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Monitoring of Eurasian otter (*Lutra lutra*) around Nyhamna (Aukra municipality) on the western coast of Norway

Final report 2015-2018

Jiska van Dijk, Juan Carrillo, Øyvind Hamre and Oddmund Kleven

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Norwegian Institute for Nature Research

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COVER PICTURE
Otters like to wash their salty fur in fresh water pools © Jiska van Dijk

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- Lokal tetthetsberegning

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Abstract

Van Dijk, J., Carrillo, J., Hamre, Ø. og Kleven, O. 2020. Monitoring of Eurasian otter (*Lutra lutra*) around Nyhamna (Aukra municipality) on the western coast of Norway. Final report 2015-2018. NINA Report 1713. Norwegian Institute for Nature Research.

The main objectives of the long-term otter monitoring in and around Nyhamna is to gain knowledge on how industrial complexes such as Nyhamna affect the otter population in the surrounding area and to estimate otter density in the study area using DNA-analysis of non-invasively collected faecal samples. The monitoring project started in 2008 (Landa et al. 2009) and surveys were repeated in 2010, 2011 and 2012. The monitoring project continued in 2015 with finances for another four-year period which ended in 2018. To monitor the local otter population and obtain an estimate of the minimum number of animals living in the study area, we apply DNA-analysis of non-invasively collected faecal samples to identify individual otters. Taking the otter individuals that were found in different samples over the year and for which we can say that they are likely to be residential otters an estimation of the local otter density was calculated.
DNA was extracted from 593 spraints and 268 of these samples resulted in a successful DNA-profile and a confirmed individual, which represents a success rate of ca. 45.2%. The 268 samples with confirmed individuals, were identified as being 20 females and 27 males. Females were often re-sampled (15 of the in total 20 females) and found in different samples in different years whereas males were less often re-sampled (nine of the in total 27 males), indicating that the female population is much more stable and resident than the more fluctuating and dispersed male population.
The territories of males and females showed to partially overlap, similarly to other studies in Europe and we estimated the otter density on Aukra to be of 3.1 resident otters per 10 km of coastline when considering the otter individuals in 2018 that were also sampled in 2017. When looking at 2018 only, a total of 20 individuals were identified including both resident and non-resident otters resulting in an estimated population density of 4.4 individuals per 10 km of coastline.
Unfortunately the samples collected on the industrial plant, inside the fence, were unsuccessful in DNA-profiling apart from one sample. It was remarkable that very few fresh spraints were found on the plant compared to the earlier study by Landa et al. (2008-2012). The intensive building activity during 2015 and early 2016 apparently resulted in less attractive otter habitat and otter activity on the plant. However as seen from the number of samples found and successfully DNA-profiled outside the fence of the industrial plant, together with our density estimation similar to the earlier study by Landa et al., the otter population may not have been affected by the constructions as such.

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Sammendrag

Van Dijk, J., Carrillo, J., Hamre, Ø. og Kleven, O. 2020. Monitoring of Eurasian otter (*Lutra lutra*) around Nyhamna (Aukra municipality) on the western coast of Norway. Final report 2015-2018. NINA Report 1713. Norwegian Institute for Nature Research.

Hovedmålene med den langsiktige oterovervåkningen i og rundt industrianlegget Nyhamna er å få kunnskap om hvordan industrikomplekser som Nyhamna påvirker lokale oterpopulasjoner og å estimere den lokale otertettheten ved hjelp av DNA-analyse av innsamlet ekskrementer. Overvåkningsprosjektet startet i 2008 (Landa et al. 2009) med gjentatte kartlegginger i 2010, 2011 og 2012. Prosjektet fortsatte i 2015 med finansiering for ytterligere fire år og ble avsluttet i 2018. For å overvåke den lokale oterpopulasjonen og få et estimat av minimum antall dyr som bor i studieområdet, brukte vi DNA-analyse av innsamlet avføring for å identifisere individuelle otere. Ved å bruke Oterindivider gjenfunnet i forskjellige prøver fra tidligere år, noe som tilsier at de sannsynligvis er etablert i områder, ble det beregnet et lokale otertetthet.

DNA ble funnet i 593 prøver hvorav 268 av prøvene resulterte i en vellykket DNA-profil og et bekreftet oterindivid. Det representerer en suksessrate på ca. 45,2%. Av de 268 prøvene ble 20 hunn- og 27 hannotre identifisert. Hunnoterindivider ble ofte gjenfunnet på nytt fra forskjellige prøver forskjellige år (15 av 20 hunnotre), mens hannotere sjeldnere ble gjenfunnet (9 av 27 hannotre). Dette indikerer at hunnotre er mye mer stabile og etablerte i området enn hannotre.

Territoriene til hanner og hunner viste seg delvis å overlappe. Dette er også vist i andre studier i Europa. Vi har estimert Aukras lokale otertetthet til 3,1 etablerte individer per 10 km kystlinje. I denne analysen inkluderte vi oterindividene som var registrert både i 2018 og 2017. Dersom man estimerer tetthet basert på alle de 20 individene som ble registrert i 2018 (både gjenfunnet og ikke gjenfunnet tidligere år), ble den estimerte lokale otertettheten 4,4 individer per 10 km kystlinje.

Bortsett fra en vellykket prøve, lyktes det ikke med DNA-profilering på de øvrige prøvene som ble samlet inn innenfor gjerdet på Nyhamna industrianlegget. Det er bemerkelsesverdig at det ble funnet svært få ferske avføringsprøver på industrianlegget sammenlignet med den forrige studien av Landa et al. (2008-2012). De intensive bygningsaktivitetene i løpet av 2015 og 2016 resulterte i mindre attraktive leveområder og mindre aktivitet av oter innenfor gjerdet. Imidlertid, sett ut fra det store antallet avføringsprøver som ble funnet utenfor industrianlegget, og vår lokal tetthetsberegning av oter som viser sammenlignbare tettheter med den forrige studien av Landa et al. fra 2012, ser det ut til at oterbestanden på Aukra sannsynligvis ikke har blitt påvirket av de intensive bygningsaktivitetene på anlegget.

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Contents

Foreword

With financial contribution from Shell AS we were able to continue our study on local otter density estimation between 2015-2018. Otter is known as an elusive species currently on the red list of Norway. Although the species may seem abundant and increasing in its population size by locals, it is difficult to obtain scientific verification of this. The methods developed in this study helps us tremendously to increase our knowledge on especially the otter along the Norwegian coast. Also the second aim of our study, i.e. to look at the impact of the extensive building activities in 2015 on the local otter population, gave us valuable insights on how otters deal with these areal changes and disturbances. We therefore want to thank Shell AS for the financial possibility and for their contribution to the realization of new knowledge on the charismatic but elusive species.

