University of South-Eastern Norway
Faculty of Technology, Natural Sciences and Maritime Sciences
Master's Thesis
Study programme: 4317
Spring 2018

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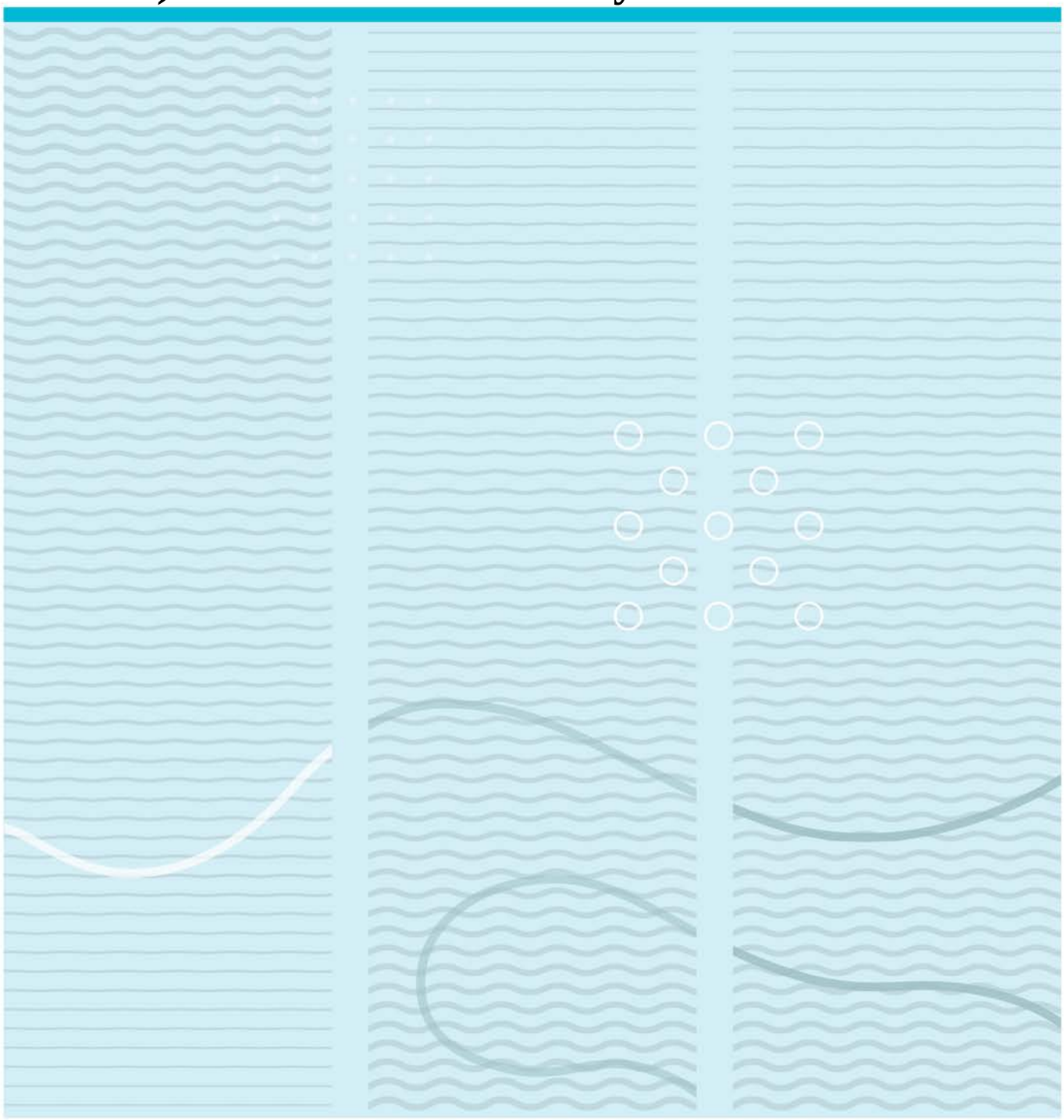
Microclimate and topography influence genetic differentiation in northern crested newt (*Triturus cristatus)* in a boreal forest ecosystem



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This thesis is worth 60 study points

Abstract

Among all vertebrates, the amphibians is the group that currently has the highest proportion of threatened species, and monitored populations have suffered large declines since 1970. The most widespread threat is loss of habitat. Habitat loss makes the reduced populations more dependent on immigration for survival. Moreover, habitat loss often leads to habitat fragmentation, which tends to increase population isolation, and thereby expose them to several genetic threats. Immigration can counteract these threats. Investigation of spatial patterns and levels of population genetic differentiation are fundamental for knowledge-based conservation measures. The northern crested newt (*Triturus cristatus*) is a pond breeding amphibian which shows a decreasing population trend in Europe, and is listed as near threatened in Norway. I studied the northern crested newt in a boreal forest ecosystem subject to fragmentation and habitat loss by clear-cutting and road-building. It was documented that the intervening landscape between breeding ponds, affect the genetic differentiation, in addition to the isolation-by-distance effect. Data and analysis indicated that both microclimate and topography may play a role. South/south-west facing slopes, slopes 30° and steeper and gravel roads in addition to geographical distance, increased genetic differentiation, i.e. reduced landscape permeability for northern crested newts. The opposite effect was observed for streams, presumably more favorable for newt dispersal. Furthermore, populations within or directly adjacent to old forest had a higher allelic diversity than populations outside these areas. Such areas may therefore be important source habitats in the conservation of northern crested newt populations.

Keywords: boreal forest, genetic differentiation and diversity, landscape genetics, microclimate, *Triturus cristatus*, topography, old growth forest

Contents

Foreword

I would like to thank my main supervisor Jan Heggenes for his guidance and help during the work with this master thesis, and for funding the project. I would also like to thank my other supervisors Kjartan Østbye, Arne Linløkken and Harald Klempe for answering questions and offering advice. I am grateful to Rob Wilson and Frode Bergan for guidance with the laboratory work, and to Andreas Zedrosser for guidance with the mixed effect model statistical analysis. Last but not least I would like to thank my family for continuous support and encouragement.

Notodden, 15.05.2018

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1 Introduction

There is a widespread decline in global biodiversity (IPBES, 2018). Among all vertebrates, amphibians is the group that currently has the highest proportion of threatened species (Baillie, Griffiths, Turvey, Loh, & Collen, 2010). In 2010, 41 % of the world's amphibian species were considered threatened and the monitored amphibian populations had experienced a massive reduction of 80 % since 1970 (Baillie et al., 2010). The most widespread threat to amphibian populations is considered to be habitat loss and caused by agricultural land use, logging, and the changing of fresh water systems (Baillie et al., 2010).

Habitat loss is a negative direct effect, but it often also leads to habitat fragmentation, and both can affect amphibian populations in several ways. Smaller habitat patches can sustain fewer individuals (Fahrig, 2003), thereby directly leading to population reduction. Habitat fragmentation, as in the increase of the amount of inhospitable environment between populations, can reduce landscape permeability and thus migration between suitable habitat patches (Wiegand, Revilla, & Moloney, 2005). Smaller populations, often resulting from habitat loss, are more vulnerable towards environmental and demographic stochastic events (e.g. Fahrig & Merriam, 1985), and reduced immigration to small populations make them more vulnerable to genetic threats (Couvet, 2002) so that the combined effect of habitat loss and fragmentation can in the worst case lead to population extinction (Keller & Waller, 2002).

There are a number of genetic threats facing small and isolated populations. Genetic drift has a stronger effect in small compared to larger populations (Allendorf, Luikart, & Aitken, 2013). When genetic drift in small populations is not compensated for by immigration, or mutations in the longer perspective, it results in a within-population net loss of alleles (Keller & Waller, 2002). This may imply a lower ability to adapt to environmental change (Frankham, 2015). Natural selection is less effective in small populations, and this can cause deleterious mutations to accumulate, increasing the genetic load of the population (Keller & Waller, 2002). Inbreeding is also a major negative genetic effect in small populations. It is caused by the lack of opportunities for mating with non-related individual, which becomes less likely the smaller the population is. This leads to a heterozygosity deficit, which increases the probability of recessive deleterious alleles being expressed (Keller & Waller, 2002).

Immigration works against these negative effects in several ways. Immigration enhance the number of individuals in the population, making it less vulnerable to stochastic and demographic events, that could have led to population extinction (Brown & Kodric - Brown, 1977). Immigration introduces genetic diversity and increase fitness by reducing the effect of inbreeding and genetic load (Couvet, 2002). Migrating individuals can also recolonize empty habitat and found new populations after a population extinction event. The number of migrants matters, because more colonizers increases the chance of a sucessful recolonization of the habitat (Ebenhard, 1991). As a rule of thumb it has been suggested that one immigrant per generation is enough (Mace & Lande, 1991), but according to Couvet (2002) this may only be enough in a larger population (N >100) with a high growth rate. Some have also argued the potentially negative effects of immigration, such as loss of local adaption, but this is usually a smaller problem than inbreeding (Ralls et al., 2017). Rather, connectivity between habitats is suggested to play a key role in preserving amphibian populations (Cushman, 2006).

The likelihood of a population to suffer from habitat isolation is connected to the balance between a species ability and propensity to move and geographical distance (the isolation-by-distance effect (Hutchison & Templeton, 1999)). However, it is also connected to the species ability to traverse matrix habitat (i.e. landscape resistance (Balkenhol, Balkenhol, Cushman, Storfer, & Waits, 2016)).This would depend both on the species vagility as such, and the ability to cross different types of environments. Amphibians in general exhibit low vagility (Bowne & Bowers, 2004), have small body sizes and have a high water loss rate under hot and dry conditions (Oke, 1987; Wells, 2007). This makes them potentially poor disperses, and matrix habitat could be important in determining the level of connectivity between habitats.

Landscape genetics is a field that combines population genetics with landscape ecology. It offers tools to investigate the effect of landscape composition and configuration on gene flow and genetic drift (Balkenhol et al., 2016). These tools have been used to study the effects of matrix habitat on genetic differentiation (the combined effect of gene flow and genetic drift) in amphibian populations. Factors that seem to increase genetic

differentiation are roads (Richardson, 2012; Sotiropoulos et al., 2013), rivers (Peter, Roland, & Andreas, 2009; Richardson, 2012), topography (Kershenbaum et al., 2014; Spear & Storfer, 2008), urban areas (Emaresi, Pellet, Dubey, Hirzel, & Fumagalli, 2011) and open fields (Greenwald, Gibbs, & Waite, 2009), while forest cover have been found to decrease population differentiation (Greenwald et al., 2009; Richardson, 2012).

Many of the above mentioned studies have been performed in areas affected by agriculture and development. In contrast, a boreal forest ecosystem including breeding ponds for amphibians may not include any major human impacts or infrastructure like agricultural fields, urban areas and major roads and highways. These factors are all important in current amphibian conservation efforts. However, other landscape features could play a role and therefore become important in conservation considerations. Factors like aspect and vegetation cover may be important when it comes to distribution of cool and humid microclimates, and thereby the accessibility of suitable habitat for amphibians (Oke, 1987; W. Peterman & Semlitsch, 2013). Streams could function as humid dispersal corridors (Emel & Storfer, 2015), steep slopes as barriers or partial barriers by evoking avoidance behavior or increasing energy cost (Lowe, Likens, McPeek, & Buso, 2006). Low soil productivity and the removal of forest canopy could result in a lack of prey, as invertebrate abundance can be negatively affected by clear-cuts and low soil pH (Atlegrim & Sjöberg, 1996; Stuen & Spidsø, 1988; Wareborn, 1992). And finally, forest gravel roads could be a barrier because of a drier microclimate caused by the removal of vegetation and canopy (Marsh & Beckman, 2004), or because of steep roadside verges that are too difficult to traverse (Marsh, Milam, Gorham, & Beckman, 2005).

Here, the northern newt was used as an amphibian model to study landscape genetic relationships; what landscape features might affect the genetic differentiation and pattern of northern crested newt populations in a boreal forest breeding pond system. Such knowledge is fundamental for effective conservation actions.

2 Methods

2.1 Study species

The northern crested newt (*Triturus cristatus*) is a pond breeding amphibian, found in Europe and parts of Asia. In the IUCN red list it is listed as least concern, but the current population trend is decreasing (Jan Willem Arntzen et al., 2009). In the Norwegian red list the crested newt was listed as Vulnerable in 2010, but was moved to Near Threatened in 2015 due to the discovery of new populations after increased surveying effort. It is still assumed to be negatively affected by the continuing destruction and degradation of breeding ponds caused by urban development, forestry practices, fish introduction and pollution. It also faces the negative effects of population isolation (Artsdatabanken, 2015).

The northern crested newt alternates between the aquatic habitat of the pond and the terrestrial habitat around the breeding pond (Dervo & Kraabøl, 2010). Most adult crested newts seems to stay at their natal pond, while some juveniles disperse to other ponds (Jarvis, 2016; Alexander Kupfer & Kneitz, 2000). The dispersing capability of the species seems to be around 1 km (e.g. 860 m (Alexander Kupfer & Kneitz, 2000), 1290 m (A. Kupfer, 1998)).

However, there does not seem to be many landscape genetic studies on the effect of landscape features on genetic differentiation and pattern in this species. With respect to larger water bodies, it was found that a large regulated river on the border between Austria and Germany represented a dispersal barrier to northern crested newts (Peter et al., 2009). A couple of studies on the effect of the local terrestrial habitat around the breeding pond suggest some landscape features may be important. In a boreal forest in Finland it was found that the northern crested newts preferred forest with a high amount of understory cover, and avoided clear-cuts (Vuorio, Tikkanen, Mehtätalo, & Kouki, 2015). Moreover, the reproductive output for northern crested newt populations was larger when ponds were surrounded by herb rich forest, while the presence of young forest (age 6-15) had a negative effect. The latter was attributed to the removal of deciduous trees, creating a drier microclimate because of increased solar radiation reaching the ground and thus affecting understory vegetation and prey abundance (Vuorio, Heikkinen, & Tikkanen, 2013). In an agricultural area in France the northern

crested newts avoided pastures and open areas, and preferred bushes, hedges and trees (Jehle & Arntzen, 2000).

2.2 Study area

Thirteen northern crested newt breeding ponds were included for field sampling in the study. The ponds are located within a land area of 10.5 x 3.5 km (Fig.2), in the municipality of Notodden (N59° 37', E9° 19'), in South-eastern Norway. The area was chosen because it represents a boreal forest ecosystem with several known breeding ponds for the northern crested newt. The study ponds were selected based on the expected dispersal capability of the species, i.e. around 1 km. Mean distance between the ponds is 3841 m (SD ± 2137 m) ranging from 677 m to 8717 m. Maximum distance to closest neighbor pond is 1761 m. To the south and west the study area is delimited by a major public highway (south) and road (west) (Fig. 1), and partly in the east by an elevated topography (Fig. 2) (not likely inhabited by northern crested newt (DN, 2008)), all representing landscape features that likely are some kind of migration barriers. The most likely migration route in and out of the area is to the north, where there are several additional northern crested newt breeding ponds, all located in the boreal forest.

The study area is dominated by conifer forest, notably Scots pine (*Pinus sylvetris*) and Norway spruce (*Picea abies*), with patches of mixed forest and deciduous forest (NIBIO, 1999), where European white birch (*Betula pubescens*) is the most common hardwood species. The topography within the study area varies from nearly flat to rather rough, and elevation ranges from 200 to 500 m.a.s.l (Fig.2). Water ways in the area is limited to streams of varying sizes.

Contemporary human impact mainly consists of active logging and associated construction of forest gravel roads, a main power line, a few scattered cabins, and fish introductions into some of the lakes and ponds in the area. A 1992 human caused accidental forest fire in the study area resulted in a loss of forest in an area of 2.25 km^2 (Fig. 1) (Slettemo, 2008). Some areas within the study area likely are of particular biological importance. Two nature reserves (2.89 km2 and 0.23 km2) were established in 2014 and 1967 to preserve old forest, and a third area is an old near-natural forest with several registered observations of old forest indicator species which are also red

listed (Fig. 1) (e.g. *Fomitopsis rosea*, *Alectoria sarmentosa*, *Phlebia centrifuga* (Artskart, 2018)). Old-near natural forest is here defined as forest only affected by selective dimension felling in the early 20th century (Sverdrup-Thygeson, Ørka, Gobakken, & Næsset, 2016)

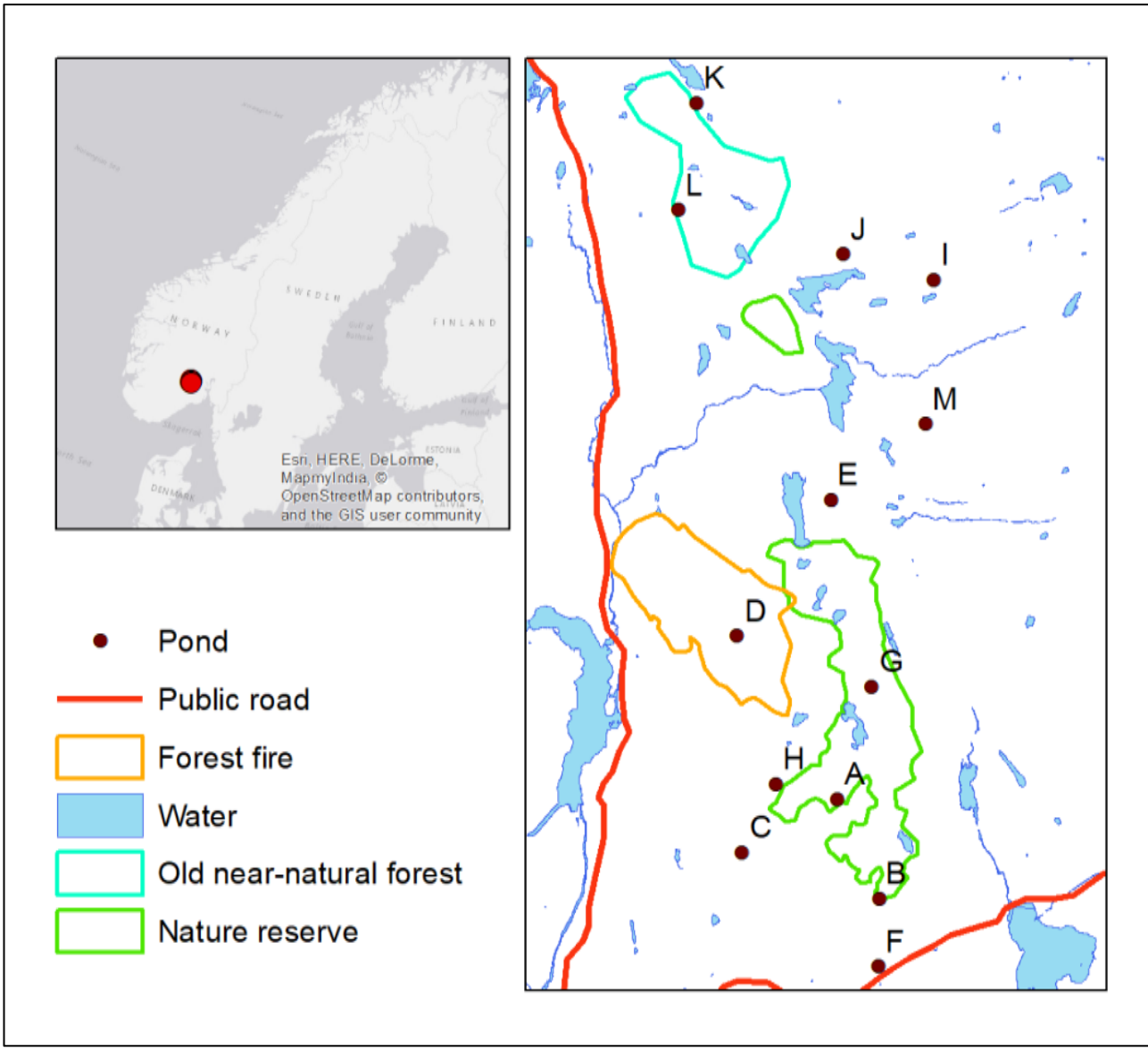

Figure 1: Study area in Notodden, Norway, with pond distribution, and some landscape features. Red lines are public roads, blue polygons and lines are water, orange line encircles an area affected by forest fire, green lines encircle nature reserves, turquoise line encircles an area with high density of red listed species (mostly wood growing fungi and lichen).

All ponds within the study area have previously been checked for the presence of northern crested newt populations, so it seems unlikely that there are any more undiscovered breeding ponds within the area (F. Gregersen, personal communication, April, 2016, and personal observations).

