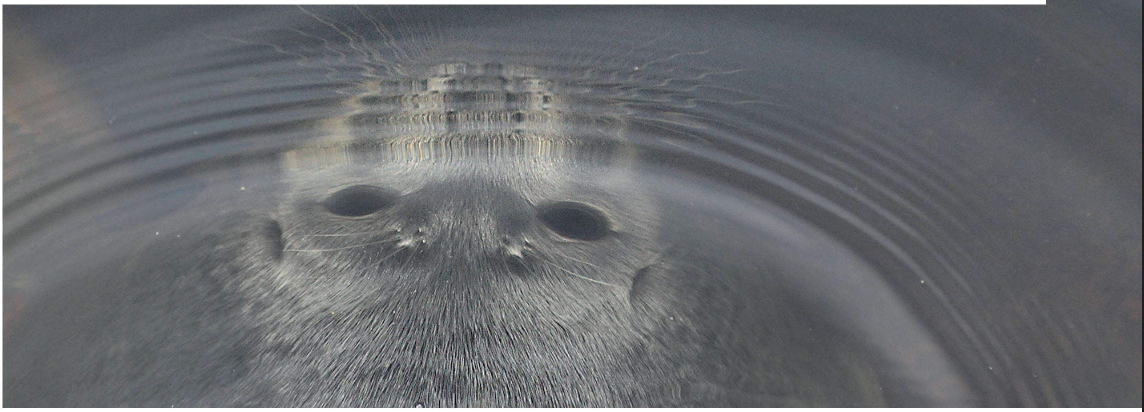
DISSERTATIONS IN
FORESTRY AND NATURAL SCIENCES

Mia Valtonen

Conservation genetics of the Saimaa ringed seal

– insights into the history of a critically endangered population



Publications of the University of Eastern Finland
Dissertations in Forestry and Natural Sciences No 159

UNIVERSITY OF
EASTERN FINLAND

MIA VALTONEN

Conservation genetics of the Saimaa ringed seal

– insights into the history of a critically endangered population

Publications of the University of Eastern Finland
Dissertations in Forestry and Natural Sciences
No 159

Academic Dissertation
To be presented by permission of the Faculty of Science and Forestry for public examination in the Auditorium N100 in Natura Building at the University of Eastern Finland, Joensuu, on October, 31, 2014, at 12 noon

Department of Biology

Grano
Joensuu, 2014
Editors: Prof. Pertti Pasanen,
Prof. Pekka Kilpeläinen, Prof. Kai Peiponen, Prof. Matti Vornanen
Cover photo: Mervi Kunnasranta
Distribution:
Eastern Finland University Library / Sales of publications
julkaisumyynti@uef.fi
www.uef.fi/kirjasto

ISBN: 978-952-61-1582-5 (printed)
ISSNL: 1798-5668
ISSN: 1798-5668

ISBN: 978-952-61-1583-2 (PDF)
ISSNL: 1798-5668
ISSN: 1798-5676

Author's address:
University of Eastern Finland
Department of Biology
P.O. Box 111
80101 JOENSUU
FINLAND
email: mia.valtonen@uef.fi

Supervisors:
Associate Professor Tommi Nyman, Ph.D.
University of Eastern Finland
Department of Biology
P.O. Box 111
80101 JOENSUU
FINLAND
email: tommi.nyman@uef.fi

Senior Researcher Mervi Kunnasranta, Ph.D.
University of Eastern Finland
Department of Biology
P.O. Box 111
80101 JOENSUU
FINLAND
email: mervi.kunnasranta@uef.fi

Docent Minna Ruokonen, Ph.D.
University of Oulu
Department of Biology
P.O. Box 3000
90014 OULU
FINLAND

Docent Jukka Palo, Ph.D.
University of Helsinki
Hjelt Institute, Department of Forensic Medicine
P.O. Box 40
00014 HELSINKI
FINLAND
email: jukka.palo@helsinki.fi

Professor Jouni Aspi, Ph.D.
University of Oulu
Department of Biology
P.O. Box 3000
90014 OULU
FINLAND
email: jouni.aspi@oulu.fi

Reviewers:
Professor David Coltman, Ph.D
University of Alberta
Department of Biological Sciences
EDMONTON, ALBERTA T6G 2E9
CANADA
email: david.coltman@alberta.ca

Senior Researcher Liselotte Andersen, Ph.D
University of Aarhus
Department of Bioscience
Grenåvej 14
8410 RØNDE
DENMARK
email: lwa@dmu.dk

Opponent:
Professor Craig Primmer, Ph.D
University of Turku
Department of Biology
20014 TURKU
FINLAND
email: craig.primmer@utu.fi

ABSTRACT

Small and isolated populations lose genetic diversity, the raw material of evolution, more rapidly than do large populations, which may make them more vulnerable to demographic and environmental stochasticity. Fragmentation of an already small population may further increase its extinction risk by intensifying such effects in the even smaller subpopulations.

