www.nina.no

1775

NINA Report

Seasonal variation of success in DNA-extraction from brown bear fecal samples

Alexander Kopatz, Oddmund Kleven, Øystein Flagstad

NINA

Norwegian Institute for Nature Research

NINA Publications

NINA Report (NINA Rapport)
This is NINA's ordinary form of reporting completed research, monitoring or review work to clients. In addition, the series will include much of the institute's other reporting, for example from seminars and conferences, results of internal research and review work and literature studies, etc. NINA

NINA Special Report (NINA Temahefte)
Special reports are produced as required and the series ranges widely: from systematic identification keys to information on important problem areas in society. Usually given a popular scientific form with weight on illustrations.

NINA Factsheet (NINA Fakta)
Factsheets have as their goal to make NINA's research results quickly and easily accessible to the general public. Fact sheets give a short presentation of some of our most important research themes.

Other publishing.
In addition to reporting in NINA's own series, the institute's employees publish a large proportion of their research results in international scientific journals and in popular academic books and journals.

Seasonal variation of success in DNA-extraction from brown bear fecal samples

Alexander Kopatz
Oddmund Kleven
Øystein Flagstad

Norwegian Institute for Nature Research

Alexander Kopatz, Oddmund Kleven, Øystein Flagstad. 2020. Seasonal variation of success in DNA-extraction from brown bear fecal samples. NINA Report 1775. Norwegian Institute for Nature Research.

Trondheim, January 2020

ISSN: 1504-3312
ISBN: 978-82-426-4532-6

AVAILABILITY
Open

PUBLICATION TYPE
Digital document (pdf)

QUALITY CONTROLLED BY
Jenny Mattisson

SIGNATURE OF RESPONSIBLE PERSON
Head of research Jonas Kindberg (sign.)

CLIENT(S)/SUBSCRIBER(S)
Norwegian Environment Agency (Miljødirektoratet)

CLIENT(S) REFERENCE(S)
19S94372

CLIENTS/SUBSCRIBER CONTACT PERSON(S)
Siv Grethe Aarnes

COVER PICTURE
Feces sample material in the field © Torbjørn Berglund

KEY WORDS
DNA, feces, large carnivores, non-invasive genetic sampling, Norway, *Ursus arctos*, wildlife monitoring

NØKKELORD
DNA, feces, ikke-invasiv prøvetaking, overvåking, rovdyr, Norge, *Ursus arctos*

CONTACT DETAILS

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

NINA Oslo
Gaustadalléen 21
NO-0349 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

www.nina.no

Abstract

Alexander Kopatz, Oddmund Kleven, Øystein Flagstad. 2020. Seasonal variation of success in DNA-extraction from brown bear fecal samples. NINA Report 1775. Norwegian Institute for Nature Research.

Brown bears (*Ursus arctos*) in Norway are monitored annually with DNA-based methods of non-invasively collected biological material for identification of individual and sex. Feces samples make up the largest part of the sample material collected throughout the year. However, feces samples collected during spring and summer specifically, have shown relatively low positive, genotyping success rates. Those samples are important to the Norwegian wildlife management, as they are often collected from sites of human-bear conflicts and other locations. In the following pilot study we explored alternative fecal storage and DNA-extraction protocols in order to evaluate how to potentially increase genotyping success rates of feces samples collected in spring and summer. We used fresh brown bear feces samples collected by experienced field personnel from May to October 2019 in Troms, Trøndelag and Hedmark. From each feces found, the trained personnel took two separate samples: one sample was stored on silica, and the other was stored in an empty tube without silica. We used a total of 94 feces samples to extract DNA using three different extraction methods, which are currently successfully applied in the DNA-based monitoring using feces in other large carnivores. After DNA-extraction, samples were genotyped with eight microsatellite markers used in the monitoring of brown bears for individual identification. For samples collected during spring and early summer (May to July), genotyping success was relatively low for all three methods. In terms of sample storage, the silica-based stored samples displayed slightly higher genotyping success rates than samples stored without silica. From late summer to autumn, success rates increased rapidly, to over 80% in October. The results showed a strong, seasonal variation in genotyping success. The low genotyping success rates of feces in spring and summer compared to e.g. hair samples collected during the same period of the year, might suggest that hair samples could offer a more informative alternative. The genotyping success rate of hair samples from 2018 seemed rather constant across the year and was above 50% during spring time. However, from August on, the genotyping success rates of feces surpassed the success rate of hair samples, and was considerably higher in September and October. For an effective, DNA-based monitoring it is crucial to identify the limiting and influencing factors affecting the success rate. In this study we focused on the sample storage and DNA-extraction. Based on our study, the tested alternatives on both storage and extraction, delivered comparable results, but with samples stored on silica having slightly higher success rates during spring.

Alexander Kopatz, Norwegian Institute for Nature Research (NINA), alexander.kopatz@nina.no
Oddmund Kleven, Norwegian Institute for Nature Research (NINA), oddmund.kleven@nina.no
Øystein Flagstad, Norwegian Institute for Nature Research (NINA), øystein.flagstad@nina.no

Sammendrag

Alexander Kopatz, Oddmund Kleven, Øystein Flagstad. 2020. Årstidsavhengig suksess med DNA-ekstraksjon fra ekskrement-prøver. NINA Rapport 1775. Norsk institutt for naturforskning.

Brunbjørn (*Ursus arctos*) i Norge overvåkes årlig med DNA-baserte metoder av ikke-invasivt innsamlet biologisk materiale for identifisering av individ og kjønn. Ekskrementprøver utgjør mesteparten av materialet som blir samlet inn i løpet av året. For ekskrementprøver samlet inn i løpet av vår og sommer har suksessraten med å fremskaffe genotyper vært relativt lav. Disse prøvene er ofte viktige for forvaltningen ettersom de gjerne samles inn på steder med konflikter mellom mennesker og bjørn, samt ved andre viktige lokaliteter. I dette pilot-studiet har vi undersøkt ulike lagringsmetoder og DNA-ekstraksjonsprotokoller for om mulig å øke suksessratene med genotyping av ekskrementprøver samlet inn i løpet av vår og sommer. Vi benyttet ferske ekskrementprøver samlet inn i perioden mai til oktober 2019 i Troms, Trøndelag og Hedmark av erfarne feltarbeidere. Fra hver ekskrement ble det samlet to prøver, en prøve som ble lagret på rør med silica og en prøve på rør uten silica. Vi inkluderte totalt 94 ekskrement-prøver, som ble ekstrahert med tre ulike metoder, som benyttes for DNA-ekstraksjon fra ekskrementprøver fra andre store rovdyr. Etter DNA-ekstraksjon ble prøvene genotypet med åtte mikrosatelitt-markører, som benyttes i forbindelse med den nasjonale overvåkingen av brunbjørn, for individbestemmelse. For prøver som ble samlet inn i løpet av vår og tidlig sommer (mai til juli) var det relativt lav suksessrate med å fremskaffe genotyper for alle tre metodene. Med hensyn til lagring så var det noe høyere genotyping-suksessrate for prøver lagret på silica sammenliknet med prøver lagret uten silica. Fra sen sommer til høst så økte suksessraten med begge lagringsmetodene til over 80% i oktober. Resultatene viste en sterk sesongavhengig variasjon i genotyping-suksessrate. Den lave suksessraten med å fremskaffe genotyper fra ekskrementprøver samlet inn i løpet av vår og sommer, sammenliknet med f.eks. hårprøver samlet inn på samme tid av året, indikerer at hårprøver er et bedre alternativ i denne tidsperioden. Suksessraten med å fremskaffe genotyper fra hårprøver samlet inn i 2018 var ganske konstant gjennom året og var over 50% for prøver samlet inn om våren. For prøver samlet inn fra og med august var det derimot høyere suksessrate med å fremskaffe genotyper fra ekskrementer sammenliknet med hår, og suksessraten var betydelig høyere for prøver samlet inn i september og oktober. For en effektiv DNA-basert overvåking er det viktig å identifisere faktorer som begrenser og påvirker suksessraten. I dette studiet fokuserte vi på lagringmedium og DNA-ekstraksjonsmetode. Basert på våre resultatervårt studie, så gav de ulike lagringsmediene og DNA-ekstraksjonsmetodene ganske like resultater, men med noe høyere suksessrate for vårprøver lagret på silica.