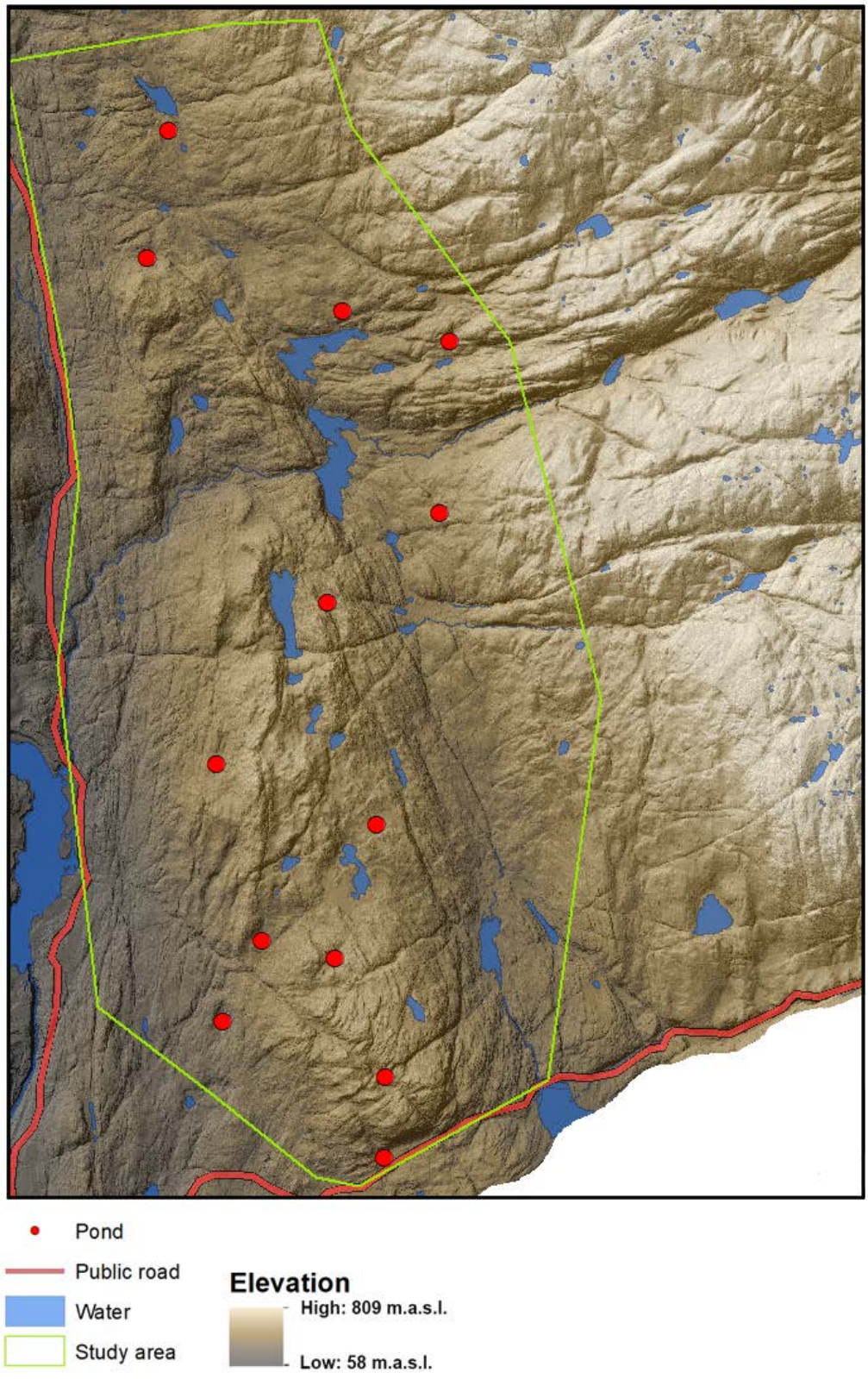

Figure 2: Topography and elevation of the study area in Notodden. Light green demarcation line = the study area, red lines = public roads, red dots = study pond, blue lines and polygons = water bodies. Elevation is illustrated with brown colour, the higher the elevation, the lighter the colour.

2.3 Sampling

Sampling of DNA was conducted during the period 20 May to 17 July, 2017. Thirteen ponds with known northern crested newt breeding populations were sampled, and each pond was sampled a minimum of two times (Tab. 1). All ponds were sampled until it was assumed that the target sample size of approximately 30 unique individuals was reached. Assumed recaptures were not resampled, but this was often hard to evaluate resulting in some individuals being sampled twice. Not all captured newts were sampled if the target size was assumed to be reached.

Adult and juvenile newts were captured using fish traps. The fish traps used has a cylindrical shape (length: 600 mm, radius: 125 mm, mesh size: 5 mm). In each end there is a funnel shaped entrance with a 15 mm wide circular opening where the newts enter. For more details see Dervo, Skei, Kooij, and Skurdal (2013). In the ponds to be sampled (Tab.1), 10 traps were placed 0.5 to 2 meters from shore, with the trap top floating in the surface. The traps were placed all around each pond with approximately 3 to 5 m between each, depending on pond size. In the smallest pond (pond F, Tab. 1) only 6 traps were used. In pond G the traps were, after four days with low capture rate, concentrated in the area with the assumed best newt habitat. The number of sampling days for each pond varied from 2 to 10.

Table 1: The thirteen study ponds with their designated symbols, place names and pond area (m2), elevation of ponds (m.a.s.l.) the number of sampling days and sample sizes after the removal of duplicates/recaptures.

Pond symbol	Place name	Pond area m2	Elevation (m.a.s.l.)	No. of sampling days	Sample size
A	Lislestultjønn	1953	400	5	34
B	Rossåstjønna	2381	409	2	39
C	Kleivtjønn	2712	242	10	35
D	Bråtelidipilen	576	360	5	39
E	Igletjønn	2549	393	7	37
F	Rossebusletta	78	314	3	4
G	Storemyr	746	402	10	12
H	Dipilen	1741	334	3	37
I	Pond north of Øvre Sveivetjønn	710	413	4	34

J	Pond north of Fiskeløys	353	355	5	36
K	Pond south of Ljostjønn	1048	322	2	34
L	Elgsliputten	2471	304	2	32
M	Geittjønn	863	397	4	35

The newts are most active at night (Bock, Hennig, & Steinfartz, 2009), so the traps were set into the pond in the afternoon/evening and recollected during the next day. Captured newts were tissue sampled for later DNA analysis, by taking non-destructive tail clips, i.e. 3-5 mm of the tail tip. These were stored individually in marked Eppendorf tubes filled with 96% ETOH. Tails regrow relatively fast. In a study on northern crested newts it was found that after clipping 12 mm of the tail, they were regrown after 1 year (J. W. Arntzen, Smithson, & Oldham, 1999). In the present study it was noticed that many newts were lacking tail tips for other reasons. This was the most commonly observed injury, and made it difficult to identify recaptured newts correctly.

Immediately after sampling, all newts were released into their pond. The total number of unique individuals sampled was 408 individuals, after removing all recaptured individuals identified by genotyping. The mean sample size from each pond was 31.4 individuals (SD ± 10.3), ranging from 4 to 39 individuals (Tab.1). Necessary permits for capture and sampling of newts and including ethical considerations, were acquired from County Governor of Telemark (20.02.2017), Norwegian Environment Agency (20.03.2017) and Norwegian Food Safety Authority (08.08.2016 \ 9118) .

2.4 Catch by Unit Effort (CPUE)

As an index of relative newt abundance or relative population sizes, CPUE was estimated for each pond based on the number of individuals captured while sampling for DNA, using the formula (adapted from (Maunder et al., 2006)):

$$CPUE = \frac{Ct}{Et}$$

Where

Ct is number of newts captures at time t (during one night).

Et is number of traps used at time t (during one night).

Each pond was visited minimum two times, and median CPUE was calculated for each pond. The captures from pond G from after the traps were concentrated in the assumed best newt habitat, were not included in the calculation.

2.5 Molecular methods

Genomic DNA was extracted from tail clips with the Qiagen blood & Tissue kit, following the manufacturer's instructions (Qiagen, 2006). Microsatellite markers were collected from two different sources: a master thesis (Håland, 2017) and a paper (Drechsler et al., 2013), and tested for their utility in population genetic studies on the northern crested newt. Both sources had developed multiplex microsatellite panels for the northern crested newt. The master thesis included two multiplex panels of a total 12 loci. The paper included three multiplex panels of a total 17 loci. This would have resulted in 29 loci, if all were found to be useful.

The two multiplexes from Håland (2017) consisted of 8 and 4 loci. Here fluorescently labelled universal primers were used to label the locus-specific primers (see Blacket et al., 2012 for universal primer sequences). The different primer and universal primer concentrations were tested to find an optimum combination for PCR (Polymerase Chain Reaction) amplification (Table 3). The four multiplexes from Drechsler et. al. (2013) consisted of 6, 6 and 5 loci. They were amplified using fluorescent labelled locus specific primers. Here, PCR reactions were run with the same concentration for all primers.

Before running the PCR reactions, some loci were excluded from further analysis. Håland (2017) tested his multiplexes on populations of northern crested newt sampled from four different locations in South-eastern Norway (total sample size = 131) and found that three of the loci in his multiplexes were monomorphic (EU760902, EU760908, KF442203). These were assumed to be monomorphic also in the present study, and were excluded. Two loci overlapped with the "Drechsler" multiplexes, and were dropped from further analysis (KF442197, AJ292517). This reduced the 2nd multiplex, originally consisting of 4 loci, to a singleplex. The remaining 7 loci from the "Håland" multiplexes were tested by running them as singleplexes or reduced multiplexes (2-3 loci) (Tab.3). After running ~100 samples, three more loci were

dropped from further analysis because of no amplification, uninterpretable amplification, and no polymorphism (EU760904, GU574495 and EU760906, respectively). The remaining 4 loci from the "Håland" multiplexes were amplified for all the collected samples (Tab.2).

The "Håland" multiplex PCRs was run with a final volume of 20 µl. PCR amplification was performed using HotStarTaq ® Master mix from Qiagen (https://corporate.qiagen.com/). Each reaction contained 1x PCR buffer (with 1.5 mM $MgCl_2$), 200 µM for each dNTP, 1 U HotStar Taq, 0.1-0.2 µM forward and reverse primers, 0.075-0.15 µM universal primers (Tab. 3) and ~ 3.6 ng/µl genomic DNA. The temperature profile for the PCR is in Table 4.

Table 2: Characterization of four polymorphic microsatellite loci for Triturus cristatus *obtained from Håland (2017) which were used to amplify all collected samples in this study. The loci are grouped after the PCR multiplex combinations, but multiplex 2 has been reduced to a singleplex.*

Locus	Primer sequence (5'–3')	Repeat motif of cloned allele	Size range of amplification product	Number of alleles	Fluorescence labelling	GeneBank accession
	Multiplex 1					
Tcri43	F: ACTCTCCTACAACTATCTCCATCTG R: GGTCGACCACCCTAACTGTTAG	$(GAAA)_{27}$	195-227	9	FAM	AJ292511
Tc52	F: AGTGCACTTACAATTCCCCTGA R: TCAATTGGTTGTAGCAGCCAGA	$(ATTG)_{17}$	127-147	6	FAM	KF442196
Tc85	F: TTGTTAGACCTCGCATCTGTTG R: GGGTGAGTAGTGCGCTTAAAAA	$(AATC)_{11}$	112-120	3	PET	KF442205
	Singleplex (reduced multiplex 2)					
Tc69	F: GTGCAATCGGTATCCAGACAAC R: GAGCTTGATCCTGGCATGAAAT	$(AGAT)_{13}$	163-171	3	FAM/NED	KF442202

Table 3: Final PCR concentrations of forward and reverse primers, and tailed universal primers for seven microsatellite loci for Triturus cristatus, *obtained from Håland (2017). Loci are grouped after multiplex combinations, but multiplex2 has been reduced to a singleplex.*

Locus	Primer conc. (F/R, µM)	Fluorescence labelling	Tailed primer conc. µM	GeneBank accession
Multiplex1				
Tcri43	0.1/0.1	FAM	0.075	AJ292511

Tc52	0.15/0.15	FAM	0.075	KF442196
TCM-414	0.1/0.1	FAM	0.075	EU760906
TCM-277	0.15-0.2/0.15-0.2	NED	0.1-0.15	EU760904
Lm_528	0.1/0.1	NED	0.1	GU574495
Tc85	0.15/0.15	PET	0.1	KF442205
Singelplex (reduced multiplex 2)				
Tc69	0.1/0.1	FAM/NED	0.1/0.1	KF442202

Table 4: The PCR temperature profile used for amplification of seven microsatellite loci gathered from Håland (2017).

Step	Time	Temperature	
Initial heat activation	15 min	95 °C	
Denaturation	15 sec	95 °C	35 cycles
Annealing	15 sec	58 °C	
Extension	30 sec	72 °C	
Final extension	5 min	72 °C	

From the "Drechsler" multiplexes three loci were dropped before running the PCR reactions (KF442196, KF442202, KF442205). This was due to them overlapping with the "Håland" multiplexes (Tab.2). One additional loci was dropped because it had been tested by Håland (2017), and found to be monomorphic (KF442203). After running the remaining 13 loci for ~95 samples, as singleplexes or reduced multiplexes (2-3 loci), two more loci were dropped from the analysis, one because of monomorphism and one because of uninterpretable amplification (KF442201 and KF442204, respectively). This resulted in the 3rd "Drechsler" multiplex being dropped completely from further analysis. What was remaining of the 1st and 2nd multiplex (6 and 5 loci) was amplified for all the samples (Tab. 5). However, the locus Tcri46 primer sequences, as described by Drechsler et. al. (2013), had to be corrected, because of an apparent mix-up of forward and reverse primers in the original article.

The "Drechsler" multiplex PCR were run with 20 µl as final volume. Each reaction contained 1x Qiagen Multiplex PCR Master Mix (which included 3mM $MgCl_2$), 0.2 µM forward and reverse primers and ~ 3.6 ng/µl genomic DNA. The temperature profile is described in Table 6. PCR products were diluted to 120 µl before running the electrophoresis.

Table 5: Characterization of 11 microsatellite loci for Triturus cristatus *obtained from Drechsler et. al. (2013), which were used to amplify all collected samples in this study. The loci are grouped after the PCR multiplex combinations.*

Locus	Primer sequence (5'–3')	Repeat motif	Size range of amplification product	Number of alleles	Fluorescence labelling	GeneBank accession
	Multiplex 1					
Tcri13	F: GTGATGGTTGCCAAGC	(GT)36	105-131	8	FAM	AJ292500
	R: GATCCAAGACACAGAATATTTAG	Interrupted				
Tcri27	F: GATCCACTATAGTGAAAATAAATAATAAG	(GAAA)27	237-281	12	FAM	AJ292517
	R: CAAGTTAGTATATGATATGCCTTTG					
Tcri29	F: CGAGTTGCCCAGACAAG	(TTTC)22(CA)11	310-338	NA	NED	AJ292505
	R: GATCACATGCCCATGGA					
Tcri35	F: CCAACTGGTATGGCATTG	(GAAA)32	203-231	8	NED	AJ292490
	R: GATCACAGAAACTCTGAATATAAGC	Interrupted				
Tcri36	F: GATCATCTGAATCCCTCTG	(GAAA)36	222-246	7	VIC	AJ292491
	R: ATACATTCATGACGTTTGG	Interrupted				
Tcri46	F: GCCTGACAAAGTAATGCTTC	(TTTC)23	272-300	8	PET	AJ292494
	R:GTTTCTTCAAGTTTCCTCTGAAGCCAG					
	Multiplex 2					
Tc50	F: GCGGATACATGGTCTTCGTT	(ACTC)18	174-298	29	PET	KF442195
	R: TTCAGTTAAAAGTGTCCTCTGTGG					
Tc66	F: CCTTTGTACACCACTGGCAAA	(ATCC)18	227-239	4	FAM	KF442197
	R: TGGTCCTATAAAGCCATCTTGG					
Tc68b	F: AAAGTGCACTCTTTCTCTGAAGC	(ATCC)24	174-206	9	FAM	KF442198
	R: TGCAAAGTGCATGTGTGACT					
Tc70	F: GGGTTGCAAAGCACCTTAAT	(ACAT)14	211-231	5	VIC	KF442199
	R: TACCTGGGTCCTCCTCCAAG					
Tc81	F: TTTAGTCTCTCCGCTCTGCAA	(AATC)13	135-152	5	VIC	KF442200
	R: AGCGGAATCTGCCTTATGGT					

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Table 6: The PCR temperature profile used to amplify 15 microsatellite loci obtained from Drechsler et. al. (2013).

Step	Time	Temperature	
Initial heat activation:	15 min	95 °C	
Denaturation	30 sec	94 °C	30 cycles
Annealing	90 sec	59 °C	
Extension	60 sec	72 °C	
Final extension	30 min	60 °C	

All PCR products were run on an Applied Biosystems 3130xl Genetic Analyzer (https://www.thermofisher.com), and the results were analyzed in GeneMapper v5 (AppliedBiosystems). Error rate was estimated by re-amplifying 10 % of the samples (arbitrarily selected).

Alleles were defined based on a cumulative line graph, which showed the stepwise change in recorded amplicon lengths for each locus. Each allele was defined based on the interval of one step in the graph. Samples with amplicon lengths near the lower and upper bound of the intervals were run twice to ensure the right allele definition. Locus Tcri29 was dropped from further analysis because of difficulties defining the alleles, leaving in all 14 loci to be used for subsequent analysis (Tab.2, Tab.5)

2.6 Preliminary statistical analysis

The 14 amplified microsatellite loci were tested for departure from Hardy Weinberg equilibrium and for linkage disequilibrium, within pond samples, using Genepop v.4.7.0 with 90 000 and 600 000 iterations respectively (Rousset, 2008). Significance of tests were assessed after the sequential Bonferroni correction procedure (Holm, 1979). The software MICRO-CHECKER v2.2.3 was used to test for null alleles, scoring errors and large allele dropouts using 10 000 iterations and $\alpha = 0.05$ (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004) . The presence of candidate loci under natural selection was investigated using BayeScan v2.1 after 5 000 000 iterations following 500 000 burn-ins (Foll & Gaggiotti, 2008).

For almost all analysis the genetic data was described as number of repeats (of the repeat motif). The exception was the analysis run in MICRO-CHECKER. Here the genetic data was described as number of base pairs. The transformation from base pairs to number of repeats was done using GenAlEx v6.503 (Peakall & Smouse, 2006, 2012). Potentially duplicated individuals were scanned in Microsoft Excel (2010), and deleted. This resulted in a total sample size of 408 unique individuals.

2.7 Summary statistics for the microsatellite loci

Locus specific Fst (Weir & Cockerham, 1984) and expected heterozygosity was calculated in SPAGeDI using 15 000 permutations (Hardy & Vekemans, 2002). Locus Tc50 was then dropped from further analysis, because of a high probability of being under natural selection. All subsequent analyses were conducted using the remaining13 microsatellite loci. The sample from pond F was reckoned to be too small to provide reliable estimates (n = 4), and was also dropped in all subsequent analysis.

2.8 Genetic and allelic diversity

Genetic diversity, i.e. heterozygosity, for the remaining pond samples were calculated in GenAlEx v.6.503 (Peakall & Smouse, 2006, 2012). This included observed heterozygosity (Ho), expected heterozygosity (He) and expected heterozygosity corrected for small samples (uHe). Allelic diversity was calculated as allelic richness with the package “diveRsity” in R (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013). Allelic richness was calculated with a resampling procedure with replacements, with a constant subsample size equal to the smallest sample in the data. This was done with and without the smallest of the remaining samples (pond G), giving a subsample size of 12 and 32, respectively. Confidence intervals (95 %) were generated using a resampling procedure and 10 000 bootstrap replicates. Confidence intervals (95%) were also generated for sample means of expected heterozygosity using a resampling procedure with replacement in the package “PopGenKit” in R, after 10 000 bootstrap replicates (Paquette, 2013). A permutation test of significant difference in expected heterozygosity between ponds, implemented in the “adegenet” package, was also performed in R (Jombart, 2008). Each population pair was tested with 999 simulations, and significance of results was evaluated after correcting for multiple tests with the sequential Bonferroni correction (Holm, 1979).