The Saimaa ringed seal (*Phoca hispida saimensis*) represents an ideal study system for investigating the genetic and demographic effects of long isolation, small population size, and spatial subdivision. This critically endangered subspecies of *c*. 300 seals inhabits the highly fragmented Lake Saimaa in southeastern Finland. The population has remained completely isolated for *c*. 9,500 years and is currently threatened by anthropogenic factors, such as high by-catch mortality and climate change. This thesis examines spatial and temporal variation in genetic diversity of the Saimaa ringed seal. For this, tissue samples collected from seal carcasses (N = 212) in 1980–2008 and placentas (N = 66) collected from birth-lair sites during 2000–2011 were examined for mtDNA and microsatellite variation. A new method of non-invasive genetic sampling was developed, demonstrating the utility of placentas for reliable DNA genotyping. The diversity of the Saimaa population was contrasted with the levels found in populations sharing the same ancestry, Baltic (*P. h. botnica*; N = 21) and Ladoga (*P. h. ladogensis*; N = 16) ringed seals.

The results show that genetic diversity of the Saimaa ringed seal is extremely low, with observed microsatellite heterozygosity for this subspecies (H_E = 0.36) being the lowest recorded within the order Pinnipedia. Effective population sizes estimated for the total population and regional subpopulations were also very low (N_E = 5–113), suggesting that the population is too small to maintain its current diversity in the long term. Although coalescent simulations indicated that most of the original diversity was lost during the long isolation, we observed a decrease in diversity also during the past decades, which suggests ongoing diversity loss in the population.

Moreover, Bayesian clustering analyses revealed significant differentiation among the breeding areas. The fine-scaled structuring of the Saimaa population is surprising, because in marine ringed seals only weak differentiation has been detected even among subpopulations located thousands of kilometres apart. In the Saimaa ringed seal, the population structure is most likely induced by the small subpopulation sizes and fragmented lacustrine habitat, but also by behavioural patterns of the seals. Overall gene flow within the lake is limited, as females are philopatric and, although males appear to be more prone to disperse, gene flow mediated by males is insufficient for counteracting the effects of genetic drift.

The findings of the present study indicate that genetic diversity of the Saimaa ringed seal will inevitably continue to decrease unless its population size can be increased substantially. Additionally, the observed fine-scaled structuring of the population raises concerns about the viability of subpopulations. Therefore, as rapid population growth is improbable in this slowly reproducing species, short-term conservation efforts (*e.g.*, translocations of adult seals) should focus on facilitating gene flow among breeding areas.

Universal Decimal Classification: 574.3, 575.113.2, 575.17, 575.22, 599.745.3

CAB Thesaurus: seals; Phoca hispida; effective population size; gene flow; genetic diversity; genetics; monitoring; placenta; mitochondrial DNA; microsatellites; heterozygosity; genotypes; population structure; spatial variation; temporal variation

Yleinen suomalainen asiasanasto: hylkeet; norppa; saimaannorppa; populaatiot; koko; rakenne; vaihtelu; genetiikka; geenit; geneettinen monimuotoisuus; genotyyppi; seuranta; näytteenotto; istukka; mitokondrio-DNA; mikrosatelliitit

Preface

There are so many people that I wish to thank for their help, encouragement and support during this long journey. First of all, I wish to acknowledge my numerous supervisors located in different parts of the country, from whom each I learned so much. I want to thank Tommi Nyman for providing me the opportunity to study Saimaa ringed seal genetics and all his help during the preparation of this thesis. Your high standards taught me what it requires to make proper science. I am grateful to Mervi Kunnasranta, whose idea this PhD project originally was. You taught me how important it is to translate my results into common sense – what do they mean and what is their real-world relevance, if any. I also wish to express my gratitude to Jukka Palo, whose PhD work provided the basis for this study. Your enthusiasm for the subject has been extremely inspiring and your endless support invaluable along the sometimes not so smooth road. I also want to acknowledge Minna Ruokonen, who patiently guided my first steps into the field of population genetics. Sadly, she passed away two years ago. Jouni Aspi replaced Minna as my supervisor. I deeply appreciate that you shared your broad experience with me, your expert advice and never-failing encouragement.

