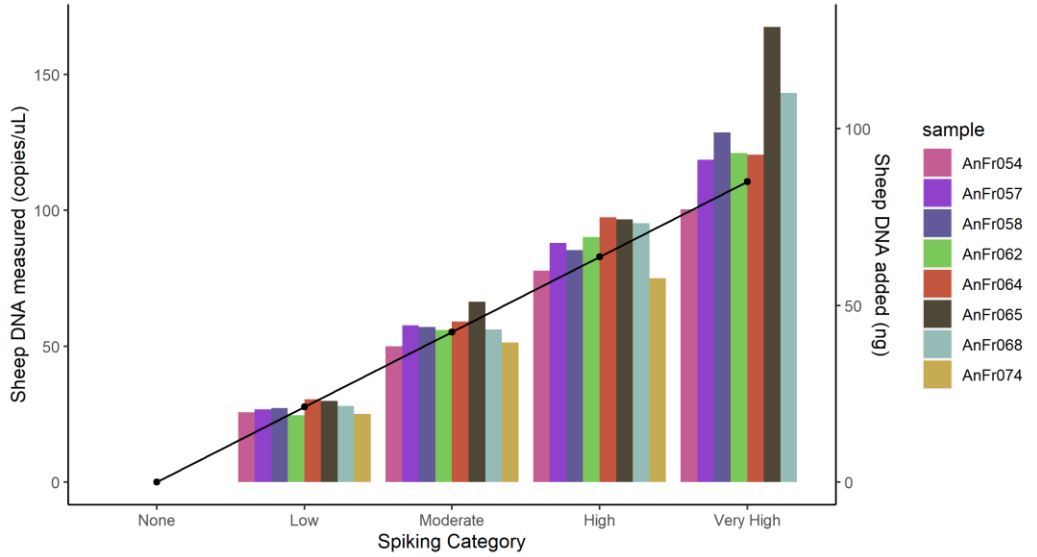

***Figure A2**. Test of the quantitative capacity of the ovisPDE marker for sheep. Brown bear faecal samples from the Scandinavian Brown Bear Research Project in central Sweden (Kopatz et al. 2021) and areas devoid of grazing sheep spiked with no, low, moderate, high, or very high amounts of sheep DNA were assayed by ddPCR. The coloured bars indicate the amount of sheep DNA measured, while the black line indicates the amount of sheep DNA added to the samples in each of the five categories (None, Low, Moderate, High, Very High).*

7.1.3 Reindeer marker development and validation

The R-FAM reindeer marker failed specificity testing, amplifying only one of three reindeer samples and could also not be used for quantification because of inconsistent amplitude measurements for the positive droplets in ddPCR (**Figures A1** and **A3A**). As such, three additional custom markers were developed targeting the cytochrome c oxidase subunit I gene and cytochrome B gene. In short, publicly available gene sequences for reindeer, moose (*Alces alces*), red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) were downloaded from public databases (BOLD, GenBank) and aligned in Geneious prime v2023.0.1 (Biomatters Ltd) to identify regions unique to reindeer that could be used as markers. We used Primer Express 3.0.1 (Applied Biosystems) to design primers and TaqMan MGB probes, hereafter referred to as reinCOI, reinCytB187, and reinCytB189. These newly developed markers were then tested against tissue samples from all four above-mentioned ungulate species, and some additional vertebrate species, to confirm their specificity. The marker reinCytB187 showed strong amplification of reindeer, but also had low levels of cross amplification with red deer and badger. Both the reincCytB189 and reinCOI markers showed strong amplification of reindeer with no or very low cross amplification of other species in the test panel. (**Figures A1** and **A3B-C**). By setting a conservative amplitude threshold for defining positive droplets in the ddPCR reaction, this cross-amplification could be identified and used to separate between reindeer and the other species in the panel. The variation in amplitude for positive droplets in the reinCytB189 marker was greater than for the reinCOI marker, making it more difficult to set a threshold for defining positive droplets. Furthermore, qPCR tests of the markers demonstrated that reinCOI has a higher efficiency of amplification than reinCytB189, making it more suited to quantifying DNA. As such, only the reinCOI marker was used in further testing.