2.9 Impact of old forest on genetic and allelic diversity

Potential population differences in estimated genetic diversity (expected heterozygosity) and allelic diversity (allelic richness) between ponds connected to areas of old forest compared to those not connected, was tested with a two-sided permutation test implemented in FSTAT v2.9.3.2 and 20 000 permutations (Goudet, 2001). Connectedness to old forest was defined as the pond being located within or in close proximity to such areas (<100 m). Areas of old forest were defined as the two nature reserves and the area of old near-natural forest with several registered observation of old forest indicator species (Fig. 1). The ponds that were defined in the group connected to old forest (group 1) were: pond A, pond B, pond G, pond H, pond K and pond L. Those that were defined as not connected to these areas (group 2) were pond C, pond D, pond E, pond I, pond J and pond M. Pond G was dropped when estimating allelic richness as the small sample size would influence the subsample size used to estimate allelic richness. The subsample size used to calculate allelic richness was therefore 32. The effect size was evaluated using Cohen's d calculated as (Y. Li, 2010):

$$\text{Cohen's d} = \frac{Ar_1 - Ar_2}{SD_{pooled}}$$

$$SD_{pooled} = \sqrt{\frac{(n_1\text{-}1)SD_1^2 + (n_2\text{-}1)SD_2^2}{(n_1 + n_2 - 2)}}$$

Where

Ar_1 is Allelic richness for group 1

Ar_2 is Allelic richness for group 2

n_1 is number of samples in group 1

n_2 is number of samples in group 2

2.10 Population structure

The software TESS v2.3.1 (Chen, Durand, Forbes, & François, 2007) and STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000) were used to infer population structure based on variation in allele frequencies, and the minimization of

within-population departure from Hardy Weinberg proportions, and linkage disequilibrium (Pritchard, Wen, & Falush, 2009). Neither software assumes predefined populations, but, unlike STRUCUTRE, TESS incorporates spatial information by assuming that neighbor populations are more similar than populations further apart (Durand, Chen, & François, 2009).

STRUCTURE has been criticized for not finding the correct population structure, when samples are unbalanced (Kalinowski, 2010; Wang, 2017). Unbalanced samples affect both the assignment of individuals to source populations, and the estimation of the optimum number of cluster (K). The main problem is that the default ancestry prior (α) assumes all source populations have an equal probability for contributing to each individual's ancestry. Another problem is the default initial α value 1.0, which can be too high and therefor impede the MCMC sampler from reaching lower α values (Wang, 2017).

Including the smallest sample (n=12) in my material, introduced the problem of unbalanced sampling. To tentatively circumvent the above-mentioned issues, the following recommended settings were used: 1. Alternative prior (α inferred for each source population). 2. Initial α =1/K = 1/12. 3. The uncorrelated allele frequency model was used, as this is more capable of dealing with unbalanced samples (Wang, 2017). STRUCTURE was then run with 10 replicates for each possible number of clusters (K), using the admixture model, the above settings and 200 000 replications of burn-in and 500 000 MCMC replicates. K was set to range from 1 - 12. The optimum number of clusters was estimated with two methods: 1. The mean likelihood of the data (mean Ln P(D)) was plotted against the different K's. The smallest K in the plateau of the plot was assumed to be the optimum estimate for K (Pritchard et al., 2009). 2. The ΔK method, which estimates optimum K based on the second order rate of change of the log probability of the data with respect to the different number of clusters (Evanno, Regnaut, & Goudet, 2005).

The mean Ln P(D) method is claimed to be the best method when working with unbalanced samples and applying the above recommended settings (Wang, 2017). The ΔK method is capable of finding the uppermost level when populations are hierarchically structured (Evanno et al., 2005), but only works well with balanced

samples (Wang, 2017). For that reason STRUCTURE was also run without the smallest sample (Pond G), with admixture model, correlated allele frequency model, initial alpha =1.0, 10 replicates per K [1-11] and 200 000 replications of burn-in and additional 500 000 MCMC replicates. Both methods for optimum K estimation was performed for both STRUCTURE runs using STRUCTURE-SELECTOR (Y. L. Li & Liu, 2018).

TESS was run with the admixture model (CAR-model), 15 replicates per K [2-12], with 50 000 replicates of burn-in and 50 000 additional MCMC replicates. Optimum K was assessed with two methods: 1. The mean DIC (Deviance Information Criterion) value was plotted for each K and it was looked for a plateau. The smallest K value in the plateau is assumed to be optimum K (Durand et al., 2009). 2. The bar graphs of individuals assignments were checked to see at what number of K no additional clusters were added (Durand et al., 2009).

Optimal alignment of replicates for the same K was obtained for the most relevant K values, and was performed in the software CLUMPP, with the Greedy algorithm (2000 repeats). All STRUCTURE replicates for chosen K's was run in CLUMPP (Jakobsson & Rosenberg, 2007), while a few TESS results showed some issues of convergence of MCMC chains (these were removed before running CLUMPP). Bar graphs of the aligned individual assignments were generated with CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015).

2.11 Genetic differentiation

Two different methods were used to estimate pairwise genetic differentiation: 1. Weir and Cockerham's Fst (1984) was calculated in SPAGeDI v1.5 (Hardy & Vekemans, 2002), significance of results were evaluated after 15 000 permutations and 95 % confidence intervals were generated by jack-knifing over loci. 2. Chord Distance Dc (Cavalli-sforza & Edwards, 1967) was calculated in FreeNA (Chapuis & Estoup, 2007), not using the INA-correction (a correction for null alleles), including 95 % bootstrap confidence intervals from 15 000 replicates. Neighbor-joining trees based on the calculated pairwise Fst and Dc were drawn using the "APE" package in R (Paradis, Claude, & Strimmer, 2004).

Fst and Dc was chosen as they both are supposed to be less locus dependent, than other similar methods (Takezaki & Nei, 1996; Whitlock, 2011).

2.12 Isolation by distance

Isolation by distance was tested using a Mantel test with 20 000 permutations using the package "ade4" in R (Thioulouse, Chessel, Dole´dec, & Olivier, 1997). Geographical distances between ponds were tested against pairwise Dc and Fst. It was also performed a Mantel test where log-transformed geographical distances were tested against linearized Fst (Fst/(1-Fst). The latter was done following the recommendation of Rousset (1997), for when testing for isolation by distance in a 2d habitat.

Isolation by distance was also tested as part of the landscape genetic mixed effect analysis described below. Here the dependency of the pairwise observations caused by the study design was accounted for by including it as a random effect in the model.

2.13 Landscape resistance and permeability

Potential effects of different landscape features on the level of population differentiation were explored statistically. To quantify the landscape variables, a strip-based approached was used. This method was developed by Emaresi et al. (2011), and is based on the concept of rectangular dispersal corridors between populations. The area of one type of landscape feature inside this rectangle relative to the total area of the rectangle is quantified. In contrast to methods that use least cost paths between habitats, this method is not dependent on the parameterization of cost values, based on a priori assumptions about dispersal strategies or abilities.

The strip-based method was tested on the alpine newt (*Ichthyosaura alpestris*), with different width to length ratio of the corridor. Based on average R^2 and AIC, it was concluded that a corridor with width : length ratio of 1 : 3 had the overall best performance because of having the highest overall explanation power (Emaresi et al., 2011). The width : length ratio of 1 : 3 was also used in the present study, assuming that the northern crested newt's dispersal strategies likely is similar to the alpine newt.

All landscape variables were quantified using ArcMap v10.4.1 (ESRI, 2015) with 3x3 m cell size. Almost all variables were quantified by first creating a raster that delimited areas of the variable of interest. The raster was then converted to shape file and each dispersal corridor was used as a "cake tin" to cut out rectangles from the shape file (using the tool "Clip"). Then the tool "Summary statistics" were used to calculate the area of the variable of interest within the rectangle. This area was divided by the total area of the rectangle, to obtain the % area of the variable.

2.13.1 Landscape variables: Relevance and quantification

Ten landscape features were quantified, and correlation among the variables was determined by calculating Spearman rank correlation coefficients. The following landscape features were quantified:

Aspect

The amount of received solar radiation depends, among other factors, on aspect. In the northern hemisphere, the south facing slopes receives the most amount of solar radiation, followed by south-west and south-east facing slopes (Oke, 1987), and southwest-facing slopes probably receive somewhat more solar radiation than south-eastern facing slopes (Hernan, Fred, & Laura, 2018).The differences in received solar radiation can create differences in microclimate. Here it was hypothesized that south/south-west facing slopes would have a drier microclimate because of higher evaporation, and thus entail more resistance to amphibian dispersal.

A digital elevation model (DEM) was created based on LAS-data (Kartverket, 2008), and were used to extract the amount of area of south/south-west facing slopes between ponds. The "Aspect" tool was used to calculate all aspects of the study area, then the "Reclassify" tool was used to extract all areas with south or south-west facing aspect (see Annex 9, Fig. A4).

Slope 20° or steeper and 30°or steeper

Slope may increase the energy cost of moving through the terrain, or could be too steep to traverse, and hence be avoided. Slopes have been found to act as dispersal barrier for

the Panamanian frog (*Atelopus varius*)(Richards-Zawacki, 2009) and the red-backed salamander (*Plethodon cinereus*)(Marsh et al., 2005).

Two slope variables were calculated based on two assumptions: 1. Slopes of 20° or more were assumed to delimit an area of more hilly terrain, including, but not limited to, steeper slopes. 2. Slopes of 30° or more would entail areas with steep slopes that could act as barriers.

The variables were quantified using the DEM created previously, and the "Slope" tool was used to calculate the slope gradients of the study area (see Annex 9, Fig. A5). The "Reclassify" tool was used to delimit areas of 20° and steeper, and 30° and steeper.

Geographical distance

Geographical distance in itself is obviously a cost to movement. This is basically the isolation by distance hypothesis, which assumes that populations closer together are more similar than those further apart (Hutchison & Templeton, 1999). This means that the variable is not a landscape variable per se, but represents the effect of the spatial distribution of ponds.

Geographical distances were calculated using the "Point distance" tool in ArcMap, with the coordinates of the center of the breeding ponds as points.

Stream distance

The high water loss rate for amphibians in hot and dry conditions (Oke, 1987; Wells, 2007) likely may resulted in a preference for, or advantage of streams as dispersal corridors. This has been shown to be the case for the Vermillion spotted newt (*Notophthalmus viridescens*) (Hurlbert, 1969).

Data describing streams in the study area was collected from FKB data (Kartverket, 2010). Streams were initially represented as polylines, but were converted to polygons by the tool "Buffer", using a 1.5 m buffer for all streams. Then this was converted to raster by the tool "Polygon to raster". The "Reclassify" tool was used to create a cost-raster, where streams had a low cost (1) and all other areas a moderately higher cost (5). Least cost paths between all pond pairs were then calculated with the tools "Path

distance" and "Cost path". This produced least cost paths that could jump between stream networks, if they were close together. This was allowed because it seemed plausible that an amphibian could traverse short distances of non-stream habitat. The least cost paths were converted to shapefiles using the tool "Raster to polyline", and length of each line segment was calculated using "Calculate geometry".

Stream area

Streams as dispersal habitat was also quantified as the amount of area of streams between ponds.

The shape file of buffered streams from the quantification of stream distances, were used. All streams were given a width of 3 m (see Annex 9, Fig. A3).

Non-forested areas 2008 and 2015

A canopy cover can affect microclimate by reducing the amount of solar radiation that reaches the ground. Thus, a forest is associated with a more humid and cooler climate, compared to open areas (Oke, 1987). The removal of forest could potentially inhibit dispersal by creating a drier microclimate. The removal of forest could also affect prey abundance (Atlegrim & Sjöberg, 1996; Stuen & Spidsø, 1988)

Because of the changing effect of clear cuts on the landscape, and the fact it takes some time before the signal is detectable in the genetic data, it is difficult to estimate what time period best represented the potential cause of the contemporary pattern of genetic differentiation. The data available for quantification was from 2008, and 2015.

Therefore, two possibilities were considered: 1.The contemporary genetic structure had after 9 years caught up with the effect of the 2008 configuration of non-forested areas, while no effect of more recent events could be detected. 2. The allele frequencies were better represented by non-forested areas in 2015 and 2008 combined. It was then assumed that the non-forested areas from 2008 had not had time to regrow into suitable dispersal habitat, or if they had, that the genetic data had not had time to catch up.

A digital surface model (DSM) was created from LAS-data (Kartverket, 2008). The DEM created earlier was then subtracted from the DSM, to create a raster of vegetation

height. This raster included also features other than vegetation, such as cabins and a powerline, but they were so few that it was ignored. Non-forested areas were defined as areas with vegetation lower than 5 m. Bogs and open surfaces were not included, as the main interest was in the effect of human activities. Thus, in essence all non-forested areas represented clear-cuts and the burned area from the fire in 1992. The “Reclassify” tool was used to delimit non-forested areas.

Since the LAS-data was created in 2008, the vegetation raster was not up to date. Forestry has continuously changed the amount of non-forested areas since then. An aerial photo from 2015 ("Ortofoto Telemark 2015 [Photo]," 2015) were used to draw in clear-cuts produced in the period 2008 to 2015. These were used to update the raster of non-forested areas from 2008, using the tools “Polygon to raster”, “Reclassify” and “Raster calculator” (see Annex 9, Fig. A4).

Forest gravel roads

Roads have been found to act as dispersal barriers for other amphibian species (e.g. Richardson, 2012; Sotiropoulos et al., 2013). The gravel roads have probably too little traffic to increase mortality directly. However, the changed microclimate at the road surface and the creation of forest edges is likely a potential barrier (Marsh & Beckman, 2004).The effect of steep roadside verges could also limit dispersal(Marsh et al., 2005).

Data describing gravel roads were collected from FKB data (Kartverket, 2010), and these were represented as polylines. The “Buffer” tool was used to convert lines to polygons, and the roads were given a width of 4.5 meter. Most of the gravel roads in the study area was built between 1960-1971 (Kartverket, 1971; Unknown, 1960) (see Annex 9, Fig. A3).

Low soil productivity

It has been found that herb rich forest is beneficial for the reproductive output of the northern crested newt (Vuorio et al., 2013). This could be because of a higher abundance of invertebrates connected to soil types with higher pH (Wareborn, 1992).Soil productivity might be important while dispersing, as areas with high soil productivity could be preferred over less nutrient rich sites.

Data describing soil productivity was gathered from FKB data (Kartverket, 2010), and areas of low soil productivity was extracted using the tool "Reclassify" (see Annex 9, Fig. A5). Low productivity was defined as the two classes representing the lowest ("impediment") and 2nd lowest ("lav bonitet") soil productivity.

2.13.2 Statistical analysis of landscape effects on genetic differentiation

The relationship between genetic structure and landscape features, including geographical distance, was tested with a maximum likelihood population effects linear mixed effects model using the package "ResistanceGA" in R (W. E. Peterman, 2018). The geographical distance and landscape variables were incorporated as fixed effects, while dependency of observations caused by the pairwise study design was incorporated into the random effects (formulas found in Clarke, Rothery, & Raybould, 2002).

Models were created based on three main hypothesis' explaining the genetic structure:

1. Prey abundance
2. Microclimate – moisture gradient
3. Topography

The null models were 1. Random pattern (only intercept) and 2. Isolation by distance (geographical distance) (Tab. 7). All models, except the null models, were run both with and without geographical distance as a fixed effect. All models were run with either pairwise Fst or Dc as the response variable. All predictor variables were standardized before the models were run, because of large differences in scale. More detailed model descriptions are in Table 7.

Table 7: A priori models and hypothesis' of the relationship between landscape variables and geographical distance, and genetic differentiation measured as Fst or Dc. Expected negative effects are denoted with (-), and positive effects with (+). Geographical distance =DIST, aspect = ASP, streams =STRM, non-forested areas 2015= OPEN, low soil productivity = PROD, gravel roads = ROAD, slope 30° or steeper = SLOP

Model	Fixed effects	Hypothesis'
		Null hypothesis
01	Only intercept	Random pattern
02	DIST	Isolation by distance

Model without DIST	Model with DIST	Fixed effects (with and without DIST included)	Hypothesis'
			Prey abundance
V1	V1d	PROD + OPEN	Areas of low prey abundance caused by low soil productivity PROD (+) and less prey due to drier microclimate OPEN (+), increases mortality or is avoided by the newts
V2	V2d	PROD	Low soil productivity PROD (+) may cause lower prey abundance, and thus affect newt dispersal. The effect of canopy removal is not detectable in the genetic data.
V3	V3d	OPEN	Low prey abundance because of drier microclimate OPEN (+). The effect of soil productivity is less important.
			Microclimate – moisture gradient
M1	M1d	ASP + ROAD + OPEN + STRM	Dry microclimates ASP (+), OPEN (+), ROAD (+) and humid corridors STRM (-), can resist or permit gene flow.
M2	M2d	ASP + OPEN + STRM	Enhanced solar radiation ASP (+) and less shadowing effect of forest canopy OPEN (+) can increase mortality or create areas that are avoided, while humid areas STRM (-) can function as corridors. Roads have no detectable effect.
M3	M3d	ASP + ROAD + STRM	Factors causing a drier microclimate ASP (+) and ROAD (+), and moist corridors STRM (-) have an effect on genetic differentiation. Non forested areas may not be as important, thus not included.
M4	M4d	ASP + STRM	High solar radiations ASP (+) can create drier microclimates, while streams can function as corridors STRM (-). Neither roads nor open areas create a detectable signal.
M5	M5d	STRM	Low drought tolerance can have created a generally preference for humid areas, which are used as corridors STRM (-).
M6	M6d	ASP + ROAD + OPEN	High solar radiation ASP (+) and human altered landscapes ROAD (+), OPEN (+), creates a more unsuitable habitat for dispersing newts. Streams do not seem to have an important function as corridors.
M7	M7d	ASP + ROAD	Lack of canopy and understory vegetation, and edge effects caused by gravel roads ROAD (+), create a drier microclimate. The same does the higher solar radiation load in south/southwest facing slopes ASP (+). Streams are not important corridors, while open areas do not create a detectable signal in the genetic data.
M8	M8d	ASP + OPEN	Dry microclimate created by high solar radiation ASP (+) and no canopy shadowing effect OPEN (+), increases genetic differentiation. Gravel roads have no detectable effect, while streams are not important as dispersal corridors.
M9	M9d	ROAD + OPEN	Human altered landscape modifies microclimate ROAD (+), OPEN (+) and thus increases genetic differentiation between populations. South/south-west facing slopes does not increase population differentiation, and stream have no important role as dispersal corridors.
M10	M10d	ASP	The amount of incoming solar radiation ASP (+) alone is the only important factor (of the tested factors) governing the dispersal between ponds.
M11	M11d	ROAD	Forest gravel roads can function as barriers, possibly because of edge effects and an altered microclimate ROAD (+). Other landscape variables are not important enough to be detectable in the genetic

			data.
			Topography
T1	T1d	SLOP + ROAD	Steep slopes (30° +) are avoided by newts SLOP (+), even road side verges can be difficult to traverse ROAD (+).
T2	T2d	SLOP	Naturally occurring steeper slopes are avoided by newts SLOP (+), but no obvious effect of road side verges.

An information-theoretic model approach with a correction for finite sample size (AICc) was used to compare models (Akaike, 1974; Burnham, 2002). AICc, ΔAICc, Akaike's weights and log likelihood were calculated using the package "AICcmodavg" in R (for formulas see Burnham, 2002; Mazerolle, 2017). Models run with the responses Fst and Dc were compared separately. According to Burnham (2002) models with a ΔAICc < 4- 7 are plausible models. For that reason a subset of models with ΔAICc < 4 were extracted for both response variables. The support of the models was evaluated using the evidence ratio described in Burnham (2002):

$$\text{Evidence ratio} = \frac{w_i}{w_j}$$

Where

w_i is the Akaike's weight for the model of interest

w_j is the Akaike's weight for the model being compared to (a lower ranking model).

The uncertainty of the estimated regression coefficients was assessed with approximate 95 % confidence intervals, attained by the rule-of-thumb formula: ± 2 x Standard Error.

As an expression of the strength of the relationship between predictor and response variables, the standardized estimated regression coefficients for the highest ranked models for both response variables was used. Standardized regression coefficients can be used to compare effect size both within the same model, and even between studies (Schielzeth, 2010). A Wald $\chi 2$ test was used to evaluate the significance of the regression coefficients, calculated in the "CAR"-package in R (Fox & Weisberg, 2011)

3 Results

3.1 Catch by Unit Effort (CPUE)

The CPUE data indicated that the most abundant ponds where pond B with a median of 26.5 (interquartile range 20.3 - 32.8), pond K with a median of 27.0 (interquartile range 26.5 - 27.5) and pond L with a median of 25.5 (interquartile range18.8 - 32.3). The ponds with the lowest CPUE were pond F with a median of 1.0 (interquartile range1.0 - 2.0) and pond G with a median of 1.5 (interquartile range 1.0 - 2.3). The others were intermediate (Fig.3, se Annex 2, Tab. A1)

The variation in number of newts captured sometimes varied a lot between traps in the same pond, both across different days and for the same day. The most extreme examples were from pond L. Here the largest difference between traps across days was 27 newts, while the largest difference between traps for the same day was 22 newts.