I thank my co-authors for their help with several issues: Hanna Buuri for her contribution to the placental lab work, Matti Heino for his huge effort in genotyping placentas and help in data analyses, and Tuomo Kokkonen, who tragically perished recently, for seamless co-operation, especially in organising the field-collection of placentas. I am grateful to Markku Viljanen for his help with the funding issues, Heikki Hyvärinen for his helpful comments, and Gernot Segelbacher for his major help and support during the final meters of the thesis. I sincerely thank David Coltman and Liselotte Andersen for their thorough reviews of this thesis. Further, I am grateful to Jukka Jernvall and Petri Auvinen for providing me the opportunity to continue and expand my research on the Saimaa ringed seal genetics.

There are a number of sponsors which made this research possible: the Maj and Tor Nessling Foundation, Saimaa Ringed Seal Genome Project, Raija and Ossi Tuuliainen Foundation, Kuopio Naturalists' Society, Nestori Foundation, Finnish Foundation for Nature Conservation, University of Eastern Finland, Finnish Graduate School in Environmental Science and Technology, Finnish Konkordia Fund and Oskar Öflunds Stiftelse are gratefully acknowledged for providing financial support for this study.

Thanks are also due to the entire staff of the Department of Biology for providing excellent conditions for my research. Particularly, I wish to thank Riitta Pietarinen for helping with the laboratory analyses, Harri Kirjavainen for his help with the tissue bank, Eija Ristola and Marja Noponen for helping with many practical issues in the lab, and Matti Savinainen and Kirsti Kyyrönen for technical support.

All people who contributed to field collection of placentas deserve big thanks, above all the divers Miina Auttila, Ismo and Paula Marttinen, Kari Ratilainen and Juha Taskinen. I also wish to acknowledge the personnel of Metsähallitus, especially Tero Sipilä, Jouni Koskela and Raisa Tiilikainen. Thanks are also due to Petri Timonen from the Finnish Game and Fisheries Research Institute for his help with the age determinations of seals and the Baltic ringed seal samples.

I have had the privilege and pleasure to study in the "Söpöjen eläinten tutkimusryhmä" with many special people, who shared the ups and downs of this journey with me: Anni, Marja, Miina and Sanna (some people may find sawflies cute...) from the very beginning, and Sari, Riikka, Meeri and Anni joining in later – my warmest thanks for your help, support and friendship. I also wish to thank the whole Saimaa field crew for the memorable springs on the lake watching and catching seals. Those lake days offered a welcome and refreshing escape away from the computer and the seals themselves reminded me of why I started this thesis to begin with. My special thanks go to

Juha for sharing many of those days with me and for teaching me the true nature of 'hyle'.

I thank all my fellow PhD students in the Biology department, both previous and current, for peer support – particularly Raisa and Ursula for refreshing company and lively discussions at lunch and coffee breaks, and Sari and Kaisa for offering a refuge at times when all went wrong. I also want to thank Jouni's group in Oulu, for welcoming me, the eastern outlier, into your group.

Many of my friends outside university have supported me all the way and lent an ear whenever I was in need of one and, equally importantly, provided me activities outside work and given me other things to think about. Especially Varpu, Suvi, Marjo, Ulla and Tiina, thank you for being there for me. I also wish to express my deepest gratitude to all of you who looked after my dog Mössi when I was travelling or needed a momentary relief at times when my everyday joy became a burden.

Finally I want to thank my family for their faith in me although I often doubted myself. I am grateful to my mother for helping me with whatever and whenever I needed and for understanding when I was too stressed and needed some space. I thank my father and Armi for always encouraging me and helping with all kinds of practical issues when visiting Helsinki and travelling abroad. I also wish to thank my brother Juho for being my personal IT-support person and for taking such good care of Mössi that he would forget my existence. My warmest thanks to my sister Niina and her husband Teijo who had their door open for me any time. Being surrounded by three lively children gave another perspective in life – a mistake in your data, the correction of which takes two days, does not seem such big an issue after all. Those three precious kids, Sara, Luka and Lumi, always remind me of the importance of being present in the present.

LIST OF ABBREVIATIONS AND SYMBOLS

a	haplotypic richness
A	number of alleles
A_R	allelic richness
CR	mitochondrial control region
F_{IS}	inbreeding coefficient; departure from Hardy-Weinberg proportions within subpopulations
F_{ST}	index of population differentiation; proportion of genetic diversity due to differences among populations
h	haplotypic diversity
hn	number of haplotypes
H_E	expected heterozygosity
H_O	observed heterozygosity
HV	Haukivesi area
IBD	isolation by distance
KV	Kolovesi
MHV	Main Haukivesi area
mtDNA	mitochondrial DNA
N	sample size
N_C	census population size
N_E	effective population size
N_P	number of polymorphic loci
NS	Northern Saimaa
pn	number of polymorphic sites
PV	Pihlajavesi area
SD	standard deviation
SS	Southern Saimaa
uh	number of unique haplotypes
yob	year of birth
yob	year of death
Φ_{ST}	index of population differentiation; proportion of genetic diversity (measured as the expected squared evolutionary distance between alleles) due to differences among populations
π	nucleotide diversity

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to by the Roman numerals I-IV.