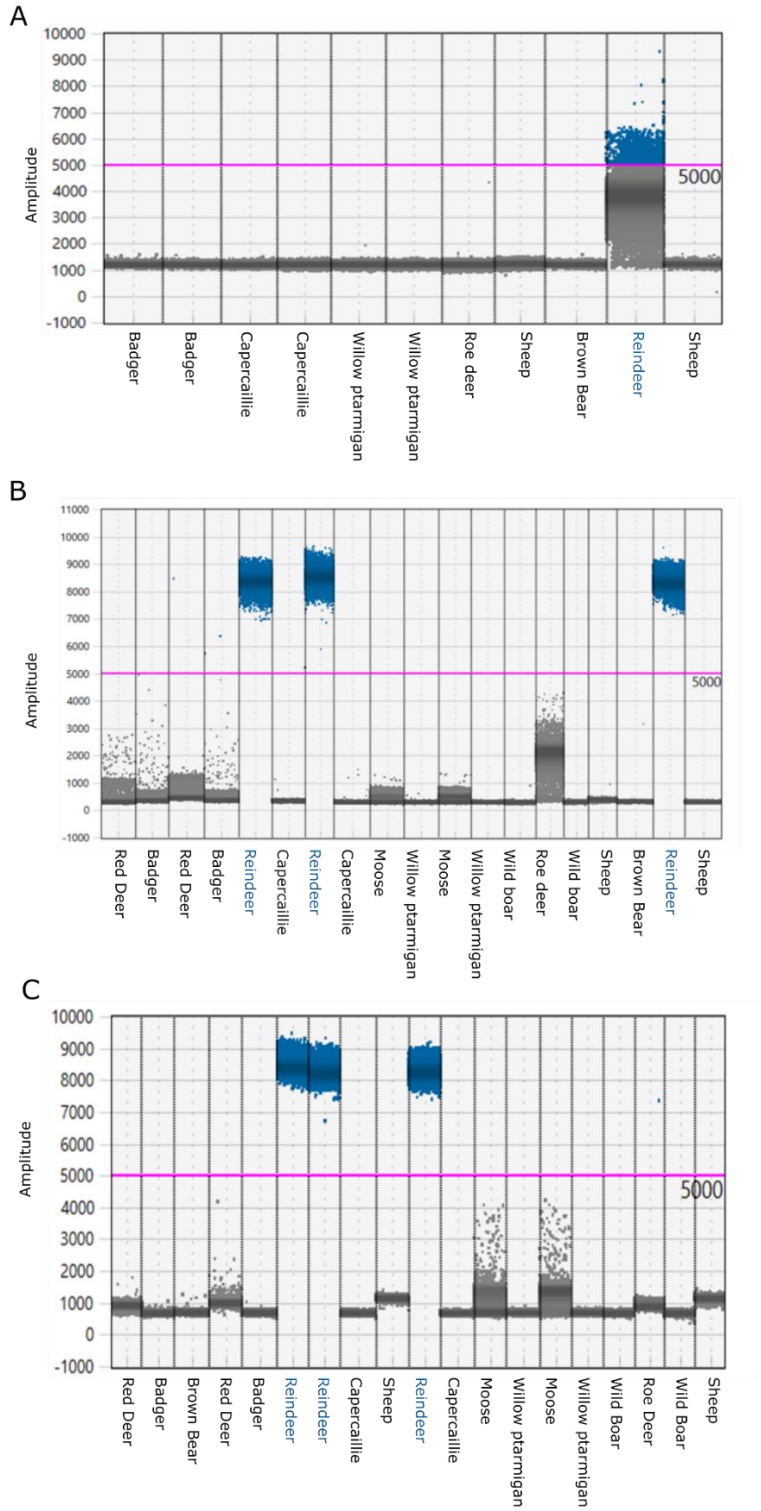

***Figure A3**. ddPCR amplitude plots of species specificity testing for the A) reinRC (Shim et al. 2011), B) reinCytB189 and C) reinCOI markers. Each column represents an assay on a particular reference sample. Each plotted dot is the result from a single droplet, with the amplitude on the y axis indicating the intensity of the droplet's fluorescence. The reinRC marker (A) shows specificity for reindeer, but high variability in amplitude, and therefore inadequate for quantification applications. The reinCytB189 (B) marker shows specificity for reindeer, but the amplitude variability observed for red deer, badger, moose, and roe deer suggests a higher likelihood for false positives than the reinCOI marker (C) where signs of cross amplification are limited to moose and red deer and are still clearly discernible from the reindeer samples.*

The reinCOI marker was then tested to confirm it not only detects reindeer but also provides a quantitative estimate of the amount of target DNA present. We first tested the marker against known concentrations of reindeer DNA from 10 ng/uL to 0.001 ng/uL using ddPCR, and then tested the same fecal samples described above. The reinCOI marker was also quantitative when tested against pure reindeer DNA (**Figure A4**) and had a detection limit of 0.001 ng/µL. However, the spiked fecal samples contained such high levels of reindeer DNA that they were beyond the limit of detection for ddPCR, with all concentrations of reindeer DNA yielding 100% positive droplets in the ddPCR reactions (data not shown). To obtain quantitative estimates for these samples, the DNA extracts would need to be diluted and again tested by ddPCR.