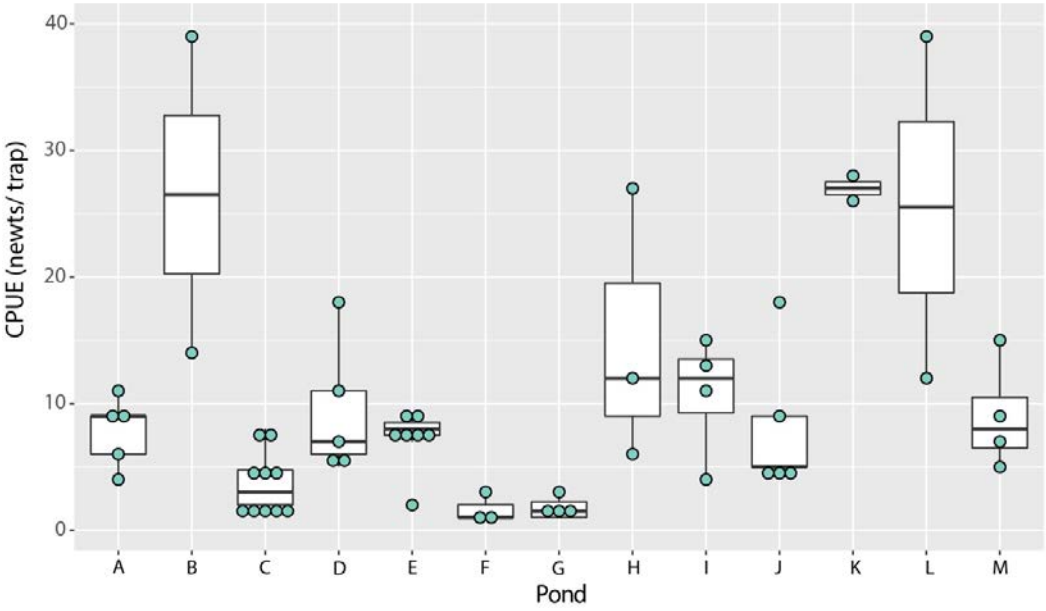

Figure 3: Catch per Unit Effort estimated as number of captured newt per trap during one night, for each pond. Combined boxplot and point plot, each green dot is one estimated CPUE value.

A heavy rain fall between 8 -10 June could possibly have resulted in a lower median CPUE for the ponds that were sampled right after this period (see Annex 1, Fig. A1). If that was the case, the following ponds would be affected: pond E, pond I, pond J and pond M.

The variability of capture within pond, both in space and time, suggests that the CPUE data should be taken only as a rough indicator of abundance.

3.2 Preliminary statistical analysis

The test implemented in Bayescan found one locus (Tc50) under balancing or purifying selection (P(α≠0)=1.00). No deviation from Hardy Weinberg equilibrium was found within samples after correcting for multiple tests (p>0.0036). One loci pair was found to be in linkage disequilibrium (p=0.00) after correcting for multiple tests (Tcri36 – Tc50, pond M). Tc50 was dropped from all subsequent analyses, except for the summary statistics described in section 3.3.

3.3 Summary statistics for the microsatellite loci

The number of alleles at each locus ranged from 3 (Tc85, Tc69) to 29 (Tc50) (Tab. 8). The contribution from each locus to population structure, estimated as Fst, varied from 0.081 (Tc69) to 0.237 (Tcri46). Fst over all loci and samples were 0.157. Genetic diversity defined as expected heterozygosity varied considerably between loci, from 0.076 (Tc69) to 0.831 (Tc50) (Tab. 8). The error rate, estimated on the basis of 10 % re-amplified samples, was 1.78 %.

Table 8: The 14 microsatellite loci used in the study, total number of alleles per locus, expected heterozygosity (He) and genetic differentiation per locus (Fst).

Locus	**Total No. Alleles**	**He**	**Fst**
Tcri43	9	0.640	0.154
Tc52	6	0.469	0.179
Tc85	3	0.456	0.138
Tc69	3	0.076	0.081
Tcri13	8	0.525	0.188
Tcri35	8	0.604	0.137
Tcri36	7	0.590	0.229
Tcri46	8	0.577	0.237

Tcri27	12	0.551	0.143
Tc50	29	0.831	0.091
Tc66	4	0.382	0.089
Tc68b	9	0.680	0.132
Tc70	5	0.305	0.121
Tc81	5	0.432	0.184

3.4 Genetic and allelic diversity

3.4.1 Expected heterozygosity

Expected heterozygosity did not vary much between ponds (Fig. 4, see also Annex 3, Tab. A2). However, the lowest value was found in pond I witch had an expected heterozygosity of 0.402, 95% CI [0.365, 0.426], and this was significantly lower than all the other ponds expect pond G ($p<001$). The 2nd lowest value was found in pond H with an expected heterozygosity of 0.463, 95% CI [0.426, 0.485], and this was significantly different from the three ponds with the highest expected heterozygosity, which were pond K (He=0.520, 95% CI [0.482, 0.540], p=0.00), pond E (He= 0.527, 95% CI [0.490, 0.549], p=0.01) and pond L (He=0.531, 95% CI [0.499, 0.543], p=0.00). Pond D had a medium expected heterozygosity, and was found to be significantly different from the pond with the highest expected heterozygosity which was pond L ($p<0.001$) (for all p-values, see Annex 4, Tab.A3).

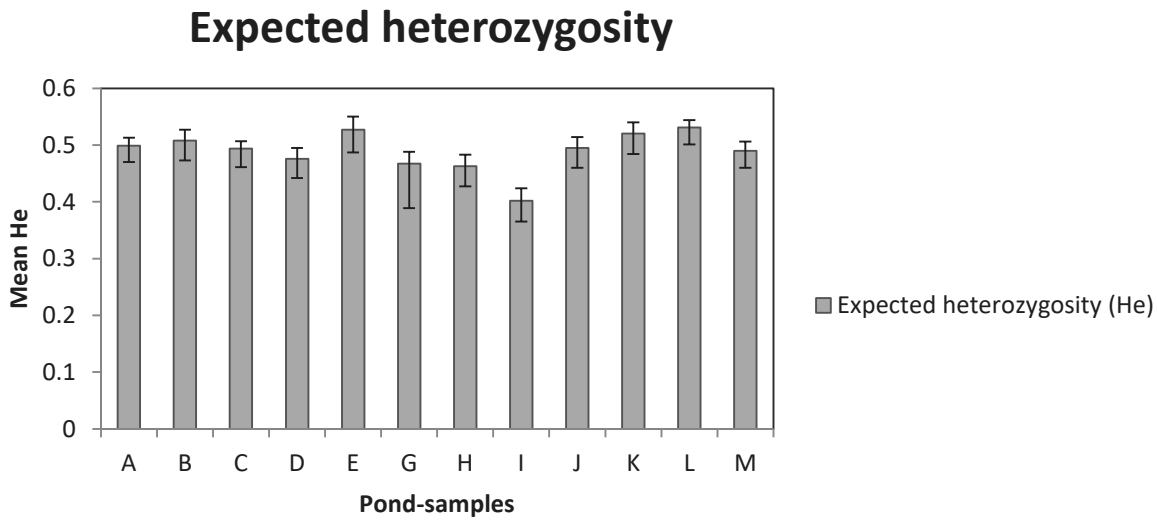

Figure 4: The mean expected heterozygosity estimated from each pond sample, with error bars showing 95 % confidence intervals.

When correcting expected heterozygosity for small sample sizes (uHe) pond G changed from having the 3rd lowest expected heterozygosity, to the 4th, exchanging place with pond D. Since pond G had the smallest sample size, it was most affected by the sample size correction, increasing from He = 0.467 to uHe = 0.483. The other pond samples got only a small increase in He value [0.006 - 0.0084], and this did not lower the estimated difference between the ponds that were significantly different (see Annex 3, Tab. A2)

3.4.2 Allelic richness

Allelic diversity was estimated as allelic richness (Ar). When using a subsample size of 12 the differences between ponds were rather small (Fig. 5). Pond I had the lowest estimate (Ar = 2.9, 95% CI [2.5, 3.2]), while pond B (Ar = 3.7, 95% CI [3.3, 4.0]) and pond K (Ar = 3.7, 95% CI [3.2, 4.4]) had the highest estimate. Allelic richness with a subsample size of 32 showed larger variation. The new range of allelic richness was 3.2 (pond I) to 4.5 (pond K). Pond I still had the lowest estimate, and pond K the highest, but the difference became more pronounced (Fig. 5).

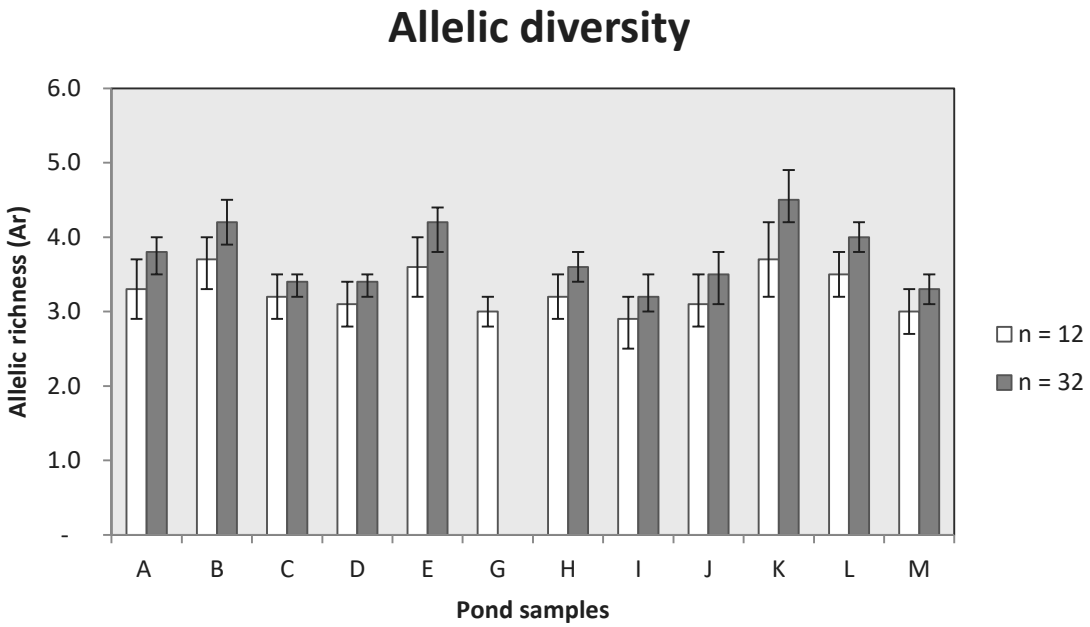

Figure 5: Estimated allelic richness for each pond sample, with subsample size =12 (white bars) and allelic richness estimated for all ponds except pond G, with subsample size = 32 (grey bars). Error bars show 95 % confidence intervals.

The calculated 95% confidence intervals suggested that pond G with allelic richness of 3.0, 95% CI [2.8, 3.2], was significantly different from pond B which had an allelic richness of 3.7, 95% CI [3.3, 4.0] for subsample size 12. All other comparisons were done for subsample size 32. The ponds with the lowest allelic richness was pond C (3.4, 95% CI [3.2, 3.5]), pond D (3.4, 95% CI [3.2, 3.5]), pond I (3.2, 95% CI [3.0, 3.5]), pond M (3.3, 95% CI [3.1, 3.5]). These ponds had non-overlapping confidence intervals with pond B (4.2, 95 % CI [3.9, 4.5]), pond E (4.2, 95% CI [3.8, 4.4]), pond K (4.5, 95% CI [4.2, 4.9]) and pond L (4.0, 95% CI [3.8, 4.2]), suggesting that they had different levels of allelic richness. Pond H and pond J had an intermediate allelic richness of 3.6, 95% CI [3.4, 3.8] and 3.5, 95% CI [3.1, 3.8], respectively, and confidence intervals were not overlapping with pond B and pond K, suggesting that they were different. All summary statistics is presented in Annex3, Tab. A2.

3.5 Impact of old forest on genetic and allelic diversity

The test performed in FSTAT showed that the ponds connected to old forest had a significantly higher allelic richness compared to the other ponds (average Ar = 3.63 and 3.24 respectively, two-sided test, p=0.034). Effect size estimated as Cohen's d was 1.15.

This is considered to be a large to very large effect (Sullivan & Feinn, 2012). The difference in expected heterozygosity, on the other hand, was not significantly different between ponds connected to old forest areas and the other ponds (average He = 0.509 and 0.488, respectively, two-sided test, p=0.372)

3.6 Population structure

The optimum number of clusters estimated from STRUCTURE results varied with the estimation procedure (Tab. 9). The mean Ln P(K) method predicted the highest number of clusters both with balanced and unbalanced sampling. With the uncorrelated allele frequency model, the number was somewhat lower than with the correlated allele frequency model (optimum K = 8 and 9-10, respectively). The tendency of the correlated allele frequency model to generate more clusters have been described in the literature, and is caused by the model itself (Falush, Stephens, & Pritchard, 2003). The ΔK procedure generated the same optimum clusters for both runs, namely four clusters. The optimum number of clusters estimated from TESS results was estimated with two different procedures described in the method-section. Both produced an optimum K estimate of 8 clusters (Tab. 9).

Table 9: The settings used when running population genetic software STRUCTURE and TESS, and the estimated optimum number of clusters obtained by several different methods. K = the range of assumed number of clusters.

K	Admixture model	Other settings		Optimum K estimation method	
STRUCTURE				Mean Ln P(K)	ΔK
1-12	Yes	Uncorrelated allele frequencies	Alpha prior = 1/12	8	4 (8)
1-11	Yes	Correlated allele frequencies	Alpha prior = 1.0	9-10	4 (8)
TESS				DIC-plot	Bar graph/ stabilized K
2-12	Yes	CAR-model		8	8

The bar graphs of average individual assignments, ranging from 4 to 8 clusters, were consistent across TESS (see Annex 5, Fig. A2) and STRUCTURE results (Fig. 6). The

difference was that STRUCTURE produced the same clusters for each replicate for each K, but had more admixture between clusters. TESS varied more when it came to how the ponds were clustered, but the average results were in alignment with the STRUCTURE results.

At the level of four clusters there were three main clusters, one in the south around the nature reserve, one in the east/north-east, and one in the north/north-west around the area with old near-natural forest. The pond at the forest fire site was a cluster on its own. The geographical distance between neighbor ponds within clusters, was sometimes longer than between neighbor ponds from different clusters. The longest distances between neighbor ponds within the same cluster were 2250 m (pond J to K) and 2040 m (pond E to G). While the closest distances between ponds from different clusters were 999 m (pond I to J) and 1298m (pond E to M).

Going from the levels of five to eight clusters (Fig. 6) more and more ponds became separated from their initial cluster. The first pond to be separated out was pond C, which no longer got placed inside the large cluster in the south. Yet it showed some admixture with pond A. Pond C pond was placed a bit downhill from the other ponds, but was only 815 meters from the nearest pond (pond H). The next pond to be separated out from its cluster was pond J, at the level of six clusters. It was no longer placed inside the north/north-west cluster. This was the pond that was furthest away from the other ponds inside the cluster (1822 m to nearest pond). Yet this pond was only 999 m from the nearest pond in the neighboring cluster (pond I). At the level of seven clusters the two ponds in the north/north-east cluster got separated. The distance between the ponds were 1543 meters, but between the ponds was also a topographically rough area. At the level of eight clusters what's remaining of the southern cluster got split in two. Pond E, H and A was placed in one cluster, and pond B and G into another (Fig. 6). Since pond G had a really low CPUE estimate, and all individuals were recaptured at least once, it was assumed that this population consisted of very few individuals. It was suspected that this pond actually contained, for the most part, individuals originating from pond B who either used the pond as a feeding site, or had tried to colonize it recently.

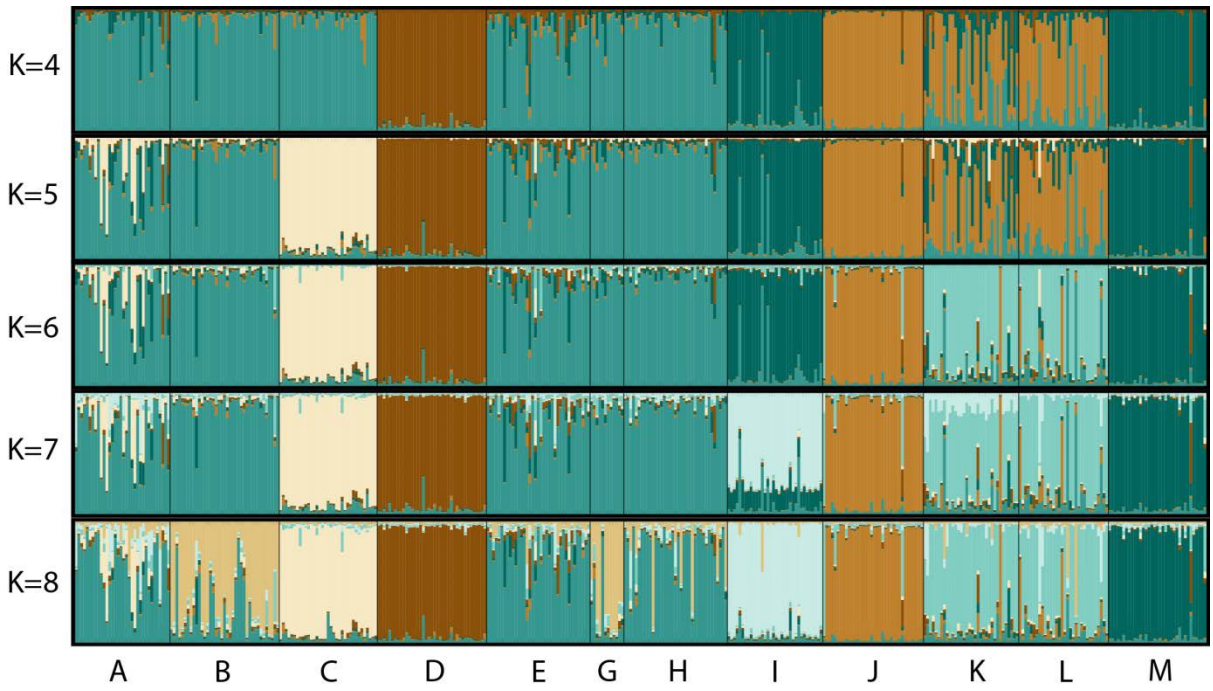

Figure 6: Results from STRUCTURE, which was run with the uncorrelated allele frequency model, and unbalanced sample sizes. The bar graph show individual assignment probabilities grouped after the pond the samples were collected from. Each horizontal bar chart represents the number of clusters (K), going from 4 through 8.

3.7 Genetic differentiation

All pairwise Fst results were significantly different from zero (p=0.00). The values ranged from 0.067, 95 % CI [0.061, 0.077] for pond E to Pond H, to 0.287, 95% CI [0.278, 0.308] for pond I to J (see Annex 6, Tab. A4). All pairwise Dc estimates were different from zero, based on 95 % confidence intervals, suggesting the presence of actual genetic differentiation. The least differentiated pond-pair was Pond E to Pond H with a Dc of 0.228, 95% CI [0.171, 0.289] and the most differentiated were Pond J to Pond M with a Dc of 0.458, 95% CI [0.352, 0.550] (see Annex 7, Tab. A5).

Neighbor-joining tree constructed on the basis of pairwise Fst and Dc showed that they coincided for most parts with the results from STRUCTURE and TESS, but not always (Fig.7). In the Fst-based tree Pond C was placed closer to the two main clusters in the north of the area, instead of with the neighboring ponds in the south. Here the Dc estimates were more consistent with STRUCTURE /TESS results, placing C inside the

larger cluster in the south. Another deviation was that the Dc-based tree associated pond D more closely to the two main clusters in the north, while the Fst-based tree placed pond D closer to the southern cluster. Here, Fst was consistent with STRUCTURE results on the level of three clusters.