Alexander Kopatz, Norsk institutt for naturforskning (NINA), alexander.kopatz@nina.no
Oddmund Kleven, Norsk institutt for naturforskning (NINA), oddmund.kleven@nina.no
Øystein Flagstad, Norsk institutt for naturforskning (NINA), øystein.flagstad@nina.no

Contents

Foreword

The brown bear is one of five species in Norway monitored under the national monitoring program for large carnivores. The Norwegian brown bear population is annually monitored using DNA-based methods on mainly non-invasively sampled biological material for identification of individual and sex. Samples are collected throughout the year; from the time brown bears emerge from their winter dens until they return into denning again. Feces samples make up the largest part of biological material collected for monitoring. However, extracting brown bear DNA of fecal samples has shown large differences in success rates likely due to the fecal contents and exposure to environmental conditions, which is also heavily dependent on location, weather and season.

Specifically, feces samples collected during spring and summer have resulted in relatively low genotyping success rates. Such sample material is often collected at sites of human-bear conflict such as livestock predation and a successful analysis of those samples is therefore important for the wildlife management.

Here, we conducted a pilot study to explore alternative fecal storage and DNA-extraction protocols to evaluate potential increase in genotyping success rates of fecal samples collected during spring and summer. Overall, the different storage methods and extraction protocols resulted in similar, although relatively low, genotyping success rates for that period of the year. Based on these findings, we discuss potential implications for the management.

Trondheim, February 2020

Alexander Kopatz

1 Introduction

The DNA-molecule in a biological sample, such as feces or hair, degrades with the time it is exposed to the environment. The longer a sample has been laying in the field uncollected, the lower the success rate for its genetic analysis. Different and changing weather conditions have substantial impact on the preservation of this large molecule (Murphy et al. 2007; Panasci et al. 2011). Warmer environments characterized not only by high temperatures but also humidity and a specific fauna of microorganisms, cause rapid degradation of DNA (Jeffrey et al. 2007; Wultsch et al. 2015). In addition, the content of feces, i.e. diet, also impacts the DNA-analyses, such as from our target species, the brown bear *Ursus arctos* (Wasser et al. 1997; Murphy et al. 2003).

Reports on the DNA-based monitoring of brown bears in Norway described large differences in the success rate after DNA analysis of feces samples from different seasons (see e.g. Aarnes et al. 2016 & 2017; Fløystad et al. 2018). Potentially, the negative results are due to the content of the sample. For instance, samples collected in 2017 in Norway that contained large proportion of grass showed a success rate of only 26% after DNA-analysis. In comparison, the overall success rate of feces samples of the same year was 59% and feces containing mainly berries was 69% (Fløystad et al. 2018). During spring and summer time, brown bears tend to feed also on grass. Open fields and grass meadows are often located near human structures and settlements, such as farms, where potentially conflicts may occur. Therefore, feces collected at those locations during that time of the year is of importance for the management. However, the low success rate does not allow for a feasible utilization of such sample material for individual identification and thus DNA-based monitoring. A similar challenge arises if a brown bear has fed on protein-rich food such as e.g. moose or livestock. In both cases, grass and meat, in addition to the impact of environmental conditions, a combination of enzymes and non-target-DNA may potentially interfere with the genetic analyses.

We conducted a pilot study to evaluate potential alternatives and improvements in laboratory procedures; specifically sample storage and DNA-extraction. Especially the latter can be crucial in the genotyping success (Flagstad et al. 1999; Goossens et al. 2000; Wehausen et al. 2004). Subsequent to the actual sampling, sample storage and DNA-extraction represent the first major steps of human involvement in DNA-based monitoring of wildlife species. Therefore, our goal was to test different storage and DNA-extraction methods in order to find out how to potentially improve the success rate when processing such samples. We used samples from spring, summer and autumn, to evaluate potential differences in sampling and thus genotyping success. An improvement would not only be of service for the monitoring of brown bears, but also of large benefit to the non-invasive genetic monitoring of other wildlife.

2 Material and Methods

2.1 Sample collection

In collaboration with the Norwegian State Nature Inspectorate (Statens naturoppsyn, SNO) and the Inland Norway University of Applied Sciences (INN University) brown bear feces samples were collected by trained and experienced field personnel from May to October 2019 in Troms in northern Norway, Trøndelag in central Norway and Hedmark in southern Norway (**Figure 1**). From each feces found in the field, the personnel took two separate samples: one sample was stored using silica (noncrystalline silica is commonly used to preserve the sample by adsorbing moisture), as done for the regular DNA-based national monitoring of brown bears in Norway, and the other sample was stored in an empty tube without silica, as currently done during the DNA-based monitoring of brown bears in Sweden. The sample material had the size of about 1 cm^3 , which correspondents to the size of a pea to a sugar-cube. All samples included a sheet with sample ID-barcode, date, location and collector's name. Samples were sent in prepared and stamped envelopes as quickly as possible to the laboratory at the Norwegian Institute for Nature Research (NINA) in Trondheim (**Figure 2**). All meta-data of the sample was stored electronically in the Norwegian-Swedish monitoring database Rovbase (www.rovbase.no). At arrival, samples were immediately frozen at -80 °C for at least three days to eliminate potential parasite species before processing (Eckert et al. 2001), and afterwards stored at -20 °C until DNA-analysis. Here on we refer to such samples with silica as "silica" and the ones without silica as "no silica". The samples arrived from June 2019 to January 2020 at the laboratory of NINA in Trondheim. A total of 153 feces was sent in.

***Figure 1**. Feces-sample registration and collection in the field. Photo: Terje Gifstad (SNO).*

2.2 Genetic analyses

The first 94 feces samples to arrive were divided into two batches of 47 samples. We applied three extraction methods for the first batch; QIAamp Fast DNA Stool Mini Kit (Qiagen), the Fast DNA SPIN Kit for Soil (MP Biomedicals) and the Maxwell 16 Tissue DNA Purification Kit in combination with the Maxwell 16 Instrument (Promega) for automated purification of genomic DNA. None of these methods are currently applied in the DNA-based monitoring of brown bears in Scandinavia. After a first evaluation, we only applied two of the methods for the second batch, due to the comparably poor performance of the Fast DNA SPIN Kit for Soil. With originally two samples taken (one on silica and one without silica), a single sample was object to six analyses during the first batch (May to July) and four analyses during the second batch (August to October) leading to a sum of 470 runs in total (**Figure 3**). DNA-extractions followed the manufacturer's instructions. A schematic overview over the analysis-pipeline can be seen in **Figure 3**.

After extraction, samples were genotyped with eight microsatellite or STR-markers (STR=short tandem repeat) and a sex-typing marker (Andreassen et al. 2012; Kopatz et al. 2012). A STR-genotype profile consisted of the eight markers which have been used for individual identification during the non-invasive genetic monitoring in Norway and Sweden previously (Andreassen et al. 2012). Specificity, sensitivity and forensic evaluation of the STR-markers are reported in Andreassen et al. (2012). All samples were analysed in two independent PCR replicates including negative and positive controls. A consensus genotype for each sample was constructed for markers with identical results in both PCR replicates. Samples with a consensus genotype containing at least six markers were defined as successfully genotyped.