I Valtonen M, Palo J U, Ruokonen M, Kunnasranta M, Nyman T. Spatial and temporal variation in genetic diversity of an endangered freshwater seal. *Conservation Genetics 13: 1231–1245, 2012.*

II Nyman T, Valtonen M, Aspi J, Ruokonen M, Kunnasranta M, Palo J U. Demographic histories and genetic diversities of Fennoscandian marine and landlocked ringed seal subspecies. *Ecology and Evolution 4: 3420–3434, 2014.*

III Valtonen M, Palo J U, Aspi J, Ruokonen M, Kunnasranta M, Nyman T. Causes and consequences of fine-scale population structure in a critically endangered freshwater seal. *BMC Ecology 14: 22, 2014.*

IV Valtonen M, Heino M, Aspi J, Buuri H, Kokkonen T, Kunnasranta M, Palo J U, Nyman T. The utility of field-collected placentas for genetic monitoring of a critically endangered freshwater seal population. Submitted manuscript.

The publications are printed with kind permission of Springer and John Wiley and Sons.

AUTHOR’S CONTRIBUTION

The present author contributed to the planning and to a minor part of the sample collection of all papers. She did the laboratory analyses for papers I–III, and was responsible for most data analyses and writing the original manuscripts for papers I, III and IV. She also participated in data analyses and writing of paper II.

Contents

1 Introduction

> *"The world is changed. I feel it in the water. I feel it in the earth.*
> *I smell it in the air. Much that once was is lost; for none now*
> *live who remember it. [...] But there were some who resisted."*
> – Galadriel (The Lord of the Rings: The Fellowship of the Ring. 2001)

The importance of genetic diversity for the persistence of species and populations is nowadays commonly recognized (McNeely *et al.,* 1990; Reed & Frankham, 2003; Frankham, 2005). Genetic diversity reflects the evolutionary potential of organisms, *i.e.*, their capability to adapt to environmental changes. Small and endangered populations usually exhibit lower levels of genetic diversity than do closely related non-endangered ones (Spielman *et al.,* 2004) and, thus, are expected to have reduced adaptation capacity in a changing environment (Willi *et al.,* 2006). Moreover, fitness of individuals is often reduced due to inbreeding (Reed & Frankham, 2003) and environmental stress (Willi *et al.,* 2006), further elevating the extinction risk of small populations.

The correlation between genetic diversity and viability of populations is, however, not always straightforward, particularly in stable and favourable environments, and there are examples of populations that thrive despite low diversity (Weber *et al.,* 2000; Reed, 2010; Kekkonen *et al.,* 2012). At present, many previously stable habitats are globally threatened by anthropogenic impacts, such as fragmentation, introduction of alien species, and climate change, that pose a challenge for many populations by altering the environmental conditions that they are adapted to. Unless a population is able to respond to environmental changes or to move to a more favourable habitat, its viability is severely compromised, which may lead to extinction (Hoffmann & Sgrò, 2011). Therefore, knowledge on the levels of, and changes in, genetic diversity of small and isolated populations facing environmental changes is essential for efficient conservation management.

1.1 GENETIC DIVERSITY IN SMALL POPULATIONS

The current genetic diversity of any given population has been shaped by evolutionary forces during the history, and is also influenced by factors such as demographic history and reproductive biology of the species. The evolutionary forces influencing genetic diversity of a population include mutation, gene flow, selection, and genetic drift (*e.g.*, Charlesworth, 2009). All genetic diversity is originally generated by mutations, but as only a minority of them are beneficial and the rate at which they occur is very low, lost adaptive diversity is regenerated extremely slowly. New alleles may also be brought into a population by immigrants arriving from other populations. Natural selection increases the frequency of alleles that are beneficial in prevailing conditions and reduces the frequency of those that are deleterious, while having no effect on neutral alleles and loci. Loss of genetic diversity is also caused by genetic drift, an inevitable, random process that causes allele frequencies to fluctuate from one generation to the next owing to sheer chance.

In small populations, genetic diversity is lost through genetic drift more rapidly than is created by mutations, as the rate at which diversity is lost is inversely proportional to population size (Willi *et al.,* 2006). At the same time, slightly negative mutations act as effectively neutral, and their fate is determined by genetic drift instead of natural selection, with the result that they may become fixed due to chance. Also inbreeding, *i.e.,* mating between related individuals, which is unavoidable in small populations, reduces individual genetic diversity (heterozygosity), although does not directly influence the number of alleles. As homozygosity increases, deleterious recessive alleles are exposed, resulting in fitness reduction of individuals. In consequence of both genetic drift and inbreeding, small populations often face an elevated risk of extinction due to reduced environmental adaptability (Frankham, 2005; Willi *et al.,* 2006; Liao & Reed, 2009) and lowered fitness of individuals (Madsen *et al.,* 1996; Reed & Frankham, 2003; Blomqvist *et al.,* 2010; Mattila *et al.,* 2012).