15th of May 2020, Jiska van Dijk

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 otters (Heggberget 2007), in addition to good fur prices, the otter population declined rapidly during the 1950's and 60's (Christensen 1995). Also, diminishing salmon and trout populations as a result of bad water quality and water acidification in southern parts of Norway can be seen as contributory causes for the decline of the otter population (Valeur 1970). In the late 1970's only remnant viable populations remained in Nordland and Hedmark counties. Since the protection law (viltloven) came into force in 1982, the population has been slowly recovering 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 NINA's database of dead otters (e.g. 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 at Nyhamna, which is 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 affect the otter population in the surrounding area. The monitoring project started in 2008 (Landa et al. 2009) and surveys were repeated in 2010 and 2011. The monitoring project continued in 2015 with finances for another four-year period which ended in 2018. To monitor the local otter population and obtain an estimate of the minimum number of animals living in the study area, we apply DNA-analysis of non-invasively collected faecal samples to identify individual otters. Including otter individuals that were repeatedly found in different samples over several years (minimal two years) and for which we can say that they are most likely residential otters an estimation of the local otter density was calculated.. This final report gives a summary of the results obtained between 2015 and 2018.

Figure 1. *Overview of the industrial area Nyhamna on the island Aukra, Aukra municipality, Møre og Romsdal county, Norway. On the main island, the adjacent archipelago and also within the Nyhamna industrial area, one can find many signs of otter activity such as spraints, otter paths, day beds and dens. Photo: Jiska van Dijk, 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 coastline of the Nyhamna facility and the north-western and north-eastern coastlines adjacent to the facility (Figures 1 and 2). 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 contain 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 mainland, is characterized by 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 coastline. The islets are partly covered with dense dwarf shrubs as mentioned above and some contain fresh water pools. An extensive kelp forest can be found especially on the north-western side. On the north-eastern side of the Aukra island kelp forest is found only directly adjacent to the shoreline. On both sides fish are abundant.

Winter temperatures are relatively mild compared to the Norwegian interior. Snow cover is normally just a few centimetres deep and lasts 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 Aukra and its location in Norway (small map). The coastline of the main island and the coastline of the adjacent small islands within 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. Captive Eurasian otters produce this jelly when they have not fed for a day or more (Carss and Parkinson 1996), but the excretion of this remains unclear (Kruuk 2006). Otter spraints and jelly were collected throughout six sampling rounds in four years: February and October 2015, March and November 2016, November 2017 and January/February 2018. We searched for otter spraints and jelly along the coastline 1) between the north-western most fence of the Nyhamna industrial facility to the leisure harbour at Juvika (see also Figure 6a and 6b), 2) between the north-eastern most fence around the Nyhamna industrial facility to Hoksnesbukta (see also Figure 6c), and 3) on the islets on the north-western side of the main island (see also Figure 6a and 6b). During each sampling round in the study area, approximately 45.5 km of coastline was covered. Due to difficulties in synchronizing optimal weather conditions (no precipitation for 48 hours prior to the field day for collecting samples and no precipitation on the field day itself) together with the permission to enter the Nyhamna facility, we were only able to search for otter spraints along the coastline at the Nyhamna facility in 2016 and 2017. We also search along the small river Seterelva, but due to clear cuts along the banks the area wasn't very attractive for otters. Because the number of spraints found on the coastline at the Nyhamna facility during these two searches was extremely low compared to outside the facility together with the logistic challenges and budget availability we had to prioritize collection of spraints outside the facility more than we anticipated on.

DNA degrades relatively fast in otter spraints, hence we targeted fresh (≤ 2 days old) spraints. Age of the spraints were estimated based on the appearance, i.e., old spraints (> 2 days) have begun to harden and dry out, giving it the colour of black tarry to pale grey, while spraints less than and including two days old are softer 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 was 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 that breaks open the 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 (<12 hours old) with the air bubbles still visible. Photo: Jiska van Dijk, NINA.*

3.2 DNA-analysis

DNA extraction

Spraint parts stored in ethanol were prioritized for DNA analysis, but when DNA analysis failed for ethanol, analysis was repeated for the spraint part stored in silica when available. Jelly samples stored in ethanol or silica were analysed in addition. 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 200 µl 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 or not. 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 provides information about the DNA concentration in a sample. Based on previous analyses 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 amplify relatively short amplicons (<250 base-pair) which

facilitates analysis of non-invasively collected samples, like otter spraints, 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.*

Statistical analysis

To evaluate which factors that could potentially affect success rates of the genotyping tests, we first assessed to which extent these rates varied by observer and field session using univariate generalized linear models with a binomial distribution. Thereafter we tested whether weather conditions two days prior and during fieldwork (wet versus dry), temperature during fieldwork and season affected success rates. We also tested whether sample-specific factors contributed to success rates; sample type (secrete and spraint, or unknown (in cases where secrete and spraints were mixed, or when it was unclear which it was)) and estimated age (0, <2 and >2 days). These tests were done with generalized linear mixed-effects models with a binomial distribution using the glmer function in the lme4 library (Bates et al. 2015) in R version 3.6.1 (R Core Team 2019). In these two models (including either weather- or sample-specific factors), we controlled for the random effects of observer and field session.

4 Results 2015-2018

4.1 Otter individual identification and local density

DNA was extracted from 746 samples which were stored in ethanol or silica. All 746 samples were successfully analysed for species identification giving 742 (99%) identified as otter and four (1%) identified as unknown species. Ninety-eight percent (n=738) of the 742 otter-samples contained a DNA concentration enough for microsatellite genotyping. Thirty-six percent (268 of 738 samples) of the microsatellite genotyped samples resulted in a successful DNA-profile. The 268 samples with a successful DNA profile represented 49 individual otters: 20 females and 27 males, and two individual otters for which the sex could not be determined (Ind016 and Ind032) (Table 1 and Figure 6a-c).

Of the 20 females, 15 individuals were repeatedly registered over more than two years of the study (Table 1). Five individual females were sampled in only one year (Table 1). In addition, DNA was extracted from one tooth found during the sampling round in November 2016, which belonged to a female that had been identified from several spraint samples in 2015, 2016 and 2018 (Ind015; Table 1, Figure 7).

For males the DNA results identified 27 different males during all six sampling periods. Only nine male individuals were repeatedly registered over more than two years of the study (Table 1), with only one individual male identified on all four years of study (Ind012; Table 1). Eighteen males from the total 27 individual males identified where only recorded during one year (Table 1). In addition, one tissue sample collected in March 2016 from a dead male otter was assigned to individual Ind040 (Table 1, Figure 8). This otter had previously not been identified.
When looking at the last sampling period in January/February 2018 we identified 20 individual otters, from which 14 animals had been identified also in 2017 (Table 1). Hence, we can assume that these 14 individuals are well-established adults (ten females, three males and one unknown), and we can estimate Aukra's otter density to be of 3.1 resident adults per 10 km of coastline (14 individuals/ 45.5 km coastline * 10).

The number of spraints found within the industrial facility was small compared to what was found both northeast and northwest of it, including the islets (Figure 6). Still, both female (Figure 7) and male individuals (Figure 8) were found to have been identified on either side of the facility. This indicates that they do move past the facility and that their habitat is connected to some extent.

