www.nina.no

NINA Kortrapport 3

Otter monitoring Nyhamna, Aukra

Results 2015

Kristine Ulvund, Jiska van Dijk, Oddmund Kleven and Sondre Dahle

NINA

Norsk institutt for naturforskning

NINAs publikasjoner

NINA Rapport
Dette er en elektronisk serie fra 2005 som erstatter de tidligere seriene NINA Fagrapport, NINA Oppdragsmelding og NINA Project Report. Normalt er dette NINAs rapportering til oppdragsgiver etter gjennomført forsknings-, overvåkings- eller utredningsarbeid. I tillegg vil serien favne mye av instituttets øvrige rapportering, for eksempel fra seminarer og konferanser, resultater av eget forsknings- og utredningsarbeid og litteraturstudier. NINA Rapport kan også utgis på annet språk når det er hensiktsmessig.

NINA Kortrapport
Dette er en enklere og ofte kortere rapportform til oppdragsgiver, gjerne for prosjekt med mindre arbeidsomfang enn det som ligger til grunn for NINA Rapport. Det er ikke krav om sammendrag på engelsk. Rapportserien kan også benyttes til framdriftsrapporter eller foreløpige meldinger til oppdragsgiver.

NINA Temahefte
Som navnet angir behandler temaheftene spesielle emner. Heftene utarbeides etter behov og serien favner svært vidt; fra systematiske bestemmelsesnøkler til informasjon om viktige problemstillinger i samfunnet. NINA Temahefte gis vanligvis en populærvitenskapelig form med mer vekt på illustrasjoner enn NINA Rapport.

NINA Fakta
Faktaarkene har som mål å gjøre NINAs forskningsresultater raskt og enkelt tilgjengelig for et større publikum. De sendes til presse, ideelle organisasjoner, naturforvaltningen på ulike nivå, politikere og andre spesielt interesserte. Faktaarkene gir en kort framstilling av noen av våre viktigste forskningstema.

Annen publisering
I tillegg til rapporteringen i NINAs egne serier publiserer instituttets ansatte en stor del av sine vitenskapelige resultater i internasjonale journaler, populærfaglige bøker og tidsskrifter.

www.nina.no

NINA Kortrapport

Otter monitoring Nyhamna, Aukra

Results 2015

Kristine Ulvund
Jiska van Dijk
Oddmund Kleven
Sondre Dahle

NINA

Norsk institutt for naturforskning

Norsk institutt for naturforskning

Ulvund, K., Van Dijk, J., Kleven, O., Dahle, S. 2017. Otter monitoring Nyhamna, Aukra. Results 2015. NINA Kortrapport 3. 18p.

Trondheim, January 2017

ISSN: 2464-2797
ISBN: 978-82-426-2862-6

TILGJENGELIGHET
Åpen

PUBLISERINGSTYPE
Digitalt dokument (pdf)

KVALITETSSIKRET AV
Roel May

ANSVARLIG SIGNATUR
Hans Christian Pedersen (signature)

OPPDRAGSGIVER/BIDRAGSYTER
Shell Norge

OPPDRAGSGIVERS REFERANSE
Contract Number – PO 4512049862, also for 2015-2018

KONTAKTPERSON HOS OPPDRAGSGIVER/BIDRAGSYTER
Mikkel Hedegaard

NØKKELORD
Møre og Romsdal fylke, Eurasisk oter, *Lutra lutra*, Ormen Lange, oterovervåking, bestandstetthet, DNA-analyser

KEY WORDS
Møre and Romsdal county, Ormen Lange, Eurasian otter, *Lutra lutra*, monitoring, population density, DNA-analysis

KONTAKTOPPLYSNINGER

NINA hovedkontor
Postboks 5685 Sluppen
7485 Trondheim
Telefon: 73 80 14 00

NINA Oslo
Gaustadalléen 21
0349 Oslo
Telefon: 73 80 14 00

NINA Tromsø
Framsenteret
9296 Tromsø
Telefon: 77 75 04 00

NINA Lillehammer
Fakkelgården
2624 Lillehammer
Telefon: 73 80 14 00

www.nina.no

Abstract

Ulvund, K., Van Dijk, J., Kleven, O., Dahle, S. 2017. Otter monitoring Nyhamna, Aukra. Results 2015. NINA Kortrapport 3. 18 p.