Gene flow counteracts the effects of genetic drift and inbreeding by equalizing differences in allele frequencies among populations (Slatkin, 1985). If a small population is further divided into even smaller subunits, gene flow among subpopulations is essential for maintaining genetic diversity and alleviating the negative genetic consequences of small population size (Keller & Waller, 2002; Tallmon *et al.,* 2004). Gene flow among and within populations may be impaired or even prevented by geographic and ecological barriers. This typically applies to island populations (Hoeck *et al.,* 2010; Runemark *et al.,* 2012), but also to species with specialised habitat requirements (Ferchaud *et al.,* 2011; Gottelli *et al.,* 2012) or limited dispersal capacity (Louy *et al.,* 2007) living in fragmented landscapes. However, species-specific behavioural patterns may also influence the level of gene flow, for example, due to sex-dependent differences in dispersal. For example, in many mammals females are philopatric, while males are more prone to disperse (Greenwood, 1980; I, III).

1.2 GENETIC MONITORING OF WILDLIFE POPULATIONS

Introduction of genetic methods into population monitoring has considerably facilitated conservation and management of elusive species and small, endangered populations. Today, molecular methods are used for assessing the levels of genetic diversity and other genetic parameters of species and populations (*e.g.,* Aspi *et al.,* 2006; Schultz *et al.,* 2009; Segelbacher *et al.,* 2014). They also provide a means for examining many aspects of the species' biology, such as dispersal, mating patterns, reproductive success, and survival (Fedy *et al.,* 2008; Brøseth *et al.,* 2010; Ford *et al.,* 2011), which are often difficult to study using traditional approaches, such as mark–recapture and telemetry methods.

Estimating the level of genetic diversity is essential for management and conservation decisions. Assessing population structure and patterns of gene flow is also important, for example, when identifying management units (Palsbøll *et al.,* 2007) and planning translocations among subpopulations (De Barba *et al.,* 2010; Latch *et al.,* 2011). In addition, identification of individuals from DNA samples can be used for estimating, for example, population census size (N_C) and individual dispersal patterns and survival (Schwartz *et al.,* 2007). Especially for species of conservation concern, effective population size (N_E) is a much more important measure than is N_C, as N_E reflects the number of individuals contributing genes to the next generation. In most natural populations, N_E is far lower than N_C (Palstra & Ruzzante, 2008; Palstra & Fraser, 2012). Moreover, investigating the level of inbreeding, kinship, mating patterns, and individual reproductive success is often possible only by using genetic data.

Genetic approaches can also be used for studying ecological and demographic changes in a population over time (Schwartz *et al.,* 2007). This requires a time series of archived genetic data (tissue samples, extracted DNA, or records of genetic information from previous studies) with information on collection time and place of samples, but also multiple samples from each period (Jackson *et al.,* 2011). Using such sample archives, it is possible to detect, for example, changes in genetic diversity of a population (Pichler & Baker, 2000, I, III), which may provide information on factors influencing the diversity and, hence, assist in designing appropriate management strategies. Technical advances have also enabled extraction of DNA from historical samples (*e.g.*, hair, feather, skin, and bone) from hundreds to thousands of years old and, thus, direct assessments of historical levels of genetic diversity (*e.g.*, Welch *et al.,* 2012; Foote *et al.,* 2012; Jansson *et al.,* 2014; Segelbacher *et al.,* 2014). However, past events can also be inferred from genetic information obtained from current samples using coalescent approaches (Nordborg, 2010, I, II).

Non-invasive samples that can be collected without catching or even seeing the animal itself, such as hair, feather, shed skin, and faeces, provide a means for studying rare, elusive and endangered species without causing disturbance, danger, or stress to the animals (Swanson *et al.,* 2006). At the same time, the use of such samples often enables obtaining large numbers of samples for monitoring purposes. Today, many terrestrial populations, including large carnivores (*e.g.*, Brøseth *et al.,* 2010; Kopatz *et al.,* 2012; Davoli *et al.,* 2013), are routinely monitored using non-invasive genetic methods. Collection of non-invasive samples in aquatic environments is often more challenging than in terrestrial habitats, but this approach is being increasingly utilised also in studies of marine mammals. For example, genetic information has been obtained from samples of shed skin in ringed seals (Martinez-Bakker *et al.,* 2013) and humpback whales (Baker *et al.,* 2013), and from faeces in dolphins (Parsons *et al.,* 2006) and marine otters (Valqui *et al.,* 2010).