Table 1. *Overview of the individual otters identified by DNA-analysis of spraint and jelly samples collected in 2015 (February and October), 2016 (March and November), 2017 (November) and 2018 (January and February) near the Nyhamna plant on the main island of Aukra (Gossa) and smaller adjacent islands, Aukra municipality. The number of DNA samples is the number of samples assigned to an individual otter. In addition, two individuals of unknown sex (Ind016 and Ind032) were identified as two different otters with unknown sex. Ind040* was only identified from one tissue sample (dead otter). In total 269 samples were assigned to the different otter individuals. Blue indicates recaptures of that particular individuals over two or more years*

	Indiv. nr.	2015 (02)	2015 (10)	2016 (03)	2016 (11)	2017 (11)	2018 (01/02)	Total nr. of samples per individual
Females	Ind003	3	2	3	2	2		12
(n=20)	Ind004	2					1	3
	Ind005	2		2	4		1	9
	Ind006	3		1	2		6	12
	Ind007	3	1					4
	Ind010	5	2		1			8
	Ind011	5	2	1			2	10
	Ind013	7			5			12
	Ind014	1	1	2	2		4	10
	Ind015	6	4	3	10		6	29
	Ind018	2						2
	Ind024		1	4		3		8
	Ind025		3	1			1	5
	Ind027		1		2	2	2	7
	Ind028		1					1
	Ind029		1	2		1	5	9
	Ind030			1	2	1		4
	Ind035				2		4	6
	Ind045					1		1
	Ind046				2			2

Table 1. continued.

Males (n=27)	Ind001	4	1		6			11
	Ind002	7			1			8
	Ind008	4						4
	Ind009	3		7				10
	Ind012	3		1	1	3	2	10
	Ind017	1	1	1	3			6
	Ind019		1					1
	Ind020		1					1
	Ind021		4	3	16			23
	Ind022		1					1
	Ind023		2					2
	Ind026		1	1				2
	Ind031			1				1
	Ind033				1			1
	Ind034				2			2
	Ind036				1			1
	Ind037				1			1
	Ind038				2	2	5	9
	Ind039				1			1
	Ind040*			1				1
	Ind041						2	2
	Ind042						1	1
	Ind043						1	1
	Ind044						1	1
	Ind047					2	1	3
	Ind048						1	1
	Ind049						4	4
Unknown sex (n=1)	Ind032			1	3		1	5
	Total	61	31	37	72	17	51	268

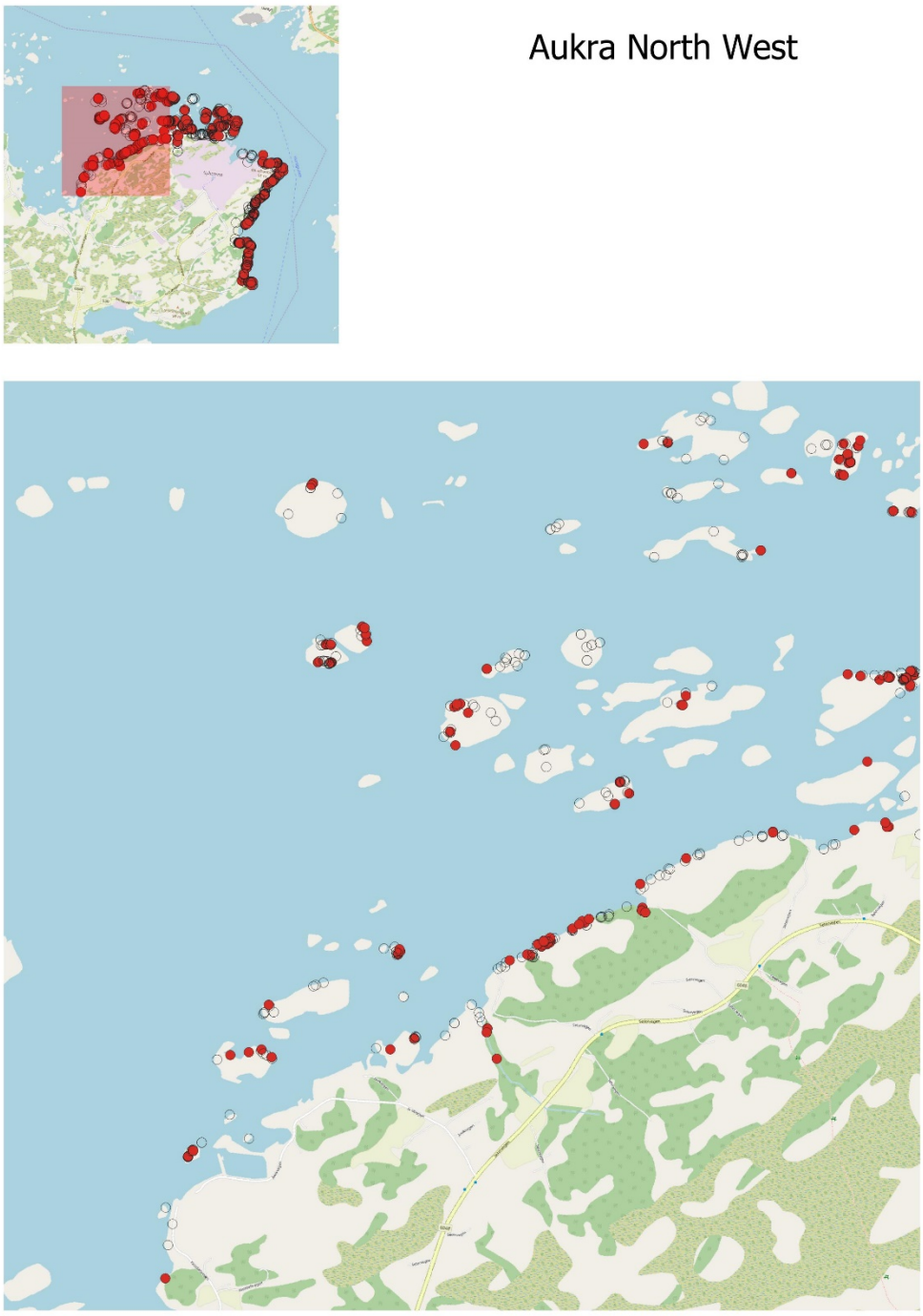
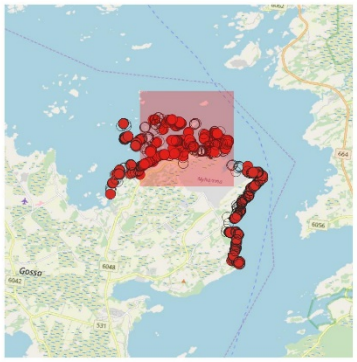


Figure 6a. *Overview of otter spraint and jelly samples collected at Aukra North West and analyzed to individual otters. The samples were collected between 2015 and 2018 near and on the Nyhamna industrial plant on the Island of Aukra (Gossa), Aukra municipality. Red circles indicate successful DNA analyses and individual recognition whereas open circles indicate unsuccessful DNA analyses.*