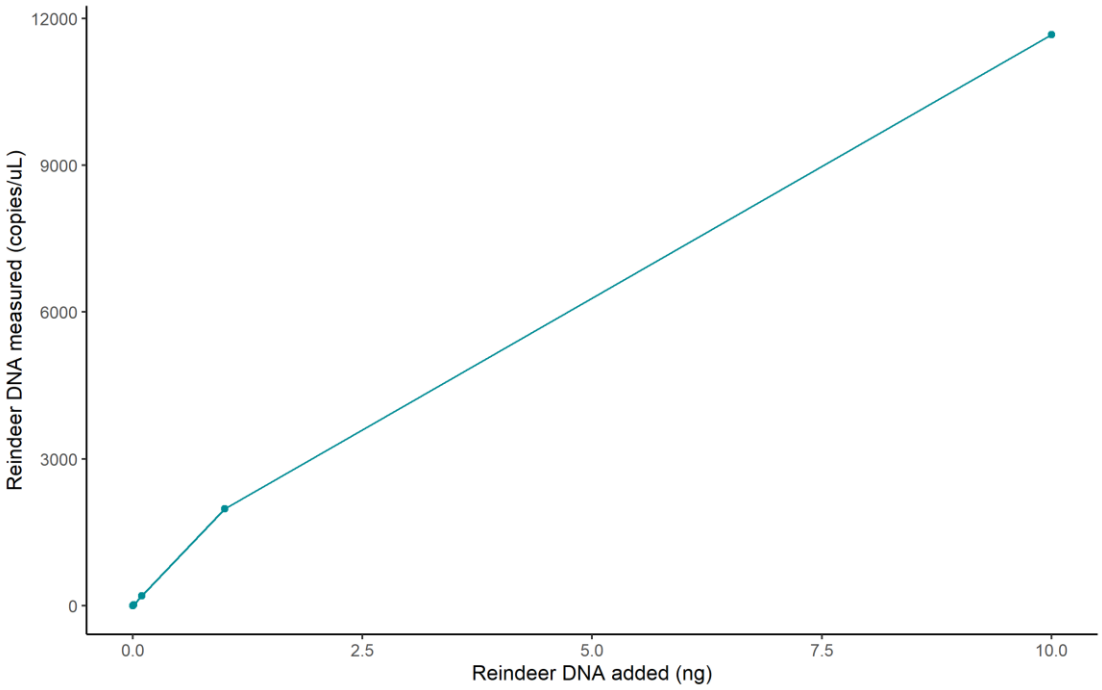

***Figure A4**. Results of the quantification testing for the marker reinCOI, where ddPCR was used to quantify the amount of reindeer DNA present in samples of known concentrations from 0.001 ng to 10 ng.*

7.2 Molecular diet analysis of faeces

***Table A1**. Sheep and reindeer content of the 124 brown bear faeces samples from Trøndelag and assessed by droplet digital PCR (ddPCR). Results are presented in order of sample number and as the number of DNA copies present per gram dry weight of faeces. Individual ID and sex are presented from genotyping of the faeces samples through the Norwegian national brown bear monitoring (Brøseth et al. 2023).*