1.3 THE STUDY SPECIES

1.3.1 The ringed seal as a species

The ringed seal (*Phoca hispida*) is a holarctically distributed species numbering a few million individuals in total, being at the same time the most northern and the most abundant of northern seals (Reeves, 1998). The species is one of the few pinnipeds capable of inhabiting fast ice areas during winter, as they can maintain breathing holes by their fore flipper claws. Not only can ringed seals survive in icy conditions, but ice and snow are indispensable for them as a breeding habitat. In comparison to other phocid seals, the ringed seal is genetically very diverse (Palo *et al.,* 2001; Davis *et al.,* 2008). Five different subspecies of ringed seal are recognised worldwide (Amano *et al.,* 2002; but see Berta & Churchill, 2012), three of which are found in Fennoscandia: the Baltic (*P. h. botnica*), Ladoga (*P. h.*

ladogensis) and Saimaa (*P. h. saimensis*) ringed seals (Hyvärinen & Nieminen, 1990).

1.3.2 History of the Saimaa ringed seal population

The current Fennoscandian ringed seal populations in Lake Saimaa, Lake Ladoga, and the Baltic Sea (Fig. 1A) descend from Arctic ringed seals (*P. h. hispida*) that colonized the Baltic basin from the Atlantic during the deglaciation, *c.* 10,000 years ago (Forstén & Alhonen, 1975; Ukkonen, 2002). Isostatic land-uplift gave rise to numerous lakes, including lakes Saimaa and Ladoga, where parts of the Baltic population were trapped. The ringed seals in Lake Saimaa have lived in complete isolation for *c.* 9,500 years, during which they have evolved into a morphologically, ecologically, and genetically distinct subspecies (Hyvärinen & Nieminen, 1990; Kunnasranta, 2001; Palo, 2003; Palo *et al.*, 2003; I, II).

During its long isolation, the Saimaa ringed seal population has undergone substantial changes in size: it has been estimated that there were a few thousand seals in the lake before human impact (Hyvärinen *et al.*, 1999), and still up to 1,000 seals at the turn of the 20th century (Kokko *et al.*, 1999). During the last hundred years, the population experienced a human-induced bottleneck: despite being placed under protection in 1955, the population continued to decrease mainly due to high by-catch mortality and environmental pollutants (Hyvärinen *et al.*, 1999) and reached its ultimate low of fewer than 150 individuals in the 1980s (Sipilä *et al.*, 1990).

1.3.3 Current status of the population

Since the end of the 20th century, the Saimaa ringed seal population has slowly increased, and it currently numbers slightly over 300 seals (Metsähallitus, 2014). However, the population is still very small and threatened by human activities (including by-catch mortality and disturbance), and also by deterioration of breeding conditions associated with warming