***Figure 2**. Example on how the samples were stored and sent from the field. Photo: Tore Solstad (SNO).*

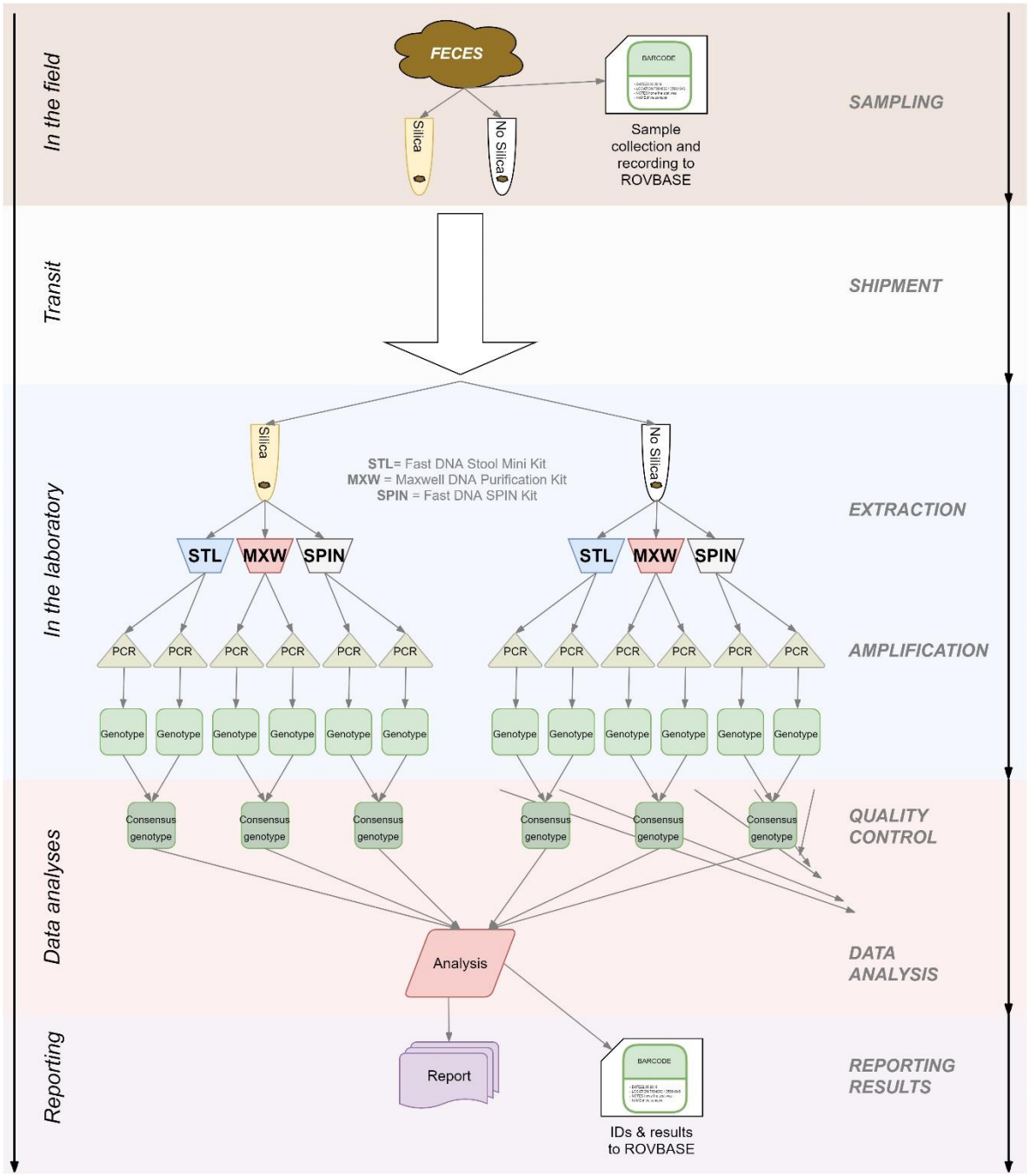

***Figure 3**. Schematic work-flow of the analysis pipeline from sample collection in the field over laboratory analyses to reporting the results of this project.*

3 Results

For samples collected during spring and early summer (May to July), feces stored on silica displayed slightly higher genotyping success rates compared with samples stored without silica (**Figure 4a** and **Figure A1-A3**). From late summer to autumn (August to October) genotyping success rates increased rapidly for both storage methods.

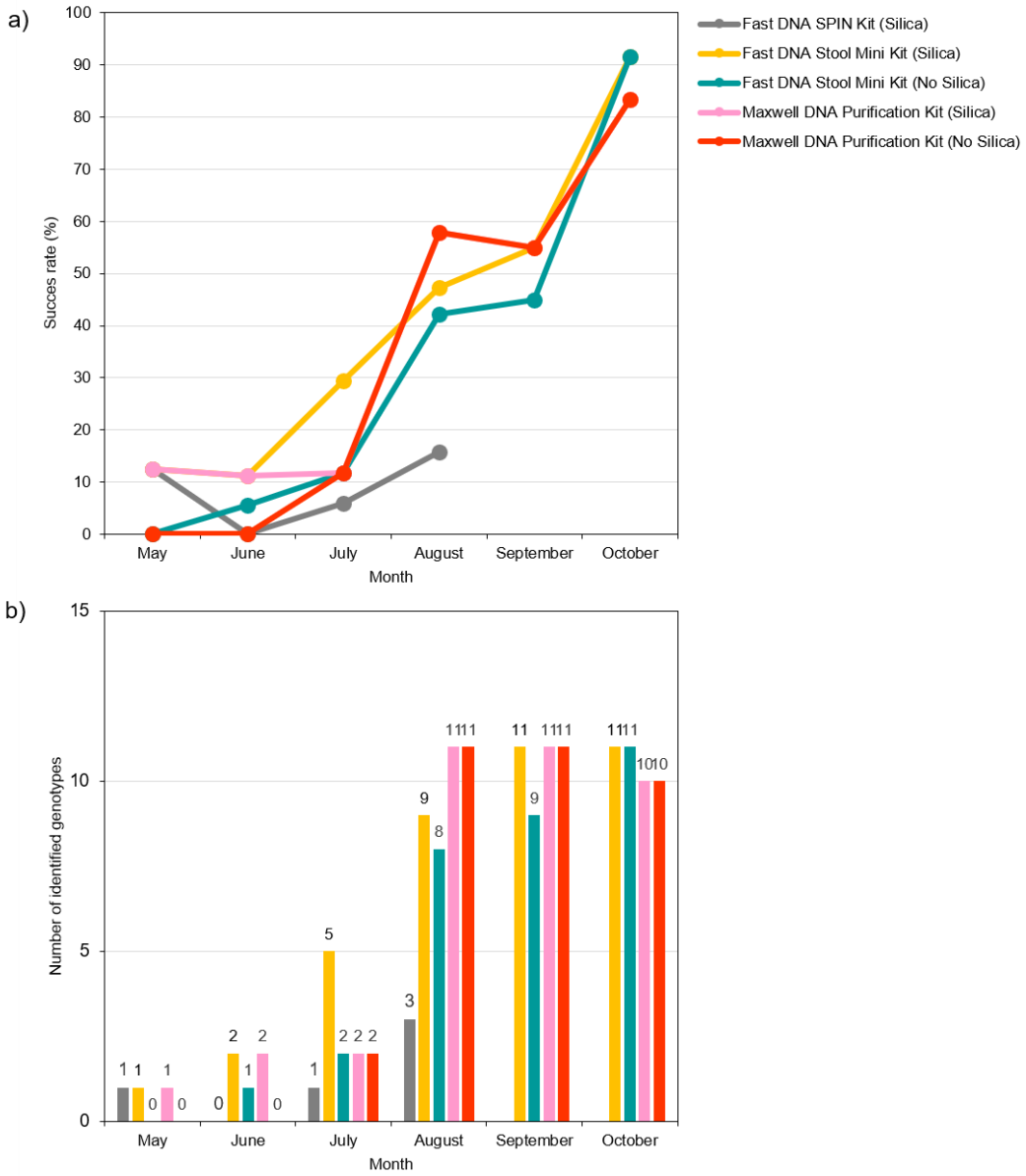

***Figure 4**. Overall genotyping success rate per extraction method and storage (a); see also Figures A1-A3; and the number of identified unique genotypes per method and storage over the months (b).*

The results of genotyping showed a clear seasonal variation in success after extraction and amplification of fecal samples collected throughout the period for each storage and extraction method (**Figure 4b**). While the success was between 0 and 30% in May, June and July, rates increased significantly for all applied methods from August to October to over 80%. Storage of the fecal sample seem to have a minor influence , as silica-stored and samples without silica had similar success rates except for the samples collected from May to July, where the samples stored on silica had a slightly higher success rate. DNA-extraction using the Fast DNA Stool Mini Kit and automated Maxwell DNA Purification Kit also displayed similar success rates, for samples with and without silica. The Fast DNA Spin Kit for Soil, however, showed the lowest success rates (**Figure 4**; see also 2.2 Genetic analyses). These results corresponded closely with the average number of genetic markers amplified and successful determination of the sex (**Figure A4 and A5**).