Sample number	Collection date	Individual ID	Sex	Reindeer	Concentration reindeer (reinCOI; copies/g)	Sheep	Concentration sheep (ovisPDE, copies/g)
B00026945	07.10.2022	BI418925 NT193	Male	Present	12066.405	Absent	0
B00041771	21.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00041778	21.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00042603	14.07.2022	BI406278 NT125	Female	Absent	0	Present	13176.771
B00042710	05.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00045755	05.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00051346	29.10.2022	Unknown	Unknown	Absent	0	Absent	0
B00051360	16.09.2022	Unknown	Unknown	Absent	0	Absent	0
B00051370	27.09.2022	BI418825 NT186	Male	Absent	0	Absent	0
B00051371	19.09.2022	BI406278 NT125	Female	Absent	0	Absent	0
B00051377	11.08.2022	BI417049 NT179	Male	Absent	0	Present	3707.264
B00052616	24.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00052618	24.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00053515	01.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00053516	01.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00053518	01.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00053519	01.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00053586	14.04.2022	Unknown	Unknown	Absent	0	Absent	0
B00053587	14.04.2022	Unknown	Unknown	Absent	0	Absent	0
B00053588	14.04.2022	Unknown	Unknown	Absent	0	Absent	0
B00053589	14.04.2022	Unknown	Unknown	Absent	0	Absent	0
B00053593	14.04.2022	Unknown	Unknown	Absent	0	Absent	0
B00054133	11.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00054136	06.10.2022	Unknown	Unknown	Absent	0	Absent	0
B00054138	05.10.2022	BI060033 NT97	Female	Absent	0	Absent	0
B00054487	11.05.2022	BI418824 NT185	Male	Absent	0	Absent	0
B00054488	11.05.2022	BI403866 NT102	Female	Present	8780648.338	Absent	0
B00055836	16.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00055838	22.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00056021	11.11.2022	Unknown	Unknown	Absent	0	Absent	0
B00056084	18.05.2022	BI418824 NT185	Male	Present	4564.180	Absent	0
B00056086	18.05.2022	Unknown	Unknown	Present	393023.982	Absent	0
B00056089	19.05.2022	BI403866 NT102	Female	Present	300870977.493	Absent	0
B00056184	28.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00056185	28.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00059233	01.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00059253	02.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00059338	26.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00060245	11.05.2022	BI418824 NT185	Male	Absent	0	Absent	0
B00063245	14.06.2022	Unknown	Unknown	Absent	0	Absent	0
B00066820	11.05.2022	BI418830 NT191	Male	Absent	0	Absent	0
B00066823	11.05.2022	Unknown	Unknown	Present	10584353.946	Absent	0
B00066824	11.05.2022	BI403866 NT102	Female	Present	13175983.987	Absent	0
B00066825	11.05.2022	BI418824 NT185	Male	Absent	0	Absent	0
B00066929	11.04.2022	Unknown	Unknown	Present	2002590.487	Absent	0
B00066944	10.06.2022	BI418828 NT188	Male	Present	212528.005	Absent	0
B00066945	19.06.2022	Unknown	Unknown	Absent	0	Absent	0
B00067830	31.08.2022	BI406284 NT131	Female	Absent	0	Absent	0
B00067865	11.04.2022	BI060035 NT99	Male	Present	513105.634	Absent	0
B00067885	11.05.2022	BI403866 NT102	Female	Present	2937981.714	Absent	0
B00071331	08.08.2022	Unknown	Unknown	Absent	0	Present	2919.043
B00071352	23.11.2022	Unknown	Unknown	Absent	0	Absent	0
B00071363	23.11.2022	BI417049 NT179	Male	Absent	0	Absent	0
B00071364	23.11.2022	BI417049 NT179	Male	Absent	0	Present	79680.408
B00071365	03.08.2022	Unknown	Unknown	Absent	0	Present	26245.060
B00071366	03.08.2022	BI417049 NT179	Male	Absent	0	Present	85013.779
B00071389	20.05.2022	BI418304 NT183 +	Female	Present	1335015.809	Absent	0