Aukra North

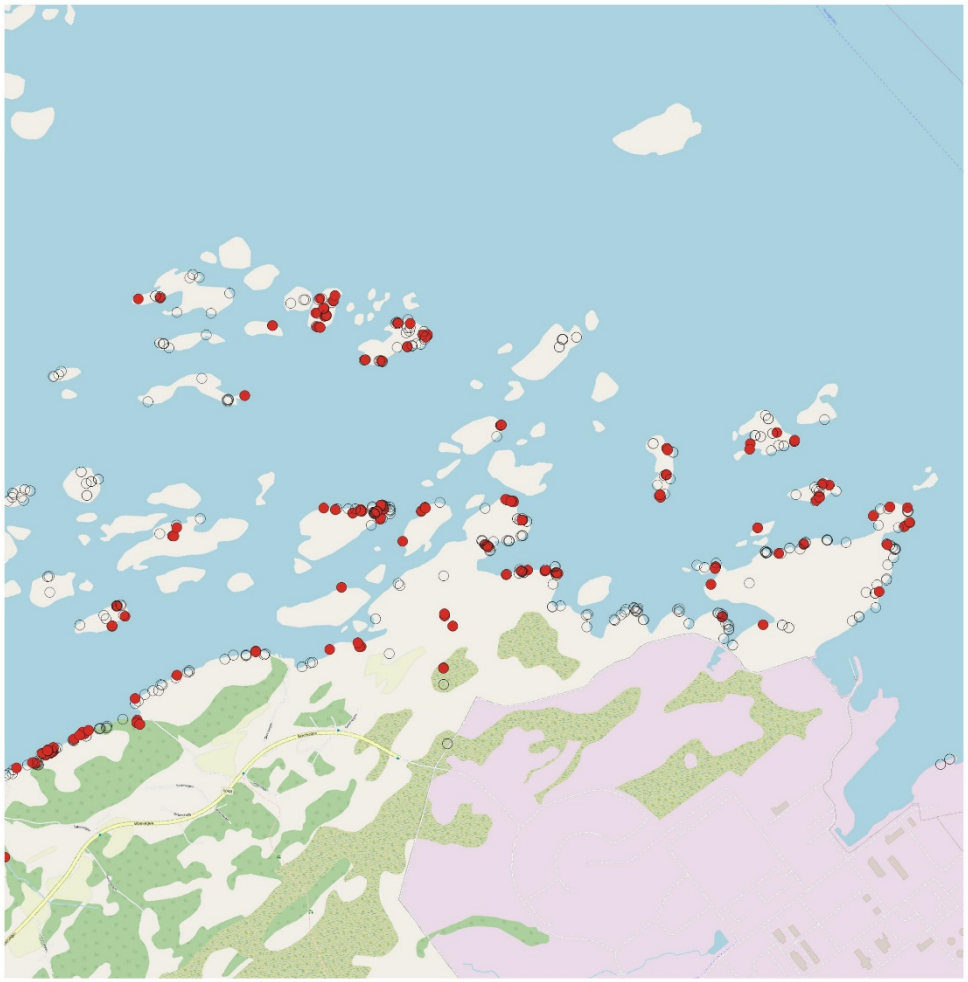


Figure 6b. *Overview of otter spraint and jelly samples collected at Aukra North and analyzed to individual otters. The samples were collected between 2015 and 2018 near and on the Nyhamna industrial plant on the Island of Aukra (Gossa), Aukra municipality. Red circles indicate successful DNA analyses and individual recognition whereas open circles indicate unsuccessful DNA analyses.*

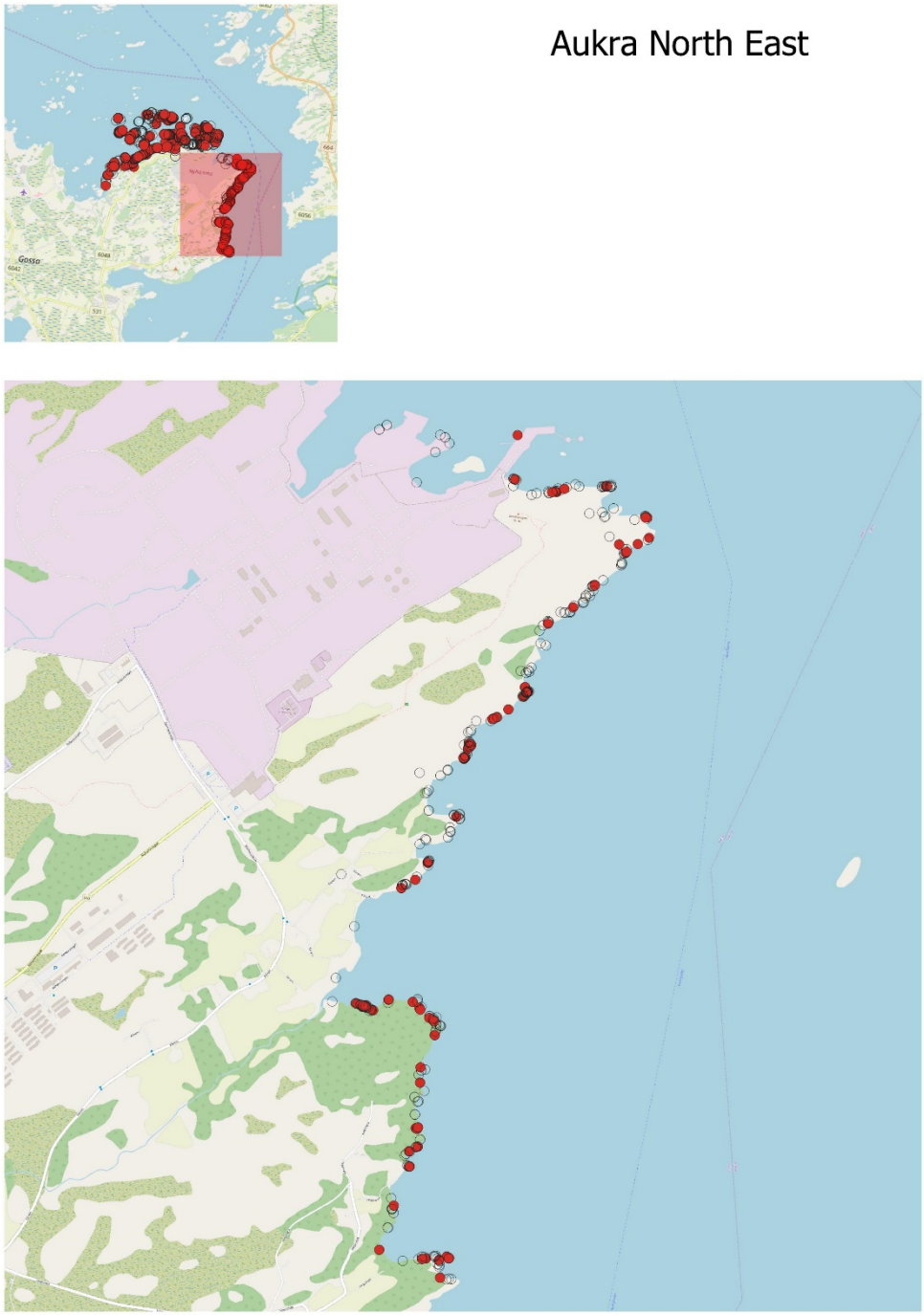


Figure 6c. *Overview of otter spraint and jelly samples collected at Aukra North East and analyzed to individual otters. The samples were collected between 2015 and 2018 near and on the Nyhamna industrial plant on the Island of Aukra (Gossa), Aukra municipality. Red circles indicate successful DNA analyses and individual recognition whereas open circles indicate unsuccessful DNA analyses.*