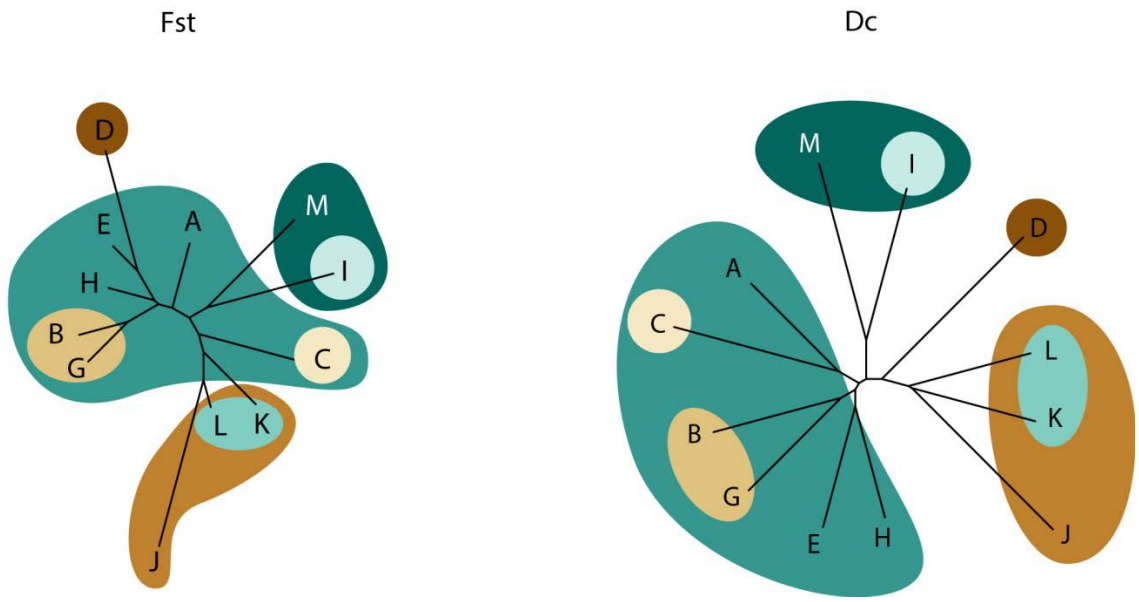

Figure 7: Neighbour joining trees based on pairwise Fst (left) and chord distance Dc (right). Coloured figures represent genetic clusters at the level of four and eight clusters, from the STRUCTURE result (Fig. 6).

3.8 Isolation by distance

The Mantel test showed no significant isolation by distance for neither response variables. Geographical distance tested against Dc had the highest correlation coefficient (r=0.211, p=0.0686), but was not significant with an α-level of 0.05. Geographical distance tested against Fst was far from significant (r=0.124, p=0.188). That was also the case for log-transformed geographical distance tested against linearized Fst (r=0.130, p=0.153)

Isolation by distance was also tested for in the linear mixed effect method, where the random effect of the study design was accounted for. Isolation by distance was significant for both types of responses, but showed a stronger effect for Dc (β =0.0160, $\chi 2$ (1, N=66) = 19.1, p=0.00001) than for Fst (β =0.0082, $\chi 2$ (1, N=66) =4.95, p=0.026).

3.9 Landscape resistance and permeability

3.9.1 Landscape variables and correlation

Some landscape variables were highly correlated, and were dropped from the analysis. The threshold for excluding a variable based on correlation was set to $r > 0.60$.

Slope 20° and steeper and slope 30° and steeper

The variable slope 20° and steeper was highly correlated with slope 30° and steeper ($r=0.88$, $p<0.001$) (see Annex 8, Tab. A6). The first variable represented both steep slopes and generally hilly areas, while the latter represented only steep slopes. It was reasoned that slopes of 20° and steeper would represent areas with increased cost of movement, and also the barrier effect of steep slopes. The latter would put more weight on the barrier effect. It was made a choice to focus on the barrier effect, thus the 20° and steeper variable was dropped.

Stream distance and geographical distance

Geographical distance and stream distance was very highly correlated ($r = 0.97$, $p < 0.001$) (see Annex 8, Tab. A6). It was reasoned that the variables were too similar to actually say something about the use of streams as corridors. Geographical distance had to be included either how, because it represented a null model for landscape resistance. Stream distance was therefore dropped.

Non-forested areas for 2008 and 2015

Non-forested areas for 2008 and 2015 was very highly correlated ($r=0.97$, $p<0.001$) (see Annex 8, Tab. A6). It was assumed that the areas clear-cut after 2008 could have contributed to the genetic differentiation found today, therefore the non-forested areas for 2015 was kept.

Stream area and gravel roads

Stream area was moderately correlated with gravel roads ($r=0.60$, $p<0.001$) (see Annex 8, Tab. A6). This fell exactly below the decided threshold for correlation. Since the

effect of streams as corridors and the effect of gravel roads as resistance to gene flow both were seen as important hypothesis, none were excluded.

3.9.2 AICc top ranked models

Dc as response variable

The highest ranked model (M3d) for Dc as response variable was the one representing the hypothesis of microclimate effects (Tab. 10). It included geographical distance, aspect, gravel roads and streams. The evidence ratio showed that it had 1.7 more support than the 2nd highest ranked model (T2d), which included only geographical distance and slope. The latter model represented the hypothesis of topographical effects on genetic differentiation.

Model M3d had all the same predictor variables as model M7d (the 4th highest ranked model), but in addition included streams. The model including streams had 2.6 more support than the one that didn't, indicating that streams improved the model to a certain degree. The 2nd and 3rd highest ranking models (T2d and T1d) had a similar weight (0.18 and 0.17) (Tab. 10). Td2 included only geographical distance and slope, while Td1 in addition to geographical distance and slope, also included roads. In other words, including roads did not improve the model.

Both null models were ranked much lower than the top models. The isolation by distance model (model 02, ranked as nr.15) performed better than the one with only intercept (model 01, ranked as nr. 30) (Tab. 10). As both models had Akaike's weight = 0.00, evidence ratio could not be calculated.

Table 10: The highest ranked a priori models and the null models ranked relative to Dc as measure of genetic differentiation. The models are ranked after the smallest AICc value. Rank = the rank based on AICc values, Model= name of the model, fixed effects = the predictor variables, K=number of parameters in the model, AICc= the AICc score, ΔAICc= the difference between AICc for the model of concern compared to the highest ranked model, wi= Akaike's weight, Cum.wi= cumulative Akaike's weight, LnL=log likelihood.

Rank	Model	FIXED EFFECTS	K	AICc	ΔAICc	wi	Cum. wi	LnL
1	M3d	DIST + ASP + ROAD+ STRM	7	-268.21	0	0.31	0.31	142.07
2	T2d	DIST + SLOP	5	-267.04	1.17	0.18	0.49	139.02
3	T1d	DIST + SLOP + ROAD	6	-266.94	1.27	0.17	0.66	140.18

4	M7d	DIST + ASP + ROAD	6	-266.23	1.98	0.12	0.77	139.82
5	M1d	DIST + ASP + OPEN + ROAD + STRM	8	-265.87	2.34	0.1	0.87	142.2
6	M6d	DIST + ASP + OPEN + ROAD	7	-264.55	3.66	0.05	0.92	140.24
		Null models						
15	02	DIST	4	-253.42	14.79	0.00	1.00	131.04
30	01	INTERCEPT	3	-239.45	28.76	0.00	1.00	122.92

Fst as response variable

The highest ranked model for Fst as response variable was also model Md3 (Tab. 11). This had a support of only 1.13 over the 2nd highest ranked model (T1), so the models were seen as competitive. Model T1 represented the topography hypothesis, and included gravel roads and slope. The combination of road and slope had 2.4 more support than the model with only slope (T2), indicating that road should be included in the model. Model M7d had the same combination of variables as model M3d, except missing the variable stream. The evidence ratio showed that adding streams had a support of 1.9.

Both null models were ranked much lower than the top ranked models (Tab. 11). The isolation by distance model (model 02, ranked as nr. 23), had a higher rank then the intercept model (model 01, ranked as nr. 29). Aikake's weights for both variables were 0.00, so evidence ratio could not be calculated.

Table 11: The highest ranked a priori models and the null models ranked relative to Fst as measure of genetic differentiation. The models are ranked after the smallest AICc value. Rank= the rank based on AICc values, Model= name of the model, fixed effects= the predictor variables, K=number of parameters in the model, AICc= the AICc score, ΔAICc= the difference between AICc for the model of concern compared to the highest ranked model, wi= Akaike's weight, Cum.wi= cumulative Akaike's weight, LnL=log likelihood.

Rank	Model	FIXED EFFECTS	K	AICc	ΔAICc	wi	Cum. wi	LnL
1	M3d	DIST + ASP + ROAD + STRM	7	-277.84	0	0.27	0.27	146.88
2	T1	ROAD + SLOP	5	-277.59	0.25	0.24	0.5	144.29
3	M7d	DIST + ASP + ROAD	6	-276.59	1.25	0.14	0.65	145.01
4	T1d	DIST + SLOP + ROAD	6	-275.98	1.86	0.11	0.75	144.7
5	T2	SLOP	4	-275.77	2.07	0.10	0.85	142.21
6	M1d	DIST + ASP + OPEN + ROAD + STRM	8	-275.32	2.52	0.08	0.92	146.92
7	M6d	DIST + ASP + OPEN + ROAD	7	-274.1	3.74	0.04	0.96	145.02
		Null models						
23	02	DIST	4	-253.57	24.27	0.00	1.00	131.11
29	01	INTERCEPT	2	-251.05	26.78	0.00	1.00	128.72

3.9.3 Strength of relationship between landscape features and genetic differentiation

The strength of the relationship between the respective landscape variables and the population genetic differentiation as the response variable (Fst and Dc) were evaluated and compared by looking at the standardized (z-transformed) regression coefficients for each fixed effect landscape variable from the top ranked models (Tab.12).

The top ranked models with Dc as response variable was model Md3, while the top ranked models for Fst as response variable was considered to be both model Md3 and T1. These two models were comparable as shown by their similar Aikake's weights (Tab. 12).

All considered regression coefficients were significant (at an α-level of 0.05), except for the stream variable in model Md3 with Fst as the response variable ($p = 0.059$). All predictor variables showed a positive relationship with the response variable, except for streams (Tab.12)

In model M3d with Dc as response variable, geographical distance had the higher estimated regression coefficient (β=0.022, 95 % CI [0.015, 0.030]), but also very similar to the estimates for aspect (β=0.015, 95 % CI [0.006, 0.024]) and road (β=0.017, 95 % CI [0.006, 0.028]) (Tab.12) (Fig.10). The absolute value of the estimated regression coefficient for the stream variable was also rather similar to the other predictor variables within the same model, but non-overlapping confidence intervals suggested it was different when the sign of the coefficient was considered (β = - 0.012, 95% CI [-0.001, -0.023]) (Fig. 10).

In model M3d with Fst as response variable, roads had the higher regression coefficient value (β=0.018, 95% CI [0.008, 0.028]), yet this was also very similar to the coefficient for aspect, (β=0.017, 95 % CI [0.009, 0.025]) and geographical distance (β=0.016, 95% CI [0.009, 0.023]) (Tab.12) (Fig. 8). Considering the 95 % confidence intervals, these

overlapped, suggesting they are not different. The regression coefficient of streams was here found to be non-significant.

In model T1 with Fst as response variable, road had a higher estimated regression coefficient (β=0.0068) than landscape hill slope (β=0.0036). However, road also had a wider confidence interval with a lower limit very close to zero (95% CI [0.0001, 0.014]) than did slope (95% CI [0.0022, 0.0050]) (Fig. 9). The 95% CI overlap suggests the correlation coefficients were not different.

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Table 12: The strength of the relationship between the top ranked models and the response variables, estimated as the regression coefficient. From left: predictor variables included in the model, the regression coefficients, 2 times the standard error, X^2 statistics, degrees of freedom, p- value and 1 SD of the variable in the original unit.

M3d - Fst	beta	2xSE	X^2	Df	Pr(> X^2)	SD -> Unit
(Intercept)	0.160	0.029				
DIST	0.016	0.0068	21.0	1	0.000005	2137.4 m
ASP	0.017	0.0081	17.8	1	0.00003	4.82 %
ROAD	0.018	0.010	13.1	1	0.00029	0.434 %
STRM	-0.010	0.011	3.6	1	0.059	0.220 %
T1 - Fst	beta	2xSE	X^2	Df	Pr(> X^2)	SD -> Unit
(Intercept)	0.117	0.034				
ROAD	0.0068	0.0067	4.1	1	0.043	0.434 %
SLOPE	0.0036	0.0014	26.7	1	0.0000002	1.81 %
M3d - Dc	beta	2xSE	X^2	Df	Pr(> X^2)	SD -> Unit
(Intercept)	0.337	0.033				
DIST	0.022	0.0072	36.6	1	<0.0000001	2137.4 m
ASP	0.015	0.0086	11.3	1	0.0008	4.82 %
ROAD	0.017	0.011	10.2	1	0.0014	0.434 %
STRM	-0.012	0.011	4.3	1	0.038	0.220 %

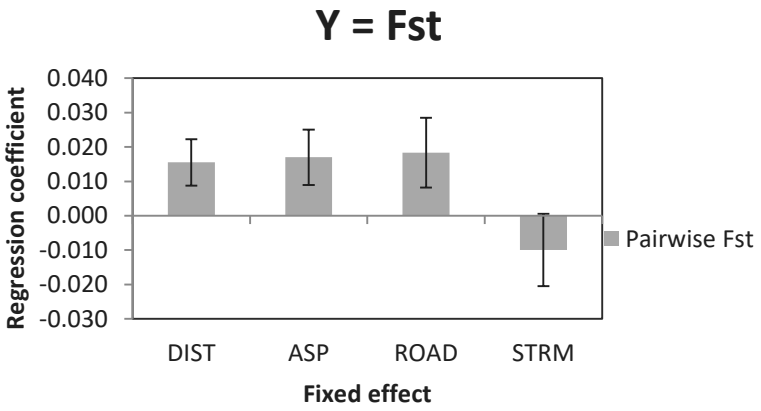

Figure 8: The standardized regression coefficients for the fixed effect in the highest ranked model, with Fst as response variable. Error bars represent 95 % confidence intervals.

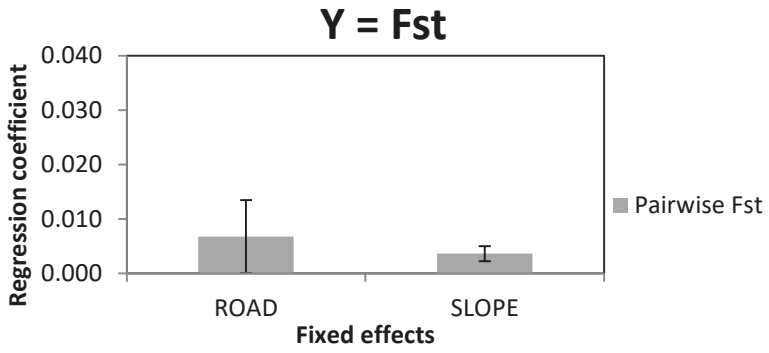

Figure 9: The standardized regression coefficients for the fixed effect from the 2nd highest ranked model, with Fst as response variable. Error bars represent 95 % confidence intervals.

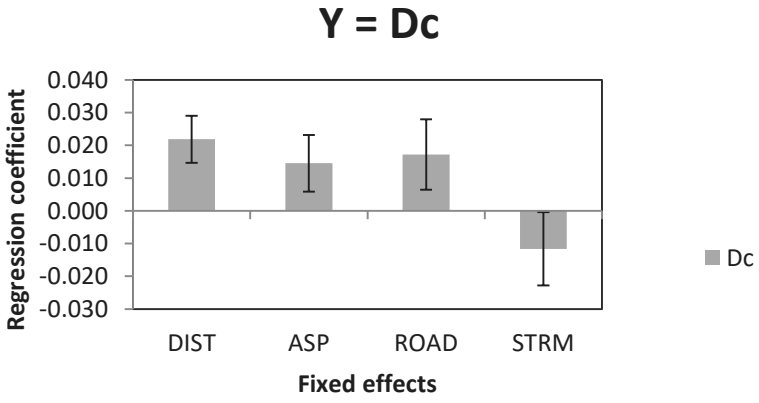

Figure 10: The standardized regression coefficients for the fixed effect for the top ranked model, with Dc as response variable. Error bars represent 95 % confidence intervals.

As mentioned, the predictor variables were standardized before the statistical analysis, meaning that the regression coefficient should be interpreted in terms of the size of the standard deviation of the different variables. In model M3d with Fst as response variable, an increase in one unit (1 SD) % roads could predict an increase in Fst of somewhere between 0.008 and 0.0028. Similarly, 1 SD increase in geographical distance would predict an increase in Fst of somewhere between 0.0092 and 0.0228, i.e.an increase of 0.43 % gravel roads would be approximately equivalent to the effect of 2137.4 m increase in geographical distance.

The potential effects of the different landscape variables may be illustrated by some extrapolation examples and their effect sizes. Say one starts with a pairwise Fst of 0.100 (a moderate level of genetic differentiation (Hartl & Clark, 2007)). An increase of 6 standard deviations of forest gravel roads (which represents an increase from 0% to 2.58 %, which is reasonable given that the maximum amount found in this study was 2.68%), would increase Fst from 0.100 to somewhere between 0.148 and 0.268. That represents an increase from moderate up to the level of nearly great to very great genetic differentiation (Hartl & Clark, 2007). To get approximately the same amount of increase in Fst by geographical distance, it would have to increase by 12 825 m (= 6 SD).

Similarly, if one compared an area of no south-facing slopes, to an area of 6 SD (28.9 %) south-facing slopes, moving from the first area to the other, Fst could be expected to increase from 0.100 to somewhere between 0.150 and 0.250. This represents an increase from moderate to great or very great genetic differentiation (Hartl & Clark, 2007). One could also compare a pond pair separated by an area of flat topography, to an area of 6 SD (10.9 %) steep slopes. Fst would then be predicted to increase from 0.100 to somewhere between 0.102 and 0.130, which is considered to be an increase only within the interval of moderate genetic differentiation, according to Hartl and Clark (2007).

Streams were not significant when Fst was used as response variable, but it was significant when Dc was the response variable. To get an idea of the effect size one could compare two ponds with the maximum value of Dc found in this study to two ponds separated by an area consisting of 6SD (1.32%) more streams. All else being equal, it could be predicted that Dc would decrease from 0.458 to somewhere between 0.452 and 0.320. In other words, it could have almost no effect, or it could actually reduce the genetic distance to under the average found in this study (average Dc = 0.337).

4 Discussion

Habitat loss, and fragmentation, represents a substantial threat to many amphibian populations (Cushman, 2006; J. Gibbs, 1998). Avoiding the negative effects of genetic drift is crucial, and it is important to know what enhances these effects, and what reduces them. This could be specific for the type of organism, the species and also the type of habitat the populations inhabit. In the present study I have studied northern crested newt populations in a boreal forest ecosystem. In the present study allelic diversity varied more between ponds, than heterozygosity. Ponds connected to old forest showed a higher allelic diversity compared to ponds in areas more affected by forestry. When it came to gene flow it was found that geographical distance, microclimate conditions and topography were significantly related to the level of genetic differentiation.

4.1 Genetic and allelic diversity across ponds

The expected heterozygosity varied less between ponds than allelic richness, but for both cases pond I were the one with lowest amount of diversity. Compared to the ponds with the highest level of expected heterozygosity, pond H, and to a certain degree pond D, also showed some lack of genetic diversity. The allelic diversity varied much more. The less diverse ponds were then pond C, pond D, pond I and pond M, which had non-overlapping confidence intervals with the four study ponds with the highest allelic diversity, suggesting that they were different.

For some reason pond I seemed to have experienced the most drastic effect of genetic drift, even though the CPUE data indicated that it had an intermediate abundance of newts. One explanation could be what looked like a low migration rate between pond I and the closest neighbor, pond J. These ponds got placed into different clusters by STRUCTURE and TESS at the level of four clusters. They also had the highest genetic differentiation of all pond pairs measured by Fst, and the 2nd highest measured by Dc. This could not be explained by geographical distance alone, since these ponds were separated by only 1 km and genetic differentiation between several other ponds at similar distances were much lower.

A low migration rate could be related to the gravel road that passes between the ponds. This amounted to the 2nd highest amount of gravel road area between the studied ponds. The other neighboring pond, pond M, might not have contributed many migrants either. The area between pond M and pond I consists of a quite rough topography. In other words, immigration into pond I could have been too low to compensate for the loss of genetic and allelic diversity caused by genetic drift (Allendorf et al., 2013).

Pond D was another pond that stood out. It had a low allelic diversity, but heterozygosity was only significantly lower than the most diverse pond (pond L), and CPUE indicated that it had an intermediate population size. However, the pond was separated out as a single cluster in STRUCTURE and TESS at the level of four clusters. One possible explanation of the discrepancy between heterozygosity and allelic richness could be that the population may have experienced a population reduction, e.g. caused by the forest fire in 1992, but that is has to some degree recovered in population size (indicated by the CPUE). In contrast to allelic diversity, heterozygosity need not be affected much if the population grows fast after the population reduction (Allendorf et al., 2013). Another possibility is that the population is in a process of losing both allelic and genetic diversity, caused by a relatively small population size and a recent reduction in migration rate. Yet, the loss of heterozygosity is not currently detectable in the genetic data, because of time lag. Time lag is more severe for heterozygosity compared to allelic diversity (Epps & Keyghobadi, 2015). The forest fire created a large forestless area around the pond, and this could have contributed to reduced gene flow, e.g. by causing a drier microclimate.