In February and October 2015, fresh otter faeces were collected at the industrial area Nyhamna, the adjacent coastal zones and on the smaller island adjacent to Aukra. DNA was extracted from 270 samples. Of these 270 samples, 95 rendered a successful otter DNA profile representing 16 females and 12 males. Of the 16 females, ten individuals were picked up only in one of the sampling periods (February or October). Six of the females were picked up in both periods. The DNA results showed 12 different males during the two sampling periods, eight in October and six in February. Only two of the males were identified in both periods. Those individuals identified in both field periods may indicate established adults, but more study years needs to be included for more certainty.

The opportunity, provided by Shell Norge AS to test this non-invasive method regarding collection and storage of otter faeces and jelly gives us a unique possibility to enhance this field and laboratory methodology. It also enables us to improve our understanding of local population density dynamics over time.

Kristine Ulvund, Jiska van Dijk, Oddmund Kleven, Sondre Dahle. Norwegian Institute for Nature Research, Postboks 5685 Sluppen, 7485 Trondheim, Norway. kristine.ulvund@nina.no; jiska.van.dijk@nina.no; oddmund.kleven@nina.no; sondre.dahle@nina.no

Content

Preface

Since 2008, the Norwegian Institute for Nature research (NINA) has conducted otter monitoring in and around the facility Nyhamna on the island of Aukra, commissioned by Shell Norge AS. The project is part of a large environmental monitoring trajectory for which also botanic and marine aspects are included, involving besides NINA also other Norwegian environmental research institutes.

Because research on the Eurasian otter (*Lutra lutra*) is extremely challenging due to its elusive character, the long-term genetic monitoring project at Aukra is a unique opportunity to develop better genetic protocols and to get more insight into population densities and demographic changes over time. The (financial) possibilities given by Shell Norge AS for conducting this research in and around the facility Nyhamna is of great importance to otter research in Europe.

Trondheim, January 2017, Jiska van Dijk (Project leader)

1 Introduction

During the first part of the 20th century, the Eurasian otter (*Lutra lutra*) was distributed throughout Norway. However, after the introduction of a bounty for killed otter and in addition to good fur prices, the otter population declined rapidly during the 1950s and 60s (Christensen 1995). In the late 1970s only remnant populations remained in Nordland and Hedmark Counties. After the protection law came into force during the 1980s, the population slowly recovered and the current distribution is restricted to northern and central parts of the country with a few individuals in the areas south of Bergen towards Oslo. Norwegian otters are presently found both in freshwater and coastal ecosystems. They are however more widespread and abundant along the coast (van Dijk et al. 2016). Due to a decline in registrations in the database of dead otters (i.e. drowned, traffic kills, (il)legal hunting, diseases), the otter is currently listed as vulnerable in the Norwegian Red List (Henriksen & Hilmo, 2015).

The island Aukra, also known as Gossa, located in Aukra municipality, Møre og Romsdal County, is especially known for its industrial area Nyhamna operated by Shell Norge AS. At Nyhamna, natural gas from the gas field Ormen Lange is led onshore, processed and redirected to Easington in England. The industrial facility has been operational since 2007. The Aukra island holds portions of good quality otter habitat and in 2009, when the Nyhamna facility had been operational for two years, Landa et al. (2009) registered 18 individual otters from DNA-analysis of otter spraint (faeces) sampled in the coastal areas in and around the Nyhamna facility.

The main goal of the current long-term otter monitoring in and around Nyhamna is to gain knowledge on how industrial complexes such as Nyhamna affects the otter population in the surrounding area. The monitoring project started in 2008 (Landa et al. 2009) and surveys were again carried out in 2010 and 2011. The monitoring project continued in 2015 with finances for another four-year period. To monitor the local otter population and obtain an estimate of the minimum number of animals living in the study area, we apply a non-invasive method through scat collection. This is followed by DNA-analyses where a capture-recapture method is used to estimate otter abundance, sex distribution and seasonal fluctuations regarding otter individuals. This yearly report will give a summary of the results obtained in 2015.