Sample number	Collection date	Individual ID	Sex	Reindeer	Concentration reindeer (reinCOI; copies/g)	Sheep	Concentration sheep (ovisPDE, copies/g)
B00071390	20.05.2022	BI418304 NT183 +	Female	Present	22099417.087	Absent	0
B00071394	20.05.2022	BI418303 NT182 +	Male	Present	8211800.781	Absent	0
B00078234	12.10.2022	Unknown	Unknown	Absent	0	Absent	0
B00079968	07.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00079987	04.05.2022	Unknown	Unknown	Absent	0	Absent	0
B00079999	01.11.2022	Unknown	Unknown	Absent	0	Absent	0
B00082225	22.06.2022	Unknown	Unknown	Absent	0	Absent	0
B00083067	11.05.2022	BI060033 NT97	Female	Present	99104678.011	Absent	0
B00083069	11.05.2022	Unknown	Unknown	Absent	0	Absent	0
B00083359	12.09.2022	BI418826 NT187	Male	Absent	0	Absent	0
B00083361	14.09.2022	BI406284 NT131	Female	Absent	0	Absent	0
B00083362	10.09.2022	BI406284 NT131	Female	Absent	0	Absent	0
B00083363	10.09.2022	BI406284 NT131	Female	Absent	0	Absent	0
B00083364	12.09.2022	BI418828 NT188	Male	Absent	0	Absent	0
B00083365	12.09.2022	BI418826 NT187	Male	Absent	0	Absent	0
B00083366	12.09.2022	Unknown	Unknown	Absent	0	Absent	0
B00084375	12.09.2022	BI418826 NT187	Male	Absent	0	Absent	0
B00084376	12.09.2022	BI406284 NT131	Female	Absent	0	Absent	0
B00084377	16.09.2022	BI418911 NT192	Female	Absent	0	Absent	0
B00084378	16.09.2022	BI418827 NT189	Male	Absent	0	Absent	0
B00084379	02.10.2022	Unknown	Unknown	Absent	0	Absent	0
B00084380	04.09.2022	BI406284 NT131	Female	Absent	0	Absent	0
B00084381	16.08.2022	BI418827 NT189	Male	Absent	0	Absent	0
B00084382	29.09.2022	BI418827 NT189	Male	Absent	0	Absent	0
B00084383	29.09.2022	Unknown	Unknown	Absent	0	Absent	0
B00084411	20.05.2022	BI418303 NT182 +	Male	Present	73995.793	Absent	0
B00084413	20.05.2022	BI418829 NT190	Female	Present	1198001.746	Absent	0
B00084414	26.08.2022	BI418825 NT186	Male	Absent	0	Absent	0
B00084415	26.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00084416	30.08.2022	BI406284 NT131	Female	Absent	0	Absent	0
B00084424	24.06.2022	Unknown	Unknown	Absent	0	Absent	0
B00084425	05.05.2022	Unknown	Unknown	Absent	0	Absent	0
B00084426	05.05.2022	Unknown	Unknown	Absent	0	Absent	0
B00084427	13.05.2022	Unknown	Unknown	Absent	0	Absent	0
B00084428	03.07.2022	BI418830 NT191	Male	Absent	0	Absent	0
B00084429	03.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00084430	03.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00084431	31.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00084432	25.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00084433	31.08.2022	BI418825 NT186	Male	Absent	0	Absent	0
B00084442	11.05.2022	BI060033 NT97	Female	Present	470208.400	Absent	0
B00084443	11.05.2022	BI060033 NT97	Female	Present	25651292.067	Absent	0
B00084444	11.05.2022	Unknown	Unknown	Present	254575808.905	Absent	0
B00084445	11.05.2022	BI060033 NT97	Female	Present	463903.367	Absent	0
B00084446	18.05.2022	BI408808 NT142 +	Female	Present	220745510.464	Absent	0
B00084461	20.05.2022	BI418304 NT183 +	Female	Present	297390.257	Absent	0
B00084462	20.05.2022	BI408808 NT142 +	Female	Present	4410408.978	Absent	0
B00084463	28.08.2022	BI406284 NT131	Female	Absent	0	Absent	0
B00084464	28.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00084465	28.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00084466	05.09.2022	BI406284 NT131	Female	Absent	0	Absent	0
B00084551	28.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00084552	16.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00084558	18.08.2022	BI060033 NT97	Female	Absent	0	Absent	0
B00084559	19.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00084560	23.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00084561	12.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00084563	10.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00084564	05.08.2022	Unknown	Unknown	Absent	0	Absent	0
B00085115	16.07.2022	Unknown	Unknown	Absent	0	Absent	0
B00086309	23.10.2022	Unknown	Unknown	Absent	0	Absent	0
B00086357	11.05.2022	Unknown	Unknown	Absent	0	Absent	0
B00086473	19.05.2022	Unknown	Unknown	Absent	0	Absent	0
J00045739	18.05.2022	Unknown	Unknown	Present	45941.052	Absent	0
J00045741	18.05.2022	Unknown	Unknown	Present	177753851.997	Absent	0
J00045742	18.05.2022	Unknown	Unknown	Present	155572.136	Absent	0
J00045744	18.05.2022	BI403866 NT102	Female	Present	24707.927	Absent	0