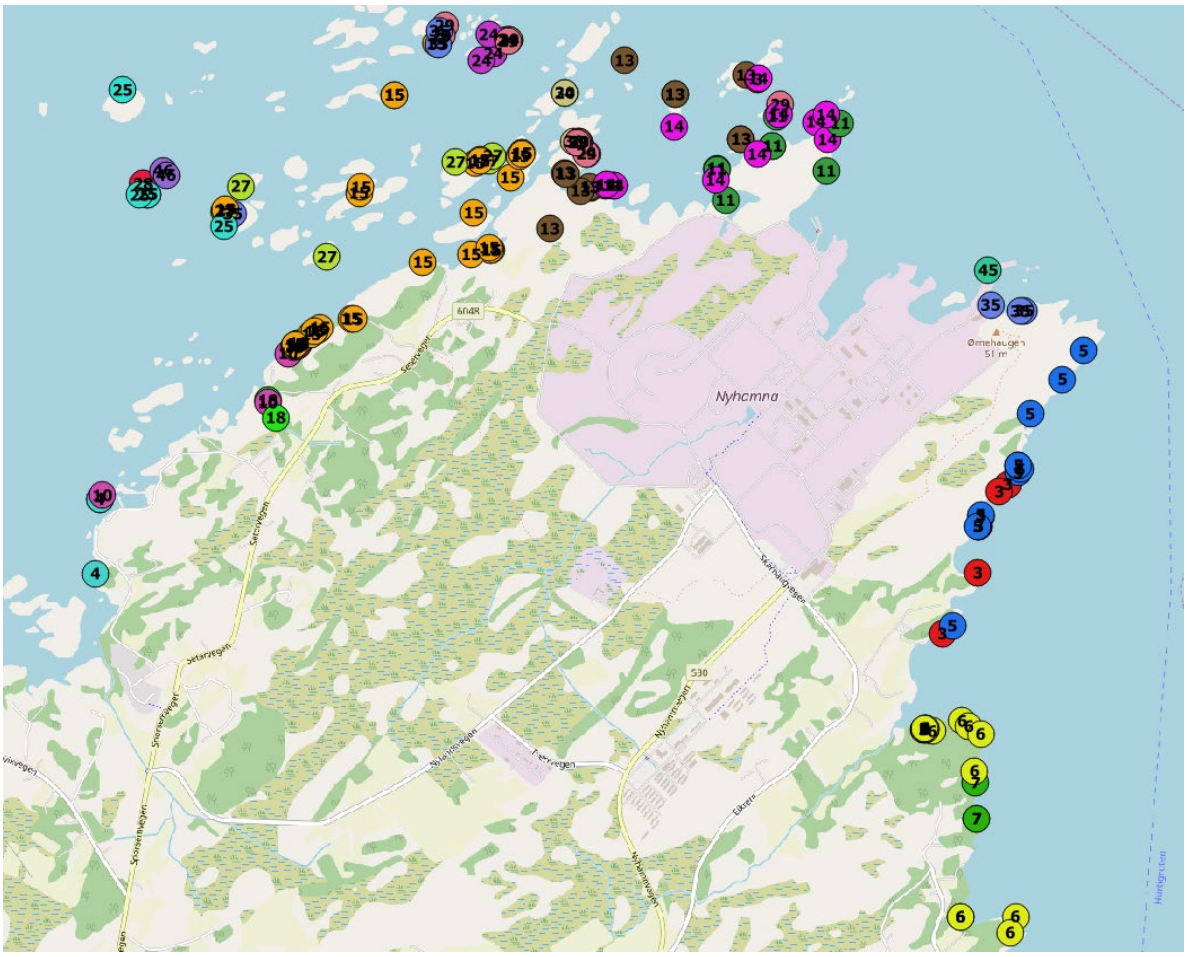


***Figure 7a**. Overview of otter spraint and jelly samples identifying otter females. The samples were collected along the shoreline of the main island and adjacent smaller islands of Aukra (Gossa), Aukra municipality between 2015 and 2018. The different colours and numbers indicate different individuals (see Table 1).*

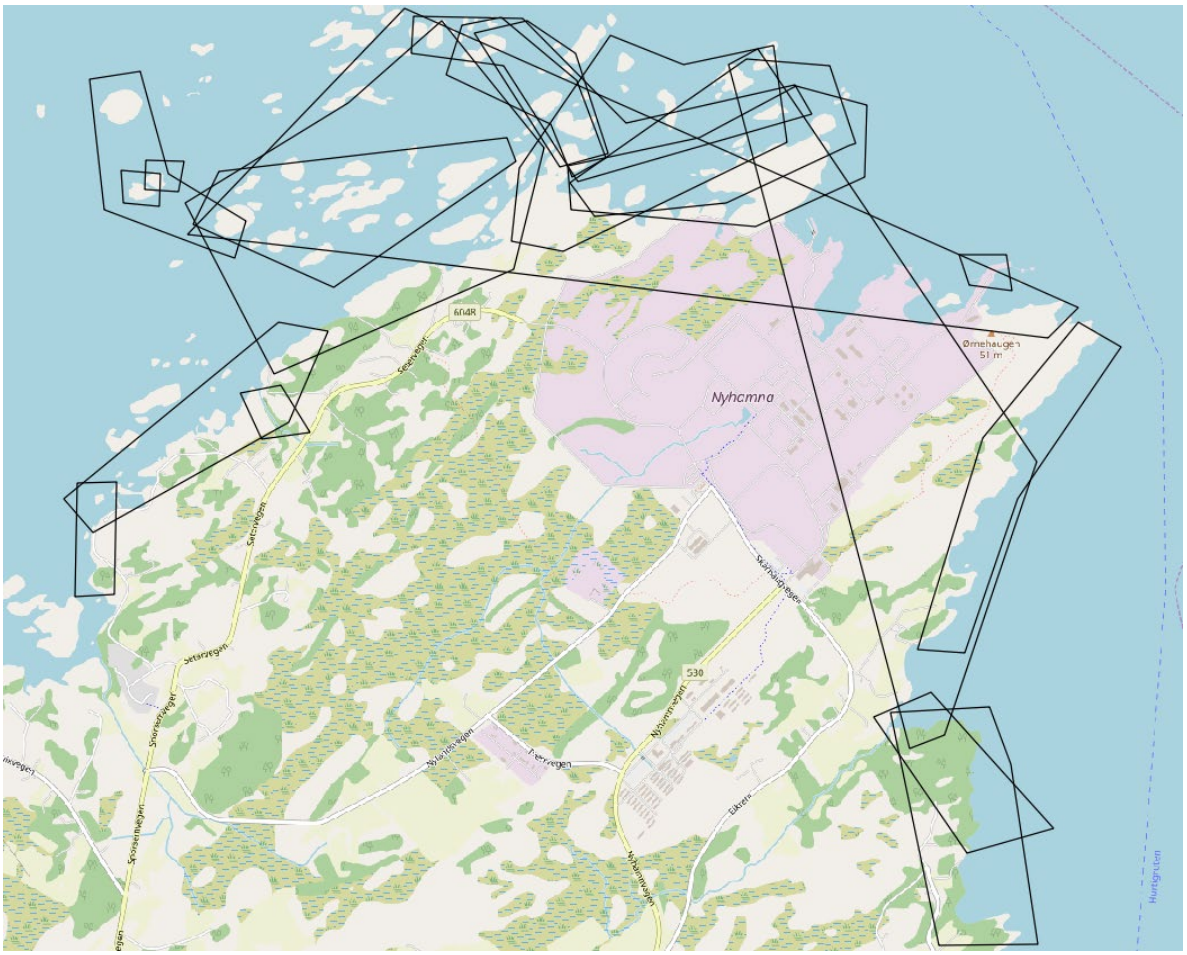


Figure 7b. *Overview of otter females overlapping areas with other otter females. Two females used both the western area and the eastern area adjacent to the industrial plant. The samples were collected along the shoreline of the main island and adjacent smaller islands of Aukra (Gossa), Aukra municipality between 2015 and 2018.*