Figure 1. Overview of the industrial area Nyhamna on the island Aukra, Aukra municipality, Møre og Romsdal county, Norway. On the main island, the archipelago close to it and also at the industrial area Nyhamna, one can find many signs of otter activity. Photo: Jiska van Djik, NINA.

Figure 2. Typical landscape along the coastline of the north-eastern part of the Aukra Island (Gossa). Photo: Sondre Dahle, NINA.

2 Study area

The study area is located on the island Aukra (also referred to as Gossa) in Aukra municipality, Møre og Romsdal County, Norway. The study area for the otter monitoring is limited to the north-eastern part of the island, and includes the coast lines of the facility Nyhamna and the north-western and north-eastern coast lines adjacent to the facility. For an overview map see Figure 3. The Nyhamna facility is an industrial area where the natural gas extracted from the Ormen Lange gas field further north comes onshore for further processing before being exported to Easington in England.

The coastal areas included in this study contains steep ridges with dense dwarf shrubs such as juniper (*Juniperus communis*), dwarf birch (*Betula nana*) and willow (*Salix* spp.) with occasional larger trees such as mountain ash (*Sorbus aucuparia*). The north-eastern side of the study area, facing the inland, is characterized with steep ridges only, interchanged by small beach areas where streams run into the sea. The north-western side of the study area, facing the open sea, is characterized by relatively less steep ridges, also interchanged by small beach like areas where small streams run into the sea, but also with a network of islets in front of the coast line. The islets are partly covered with dense dwarf shrubs as mentioned above and some contain fresh water ditches. An extended kelp forest can be found especially on the north-western side. On the north-eastern side of the island Aukra only kelp forest is found directly adjacent to the shore line. On both sides fish is abundant.
Winter temperatures are relatively mild compared to inland Norway and snow cover is normally a few centimetres deep and last only for a few days before rain takes over again. On the other hand, windy and rainy weather occur frequently especially during autumn, winter and spring.

Figure 3. Map of Norway and where the island of Aukra is situated. The coast lines of the main island and the coast lines of the adjacent small islands in the red circle were included in the study.

3 Method

3.1 Collecting otter spraints and jelly

The faeces from otter is commonly referred to as spraints. In addition, Otters also excrete a jelly-like substance from the intestines, hereafter called jelly. Otter spraints and jelly were collected in February and October 2015 for DNA extraction. We searched for otter spraints and jelly along 1) the coastal line between the north-western most fence of the Nyhamna industrial facility to the leisure harbour at Juvika, 2) the north-eastern most fence around the Nyhamna industrial facility to Hoksnesbukta, and 3) on the islets on the north-western side of the main island. Due to difficulties in synchronizing optimal weather conditions (no precipitation for 48 hours prior to the field day for collecting samples) with the permission to enter the Nyhamna facility we only managed to search for otter spraints along the coastal lines at the Nyhamna facility in October.

DNA degrades relatively fast in otter spraints, hence we targeted fresh (less than and including 2 days old) spraints. Age of the spraints were estimated based on the appearance, i.e., old spraints (over 2 days old) harden quickly and dry out, giving it the colour of black tarry to pale grey, while spraints less than and including 2 days old are more soft and the black tarry mucous have not hardened yet. Very fresh spraints from the night before often still have air bubbles visible (Figure 4).

Ethanol and silica beads are both commonly used as storage media for scats in wildlife research. To test which of these storage media that conserve otter spraints best, we sampled two pieces of each otter spraint and stored one piece in 96% ethanol (Figure 5) and the other in a container with silica beads. This duplicate sampling were conducted for most otter spraints, while for a few spraints only one sample was obtained and stored in either ethanol or silica beads. Otter jelly samples were stored in either lysis buffer (i.e. a salt solution used for the purpose of breaking open cells for use in DNA analysis), ethanol or silica beads. The geographic position for each sample was recorded using a handheld GPS (outside the industrial facility) or map (inside the industrial facility). In addition to sampling otter spraints and jelly, we also registered day-beds and denning areas.

Figure 4. An example of a very fresh otter spraint from the night before (0 days old) with the air bubbles still visible. Photo: Jiska van Dijk, NINA.

3.2 DNA-analyses

DNA extraction

DNA from otter spraint and jelly samples was extracted using 'FastDNA SPIN Kit for Soil' and associated protocol (http://www.mpbio.com). The DNA was eluted in 200µl elution buffer (Buffer AE; Qiagen) and stored at -20 °C.