The ponds with the highest estimated allelic richness were pond B and E in the southern cluster and pond K and L in the north-western cluster. All four ponds belonged to the clusters that were connected to the old forest areas, though pond E was placed 400 meters outside of the nature reserve. While pond K, L and B had the highest CPUE and therefore might be able to best maintain a high allelic diversity (Allendorf et al., 2013), pond E only had an intermediate CPUE. However, this pond seemed have experienced some migration from the north-eastern cluster (Fig. 6). It is also the possibility that the CPUE is too low in pond E, because some of the sampling happened right after a heavy rainfall.

Loss of heterozygosity in neutral molecular markers is correlated with loss of fitness, due to both being related to inbreeding and genetic drift (Reed & Frankham, 2003). Reduced fitness can manifest in different ways. In amphibians a loss of heterozygosity has been found to affect larvae growth and survival and increase frequency of malformation. It have also been found to affect oxygen consumption, clutch size, hatching success, adult survival and increased vulnerability to UV-B radiation (O'brien & Allentoft, 2010). The reduction in heterozygosity need not be substantial before fitness is reduced. According to Ralls et al. (2017), only 10% reduction could be enough to reduce fitness considerably.

Allelic diversity and heterozygosity in neutral markers can also predict adaptive response to selection. This is caused by the information the markers convey about population structure, and not a direct relationship with factors affecting adaption (Caballero & García-Dorado, 2013). That said, the discovery of lower heterozygosity and/or allelic richness in some of the ponds in Notodden could signal a negative trend connected to reduced fitness or loss of adaptive potential.

4.2 The impact of old forest on genetic and allelic diversity

When the ponds were grouped based on connectedness to old forest, STRUCTURE/TESS results were not considered. Therefore Pond E was grouped as a pond not connected to old forest, based on the geographical distance to the nature reserve. However, STRUCTURE/ TESS result suggested that this pond was actually quite closely connected to the other ponds within and around the nature reserve. Even without pond E in the old forest group, there was a significant difference in allelic diversity between the ponds grouped as old forest ponds, and those that were not. On the other side, genetic diversity defined as expected heterozygosity was not significantly different.

One possible explanation for this discrepancy is that forestry activities could have led to reduced population sizes and/or migration rates, making the populations more exposed to the effect of genetic drift. Alleles are lost more rapidly when population sizes is reduced, compared to heterozygosity (Hedrick, 2011), and according to Epps and Keyghobadi (2015) it takes less time before the effect of some disturbance affects

allelic richness, compared to heterozygosity. This means that heterozygosity in lesser degree mirror the contemporary landscape, and could be in the process of decline. Another possibility is that the populations outside of old forest areas experience more frequently events of low population size, yet are able to grow fast enough to that heterozygosity is not substantially affected (Allendorf et al., 2013). This has to do with the fact that allelic richness is more connected to the size of the reduced population, while loss of heterozygosity is also dependent on the duration of the period of low population size (Hedrick, 2011). Both scenarios, though, would lead to a loss of alleles and thus evolutionary potential (Caballero & García-Dorado, 2013).

Since the northern crested newt seems to prefer forest around their breeding ponds (Vuorio et al., 2015), and population differentiation seems to increase with drier microclimates and presence of gravel roads, it seems reasonable to assume that the areas of old forest represents a less fragmented habitat, compared to the rest of the study area. Several studies have found a lower genetic and allelic diversity in amphibian populations located in fragmented habitats (e.g. Cosentino, Phillips, Schooley, Lowe, and Douglas (2012); Hitchings and Beebee (1997); Johansson, Primmer, Sahlsten, and Merilä (2005)). However, these studies focused on the effect of habitat fragmentation caused by urbanization or agricultural land use, and may not be applicable for this study. On the other hand, a study in British Colombia on the costal giant salamander (*Dicamptodon tenebrosus*) found that allelic richness and heterozygosity was positively correlated with the age of the forest stands, where allelic richness showed a higher correlation (r2=0.59) compared to heterozygosity (r2=0.37). Old growth forest showed the highest amount of both measures of genetic variability. The results were attributed to effect of forestry harvest on population size and consequently a higher impact of genetic drift (Curtis & Taylor, 2004).

To sum up, old forest represents areas that have not been disturbed for a long time. Forest removal can affect both reproductive output for the northern crested newt (Vuorio et al., 2013), and possibly migration success due to the creation of a drier microclimate. Populations in undisturbed areas could thus be less prone to reduction of population size and/or a lowering of gene flow rate, which can cause genetic drift to erode the allelic and genetic diversity and create divergent allele frequencies between populations.

4.3 Spatial effect on genetic differentiation

Isolation by distance

There was not found a significant correlation between geographical distance and genetic differentiation, when using a Mantel test. However, there was found a significant isolation by distance effect for both response variables (Fst and Dc), when testing it in a mixed effect model.

According to Hutchison and Templeton (1999) isolation by distance emerges when gene flow is higher among neighboring populations, compared to more distant populations. They mention two scenarios where isolation by distance no longer is present. The first scenario is when there is generally strong gene flow that homogenizes allele frequencies between all populations. The other scenario is when the populations get isolated so that genetic drift makes allele frequencies drift in independent directions. Given that isolation by distance was found to be significant in this master study, it was assumed that the more extreme scenarios of strong gene flow or no gene flow could be excluded. The hypothesis of isolation by distance is often tested with the Mantel test. This was the case for a study of the northern crested newt in an agricultural landscape in Germany/Austria, where they found no significant result (Peter et al., 2009). The same was the case for a study of the northern crested newt in Flanders, also this with a non-significant result (Schön et al., 2011). However, a study in France found significant isolation by distance effect for this species inhabiting man made cattle ponds (Jehle, Wilson, Arntzen, & Burke, 2005). All three studies used the Mantel test. The fact that the Mantel test generated negative result, in my study, while the mixed effect model did not, shows how the result might be a result of method choice rather than an actual genetic pattern.

The spatial distribution of breeding ponds is important, especially under isolation by distance scenario. A loss of breeding habitat, for example caused by wetland drainage or fish introduction(Malmgren, 2001), would increase distance between breeding populations. In the worst case this could isolate populations because of distance to nearest neighbor being too large for gene flow to occur. For this reason even smaller

wetlands should be preserved, and should not be seen as expandable (Semlitsch & Bodie, 1998).

4.4 Landscape effects on genetic differentiation

In the statistical analysis of landscape effects on genetic differentiation, the isolation by distance hypothesis was used as a null model. It was found that the null model performed a lot worse than the models which also included landscape variables. This implies that the intervening landscape between ponds have played a role in shaping the pattern of genetic differentiation. This was also consistent with the results from STRUCTURE and TESS. Here, geographical distance between ponds in the same clusters was sometimes much larger than between ponds from different clusters.

Microclimate effects

The model with the highest rank (based on AICc score), for both Fst and Dc as response variable, was the one representing the microclimate hypothesis. All variables related to microclimate were included, except for non-forested areas. The reason that non-forested areas were not included could have something to do with the unstable nature of the variable, due to the continuing removal of forest and regrowth of old clear cuts.

Aspect

In the present study it was found that south/south-west facing slopes was related to higher genetic differentiation. Since many amphibians, including the genus Triturus, have a poor ability to regulate water loss (Wells, 2007), drier microclimates could pose a problem. The amount of received solar radiation is governed by several factors, including aspect (Oke, 1987). In the study area the highest amount of solar radiation is received in the south/south-west facing slopes (Hernan et al., 2018; Oke, 1987) and this could have caused these areas to have a drier microclimate, due to higher evaporation (Oke, 1987).

A high water loss rate in hot and dry conditions (Wells, 2007) could have led to a general preference for moist and cool microclimate amongst amphibians. This were the case for the western slimy salamander (*Plethodon albagula*) in Missouri, and for several salamander species in North Carolina (Harper & Guynn, 1999). When it comes to the effect of solar radiation load on gene flow, it has been found that high radiation load had a negative effect on gene flow for the southern torrent salamander *(Rhyacotriton*

variegatus) in California (Emel & Storfer, 2015) and the coastal tailed frog (*Ascaphus truei*) in Washington (Spear & Storfer, 2008).

Increased solar radiation load can also lead to increased daytime temperatures, yet it seems unlikely that this is the main problem for the northern crested newt, as this species is most active at night (Dervo, Bærum, Skurdal, & Museth, 2016; Malmgren, 2007).

Forest gravel roads

Another landscape variable that was found to be significantly related to increased genetic differentiation was forest gravel roads. Roads as such, have been found to be a barrier for amphibians, e.g. the smooth newt (*Lissotriton vulgaris)* (Sotiropoulos et al., 2013) and the spotted salamander (*Ambystoma maculatum*)(Richardson, 2012). Roads can increase mortality through the direct effect of vehicles killing or injuring the amphibians (Mitchell, Brown, & Bartholomew, 2008). A synthesis on amphibian road mortality concluded that the Triturus genus was one of the more common victims of road kill in rural areas (Elzanowski, Ciesiołkiewicz, Kaczor, Radwańska, & Urban, 2009). However, the probability of getting killed why crossing a road is connected to the amount of traffic in relation to the active periods of the amphibian species (Hels & Buchwald, 2001). The forest gravel roads in the study area are mostly used for outdoors activities like fishing, hunting and hiking. Now and then it is used for timber transport during logging. In other words, the activity is mostly located to daytime, while the northern crested newt is mostly a night active animal (Dervo & Kraabøl, 2010). The low amount of traffic makes it unlikely that this could explain the significant relationship of gravel roads on genetic differentiation.

Another explanation could be the effect of forest roads when it comes to microclimate. The construction of roads entails forest removal, which creates forest edges. Such edge effects is caused by an increased vapor concentration gradient and heat supply, i.e. increased evaporation, when air from hot and dry surfaces meets a more humid vegetated surface (Oke, 1987). In Virginia studied red-backed salamanders were observed less frequently in the edges around forest roads (up to 20 m from the roads). This was related to a decrease in soil moisture caused by the forest edges (Marsh & Beckman, 2004). It has also been found that landscape permeability for several

amphibian species is reduced more along forest-road edges, compared to edges between forest and open land (J. P. Gibbs, 1998).

Streams

The stream variable was also included in the top ranked model representing microclimate effects on genetic differentiation. They seemed to be related to a decrease in genetic differentiation, yet the effect was not significant for Fst as response variable. When Dc was used as response variable, the effect was significant, but the confidence interval was wide. The lower limit was very close to zero. Moreover, the model including the stream variable had 2.6 times more support (Dc as response variable), compared to the model with all the same variables but streams excluded. This indicates that streams can have an effect, but the amount is uncertain, and should be investigated further.

For the southern torrent salamander gene flow have been found to be limited by the lack of streams (Emel & Storfer, 2015). When it comes to other types of water ways large and medium rivers have been shown to have a negative effect on genetic connectivity for the spotted salamander and the northern crested newt (Peter et al., 2009; Richardson, 2012). Rivers and streams can be seen as lying on an continuum between barrier and corridor (Puth & Wilson, 2001). Whether the water way is experienced as a corridor or a barrier, depends on both the species mobility and the width and length of the water way and the amount of water flow (Puth & Wilson, 2001). The latter can also fluctuate with time. The study area in Notodden does not contain any rivers, and since the effect of streams seemed to decrease genetic differentiation, they are likely not large enough to be experienced as general barriers. Even though the largest streams during snow melt or heavy rain, possibly could be difficult to cross.

If streams function as corridors, configuration could matter. A stream going from one pond to another could be a more efficient corridor, than streams crossing the area between ponds. For this reason, quantifying the % area of streams between ponds might not be the best way to test the effect of streams as corridors.

Non-forested areas

Non-forested areas did not turn up as a significant landscape variable. This was unexpected due to the fact that clear cutting seems to affect many amphibian species negatively (deMaynadier & Hunter, 2011; Semlitsch et al., 2009; Tilghman, Ramee, & Marsh, 2012). And because northern crested newt seem to avoid clear-cuts and other non-forested areas in their terrestrial habitat around the breeding pond (Alexander Kupfer & Kneitz, 2000; Vuorio et al., 2015). Forest have also been found to be positively related to gene flow for several salamander species , such as the southern torrent salamander (Emel & Storfer, 2015), spotted salamander (Richardson, 2012), the alpine newt (Emaresi et al., 2011) and the marbled salamander (*Ambystoma opacum*) (Greenwald et al., 2009). Most of these studies have tested the effect of forest in contrast to other landscape types, such as agricultural areas, open fields and so on. This would represent a more stable pattern, than the configuration of non-forested areas caused by forest harvest. An area could be used for dispersal for years, and suddenly become a barrier after logging have taken place, yet the effect would not be immediately detectable in genetic data because of time lag. For example, the time lag between forest harvest and genetic response (measured as G'st) was 20-40 years for the coastal tailed frog (*Ascaphus truei*) (Spear & Storfer, 2008). When forest grows back in previous clear-cuts they could potentially become permeable for dispersal again, this effect would also be afflicted by time lag, and even more seriously so than the creation of new barriers, according to Landguth et al. (2010).

There is also the possibility that clear-cuts don't make up a problem for dispersing newts. This seems rather unlikely, when considering that all the other tested variables connected to the dryness/moistness of microclimate turned out to be significant. Clear-cuts are expected to be drier, but this could depend on the level of revegetation. Right after logging there can be a period of more soil moisture in clear-cuts because there is no vegetation to deplete soil water. This effect however disappears when the area is revegetated, and then soil moisture becomes lower in clear-cut areas compared to forested areas (Adams, Flint, & Fredriksen, 1991).

Aspect, slope and streams are rather stable features, and the genetics of the newts probably had time to align with the effect of these features. Most of the gravel roads in the area were constructed sometime between 1960 and 1971, which seems to be enough

time to create a genetic signal. In contrast, there has been a continuously change in the configuration of clear-cuts. New areas have been logged and old clear-cuts have regrown. This could have been a too dynamic feature to be detectable with the method used here.

Topographic effects

The 2nd highest ranked model had a similar Akaike's weight as the highest ranked model when using Fst as response variable. This model represented the hypothesis of topography effects on gene flow. Slopes of 30° and steeper showed a significant positive relationship to genetic differentiation. Yet the regression coefficient indicated a weaker effect for slope than for the other variables.

Slopes 30° and steeper

Slopes have been found to have an effect in other amphibian species too. The genetic differentiation of peripheral coastal giant salamander (*Dicamptodon ten*eb*rosus*) populations in British Columbia was positively related to slope. Yet the effect was not seen for core populations. The difference was attributed to smaller populations sizes and lower connectivity in the peripheral populations, which made them more vulnerable to resistance of the intervening landscape between habitats (Dudaniec, Spear, Richardson, Storfer, & Salamin, 2012). For the Panamanian frog a positive correlation between genetic differentiation and slope was also found. The analysis tested cost distance against pairwise Fst, where cost was set to increase linearly with the increase of slope steepness. The relationship showed that the correlation increased most rapidly going from no slopes to about 20 times higher cost value (indicating a rougher terrain), yet the correlation continued to increase with increasingly steeper slopes, where maximum correlation was found at the steepest slopes in the area (cost=100) (Richards-Zawacki, 2009). Slope steepness was also found to be positively related to genetic differentiation for the fire salamander (*Salamandra infraimmaculata)*in Northern Israel (Kershenbaum et al., 2014).

Even though the cut off value was set at 30° and higher my study, it was highly correlated with the presence of slopes of 20° and higher. This makes it harder to say exactly how steep the slopes must be to make a difference. And even though topography had an effect in Notodden, there is the possibility that it is not as important in areas with

larger newt populations with higher general connectivity, as was the case for the coastal giant salamander (Dudaniec et al., 2012).

Forest gravel roads

Gravel roads were also significant in the model representing the topography hypothesis, even though the lower limit of the 95 % confidence interval was very close to zero. A study on red-backed salamander found that when they displaced the salamanders and measured the return rates, forest roads (width 5-8 meters) reduced the return rate by 51 %. The effect was enhanced by steep roadside verges (35 ° and steeper), especially for newts moving downhill (Marsh et al., 2005).

The fact that gravel roads were included in both the microclimate model and the topography model indicates that gravel roads can affect genetic differentiation in more than one way. Yet, based on the regression coefficient the effect it has on microclimate seems to be more important.

Prey abundance

The only hypothesis that was not included in the top ranked models, were the one representing prey abundance. It was assumed that low soil productivity and high solar radiation load could decrease the amount of prey for the northern crested newt. It has been found that that the reproductive output for the northern crested newt can be positively related to the amount of herb rich forest around the pond. And that young forest stands (age 6-15) had a negative effect. This was attributed to the different forest type's ability to offer prey and hiding places (Vuorio et al., 2013). Based on this it was assumed that low soil productivity would generate a lack of herb rich forest, and lower prey abundance. The northern crested newts while on land, prey on insects, spiders and earthworms (DN, 2008). It has been found that clear-cuts can reduce abundance of invertebrates (Atlegrim & Sjöberg, 1996; Stuen & Spidsø, 1988). For that reason it was also assumed that clear-cuts would affect prey abundance negatively, and thus gene flow.

However, in the present study it was not found any indication on the effect of prey abundance on genetic differentiation. It might be because there are other factors that are more important, but also that the quantification of the two variables had some issues. The problem with non-forested areas has already been discussed. The quantification of

soil productivity could be affected by the fact that low soil productivity was correlated with the presence of forest canopy. Areas with high soil productivity, hence more prey, are more often exposed to the impact of forestry, thus less prey. So the pattern gets muddled, and the signal harder to detect. There is also the possibility that prey abundance is at a tolerable level even in low productivity areas.

5 Conclusion

Both habitat loss and habitat fragmentation can be a challenge for many animal populations (Cushman, 2006). An important factor of sustaining populations in areas afflicted by this, is maintaining gene flow. This requires knowledge about the effect of the intervening landscape on dispersal.

There exists a growing amount of literature on how landscape affects genetic differentiation of amphibian populations. Many have focused on landscapes with intervening urban or agricultural areas. Few have focused on the northern crested newt. This species inhabit ponds both in agricultural landscapes and in forested areas. Forest areas might include other factors affecting gene flow than what is known from agricultural areas. Such factors must be acknowledged so that right measures can be taken to preserve these populations. This could also be relevant for other amphibian species living in (boreal) forest ecosystems.

In my study it was found that geographical distance, microclimate and topography between ponds affected the genetic differentiation of northern crested newt populations in a boreal forest ecosystem. Factors that seemed to decrease gene flow was the amount of area with south/south-west facing slopes, gravel roads, slopes of 30° and steeper and geographical distance, while streams seemed to increase landscape permeability. It was also found that populations located within or near old forest had a significant higher allelic diversity, than populations in more managed forest. This was attributed to the fact that these ponds were less affected by forestry harvest and road constructions.

Management implications

When planning any future activities in areas inhabited by northern crested newt, it is important not to disrupt existing newt dispersal between ponds. Ponds already separated by naturally occurring features such as steep and south-facing slopes, are likely more vulnerable to human impact. The combination of south facing slopes and clear-cuts seems like a particular bad combination, as the already high solar radiation load in south facing slopes, will be even higher if canopy is removed. Another problematic case is the construction of gravel roads through an area used for newt dispersal. Rather, dispersal corridors should be maintained that are wide enough to maintain a humid microclimate, preferably in areas with gentle topography.

Maintaining gene flow becomes more important the smaller the populations are. To ensure robust population it is important to preserve both the aquatic and the terrestrial habitat around breeding ponds. This is in addition to preserving a sufficient level of gene flow. One effective measure is to protect larger areas of old forest that also contains northern crested newt habitat and areas used for newt dispersal. As a bonus this will also help preserve other species dependent on this ecosystem.

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Annex 1: Precipitation level in relation to number for captured newts

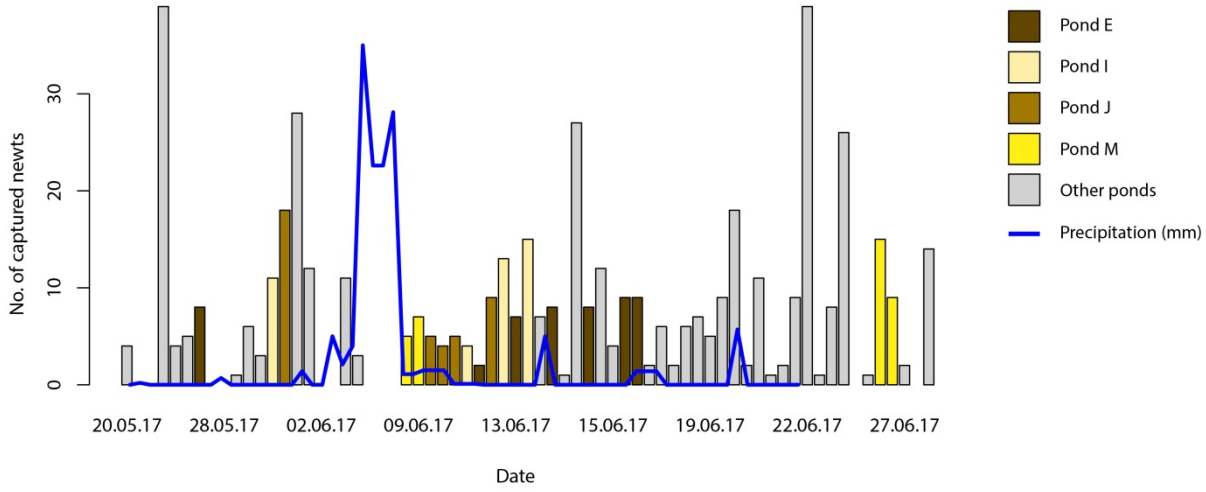

Figure A11: The bar graph represents number of captured newts per day, blue line is the amount of precipitation per day (mm) (yr.no). Samples from pond E (dark brown), pond I (light yellow), pond J (light brown) and pond M (bright yellow) is coloured to show the pattern between number of captured newts, and amount of precipitation received.