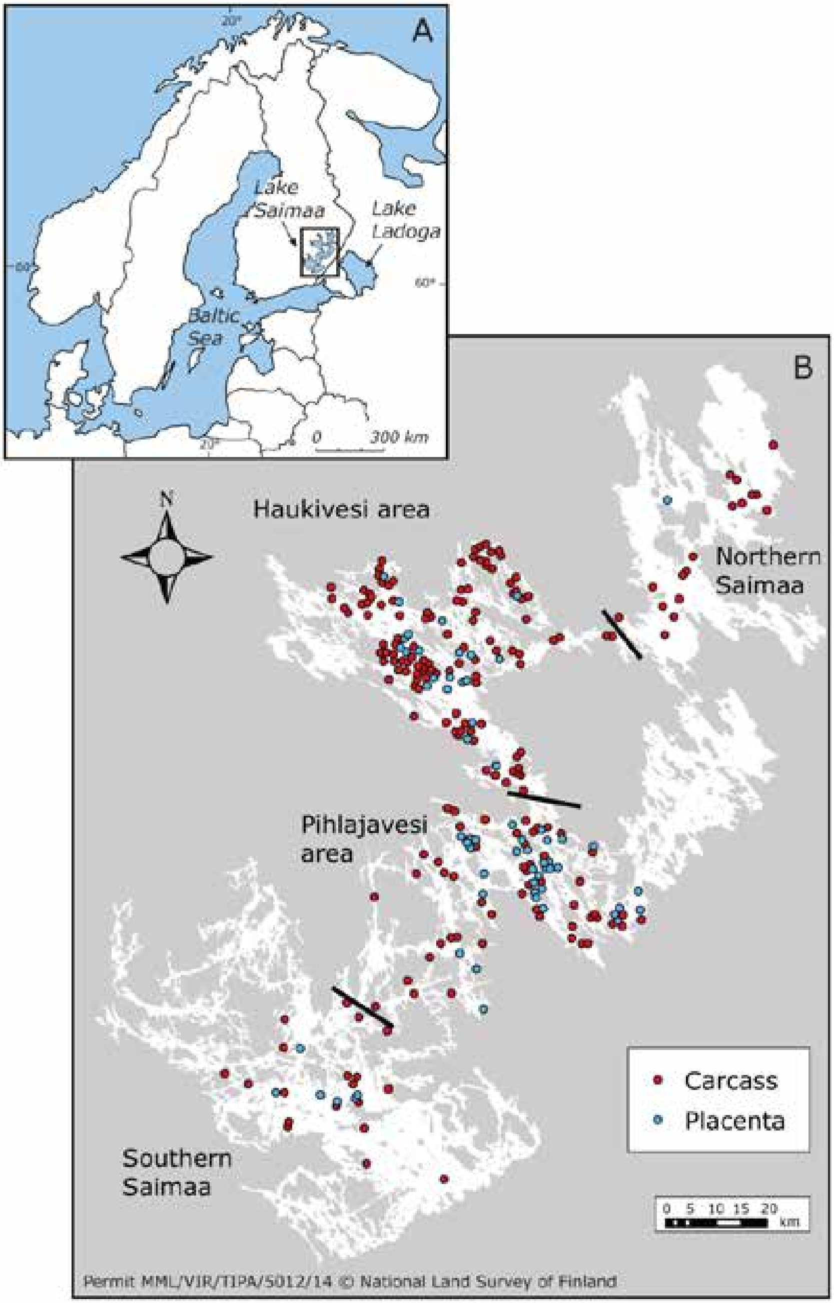


Figure 1. *The three water basins inhabited by ringed seals in Fennoscandia (A) and collection locations of Saimaa ringed seal specimens and the initial regional division of Lake Saimaa used in this study (B). Dot colours denote the type of the sample: red = carcass, blue = placenta.*

winters. Hence, the subspecies is classified as critically endangered (Rassi *et al.,* 2010; Kovacs *et al.,* 2012).

As is often the case in small and isolated populations, the level of genetic diversity of the Saimaa ringed seal is extremely low (Palo, 2003; Palo *et al.,* 2003; Martinez-Bakker *et al.,* 2013; I–III), which may have an effect on the long-term survival prospects of the subspecies. It has also been assumed that the population may be divided into several semi-isolated subpopulations, since its habitat, Lake Saimaa, is naturally fragmented, with only narrow inlets connecting the main water basins (Fig. 1B). Additionally, behavioural studies have shown that although the seals are potentially very mobile, they exhibit a high degree of site fidelity, and no long-distance migrations among different breeding areas have been observed (Kunnasranta, 2001; Koskela *et al.,* 2002; Niemi *et al.,* 2012; 2013a; 2013b). Division of this small population into even smaller units may hasten the loss of the remaining genetic diversity and, thus, make the Saimaa ringed seal even more vulnerable to environmental changes. However, in their study based on microsatellite variation of the Saimaa ringed seal, Palo *et al.,* (2003) found no evidence of significant differentiation between the northern and southern parts of the lake, but this could be due to the limited numbers of markers and samples in the analysis. Therefore, more extensive surveys were needed for assessing the current levels of divergence among regional subpopulations, and also for evaluating the effect of the anthropogenic bottleneck on the genetic diversity of the population.

1.4 AIMS OF THE STUDY

The main aims of this work were to examine spatial and temporal changes in genetic diversity and population structure of the Saimaa ringed seal. This knowledge is essential in designing and allocating conservation measures for this critically endangered population. The specific objectives were to:

1. Study the genetic diversity of the Saimaa ringed seal in relation to larger populations of the same origin, *i.e.*, the Baltic and Ladoga ringed seals (I, II)
2. Examine genetic structure and gene flow within the lake (I, III)
3. Investigate temporal changes in genetic diversity of the population (I–III)
4. Develop a method for genetic identification of Saimaa ringed seal individuals (IV)
5. Study the utility of non-invasively collected placentas for genetic monitoring of the population (IV)

2 Materials and methods

A general outline of the materials and methods is presented here. Detailed descriptions of laboratory procedures and analytical methods are found in the original papers I–IV.

2.1 SAMPLES

The majority of the Saimaa ringed seal specimens used in this study were tissue samples that had been collected from carcasses found in different parts of Lake Saimaa during the years 1980–2008 (*N* = 212; I–VI). The samples had been deposited into a tissue bank maintained by the University of Eastern Finland and Natural Heritage Services of Metsähallitus, and stored at –20°C.