Species and sex determination

As spraints from otter and American mink (*Neovison vison*) can be difficult to distinguish in the field, and because American mink are present in our study area, we applied a recently developed quantitative polymerase chain reaction (qPCR) method (O'Neill et al. 2013) to determine whether the spraint originated from otter. Sex was also molecularly determined by using a quantitative polymerase chain reaction (qPCR) method, as described by O'Neill et al. (2013).

Microsatellite analysis

The qPCR method provide information about the DNA concentration in a sample. Based on previous analyses (own unpublished data) we have found that a DNA concentration above a certain threshold is required for successful DNA-profiling. Hence, data generated from the species-typing qPCR assay were used to determine which samples that contained a sufficient DNA concentration for microsatellite genotyping. Samples with a sufficient DNA concentration were amplified using PCR for a marker set consisting of 10 variable microsatellite-markers previously developed from the otter (Lut435, Lut453, Lut604, Lut715, Lut717, Lut733, Lut782, Lut818, Lut832 and Lut833; Dallas & Piertney 1998). The selected microsatellites amplifies relatively short amplicons (<250 base-par) which facilitates analysis of non-invasively collected samples, like otter scats, as amplification success and allele-dropout are affected by amplicon size when genotyping DNA from non-invasively collected samples (Broquet et al. 2007). To streamline analysis and reduce costs the microsatellite markers

were amplified in two multiplex sets with four and six markers, respectively. Alleles were separated using capillary electrophoresis on an ABI 3130xl Genetic Analyzer and sizes assigned using GeneMapper software (Applied Biosystems). DNA from each sample was analysed in three (or more if required) independent PCR replicates and a consensus genotype was constructed based on the following criteria: loci with a heterozygote result had to show this in two independent PCRs while loci with a homozygote result had to show this in three independent PCRs. Samples with a consensus genotype containing at least seven loci were used for individual identification. Unique genotypes were identified by using allelematch (Galpern et al. 2012).

Figure 5. Fresh otter spraints collected on ethanol. Photo: Sondre Dahle, NINA.

4 Results

In total, we extracted DNA from 270 spraint and jelly samples. Of the 270 samples, 259 (96%) were identified as otter and 199 contained a DNA concentration sufficient for microsatellite genotyping (see methods). Almost half (95/199) of the genotyped samples resulted in a successful DNA-profile. The 95 samples with a successful DNA profile represented 16 females and 12 males (Table 1 and Figure 6). Of the 16 females, there were five individual females that were only identified in either February or October respectively (Table 1, Figure 3). Six individual females were identified from DNA in both periods (Table 1, Figure 7). The DNA results showed 12 different males during the two sampling periods, eight in October and six in February. Only two of the males were identified in both periods (Table 1, Figure 8).

Otter jelly

Five otter jelly samples were analysed after being stored on both silica and lysis buffer. Of these five, only one sample could be identified to an individual otter. Both samples on silica and buffer worked. Four jelly samples were divided into two samples and stored separately on both ethanol and silica. Of these, one sample on ethanol and one sample on silica could be identified to individual otters. Because too few jelly samples were collected, we cannot conclude on the best sampling method for otter jelly.

Otter spraints

Seventyone otter spraints were included to test which storage medium that best preserved DNA. Of the 71 sample pairs, 23 samples stored in ethanol resulted in a DNA-profile, while only 11 samples stored on silica resulted in a DNA-profile (McNemar's test; P=0.002). One sample pair only rendered results from the silica-stored sample. For 12 sample pairs, only the samples stored on ethanol gave results, and for the remaining 10 both samples gave results. The percentage of functioning samples in the two periods were 40% in February and 32% in October.

Table 1. Overview of the individual otters identified by DNA-analysis of spraint and jelly samples collected in February and October 2015 near the Ormen Lange gas field on the main island of Aukra (Gossa) and smaller adjacent islands, Aukra municipality. In bold are the otter individuals identified both in February and in October.