Annex 2: Data basis for calculating CPUE

Table A13: The basis for the estimation of CPUE. The number of captures newts and sampling days per pond, median CPUE and the inter quartile range (IQR). Not all sampled newts from pond G were included. The reason was that after 4 days with low catch numbers, the traps were placed close together in the assumed best newt habitat, to increase capture rate. These numbers were not seen as representative for the whole pond and were excluded. The total number of captured newts and sampling days in pond G is described within parenthesis.

Pond	Total no. captures	Effort (no. of days)	Median CPUE	IQR
A	39	5	9	6 - 9
B	53	2	26.5	20.25 - 32.75
C	37	10	3	2 - 4.75
D	47	5	7	6 - 11

E	51	7	8	7.5 - 8.5
F	5	3	1	1 - 2
G	7 (27)	4 (10)	1.5	1 - 2.25
H	45	3	12	9 - 19.5
I	43	4	12	9.25 - 13.5
J	41	5	5	5 - 9
K	54	2	27	26.5 - 27.5
L	51	2	25.5	18.75 - 32.35
M	36	4	8	6.5 - 10.5

Annex 3: Summary statistics for microsatellite loci and populations

Table A2: Summary statistics per locus and per pond, and averages per pond and overall. N=sample size, Na= number of alleles, Ho = observed heteroxygosity, He = expected heterozygosity, uHe = expected heterozygosity corrected for small sample sizes, Ar (resamp. 12) = Allelic richness with subsample size 12, Ar (resamp. 32) = Allelic richness with subsample size 32.

Pond	Locus	N	Na	Ho	He	uHe	Ar (resamp 12.)	Ar (resamp. 32)
A	Locus1	34	8.0	0.618	0.658	0.668	5.3	7.0
A	Locus2	34	3.0	0.353	0.397	0.403	2.3	2.6
A	Locus3	34	3.0	0.588	0.629	0.639	3.0	3.0
A	Locus4	34	1.0	-	-	-	1.0	1.0
A	Locus5	34	4.0	0.765	0.660	0.670	3.9	4.0
A	Locus6	34	7.0	0.735	0.767	0.778	5.8	6.7
A	Locus7	34	4.0	0.765	0.662	0.672	3.3	3.6
A	Locus8	34	3.0	0.412	0.501	0.509	2.7	2.9
A	Locus9	34	6.0	0.529	0.479	0.486	4.4	5.7
A	Locus10	34	3.0	0.382	0.314	0.319	2.3	2.6
A	Locus11	34	5.0	0.706	0.703	0.714	4.6	4.9
A	Locus12	34	3.0	0.353	0.302	0.307	2.6	2.9
A	Locus13	34	2.0	0.353	0.415	0.421	2.0	2.0
A	Average	34.0	4.0	0.505 , 95% CI [0.466-0.543]	0.499 , 95% CI [0.468-0.512]	0.507	3.3 , 95% CI [2.9-3.7]	3.8 , 95% CI [3.5-4.0]
B	Locus1	39	7.0	0.821	0.808	0.818	6.2	6.8
B	Locus2	39	4.0	0.615	0.617	0.625	3.3	3.6
B	Locus3	39	3.0	0.538	0.503	0.509	2.9	3.0
B	Locus4	39	2.0	0.051	0.050	0.051	1.5	1.8
B	Locus5	39	3.0	0.462	0.416	0.421	2.7	3.0
B	Locus6	39	5.0	0.615	0.678	0.686	4.3	4.8
B	Locus7	39	5.0	0.667	0.741	0.750	4.4	4.8
B	Locus8	39	7.0	0.641	0.652	0.661	5.0	6.0
B	Locus9	39	8.0	0.641	0.629	0.637	5.5	7.0

B	Locus10	39	3.0	0.410	0.463	0.469	2.7	3.0
B	Locus11	39	6.0	0.590	0.689	0.698	4.9	5.7
B	Locus12	39	2.0	0.077	0.074	0.075	1.6	1.9
B	Locus13	39	4.0	0.333	0.288	0.292	2.7	3.4
B	Average	39.0	4.5	0.497 , 95% CI [0.452-0.544]	0.508 , 95% CI [0.474-0.527]	0.515	3.7 , 95% CI [3.3-4.0]	4.213 , 95% CI [3.9-4.5]
C	Locus1	35	5.0	0.657	0.722	0.733	4.7	5.0
C	Locus2	35	3.0	0.543	0.622	0.631	3.0	3.0
C	Locus3	35	3.0	0.371	0.508	0.515	2.9	3.0
C	Locus4	35	1.0	-	-	-	1.0	1.0
C	Locus5	35	4.0	0.686	0.675	0.684	3.7	3.9
C	Locus6	35	4.0	0.857	0.724	0.735	3.9	4.0
C	Locus7	35	4.0	0.514	0.533	0.541	3.7	4.0
C	Locus8	35	4.0	0.400	0.440	0.447	3.0	3.6
C	Locus9	35	6.0	0.686	0.716	0.726	4.5	5.2
C	Locus10	35	2.0	0.086	0.082	0.083	1.7	1.9
C	Locus11	35	6.0	0.857	0.794	0.806	5.4	5.8
C	Locus12	35	2.0	0.314	0.337	0.342	2.0	2.0
C	Locus13	35	2.0	0.314	0.265	0.269	2.0	2.0
C	Average	35.0	3.5	0.483 , 95% CI [0.450-0.519]	0.494 , 95% CI [0.462-0.509]	0.501	3.2 , 95% CI [2.9-3.5]	3.4 , 95% CI [3.2-3.5]
D	Locus1	39	4.0	0.436	0.421	0.427	3.5	3.9
D	Locus2	39	4.0	0.538	0.531	0.538	3.3	3.9
D	Locus3	39	3.0	0.462	0.436	0.441	2.6	2.9
D	Locus4	39	2.0	0.308	0.260	0.264	2.0	2.0
D	Locus5	39	4.0	0.487	0.507	0.513	3.5	3.9
D	Locus6	39	2.0	0.538	0.499	0.505	2.0	2.0
D	Locus7	39	3.0	0.333	0.288	0.291	2.6	2.9
D	Locus8	39	6.0	0.769	0.712	0.721	5.1	5.8
D	Locus9	39	6.0	0.769	0.679	0.688	5.1	5.8
D	Locus10	39	2.0	0.282	0.242	0.245	2.0	2.0
D	Locus11	39	5.0	0.821	0.745	0.755	4.3	4.6
D	Locus12	39	3.0	0.385	0.400	0.405	2.3	2.6
D	Locus13	39	2.0	0.462	0.473	0.480	2.0	2.0
D	Average	39.0	3.5	0.507 , 95% CI [0.469-0.543]	0.476 , 95% CI [0.444-0.496]	0.483	3.1 , 95% CI [2.8-3.4]	3.4 , 95% CI [3.2-3.5]
E	Locus1	37	7.0	0.459	0.484	0.490	4.7	6.1
E	Locus2	37	4.0	0.649	0.653	0.662	3.8	4.0
E	Locus3	37	3.0	0.568	0.571	0.579	3.0	3.0
E	Locus4	37	2.0	0.054	0.053	0.053	1.5	1.8
E	Locus5	37	5.0	0.486	0.503	0.510	3.3	4.1
E	Locus6	37	4.0	0.568	0.539	0.547	3.2	3.6
E	Locus7	37	5.0	0.649	0.681	0.690	3.9	4.5
E	Locus8	37	5.0	0.676	0.619	0.628	4.0	4.8

E	Locus9	37	7.0	0.676	0.685	0.695	5.6	6.6
E	Locus10	37	3.0	0.703	0.591	0.599	3.0	3.0
E	Locus11	37	7.0	0.838	0.711	0.720	5.6	6.4
E	Locus12	37	3.0	0.135	0.173	0.176	2.2	2.6
E	Locus13	37	4.0	0.405	0.592	0.600	3.3	3.8
E	Average	37.0	4.5	0.528 , 95% CI [0.489-0.566]	0.527 , 95% CI [0.490-0.549]	0.535	3.6 , 95% CI [3.2-4.0]	4.2 , 95% CI [3.8-4.4]
G	Locus1	12	4.0	0.750	0.726	0.757	4.0	
G	Locus2	12	2.0	0.250	0.413	0.431	2.0	
G	Locus3	12	3.0	0.417	0.455	0.475	2.9	
G	Locus4	12	1.0	-	-	-	1.0	
G	Locus5	12	3.0	0.167	0.156	0.163	2.3	
G	Locus6	12	3.0	0.833	0.622	0.649	3.0	
G	Locus7	12	4.0	0.583	0.583	0.609	3.8	
G	Locus8	12	5.0	0.917	0.767	0.801	4.9	
G	Locus9	12	4.0	0.750	0.608	0.634	3.8	
G	Locus10	12	3.0	0.500	0.403	0.420	2.9	
G	Locus11	12	4.0	0.583	0.559	0.583	3.8	
G	Locus12	12	3.0	0.417	0.406	0.424	2.9	
G	Locus13	12	2.0	0.333	0.375	0.391	2.0	
G	Average	12.0	3.2	0.500 , 95% CI [0.436-0.564]	0.467 , 95% CI [0.393-0.490]	0.487	3.0 , 95% CI [2.8-3.2]	
H	Locus1	37	6.0	0.459	0.621	0.629	4.6	5.5
H	Locus2	37	3.0	0.486	0.449	0.455	2.8	3.0
H	Locus3	37	3.0	0.568	0.581	0.589	3.0	3.0
H	Locus4	37	1.0	-	-	-	1.0	1.0
H	Locus5	37	3.0	0.297	0.333	0.337	2.5	2.8
H	Locus6	37	5.0	0.676	0.663	0.672	4.1	4.6
H	Locus7	37	5.0	0.676	0.634	0.643	3.9	4.7
H	Locus8	37	6.0	0.676	0.678	0.688	4.7	5.5
H	Locus9	37	3.0	0.162	0.151	0.153	2.1	2.6
H	Locus10	37	3.0	0.378	0.343	0.348	2.8	3.0
H	Locus11	37	6.0	0.757	0.718	0.728	4.4	5.2
H	Locus12	37	3.0	0.432	0.431	0.437	2.9	3.0
H	Locus13	37	4.0	0.486	0.416	0.422	3.2	3.6
H	Average	37.0	3.9	0.466 , 95% CI [0.417-0.514]	0.463 , 95% CI [0.426-0.485]	0.469	3.2 , 95% CI [2.9-3.5]	3.6 , 95% CI [3.4-3.8]
I	Locus1	34	6.0	0.706	0.742	0.753	4.9	5.6
I	Locus2	34	3.0	0.147	0.165	0.167	2.4	2.9
I	Locus3	34	3.0	0.088	0.085	0.086	1.8	2.5
I	Locus4	34	2.0	0.088	0.136	0.138	1.8	2.0
I	Locus5	34	4.0	0.294	0.403	0.409	3.1	3.6
I	Locus6	34	5.0	0.706	0.703	0.713	4.5	4.9
I	Locus7	34	5.0	0.618	0.523	0.531	4.0	4.6

I	Locus8	34	3.0	0.382	0.483	0.490	2.3	2.6
I	Locus9	34	5.0	0.382	0.378	0.384	3.8	4.6
I	Locus10	34	2.0	0.206	0.230	0.233	2.0	2.0
I	Locus11	34	3.0	0.618	0.552	0.560	2.8	3.0
I	Locus12	34	2.0	0.353	0.360	0.365	2.0	2.0
I	Locus13	34	2.0	0.588	0.472	0.479	2.0	2.0
I	Average	34.0	3.5	0.398 , 95% CI [0.357-0.439]	0.402 , 95% CI [0.365-0.426]	0.408	2.9 , 95% CI [2.5-3.2]	3.2 , 95% CI [3-3.5]
J	Locus1	36	6.0	0.833	0.742	0.753	4.9	5.5
J	Locus2	36	4.0	0.556	0.555	0.563	3.8	4.0
J	Locus3	36	3.0	0.778	0.660	0.670	3.0	3.0
J	Locus4	36	1.0	-	-	-	1.0	1.0
J	Locus5	36	4.0	0.889	0.661	0.671	3.3	3.6
J	Locus6	36	4.0	0.306	0.373	0.378	2.6	3.2
J	Locus7	36	4.0	0.639	0.697	0.707	3.9	4.0
J	Locus8	36	4.0	0.083	0.107	0.108	2.1	3.0
J	Locus9	36	6.0	0.389	0.449	0.455	3.9	5.0
J	Locus10	36	3.0	0.500	0.599	0.608	3.0	3.0
J	Locus11	36	5.0	0.861	0.707	0.717	4.2	4.6
J	Locus12	36	2.0	0.306	0.330	0.335	2.0	2.0
J	Locus13	36	3.0	0.722	0.554	0.562	3.0	3.0
J	Average	36.0	3.8	0.528 , 95% CI [0.500-0.556]	0.495 , 95% CI [0.460-0.516]	0.502	3.1 , 95% CI [2.8-3.5]	3.5 , 95% CI [3.1-3.8]
K	Locus1	34	7.0	0.529	0.522	0.529	5.2	6.5
K	Locus2	34	5.0	0.500	0.660	0.670	4.0	4.7
K	Locus3	33	3.0	0.455	0.525	0.533	2.3	2.6
K	Locus4	34	2.0	0.353	0.291	0.295	2.0	2.0
K	Locus5	34	6.0	0.588	0.686	0.696	4.1	5.1
K	Locus6	34	6.0	0.500	0.467	0.474	3.8	4.8
K	Locus7	34	7.0	0.588	0.532	0.540	4.6	5.8
K	Locus8	34	5.0	0.559	0.582	0.590	4.1	4.8
K	Locus9	34	8.0	0.794	0.625	0.634	5.3	6.8
K	Locus10	34	4.0	0.412	0.430	0.437	3.4	3.9
K	Locus11	34	6.0	0.588	0.637	0.646	4.2	5.2
K	Locus12	34	4.0	0.324	0.323	0.328	2.8	3.5
K	Locus13	34	4.0	0.529	0.476	0.483	2.6	3.2
K	Average	33.9	5.2	0.517 , 95% CI [0.485-0.548]	0.520 , 95% CI [0.482-0.540]	0.527	3.7 , 95% CI [3.2-4.2]	4.5 , 95% CI [4.2-4.9]
L	Locus1	32	6.0	0.750	0.725	0.737	5.1	5.8
L	Locus2	32	3.0	0.406	0.366	0.372	2.9	3.0
L	Locus3	32	3.0	0.500	0.542	0.551	3.0	3.0
L	Locus4	32	2.0	0.219	0.195	0.198	1.9	2.0
L	Locus5	32	5.0	0.656	0.689	0.700	3.8	4.5
L	Locus6	32	5.0	0.688	0.700	0.711	4.3	4.9

L	Locus7	32	5.0	0.656	0.581	0.590	3.9	4.8
L	Locus8	32	6.0	0.719	0.724	0.735	4.8	5.5
L	Locus9	32	6.0	0.563	0.618	0.627	4.1	5.2
L	Locus10	32	3.0	0.375	0.338	0.344	2.7	3.0
L	Locus11	32	6.0	0.719	0.730	0.742	4.9	5.6
L	Locus12	32	3.0	0.188	0.201	0.204	2.5	2.9
L	Locus13	32	2.0	0.563	0.492	0.500	2.0	2.0
L	Average	32.0	4.2	0.538 , 95% CI [0.498-0.579]	0.531 , 95% CI [0.499-0.543]	0.539	3.5 , 95% CI [3.2-3.8]	4.0 , 95% CI [3.8-4.2]
M	Locus1	35	5.0	0.657	0.738	0.749	4.2	4.6
M	Locus2	35	4.0	0.429	0.449	0.456	3.0	3.7
M	Locus3	35	2.0	0.057	0.056	0.056	1.5	1.8
M	Locus4	35	1.0	-	-	-	1.0	1.0
M	Locus5	35	4.0	0.457	0.480	0.487	3.2	3.6
M	Locus6	35	5.0	0.629	0.710	0.720	4.1	4.6
M	Locus7	35	4.0	0.771	0.679	0.689	3.8	4.0
M	Locus8	35	4.0	0.571	0.666	0.675	3.7	3.9
M	Locus9	35	5.0	0.514	0.554	0.562	3.9	4.5
M	Locus10	35	2.0	0.629	0.467	0.474	2.0	2.0
M	Locus11	35	4.0	0.514	0.552	0.560	3.7	4.0
M	Locus12	35	3.0	0.657	0.633	0.642	3.0	3.0
M	Locus13	35	2.0	0.543	0.396	0.401	2.0	2.0
M	Average	35.0	3.5	0.494 , 95% CI [0.455-0.534]	0.490 , 95% CI [0.460-0.506]	0.498	3.0 , 95% CI [2.7-3.3]	3.3 , 95% CI [3.1-3.5]
ALL	Locus1	404	5.9	0.640	0.659	0.670	4.8	5.8
ALL	Locus2	404	3.5	0.456	0.490	0.498	3.0	5.3
ALL	Locus3	404	2.9	0.449	0.463	0.470	2.7	5.7
ALL	Locus4	404	1.5	0.089	0.082	0.083	1.4	5.3
ALL	Locus5	404	4.1	0.520	0.514	0.522	3.3	4.2
ALL	Locus6	404	4.6	0.638	0.620	0.631	3.8	4.5
ALL	Locus7	404	4.6	0.622	0.594	0.604	3.8	4.0
ALL	Locus8	404	4.8	0.567	0.578	0.588	3.9	4.2
ALL	Locus9	404	5.8	0.571	0.547	0.557	4.3	2.9
ALL	Locus10	404	2.8	0.405	0.375	0.382	2.5	5.7
ALL	Locus11	404	5.3	0.704	0.675	0.686	4.4	5.8
ALL	Locus12	404	2.8	0.328	0.331	0.337	2.4	5.8
ALL	Locus13	404	2.8	0.469	0.435	0.442	2.4	4.6
ALL	Average	404.0	3.9	0.497	0.489	0.498	3.3	4.0

Annex 4: P-values from test of significant difference in expected heterozygote between ponds.

Table A3: The p-values from the test of significant difference in estimated expected heterozygosity between ponds. Bold values are significant for α = 0.05 with sequential Bonferroni corrections.