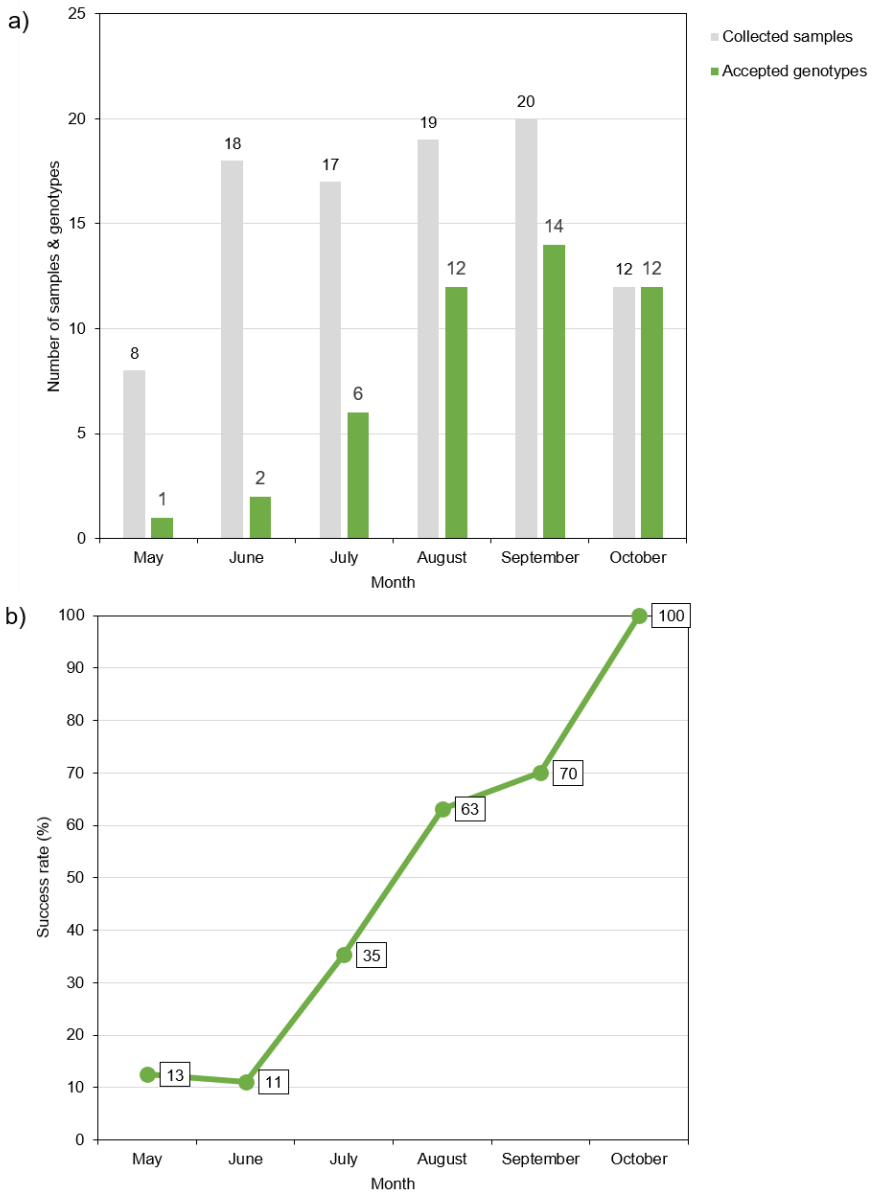

***Figure 5**. Number of samples collected and number of individual genotypes identified based on six STR-markers overall per month (a) and overall success rate across methods and storage (b).*

The number of identified individuals (genotypes) was low from May to July compared to the number of samples collected and then increased from August on (**Figure 5**). From August to October a larger proportion of feces samples were successfully genotyped using both the Fast DNA Stool Mini Kit and the automated Maxwell DNA Purification Kit. Again, the number of positively genotyped markers, including sex-marker, and thus identified individuals increased from spring to autumn (**Figure A4 and A5**). The identified individuals or genotypes were slightly inconsistent across both methods; e.g. while an individual was not identified using one method, the other method identified the individual.

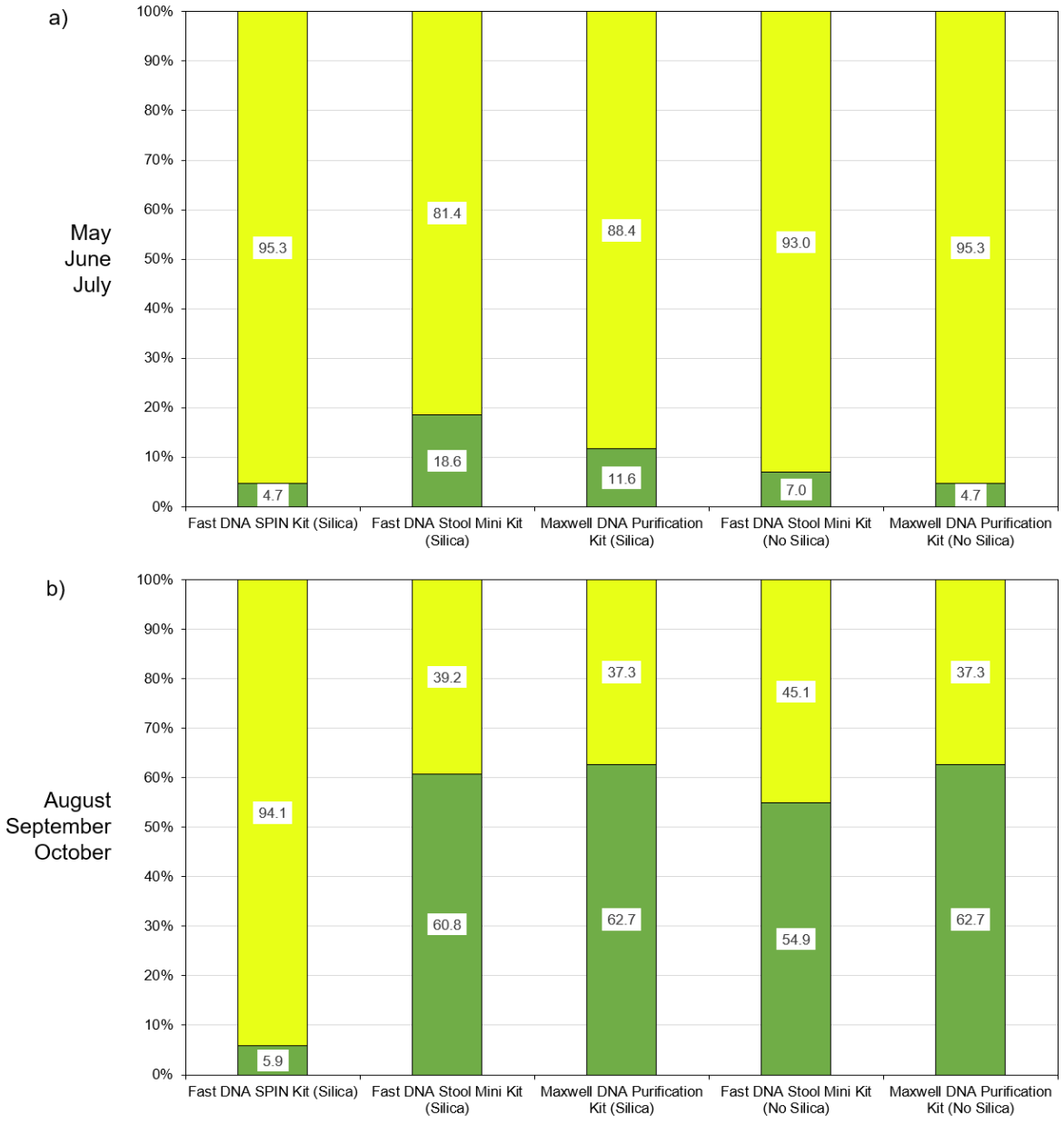

***Figure 6**. Genotyping success rates for each DNA-extraction method from May to July (a) and August to October (b) with the green colour indicating successfully genotyped samples while yellow represents the proportion of negative samples.*

When comparing the genotyping success rates after different DNA-extraction methods were applied and also different sample storage, all approaches showed very low success rate (5 to 19%) between May and July (**Figure 6a**). Success rate increased to 55 to 63% for samples collected between August and October (**Figure 6b**). The Fast DNA Spin Kit for Soil displayed overall a low success rate compared to the other two methods. However, the kit was only applied on a few samples from August, which will likely biased the result on success rate for this period. Overall, DNA-extraction success and genotyping success across all methods and storage increased significantly from spring to autumn (**Figure 5b**). While the genotyping success rate was low in May (13%) and June (11%), the rate increased to 35% in July, 63% in August and 70% in September, displaying a clear seasonal effect on the results.