Females			***Males***		
Id number	***Month***	***No. of DNA samples***	***Id number***	***Month***	***No. of DNA samples***
Ind003	**February**	**3**	**Ind001**	**February**	**4**
	October	**2**		**October**	**1**
Ind004	February	2	Ind002	February	7
Ind005	February	2	Ind008	February	4
Ind006	February	3	Ind009	February	3
Ind007	**February**	**3**	Ind012	February	3
	October	**2**	**Ind017**	**February**	**1**
Ind010	**February**	**5**		**October**	**1**
	October	**2**	Ind019	October	1
Ind011	**February**	**5**	Ind020	October	1
	October	**2**	Ind021	October	4
Ind013	February	6	Ind022	October	1
Ind014	**February**	**1**	Ind023	October	2
	October	**1**	Ind026	October	1
Ind015	**February**	**5**			
	October	**4**			
Ind018	February	2			
Ind024	October	2			
Ind025	October	3			
Ind027	October	1			
Ind028	October	1			
Ind029	October	1			

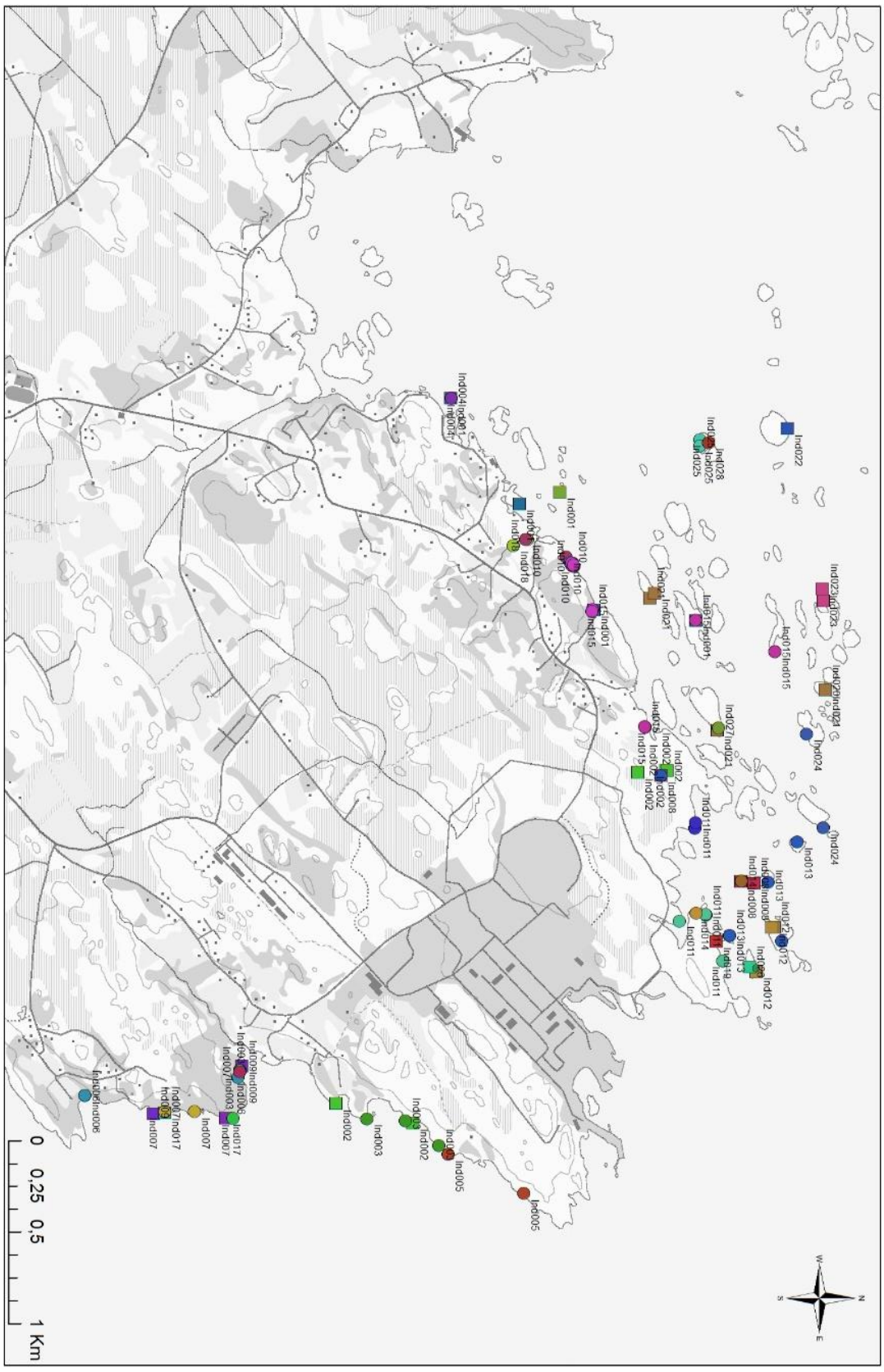

Figure 6. Overview of otter spraint and jelly samples analyzed to individual otters. The samples were collected in February and October 2015 near the Ormen Lange gas field on the Island of Aukra (Gossa), Aukra municipality. Circles indicate females, squares indicate males, while different colours indicate different individuals.

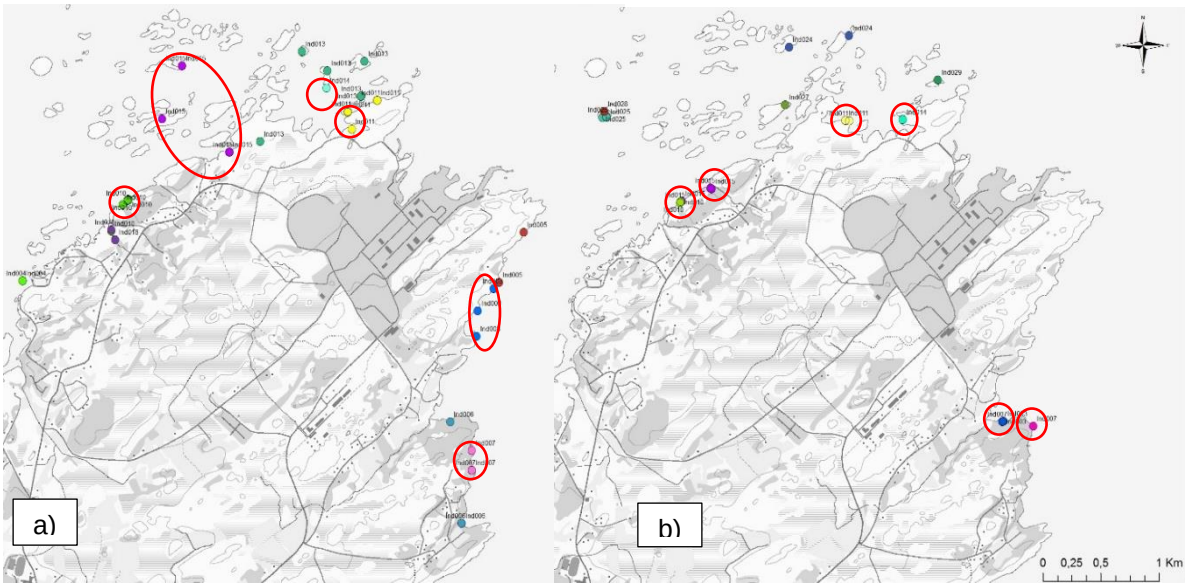

Figure 7. Overview of otter spraint and jelly samples identifying otter females. The samples were collected along the shoreline of the main island and adjacent smaller islands of Aukra (Gossa), Aukra municipality in February (a) and October (b) 2015. The different colours indicate different individuals. Red circles marks individuals found in both February and October 2015.