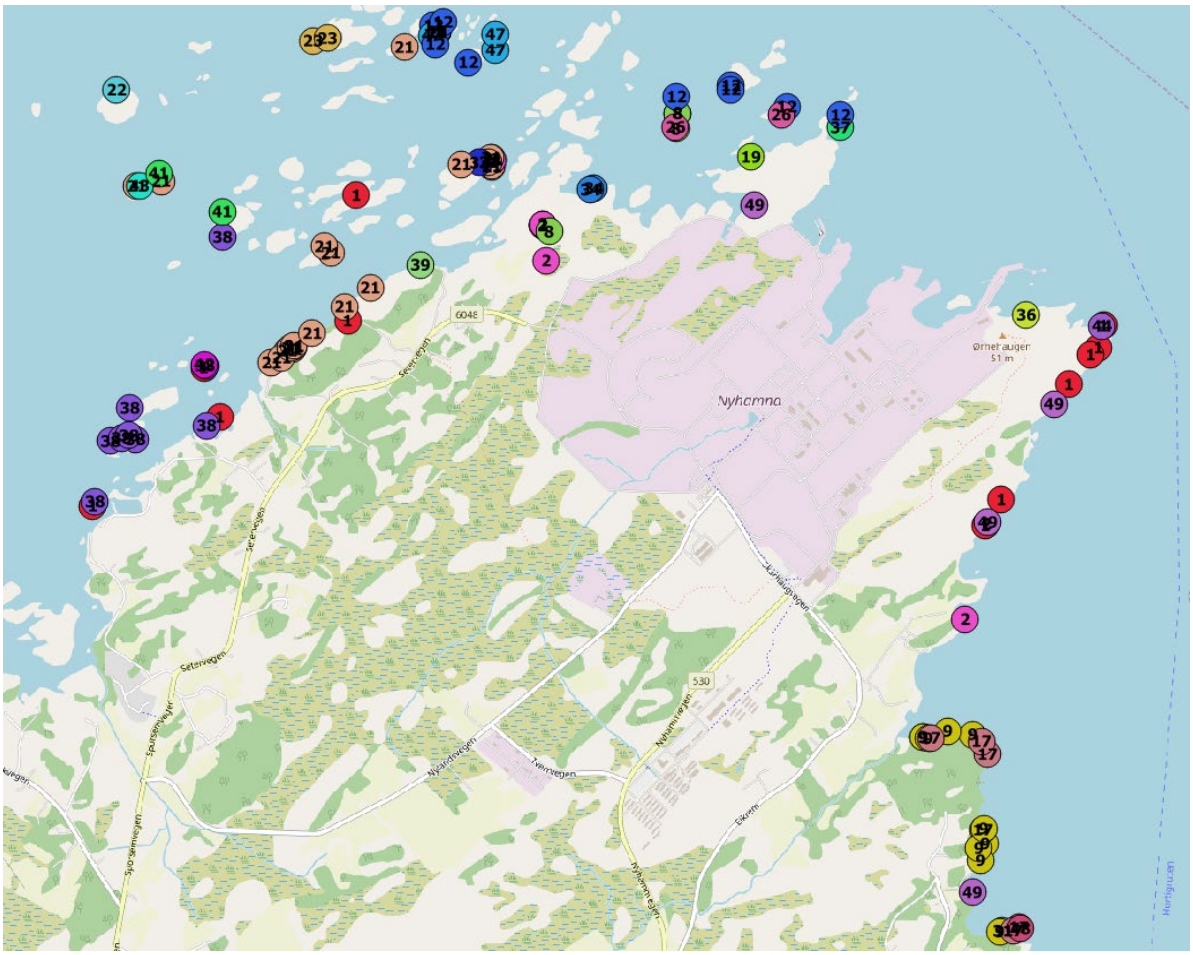


Figure 8a. *Overview of otter spraint and jelly samples identifying otter males. The samples were collected along the shoreline of the main island and adjacent smaller islands of Aukra (Gossa), Aukra municipality between 2015 and 2018. The different colours indicate different individuals (see Table 1).*