7.3 Sheep and reindeer consumption by individual brown bears

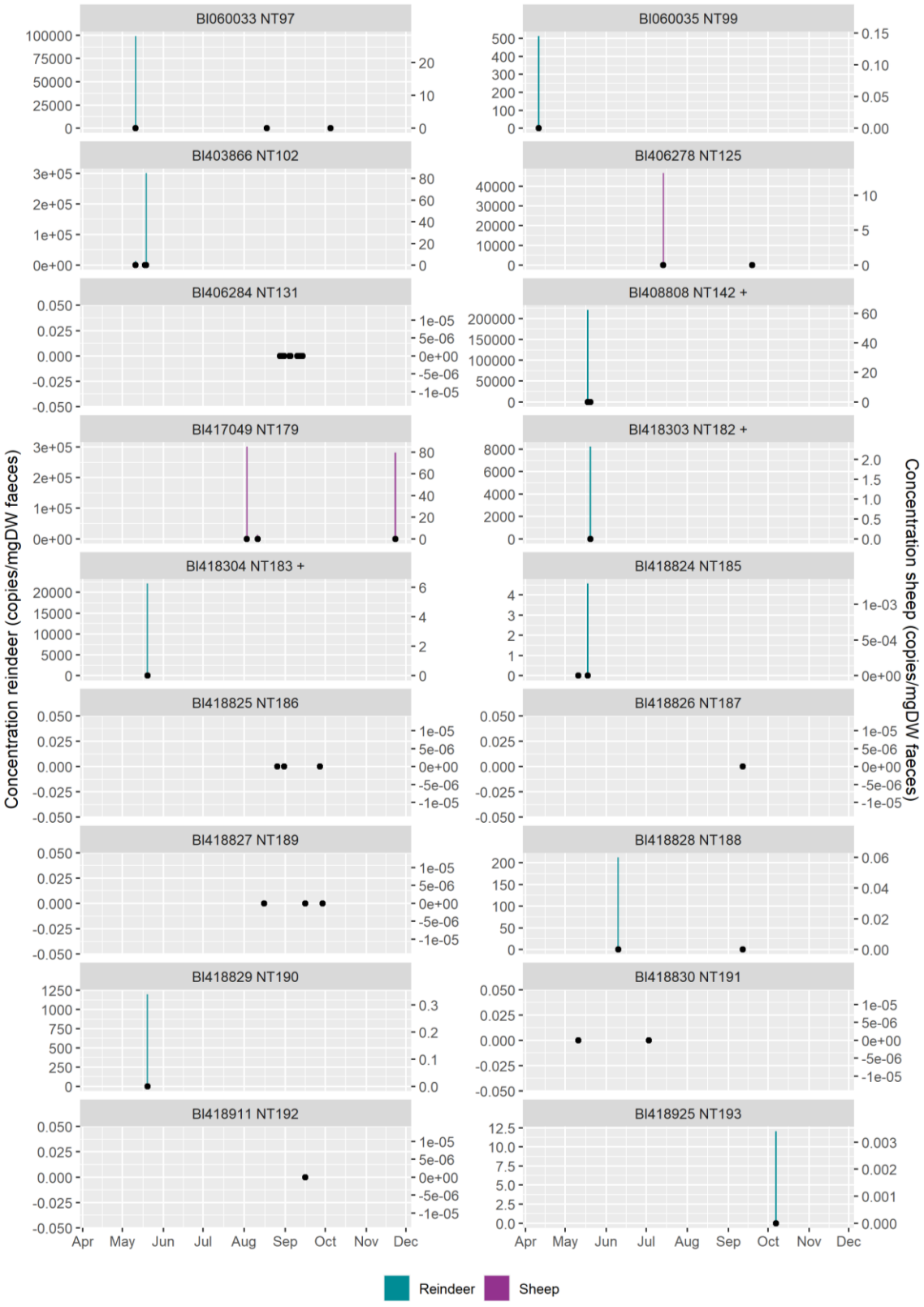

***Figure A5**. Sheep and reindeer consumption for each identified individual brown bear sampled in Trøndelag, Norway in 2022 based on ddPCR analysis of faecal samples collected through the Norwegian Large Predator Monitoring program. Y-axis scales are different for sheep and reindeer and are adjusted for each individual to improve visualization of the results.*

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Norwegian Institute for Nature Research
NINA head office
Postal address: P.O. Box 5685 Torgarden,
NO-7485 Trondheim, NORWAY
Visiting address: Høgskoleringen 9, 7034 Trondheim
Phone: +47 73 80 14 00
E-mail: firmapost@nina.no
Organization Number: 9500 37 687
http://www.nina.no

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