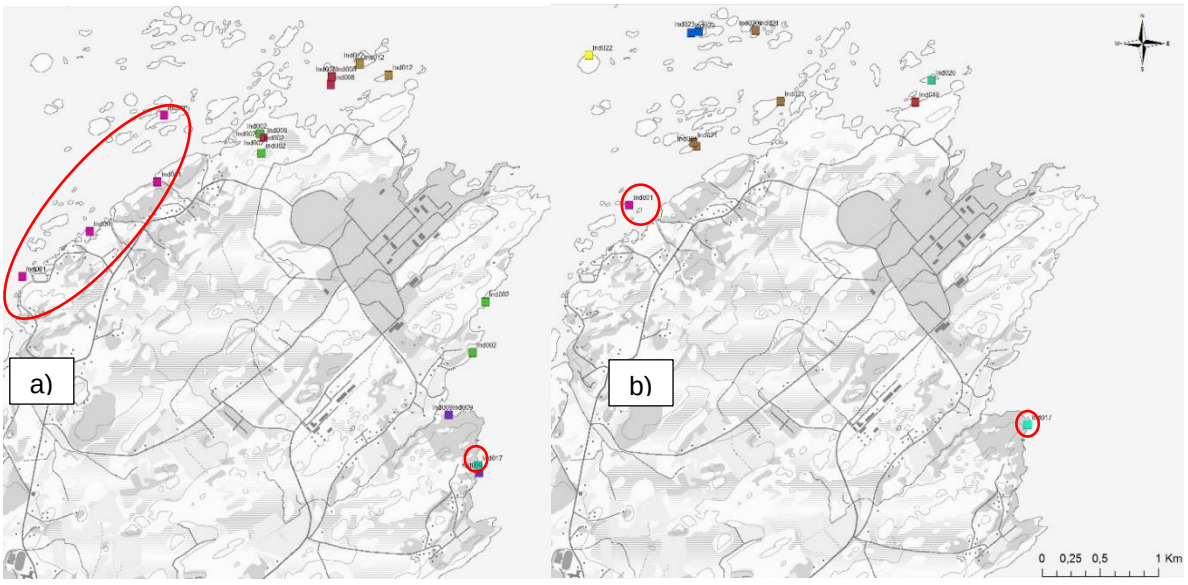

Figure 8. Overview of otter spraint and jelly samples identifying otter males. The samples were collected along the shoreline of the main island and adjacent smaller islands of Aukra (Gossa), Aukra municipality in February (a) and October (b) 2015. The different colours indicate different individuals. Red circles marks individuals found in both February and October 2015.

5 Discussion

In Norway, the distribution of the Eurasian otter is restricted to the northern and central parts of the country with few individuals south of Bergen. Along the coast, especially north of Bergen, the otter is relatively widespread and abundant (van Dijk et al. 2016). Otters were protected from hunting in 1982 after being hunted to near extinction (Christensen 1995; Heggberget 1996). Since 1982, the otter has made a gradual recovery and has become a more common sight along the coast. Although the otter has become more widespread along the coast in Norway, it is still listed as vulnerable in the Norwegian Red List (Henriksen & Hilmo 2015). This is partly due to the recorded reduction in otter registrations through the national otter monitoring. The national monitoring of otters is based on observations recorded in "Oterfallviltbasen" (1987-2016), "Hjorteviltregisteret" (1987-2014) and online in "Artsobservasjoner.no" (1987-2014). In later years, especially the number of dead otters registered in the database has been decreasing. Whether this is because the actual population is declining or because people are less eager to register otters is unknown. It is therefore of utmost importance to get more knowledge on local otter densities and demographic changes over time, which in turn can be used to estimate otter densities over larger regions. The sampling of DNA from otter spraint and jelly on the island Aukra gives a unique opportunity to gather data on otters using a non-invasive method. DNA genotyping of otter spraints potentially provides estimates of population size, home ranges, dispersal and genetic diversity.

Analysis

The spraints and jelly samples collected at Aukra in February and October 2015 held relatively good quality DNA. Of the 270 collected samples, 96% were identified as otter. The remaining four percent could be either mink or non-working samples. From the 259 samples identified as otter, 76% (199/259) contained a DNA concentration sufficient for microsatellite genotyping, i.e. individual identification. About half of these samples (95/199) resulted in a successful DNA-profile and a confirmed individual. This gives a success rate of ca. 31%. There was no significant difference in the number of functioning samples in the two periods (40% in February and 32% in October). This result is comparable to work conducted on river otters (Lerone et al. 2014). The American mink is also present on and around the island. Spraints from mink can easily be confused with otter spraints in the field. Lab-analysis (qPCR) were therefore performed to confirm that the spraints and jelly were from otters (O'Neill et al. 2013). The analysis does not distinguish between non-working samples and mink spraints. We therefore cannot give a number on potential mink spraints sampled.