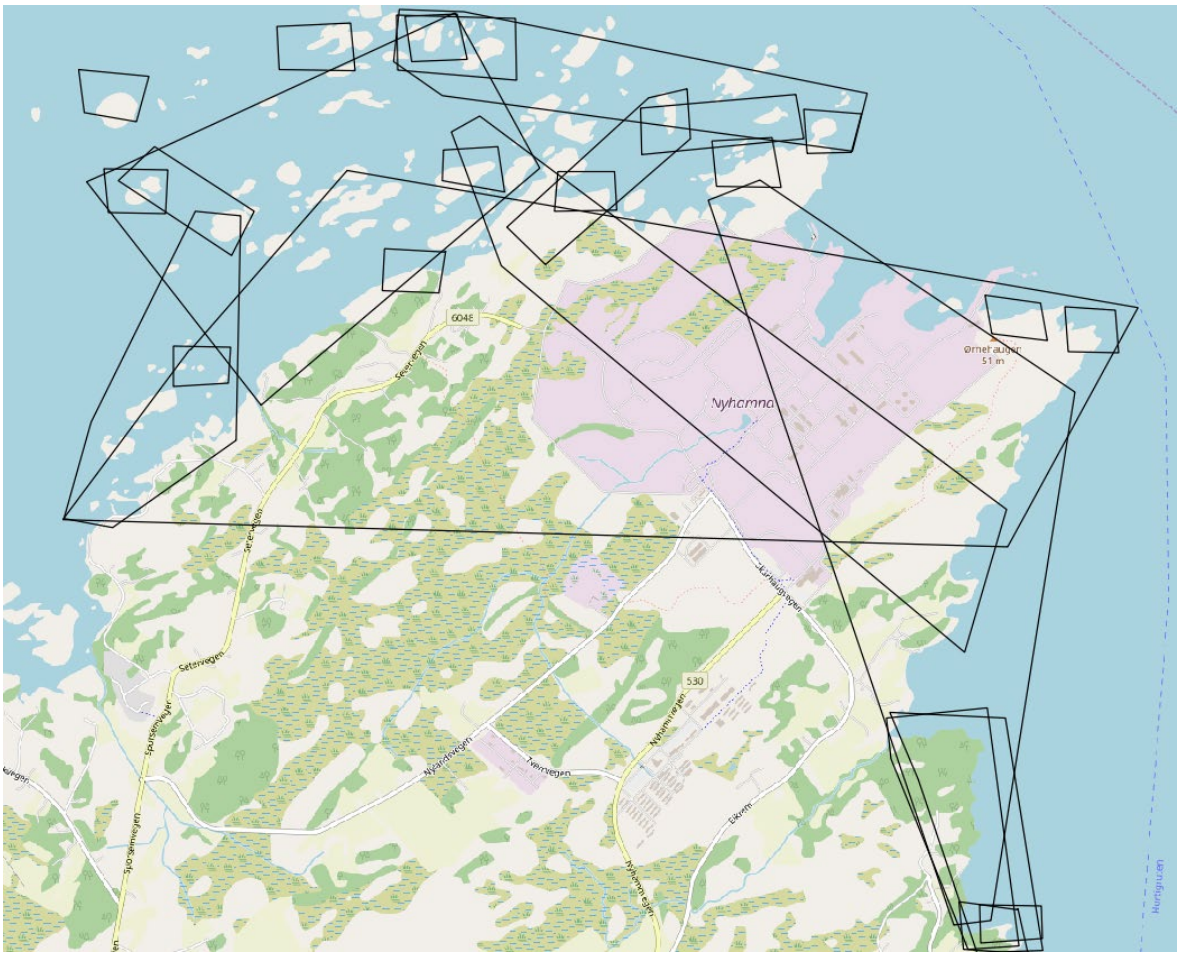


Figure 8b. *Overview of otter males overlapping with other otter males. Three males used both the western and eastern area adjacent to the industrial plant. The samples were collected along the shoreline of the main island and adjacent smaller islands of Aukra (Gossa), Aukra municipality between 2015 and 2018.*

4.2 Storage medium

Combined for the years 2015 and 2016, 71 otter spraints were included to test which storage medium best preserved DNA. Of the 71 sample pairs, 23 samples stored in ethanol resulted in a DNA-profile (32%), while 11 of the samples stored on silica resulted in a DNA-profile (15%) (McNemar's test; P=0.002). Because of this result, samples collected in 2017 and 2018 where exclusively stored in ethanol. For both sexes, the percentage of samples successfully providing a DNA-profile regardless of the storage method in the six periods were 42% in February 2015, 35% in October 2015, 34% in March 2016, 74% in November 2016, 57% in November 2017, 40% in January 2018 and 49% in February 2018. Overall, the genotyping success rate was 46%.

4.3 Recapture of individuals

The study area of Aukra was first sampled for otter DNA in February and October 2015. From these two sampling rounds we identified 28 individual otters, 12 males and 16 females (Ulvund

et al. 2017). The DNA samples collected in 2015 were then compared to the samples found in the next three years to monitor the otter population in Aukra. Of the 28 otters identified in 2015, 19 individual otters (68%) were also identified from the faeces samples collected in March and/or November 2016 (Tables 1 and 2). Twelve of these were females and seven were males. Only six (21%) of the originally identified individuals in 2015 were re-sampled in November 2017, represented by four females and two males (Tables 1 and 2). During the January/February period of 2018, up to 10 individuals (36%) previously sampled in 2015 where identified, accounting for nine females and one male (Tables 1 and 2). In 2016, 11 previously non-sampled individuals were identified (two females, eight males and one unknown sex), out of which two (18%) where subsequently re-sampled in November 2017 (one female and one male; Tables 1 and 2). In 2018, 3 (27%) of the 11 individuals first sampled in 2016 were identified (one female, one male and one unknown sex; 1 and 2). Two previously non-sampled individuals were identified in November 2017 (one female and one male), from which only the male was subsequently re-sampled in January/February 2018 (Tables 1 and 2). The last study period of January/February 2018 yielded six individuals never previously sampled, all of them being males (Tables 1 and 2). Out of the 21 individuals only sampled in one of the six study periods, four were females and 18 were males (Table 1).

4.4 Factors determining the success of DNA genotyping

Genotyping success rates varied between observers (F = 55.903) and between field sessions (F = 46.988), which we controlled for in the following analyses. Model analysis on the external factors affecting the outcome of DNA genotyping tests showed that weather conditions two days prior to or during fieldwork (dry versus wet) did not significantly affect genotyping success rates (respectively, F = 0.356 and F = 0.083). Temperature during fieldwork had a minor negative effect on genotyping success rates (F = 0.995), with temperatures below zero having higher success rates. The season in which samples were collected did however affect the outcome of DNA genotyping tests (F = 8.263), with samples gathered in autumn yielding a significant higher success rate compared to samples gathered in winter (autumn: 0.61; winter: 0.37). Model analysis on the sample-specific factors, indicated that the age of the samples estimated in the field had little effect on genotyping tests (F = 0.345), with slightly higher success rates for fresh samples (0.54 versus 0.46 for older samples). The type of sample significantly affected genotyping tests (F = 4.573), with secrete having higher success rates compared to spraints (secrete: 0.70, spraint: 0.47, unknown: 0.32).

Table 2. *The number of individual otters identified in each period and the number of "recaptures" in October 2015, March and November 2016, November 2017 and January/February 2018. In parenthesis are the number of individuals that have not been identified in previous sampling rounds. The relative low numbers for 2017 was because of bad weather and motor boat problems which resulted in less kilometre covered then in the other years.*

	2015	**2016**		**2017**	**2018**	
	Feb	**Oct**	**Mar**	**Nov**	**Nov**	**Jan/Feb**
No. of otters identified	17	19 (11)	16 (4)	21 (7)	10 (2)	20(6)
Recapture from February 2015		8	9	11	3	7
Recapture from October 2015			5	2	5	6
Recapture from March 2016				1	5	9
Recapture from November 2016					6	9
Recapture from November 2017						5

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, otters are 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 species 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 program. The national monitoring of otters is based on observations recorded in “Oterfallviltbasen” (1987-2019), “Hjorteviltregisteret” (1987-2019) and online in “Artsobservasjoner.no” (1987-2019). In later years, the number of dead otters registered in the “Oterfallviltbasen” database has seen a particular decrease. 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 the species using a non-invasive method. Indeed, DNA genotyping of otter spraints potentially provides estimates of population size, home ranges, dispersal and genetic diversity when carried out over several years.

The spraint samples collected at Aukra between 2015-2018 held relatively good quality DNA. Of the 593 successfully analysed samples for species identification, 99% were identified as otter. The remaining 1% percent resulted in non-working samples. From the 586 samples identified as otter, 99% contained a DNA concentration enough for microsatellite genotyping, i.e. individual identification. However only 45.2% (n=268) of these samples resulted in a successful DNA-profile and a confirmed individual., and is 10 % higher compared with previous research on the species (Lerone et al. 2014). The American mink being also present in 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 and not from American mink (O’Neill et al. 2013). Because this analysis does not distinguish between non-working samples and mink identification, we are unable to report the number of eventual mink scats that were sampled.

The 738 samples with a successful DNA profile represented a total of 20 females and 27 males identified throughout four years and six study periods (Table 1 and Figure 6a-c). Out of the 20 females, nine were identified during all 4 years of study, and only one male was consistently identified throughout the same period of time (Table 1) which indicates that the field method together with DNA profile analyses is a good way to closely monitor local otter population dynamics over years. In general individuals happened to be resampled for most of the study period, and in total 13 females and two males identified during the three or four years that the monitoring lasted (Table 1). The fact that eight females were identified on inconsecutive years (i.e. missing in certain years but resampled later again) may point to the possible departure and return of those individuals, or eventual failure to resample accurately during the missing years (Table 1). On the other hand, the identification of new individuals throughout the study period suggests

possible demographic changes in Aukra's otter population, such as immigration or new-born cubs. Otter can reproduce and give birth to cubs all year round, but given that DNA-analysis only provide individual profiles with no information on age, it is not possible to verify the occurrence of new litters. With our current study methods, only deaths could be registered with certainty when an otter carcass has been sent in to us, exemplified by the identification of Ind040 which was from a dead male carcass.