When plotted on a map, the successfully genotyped versus the negative samples did not show any dependency on geographical location and were not indicative of a potential latitudinal gradient (**Figure 7**) in success rate. Even though there has been a great variation in time passed before a sample arrived at the laboratory, 2 to 21 days, the success rate was not dependent on the time period between sampling and arrival at the laboratory (P=0.8).

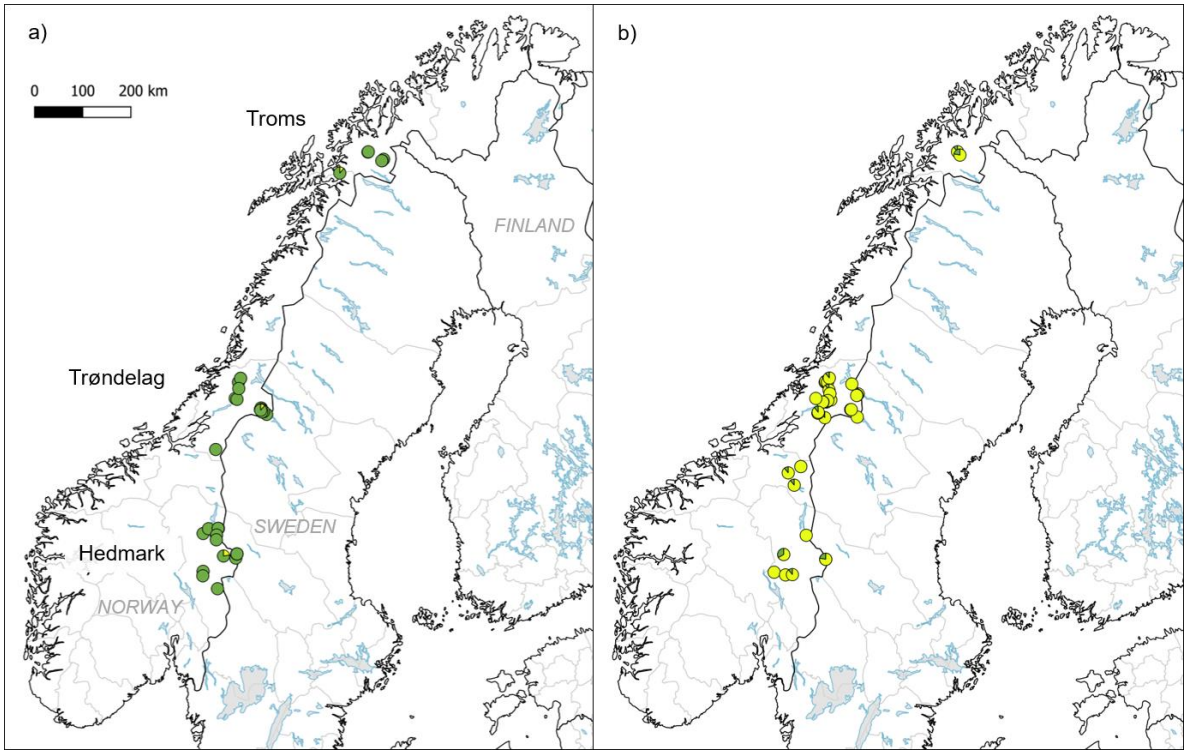

***Figure 7**. Location of the samples used in the analyses: successfully genotyped samples with accepted results (minimum 6 STR-markers) on the left (a); negative samples on the right (b). Cake symbols are representative of the number of markers successfully amplified in green or not in yellow.*

4 Discussion

For feces collected during spring, the once stored on silica had slightly higher genotyping success rates compared with samples stored without silica. From late summer to autumn, the success rate of both storage methods increased and feces collected with and without silica reached success rates of over 80% in October. Hence, our results on samples without silica are comparable to the success achieved in the DNA-based monitoring using feces in Sweden (Naturhistoriska riksmuseet 2018).

DNA-extraction and successful genotyping of brown bear feces showed a strong seasonal variation, whereas differences between storage with or without silica appeared to be small. Also the differences between the two extraction methods, the QIAamp Fast DNA Stool Mini Kit and the Maxwell 16 Tissue DNA Purification Kit in combination with the Maxwell 16 Instrument, were small and results almost similar. The Fast DNA Spin Kit for Soil displayed overall a low success rate compared to the other two methods, thus we had chosen not to apply it further after testing its performance on silica-stored feces in the first batch of 47 samples.

Interestingly, the time a sample was in transit to the laboratory seem to have no, or at least no detectable, negative effect on the extraction and genotyping success. However, we advocate caution regarding the interpretation of the results, given the limited sample size. Instead, and based on the results of numerous studies on the preservation of feces and stool samples from the field, storage and transportation time until genetic analysis should be kept to a minimum to avoid any negative impact on the material (see e.g. Bourgeois et al. 2019). Although based on a limited data set, we did not find any geographical pattern in the difference of success rates. Clearly, the DNA-based monitoring appears to work during late summer and in particular during autumn .

Overall, the success rate for positive DNA and individual identification from brown bear feces is lower than in other large carnivores such as wolverine and wolf (**Table 1**). Although the time period of sampling among these species differs within the year, e.g. feces from wolverines and wolves are mainly collected during winter, the relatively low success rates, especially in spring and early summer, suggest a combination of several factors affecting the sample quality during this time. First, some of the samples collected in spring may be from the previous year and were preserved under snow cover until spring. Second, the diet of the brown bears during this period is likely affecting sample quality. Third, environmental conditions affect preservation of the sample and thus of the DNA. However, the influence of such factors in the Scandinavian environment on the feces and thus genotyping remains unknown. Fourth, there is a growing number of studies indicating that the sampling itself can have significant effect on the genotyping success as epithelial cells, the cells containing the DNA of the target species, are not distributed evenly in the feces (Stenglein et al. 2010; Gulsby et al. 2016; Wultsch et al. 2015; Bourgeois et al. 2019).

To conclude, we still do not know much about the relative importance of the factors described above, and, how they combine to affect the degradation of the DNA-molecule in the sample. The time a sample is exposed to the environment before collected will affect success rate, but this we cannot control for. Similarly, variation in local weather conditions will have impacts which are hard, or impossible, to estimate. Although we have knowledge that the DNA degrades in the field we are still lacking information on the magnitude of the effects all these factors are causing under certain conditions.

***Table 1**. Genotyping success rate of analysed feces samples of wolf, wolverine and brown bear in Norway for the latest, completed sampling season (data from Rovbase).*

	Sampling season	Samples analyzed	Positive samples	Success rate
Brown bear	2018	723	292	**40%**
Wolf	2019	725	522	**72%**
Wolverine	2019	1617	1127	**70%**

Implication for the management

For an effective DNA-based monitoring, it is crucial to identify limiting and influencing factors. In this study we focused on the steps after the sample is collected; storage and DNA-extraction. The tested alternatives on both storage and extraction, delivered comparable results, where samples stored on silica having slightly higher success rates during spring. Our results suggest that other factors, of which dietary contents may be particularly important (see e.g. Fløystad et al. 2018), influence the success of samples collected in spring and summer.