The 95 samples with a successful DNA profile represented 16 females and 12 males (Table 1 and Figure 6). Of the 16 females, six were identified in both periods, whereas five individuals were picked up in only one of the periods, respectably. (Table 1, Figure 7). The DNA results showed 12 different males during the two sampling periods, eight in October and six in February. Only two of the males were identified in both periods (Table 1, Figure 8). The fact that ten out of 16 females and ten out of 12 males were identified from DNA-samples in either February or October, but not both periods, may point to possible demographic changes in the otter population on Aukra (i.e. individuals leaving the area or die) or possible failure to monitor all present individuals. As the otters reproduce all year round, they give

birth to cubs also all year round. It is therefore still too early to conclude that the high proportion of different individuals identified in the two periods can be explained by demographic changes or error in monitoring level. As DNA-analysis only gives us the individual otter, but no information on age, we cannot rule out the possibility that some of the otters that were identified in February were cubs born between October the year before and February. However when more results from the years to come become available, we may see a pattern in established otters (likely adults) versus non-established otters (likely cubs and young adults). With continued sampling of otter spraints and jelly we will also be able to analyse the survival rate of otters on Aukra.

Storage medium

It is difficult to obtain good enough quality on DNA extracted from otter spraints. The sampling of only fresh spraints under favourable weather conditions (i.e. dry stable weather, preferably around 0 °C) and correct storage is therefore important. We used 71 otter spraints to test which storage medium best preserved the DNA. From the paired sampling analysis, we found a clear indication that spraint samples from otter are best preserved using ethanol. To reduce the evaporation of ethanol, the samples should be stored in the freezer.

Five jelly samples were collected and stored on both ethanol and silica. However, only one of the samples could be identified to an individual otter. In this case, both the parts of the sample (on ethanol and silica beads) gave a result. We can therefore not conclude on the best storage medium for jelly samples.

6 References

Broquet T, Menard N & Petit E (2007) Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conserv Genet 8(1):249–260.

Christensen, H (1995) Determinants of otter *Lutra lutra* distribution in Norway. Effects of harvest, polyclorinated biphenyls (PCBs), human population density and competition with mink *Mustela vison*. Dr. scient. thesis. - Department of zoology, University of Trondheim, Trondheim.

Dallas JF & Piertney SB (1998) Microsatellite primers for the Eurasian otter. Mol Ecol 7 (9):1248-1251.

Galpern P, Manseau M, Hettinga P, Smith K & Wilson P (2012) Allelematch: an R package for identifying unique multilocus genotypes where genotyping error and missing data may be present. Mol Ecol Res 12 (4):771-778. doi:10.1111/j.1755-0998.2012.03137.x

Heggberget, TM (1996) En kunnskapsoversikt for eurasiatisk oter Lutra lutra; grunnlag for en forvaltningsplan. – NINA Oppdragsmelding 439: 1-29.

Henriksen S & Hilmo O (2015) Rødlista - hva, hvem, hvorfor? Norsk rødliste for arter 2015. Artsdatabanken <http://www.artsdatabanken.no/Rodliste/HvaHvemHvorfor>.

Lerone L, Mengoni C, Carpaneto GM, Ettore R & Loy A (2014) Procedures to genotype problematic non-invasive otter (*Lutra lutra*) samples. Acta Theriol (2014) 59: 511. doi:10.1007/s13364-014-0195-8

O'neill D, Turner PD, O'meara DB, Chadwick EA, Coffey L & O'reilly C (2013) Development of novel real-time TaqMan®PCR assays for the species and sex identification of otter (Lutra lutra) and their application to noninvasive genetic monitoring. Molecular Ecology Resources 13(5): 877-883.

Van Dijk J, May R, Hamre Ø & Solem MI (2016) Kartlegging av oterfallvilt for perioden 2011-2015 og verifisering av otertilstedeværelse i ulike deler av Norge. NINA Rapport 1229. 32 s.

ISSN: 2464-2797
ISBN: 978-82-426-2862-6

Norsk institutt for naturforskning
NINA Hovedkontor
Postadresse: Postboks 5685 Sluppen, 7485 Trondheim
Besøks-/leveringsadresse: Høgskoleringen 9, 7034 Trondheim
Telefon: 73 80 14 00, Telefaks: 73 80 14 01
E-post: firmapost@nina.no
Organisasjonsnummer 9500 37 687
http://www.nina.no

Samarbeid og kunnskap for framtidas miljøløsninger