Despite the limitations for non-invasive monitoring such as the sampling of only fresh spraints and jelly during dry and cold weather, six sampling rounds in four years allowed our results to uncover relevant individual signatures. Eurasian otters reaching reproductive maturity at two years old (Heggberget and Christensen 1994), it is conceivable that individuals sampled during two or more yearly periods consist of adult otters that are established and resident in the area. The recording of GPS-positions of all collected samples allowed the spatial visualization of individual territories for otters sampled for at least two years (Figures 6, 7 and 8). The territories of males and females showed to partially overlap, similarly to other studies conducted in southern otter populations in Portugal (Quaglietta et al. 2015). However, there appears to be a larger overlap in territories between female otters, and the territories of males and females also tend to overlap more than for instance in the study in Portugal (fresh water system). Future research prospects could examine relatedness between individuals in Aukra to determine if territorial overlap can be explained by parental kinship, and whether this overlap is maintained throughout the long-term or is rather temporary. With continued sampling of otter spraints in ethanol, we will also be able to analyse the survival rate of otters in Aukra and have more reliable estimations of local density.

Based on the 20 individual otters identified in January/February 2018 and of which 14 animals were also identified in 2017 we estimated Aukra's otter density to be of 3.1 resident adults per 10 km of coastline. However this is likely to be a lower threshold because the 2017 sampling period resulted in relatively few individuals compared to 2015, 2016 and 2018 due to bad weather and motor boat problems. When looking only at 2018 where 20 individuals (Table 2) were captured the Aukra's otter population density including established adults and non-established young animals could be accounting for 4.4 individuals per 10 km of coastline (upper threshold). Previous monitoring in Aukra (Shell contract 2008-2012) having resulted in density estimations of 3.9-5.4 well-established adults per 10 km (Landa et al. 2008), we believe this to be comparable to the more recent estimates presented here. In Nordland, an earlier study in Vega during the nineties showed a density estimation of 4.1-5.9 adult otters per 10 km coastline. So far, these results show that otter densities in Aukra have remained stable throughout a period of ten years (2008-2018), and that the intensive building constructions during 2015 and early 2016 has likely not affected the local otter population as such.

The sampling effort at the industrial plant didn't result into successful DNA genotyping apart from one. This individual was re-sampled also outside the plant which indicates that the otters are using the plant and adjacent water to cross from east to west. The number of spraints found in the remaining otter habitat was very little compared to the study between 2008 and 2012. From this we suggest that the intensive building constructions have resulted in less attractive habitat for otters at the industrial plant and that otters have left this area at least for using the area to

deposit their spraints. The otter spraint collection outside the industrial plant and its successful DNA genotyping reveals that the building construction on the plant has had little effect on the local otter population.

The evaluation indicated that genotyping success rates were significantly higher during autumn, with freezing temperatures and for secrete. Freshness of the samples nor weather condition two days prior to and during fieldwork had little effect on genotyping success rates. This result may however be biased towards the general protocol in planning for fieldwork during periods with dry weather conditions. It may be that during autumn weather is more stable compared to winter, necessitating fieldwork during short spells of dry weather. Nearly 75% of the samples were collected in dry weather conditions two days prior to fieldwork (autumn and winter), the remaining 25% were collected in wet weather conditions during winter. Given the difficulty to obtain good enough samples for identifying individuals via DNA genotyping (i.e. weather conditions together with age of the spraints), we believe that a better understanding of our current sampling methods could refine the quality of our data for future prospects. The sampling of only fresh spraints (i.e. 0-24 hours old only) under favourable weather conditions (i.e. dry stable weather, preferably below 0 °C) and correct storage (i.e. ethanol) is therefore important. We used 71 otter spraints to test which storage medium best preserved the DNA between silica beds and ethanol in 2015 and 2016. From the paired sampling analysis, we found that DNA is better preserved when stored in ethanol compared with silica beads. This suggests that otter spraints should be stored in ethanol rather than silica beads, and that samples should be preserved in a freezer to reduce ethanol evaporation. Additionally, samples collected in autumn might have higher DNA genotyping success than samples collected in the winter, as suggested by our GLM model analysis. However, given some missing data (e.g. no samples were taken with dry weather before fieldwork and wet weather during fieldwork and none of the samples collected in the fall were gathered under wet weather) it was not possible to analyse the interactions between these effects. In addition age of the otter spraints (those spraints with the age of <24 hours versus those spraints with the age between 24 hours and 48 hours) may also be a relevant effect on the outcome of genetic tests, but during field collection no distinction between these two groups was noted. For future density estimation projects, we propose to collect pictures and estimate spraint age from these pictures (0-12h, 12-24h, >24h) in addition, as suggested by Lerone and collaborators (2014).

6 References

Broquet, T., Menard, N. and 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.

Bates, D., Maechler, M., Bolker, B. and Walker, E. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**(1), 1-48. doi:10.18637/jss.v067.i01.

Carss, D. N., & Parkinson, S. G. (1996) Errors associated with otter *Lutra lutra* faecal analysis. I. Assessing general diet from spraints. *Journal of Zoology*, ***238***(2), 301-317.

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, J.F. and Piertney, S.B. (1998) Microsatellite primers for the Eurasian otter. *Mol Ecol* **7** (9):1248-1251.

Galpern, P., Manseau, M., Hettinga, P., Smith K. and 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.

Heggberget, T. M., and Christensen, H. (1994) Reproductive timing in Eurasian otters on the coast of Norway. Ecography **17**:339-348.

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

Heggberget, T.M. (2007) Kalking av sure vassdrag, re-etablering av oter, mink og vannspissmus – Sluttrapport. – NINA Rapport 245. 49 s.

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

Kruuk, H. (2006). Otters: ecology, behaviour, and conservation: Oxford University Press. 263 pp.

Landa, A., Flagstad, Ø., Hanssen, F., Syslak, L., Balstad, T. and Heggberget, T.M. (2009) Oterovervåking Ormen Lange 2008. - NINA Rapport 408. 18 s.

Lerone, L., Mengoni, C., Carpaneto, G.M., Ettore, R. and 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

Lov om jakt og fangst av vilt (viltloven). LOV-1981-05-29-38. Hentet fra: https://lovdata.no/dokument/NL/lov/1981-05-29-38#KAPITTEL_1

O'neill, D., Turner, P.D., O'meara, D.B., Chadwick, E.A., Coffey, L. and 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.

Quaglietta, L., Hájková, P., Mira, A. and Boitani, L. Eurasian otter (*Lutra lutra*) density estimate based on radiotracking and other data sources. *Mammal Research* **60**, 127-137, doi:10.1007/s13364-015-0216-2 (2015).

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical.Computing, Vienna, Austria. URL https://www.R-project.org/.

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

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

Valeur, P. 1970. Oter (*Lutra lutra*). – Kristiansand Museums Årbok 1970:17-29.

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