DNA-based monitoring of wildlife is dependent on a sufficient amount of biological samples collected for genetic analysis. Feces are collected mostly in autumn. The low success rates when analysing feces may suggest that e.g. hair samples may offer an alternative, in spring and early summer, as the success rate of these samples appears to be rather constant across the year (Rovdata, *unpublished data*). When comparing the overall success rate on feces in this study versus the genotyping success rate of hair samples collected in 2018, the analysis of feces surpasses the genotyping success rates of hair samples in August, and is considerably higher in September and October (**Figure 8**). However, the results may not be directly comparable due to differences in the laboratory protocols of the genetic analyses.

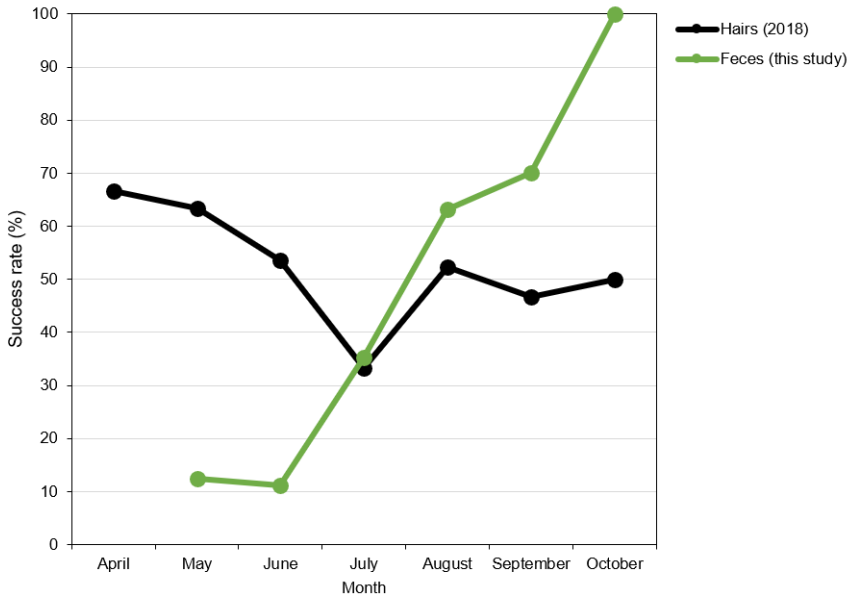

***Figure 8**. Genotyping success rates of brown bear hairs (collected in 2018, data from ROVBASE) and feces samples (this study).*

5 Acknowledgements

We would like to thank Jan Paul Bolstad, Tore Solstad and involved personnel from the Norwegian State Nature Inspectorate (Statens naturoppsyn) as well as Petter Wabakken and Erling Maartmann and their students from the Inland Norway University of Applied Sciences (INN University) for their effort of collecting the samples in the field and support. Line Birkeland Eriksen, Merethe Hagen Spets and Sten Even Erlandsen from the Norwegian Institute for Nature Research (NINA) for the genetic analyses and Henrik Brøseth from Rovdata for support and constructive comments.

6 References

Aarnes, S.G., Brøseth, H., Bakke, B.B., Fløystad, I., Eiken, H.G., & Hagen, S.B. 2017. Populasjonsovervåking av brunbjørn. DNA-analyse av prøver innsamlet i Norge i 2016. NINA Rapport 1340. Norsk institutt for naturforskning.

Aarnes, S.G., Tobiassen, C., Brøseth, H., Bakke, B.B., Eiken, H.G., & Hagen, S.B. 2016. Populasjonsovervåking av brunbjørn. DNA-analyse av prøver innsamlet i Norge i 2015. NIBIO Rapport 56. Norsk institutt for bioøkonomi.

Andreassen, R., Schregel, J., Kopatz, A., Tobiassen, C., Knappskog, P.M., et al. 2012. A forensic DNA profiling system for Northern European brown bears (*Ursus arctos*). Forensic Science International: Genetics 6(6): 798-809.

Bourgeois, S., Kaden, J., Senn, H., Bunnefeld, N., Jeffery, K.J., et al. 2019. Improving cost-efficiency of faecal genotyping: New tools for elephant species. PLOS ONE 14(1): e0210811.

Eckert, J., Gemmell, M.A., Meslin, F.X., and Pawlowski, Z.S. 2001. WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. Organization for Animal Health and World Health Organization.

Flagstad, Ø., Roed, K., Stacy, J.E., & Jakobsen, K.S. 1999. Reliable noninvasive genotyping based on excremental PCR of nuclear DNA purified with a magnetic bead protocol. Molecular Ecology 8(5): 879-883.

Fløystad I., Brøseth H., Banken Bakke B., Eiken H.G. & Hagen S.B. 2018. Populasjonsovervåking av brunbjørn. DNA-analyse av prøver innsamlet i Norge i 2017. NINA Rapport 1494. Norsk institutt for naturforskning.

Goossens, B., Chikhi, L., Utami, S.S., de Ruiter, J., & Bruford, M.W. 2000. A multi-sample, multi-extracts approach for microsatellite analysis of faecal samples in an arboreal ape. Conservation Genetics 1(2): 157-162.

Gulsby, W.D., Killmaster, C.H., Bowers, J.W., Laufenberg, J.S., Sacks, B.N., Statham, M.J. and Miller, K.V. 2016. Efficacy and precision of fecal genotyping to estimate coyote abundance. Wildlife Society Bulletin 40(4): 792-799.

Jeffery, K.J., Abernethy, K.A., Tutin, C.E., & Bruford, M.W. 2007. Biological and environmental degradation of gorilla hair and microsatellite amplification success. Biological Journal of the Linnean Society 91: 281-294.

Kopatz, A., Eiken, H.G., Hagen, S.B., Ruokonen, M., Esparza-Salas, R., et al. 2012. Connectivity and population subdivision at the fringe of a large brown bear (*Ursus arctos*) population in North Western Europe. Conservation Genetics 13(3): 681-692.

Murphy, M.A., Waits, L.P., & Kendall, K.C. 2003. The influence of diet on faecal DNA amplification and sex identification in brown bears (*Ursus arctos*). Molecular Ecology 12(9): 2261-2265.

Murphy M., Kendall K.C., Robinson A. & Waits L.P. 2007. The impact of time and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. Conservation Genetics 8(5): 1219-1224.

Naturhistoriska riksmuseet 2018. Teknisk rapport – Bjørnspillningsinventering 2017. Rapport Dnr 4.1-17-2017. Centrum för Genetisk identifiering.

Panasci, M., Ballard, W.B., Breck, S., Rodriguez, D., Densmore, L.D., Wester, D.B., & Baker, R.J. 2011. Evaluation of fecal DNA preservation techniques and effects of sample age and diet on genotyping success. Journal of Wildlife Management 75(7): 1616-1624.

Stenglein, J.L., De Barba, M., Ausband, D.E., & Waits, L.P. 2010. Impacts of sampling location within faeces on DNA quality in two carnivore species. Molecular Ecology Resources 10: 109-114.

Wasser, S.K., Houston, C.S., Koehiler, G.M., Cadd, G.G., & Fain, S.R. 1997. Techniques for applications of faecal DNA methods to field studies of Ursids. Molecular Ecology 6(11): 1091-1097.

Wehausen, J.D., Ramey, R.R. II, & Epps, C.W. 2004. Experiments in DNA extraction and PCR amplification from Bighorn Sheep feces: the importance of DNA extraction method. Journal of Heredity 95(6): 503-509.

Wultsch, C., Waits, L.P., Hallerman, E.M., & Kelly, M.J. 2015. Optimizing collection methods for non-invasive genetic sampling of Neotropical felids. Wildlife Society Bulletin 39(2): 403-412.