	A	B	C	D	E	G	H	I	J	K	L
B	0.59										
C	0.65	0.37									
D	0.17	0.08	0.44								
E	0.14	0.40	0.02	0.02							
G	0.28	0.24	0.55	0.94	0.12						
H	0.05	0.03	0.11	0.48	**0.00**	0.95					
I	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.17	**0.00**				
J	0.71	0.34	0.84	0.18	0.02	0.16	0.04	**0.00**			
K	0.28	0.58	0.15	0.02	0.59	0.03	**0.01**	**0.00**	0.23		
L	0.04	0.22	0.03	**0.00**	0.89	0.05	**0.00**	**0.00**	0.04	0.40	
M	0.59	0.27	1.00	0.50	0.04	0.25	0.12	**0.00**	0.58	0.18	0.03

Annex 5: Individual assignments to population clusters estimated in TESS

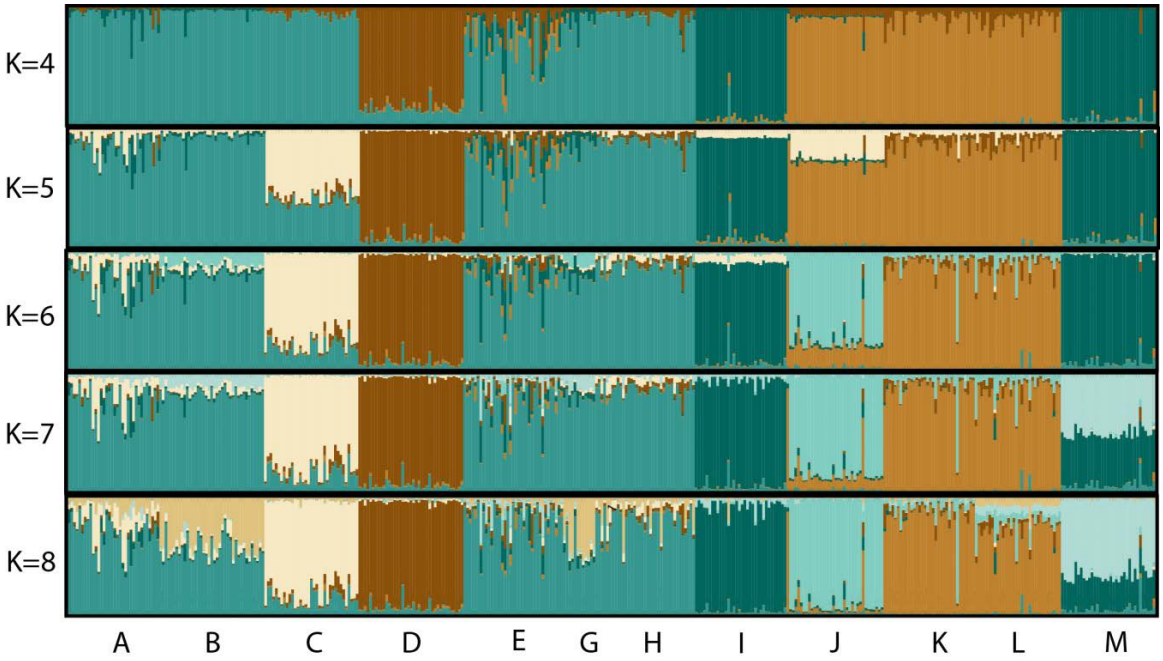

Figure 12: The averaged results from TESS. The bar graphs show individual assignment probabilities for each pond sample. Each horizontal bar chart represents the number of clusters (K), going from 4 to 8.

Annex 6: Genetic differentiation estimated as pairwise Fst

Table A4: Pairwise Fst for each pair of pond samples. Upper triangle = estimated Fst values. Lower triangle=estimated 95 % confidence intervals.

Fs	A	B	C	D	E	G	H	I	J	K	L	M
A		[0.102, 0.120]	[0.120, 0.140]	[0.168, 0.189]	[0.095, 0.114]	[0.107, 0.145]	[0.071, 0.089]	[0.136, 0.158]	[0.205, 0.230]	[0.135, 0.157]	[0.112, 0.132]	[0.172, 0.196]
B	0.109		[0.164, 0.184]	[0.157, 0.176]	[0.075, 0.091]	[0.057, 0.090]	[0.087, 0.103]	[0.181, 0.203]	[0.204, 0.227]	[0.153, 0.174]	[0.124, 0.142]	[0.170, 0.190]
C	0.128	0.171		[0.207, 0.231]	[0.149, 0.169]	[0.160, 0.200]	[0.180, 0.202]	[0.197, 0.221]	[0.211, 0.236]	[0.132, 0.151]	[0.134, 0.154]	[0.159, 0.180]
D	0.176	0.164	0.215		[0.098, 0.115]	[0.165, 0.203]	[0.139, 0.157]	[0.191, 0.213]	[0.247, 0.274]	[0.176, 0.198]	[0.170, 0.191]	[0.214, 0.239]
E	0.102	0.082	0.157	0.105		[0.116, 0.152]	[0.061, 0.077]	[0.179, 0.203]	[0.195, 0.219]	[0.103, 0.121]	[0.141, 0.162]	[0.147, 0.168]
G	0.122	0.071	0.175	0.179	0.130		[0.076, 0.112]	[0.181, 0.227]	[0.203, 0.249]	[0.139, 0.180]	[0.076, 0.111]	[0.132, 0.170]
H	0.078	0.093	0.187	0.145	0.067	0.091		[0.147, 0.169]	[0.230, 0.255]	[0.146, 0.167]	[0.128, 0.148]	[0.147, 0.168]
I	0.144	0.189	0.205	0.199	0.187	0.198	0.155		[0.278, 0.308]	[0.160, 0.183]	[0.127, 0.149]	[0.159, 0.182]
J	0.213	0.212	0.219	0.255	0.203	0.219	0.238	0.287		[0.159, 0.183]	[0.124, 0.145]	[0.271, 0.301]
K	0.143	0.160	0.139	0.184	0.110	0.154	0.154	0.168	0.168		[0.092, 0.111]	[0.162, 0.186]
L	0.120	0.131	0.141	0.177	0.149	0.091	0.136	0.135	0.132	0.099		[0.160, 0.182]
M	0.181	0.177	0.166	0.222	0.154	0.147	0.155	0.168	0.280	0.170	0.168	

Annex7: Genetic differentiation estimated as chord Distance (Dc)

Table A5: Chord distance Dc for each pair of pond samples. Upper triangle = estimated Dc values. Lower triangle=estimated 95 % confidence intervals.

Dc	A	B	C	D	E	G	H	I	J	K	L	M
A		0.284	0.280	0.367	0.298	0.279	0.245	0.317	0.394	0.332	0.322	0.376
B	[0.225, 342]		0.319	0.372	0.266	0.250	0.273	0.364	0.385	0.331	0.305	0.378
C	[0.209, 0.348]	[0.248, 0.383]		0.405	0.348	0.337	0.327	0.392	0.401	0.333	0.345	0.373
D	[0.272, 0.457]	[0.281, 0.461]	[0.313, 0.494]		0.309	0.373	0.353	0.377	0.393	0.335	0.348	0.443
E	[0.247, 0.349]	[0.197, 0.340]	[0.264, 0.428]	[0.230, 0.393]		0.306	0.228	0.343	0.368	0.294	0.301	0.342
G	[0.176, 0.377]	[0.180, 0.323]	[0.241, 0.426]	[0.261, 0.487]	[0.233, 0.379]		0.237	0.342	0.396	0.334	0.298	0.359
H	[0.172, 0.320]	[0.214, 0.330]	[0.236, 0.413]	[0.276, 0.445]	[0.171, 0.289]	[0.156, 0.318]		0.319	0.393	0.333	0.322	0.355
I	[0.259,0.380]	[0.307, 0.421]	[0.307, 0.477]	[0.270, 0.482]	[0.262, 0.427]	[0.280, 0.409]	[0.257, 0.396]		0.444	0.327	0.330	0.322
J	[0.273, 0.516]	[0.306, 0.463]	[0.300, 0.497]	[0.300, 0.488]	[0.269, 0.465]	[0.304, 0.480]	[0.290, 0.490]	[0.333, 0.547]		0.311	0.310	0.458
K	[0.270, 0.403]	[0.274, 0.384]	[0.276, 0.390]	[0.231, 0.442]	[0.229, 0.363]	[0.265, 0.405]	[0.268, 0.401]	[0.247, 0.405]	[0.244, 0.378]		0.250	0.344
L	[0.238, 0.408]	[0.227, 0.379]	[0.264, 0.421]	[0.229, 0.465]	[0.220, 0.380]	[0.208, 0.391]	[0.255, 0.385]	[0.249, 0.407]	[0.248, 0.370]	[0.180, 0.325]		0.370
M	[0.294, 0.446]	[0.292, 0.465]	[0.270, 0.475]	[0.363, 0.521]	[0.267, 0.415]	[0.263, 0.452]	[0.274, 0.426]	[0.244, 0.398]	[0.352, 0.550]	[0.288, 0.400]	[0.317, 0.424]	

Annex 8: Correlation between landscape variables

Table 14The spearman rank correlation coefficients for all landscape variables (upper triangle), and significance level (lower triangle). Alpha-level: no stars = not significant, one star = 0.05, two stars = 0.01, three stars = 0.001.

	Aspect	Slope 20° and steeper	Slope 30° and steeper	Geographical distance	Stream distance	Non-forested areas (2008)	Non-forested areas (2015)	Gravel roads	Soil productivity	Stream area
Aspect	1.00							***	***	***
Slope 20° and steeper	0.10	1.00	***			***	***		***	
Slope 30° and steeper	-0.08	**0.88**	1.00			***	***		***	**
Geographical distance	-0.16	0.03	0.13	1.00	***				*	
Stream distance	-0.19	0.08	0.17	**0.97**	1.00	*			**	
Non-forested areas (2008)	0.02	0.43	0.43	0.20	0.25	1.00	***		***	
Non-forested areas (2015)	0.10	0.48	0.45	0.17	0.22	**0.97**	1.00		***	
Gravel roads	0.45	0.11	-0.04	0.04	-0.01	-0.15	-0.07	1.00	***	***
Soil productivity	0.40	-0.44	-0.51	-0.26	-0.32	-0.48	-0.44	0.49	1.00	***
Stream area	0.54	-0.17	-0.36	0.01	-0.02	-0.16	-0.12	**0.60**	0.44	1.00

Annex 9: Distribution of landscape features in the study area

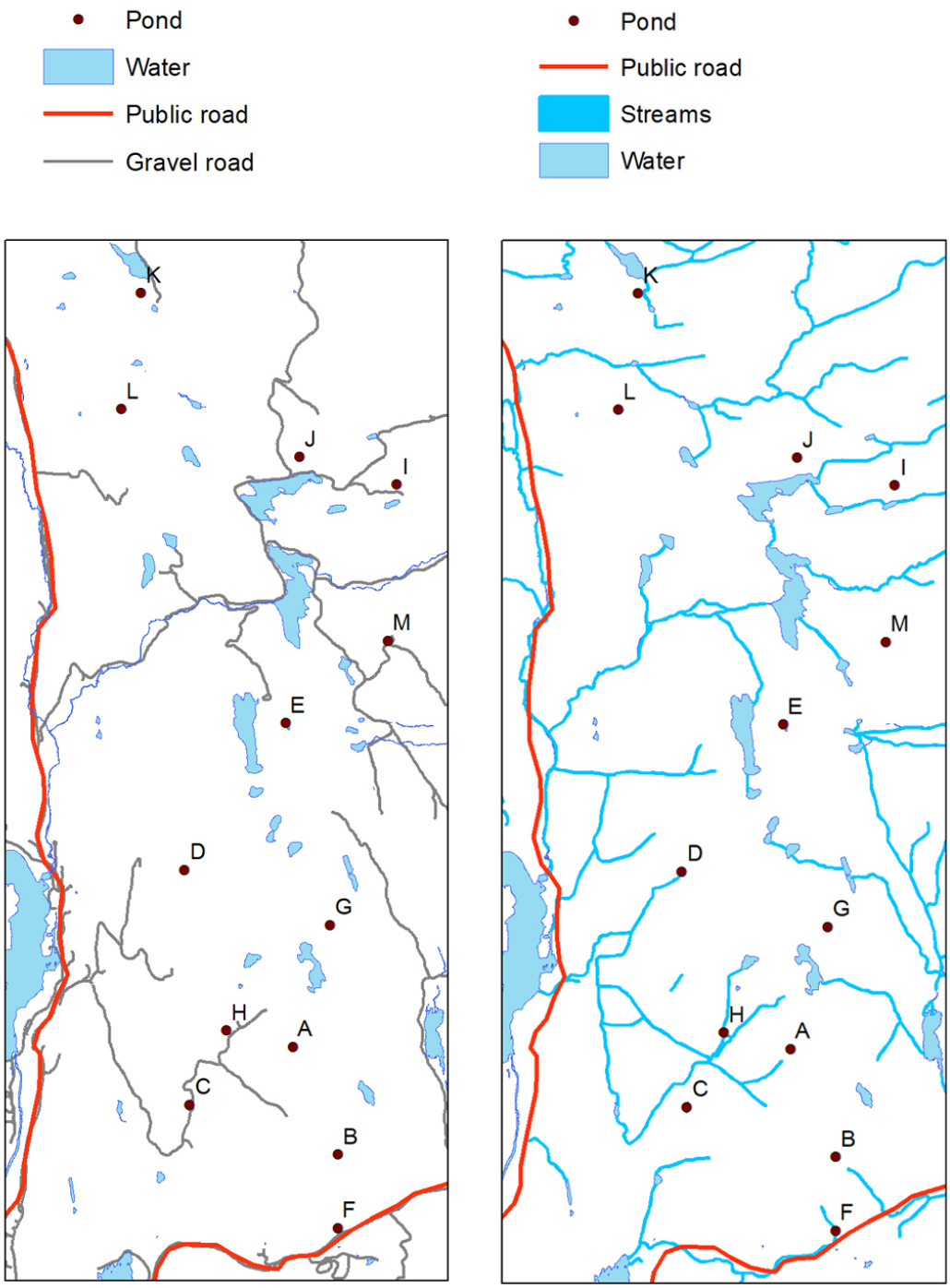

Figure A3: Map on the left: The distribution of forest gravel roads (grey lines). Map on the right: The distribution of streams (blue lines). For both maps: Public roads (red lines), water bodies (blue polygons), and ponds (dark red dots).

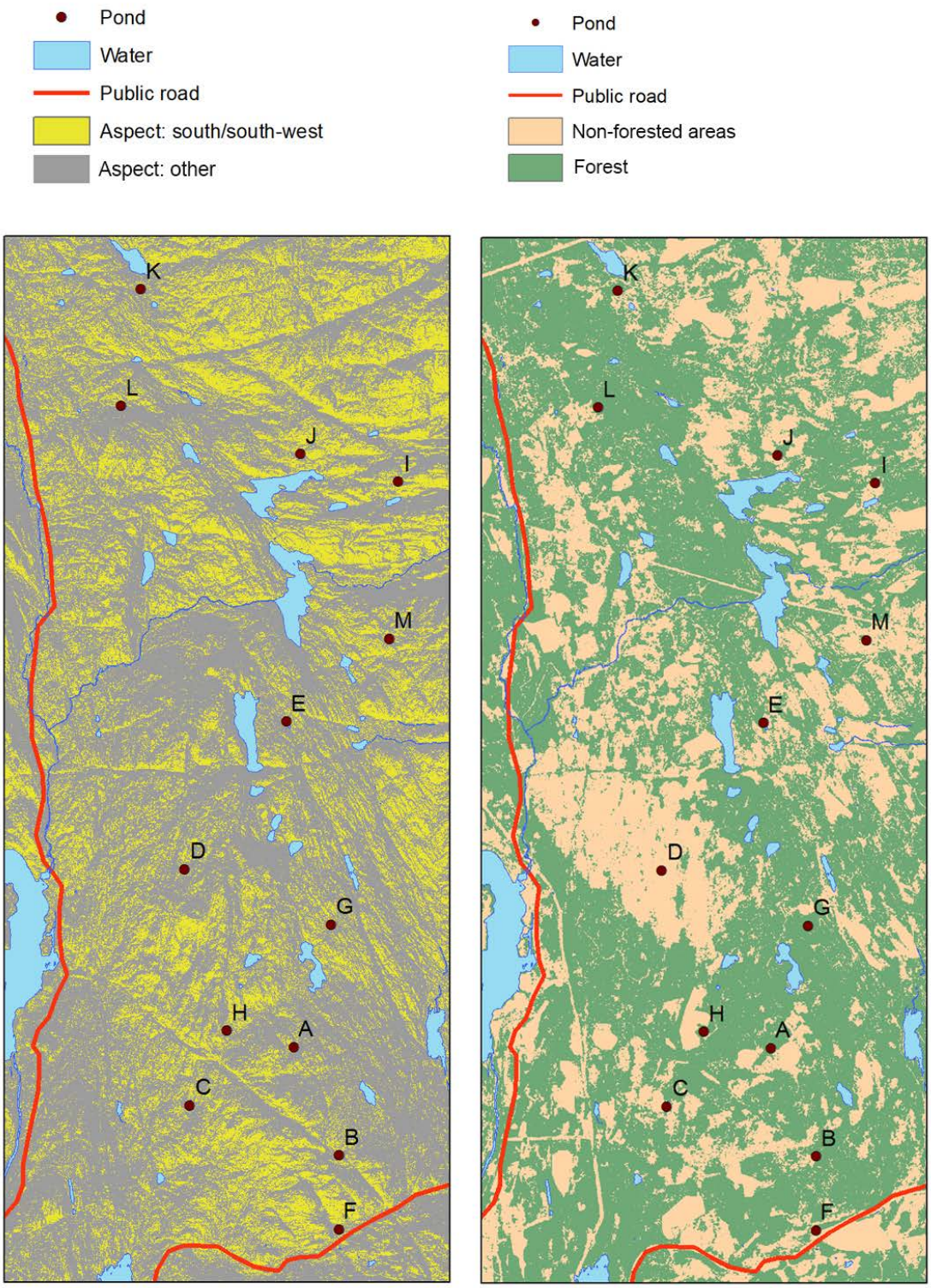

Figure A4: Map on the left: Distribution of south/south-west facing slopes (yellow polygons). Map on the right: Distribution of non-forested areas up until 2015 (beige polygons). For both maps: Public roads (red lines), water bodies (blue polygons), and ponds (dark red dots).

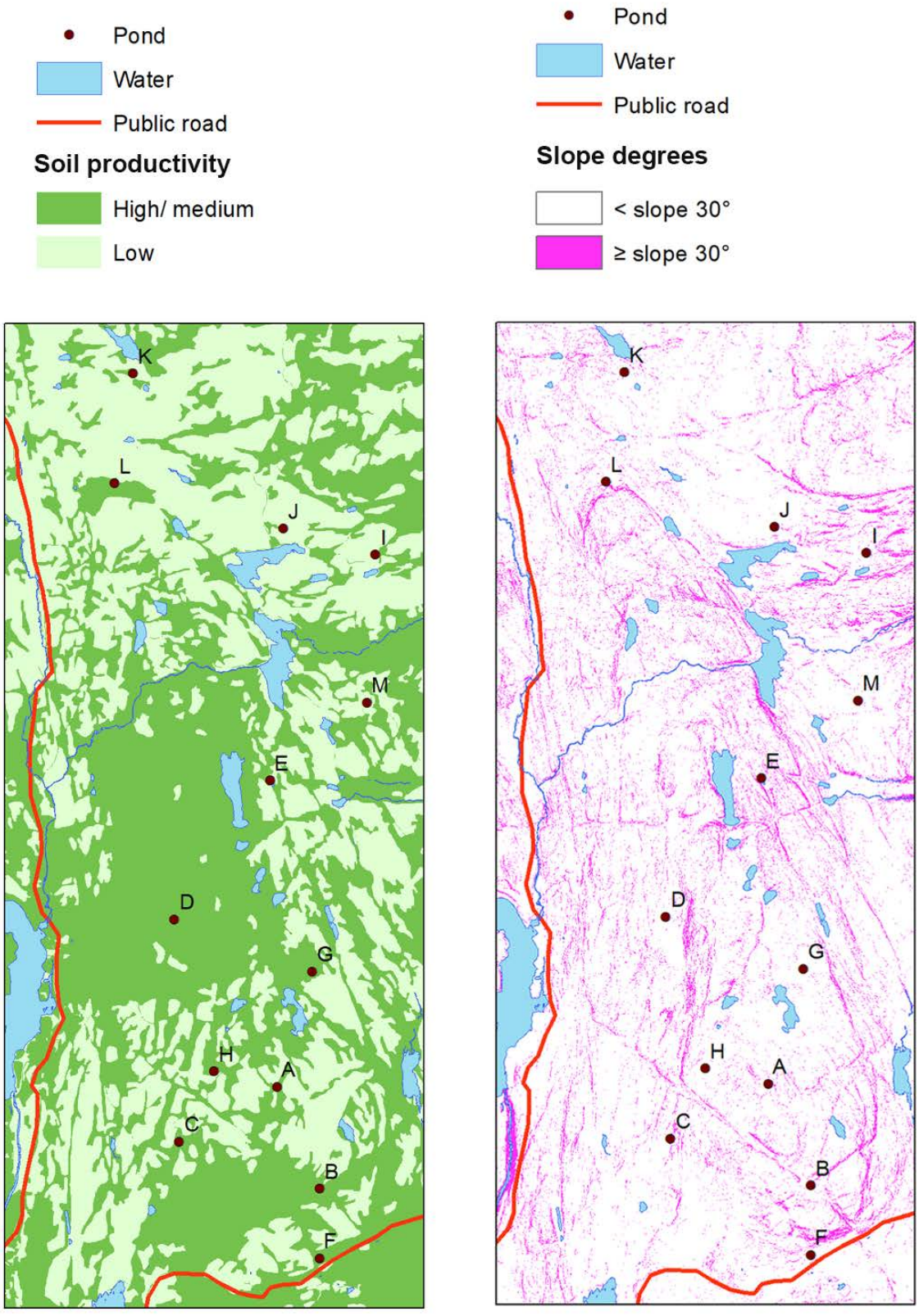

Figure A13: Map on the left: The distribution of areas with low soil productivity (light green polygons). Map on the right: The distribution of areas with slopes 30° and steeper (pink polygons). For both maps: Public roads (red lines), water bodies (blue polygons), and ponds (dark red dots).