7 Appendices

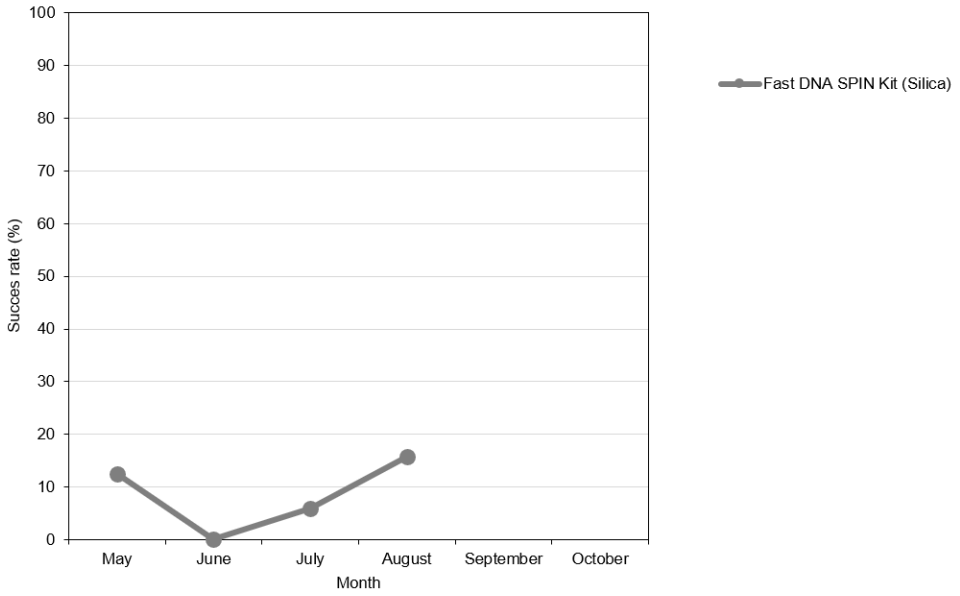

***Figure A1**. Genotyping success rate using the Fast DNA SPIN Kit for Soil (MP Biomedicals) to extract DNA from brown bear feces samples collected from May to August and stored on silica.*

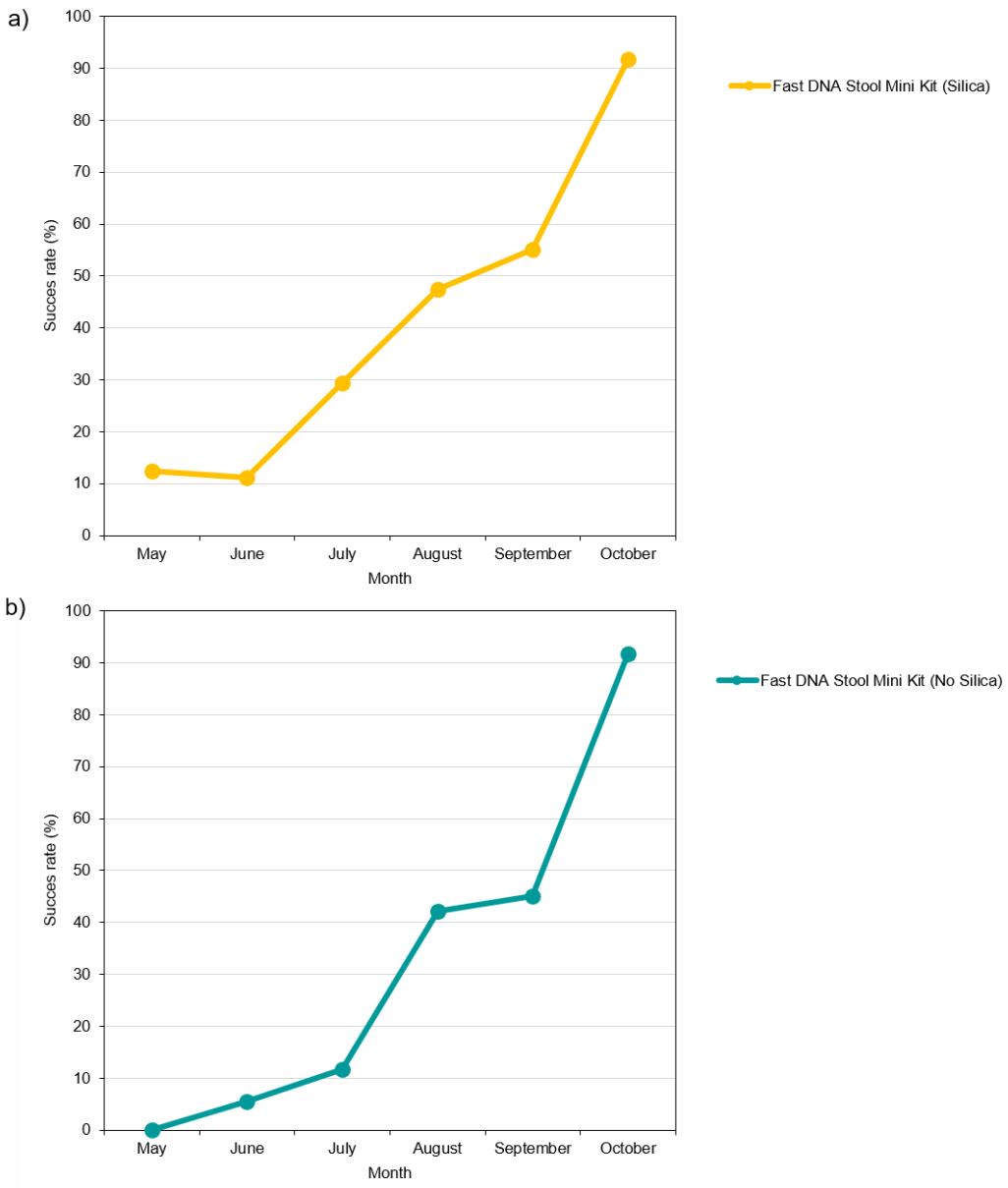

***Figure A2**. Genotyping success rate using the QIAamp Fast DNA Stool Mini Kit (Qiagen) to extract DNA from brown bear feces samples collected from May to October and stored on silica (a) and without silica (b).*

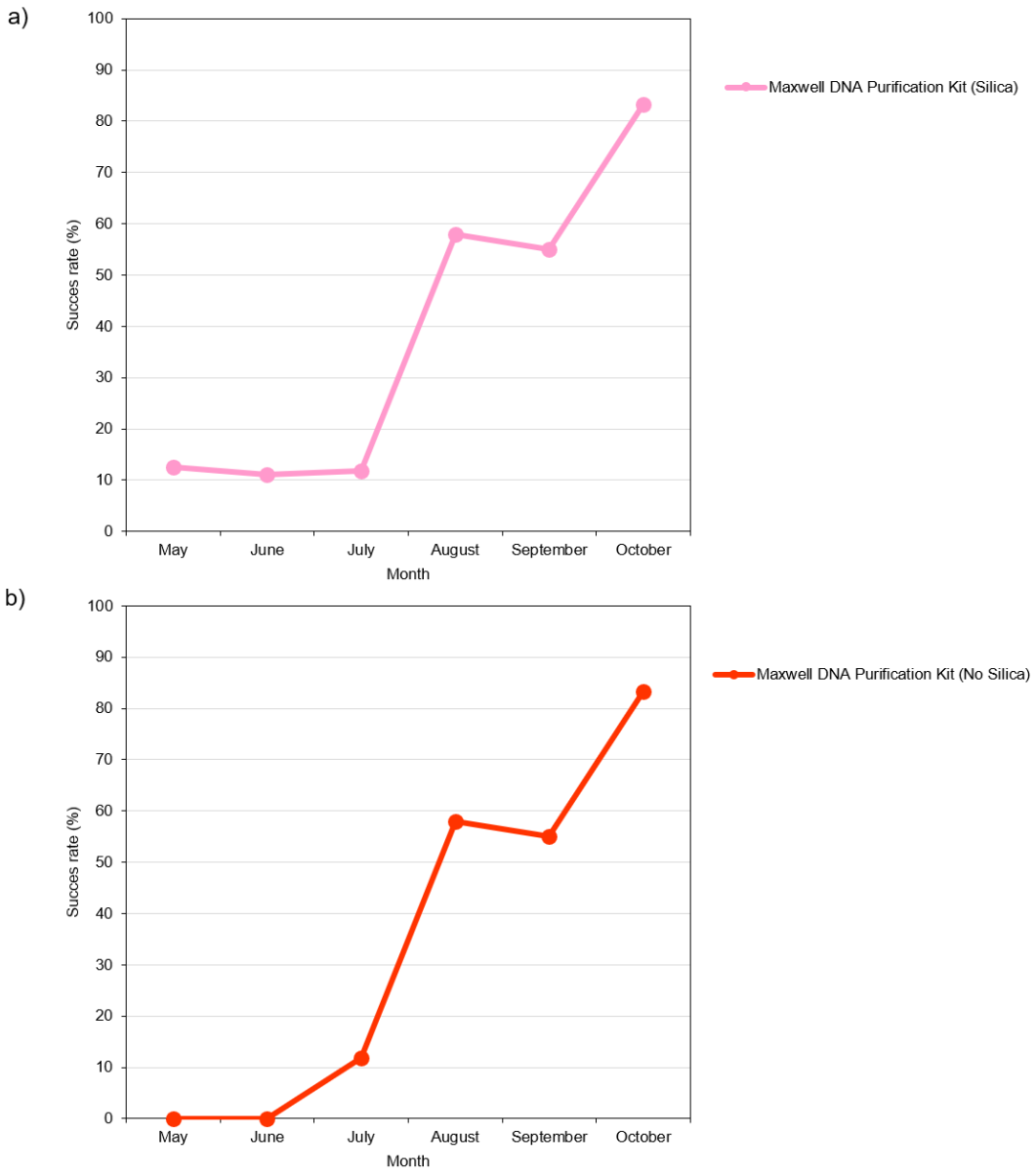

***Figure A3**. Genotyping success rate using the Maxwell 16 Tissue DNA Purification Kit in combination with the Maxwell 16 Instrument (Promega) to extract DNA from brown bear feces samples collected from May to October and stored on silica (a) and without silica (b).*

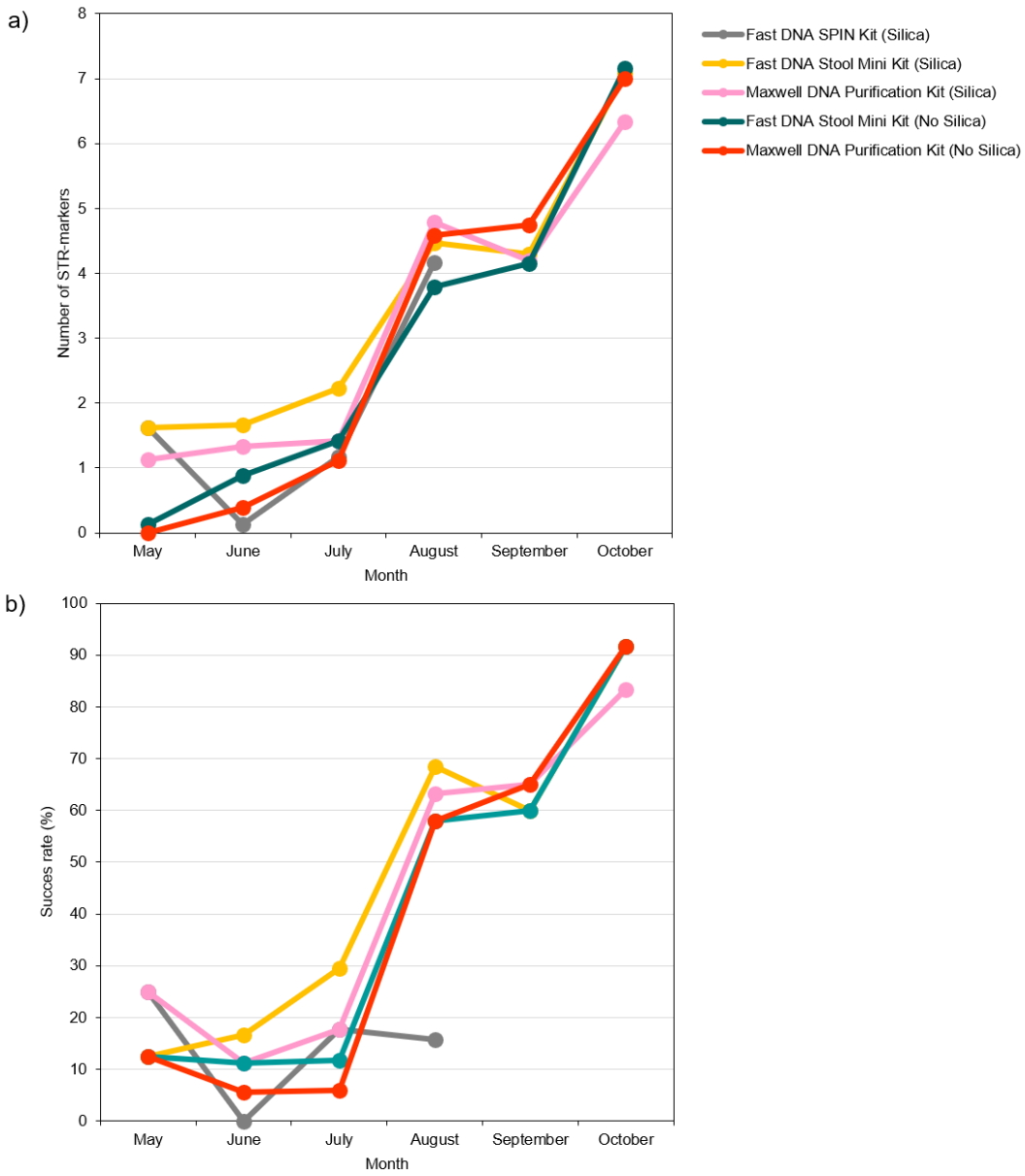

***Figure A4**. Average number of STR-markers amplified across methods and storage and time period (a) and positive identification-rate of the sex-marker across the study period and methods and storage (b).*

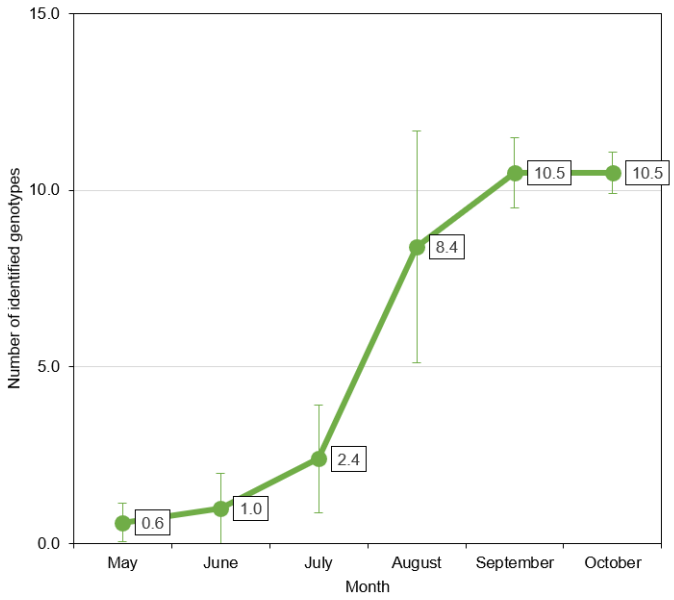

***Figure A5**. Average number of individual genotypes (incl. standard deviation) identified over all methods and time period.*

www.nina.no

The Norwegian Institute for Nature Research, NINA, *is as an independent foundation focusing on environmental research, emphasizing the interaction between human society, natural resources and biodiversity.*

NINA was established in 1988. The headquarters are located in Trondheim, with branches in Tromsø, Lillehammer, Bergen and Oslo. In addition, NINA owns and runs the aquatic research station for wild fish at Ims in Rogaland and the arctic fox breeding center at Oppdal.

NINA's activities include research, environmental impact assessments, environmental monitoring, counselling and evaluation. NINA's scientists come from a wide range of disciplinary backgrounds that include biologists, geographers, geneticists, social scientists, sociologists and more. We have a broad-based expertise on the genetic, population, species, ecosystem and landscape level, in terrestrial, freshwater and coastal marine ecosystems.

1775

NINA Report

ISSN: 1504-3312
ISBN: 978-82-426-4532-6

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

NINA

Cooperation and expertise for a sustainable future