Systematic searches for Saimaa ringed seal placentas were conducted in three consecutive springs during 2009–2011 (I, IV), as placentas can often be found from the vicinity of birth lairs situated along shorelines of islands and islets (Sipilä 2003) after the breeding season. A total of 59 placentas were found from 124 known birth lair sites, *i.e.*, from nearly half of the inspected sites. Placentas collected during the years 2000–2007 were used as additional samples (*N* = 7; IV).

Tissue samples from Baltic (*N* = 21, provided by the Finnish Game and Fisheries Research Institute) and Ladoga (*N* = 16, obtained from the tissue bank maintained by the University of Eastern Finland and Natural Heritage Services of Metsähallitus) ringed seals were used as reference, in order to compare the level of genetic diversity in the Saimaa population to those of the larger populations of the same origin (I, II).

2.1.1 Sample division

The Saimaa ringed seal specimens were initially divided into four regional samples based on the topography of the lake (Northern Saimaa, Haukivesi area, Pihlajavesi area, Southern Saimaa), as well as into three temporal samples based on the collection decade of the seals (1980s, 1990s, 2000s; I, III). A decade is close to the estimated 11-year generation time of ringed seals (Palo *et al.*, 2003 after Smith, 1973) and, therefore, was considered appropriate for examining temporal changes in the genetic composition of the Saimaa population. In some analyses (I, III), the temporal division was based on the birth decade of seals, yielding five temporal samples (1963–1969, 1970s, 1980s, 1990s, 2000s; I, III).

2.2 MOLECULAR MARKERS

The molecular markers used in this study were mitochondrial DNA (mtDNA) sequences and nuclear microsatellites, both of which are considered neutral, *i.e.*, they are typically not affected by selection. MtDNA is a haploid, circular molecule located in mitochondria, and many copies are found in each cell (Ballard & Whitlock, 2004). In most animals, mtDNA is maternally inherited, meaning that it is transmitted from mothers to their offspring and, thus, mtDNA can be used to study female lineages. The control region (CR) is a non-coding region that is involved in regulation of mtDNA replication. Due to its high mutation rate, the CR usually shows a high level of polymorphism and, hence, multiple genetic lineages are often found both within and among populations (I). MtDNA is therefore widely used in phylogeographic and population-genetic studies. The effective population size of the haploid and uniparentally inherited mtDNA is only a quarter of that of diploid nuclear DNA (Ballard & Whitlock, 2004), which makes it particularly sensitive to demographic changes.

Microsatellites are tandemly repeated DNA sequences that consist of 1–6 base pairs and are found at a high frequency throughout nuclear genomes (Schlötterer, 2000). Polymorphism in microsatellites mainly results from variation in allelic length, which is due to differing numbers of repeats among alleles. Microsatellites have a high mutation rate as compared to base substitution rates in nuclear DNA. However, the flanking sequences surrounding microsatellite loci are often conserved, enabling the use of similar microsatellite-amplifying primers across related species (II–IV). Microsatellites are biparentally inherited, *i.e.*, each individual receives one allele from each parent, providing information on both maternal and paternal contributions to gene flow within and among populations. Because of their codominant inheritance and typically high polymorphism, microsatellites are frequently used as markers in population-genetic studies as well as in identification of individuals and analyses of kinship (see, *e.g.*, Chistiakov *et al.*, 2006).

2.3 GENETIC ANALYSES

2.3.1 Genetic diversity and inbreeding coefficient

Genetic diversity was estimated for the three Fennoscandian ringed seal subspecies and for regional and temporal samples of the Saimaa population. MtDNA diversity was estimated by numbers of different haplotypes (hn), unique haplotypes (uh), and polymorphic loci (pn), haplotypic richness (a), as well as haplotype (h) and nucleotide (π) diversities (I, III, IV). Haplotypic richness is the mean number of haplotypes per locus estimated using the rarefaction method (Kalinowski, 2004) taking the sample size into account. Haplotype diversity reflects numbers and frequencies of different haplotypes, and nucleotide diversity differences between haplotypes.

Microsatellite diversity was estimated by numbers of polymorphic loci (N_{P}) and alleles (N_{A}), rarefied allelic richness (A_{R}), and observed (H_{O}) and expected (H_{E}) heterozygosities (II–VI). Observed heterozygosity is the observed proportion of heterozygous individuals at a given locus, while expected heterozygosity reflects the proportions estimated based on allele frequencies in the focal population. The level of inbreeding within each subspecies and Saimaa subsample was assessed by the inbreeding coefficient (F_{IS}), *i.e.*, the probability that both alleles at given locus of an individual are identical by descent (Wright, 1951).

2.3.2 Present and historical effective population sizes

Effective population size (N_E) is the size of an idealized Fisher–Wright population (*i.e.*, a population with constant size, equal sex ratio, random mating, equal reproductive success of individuals, and non-overlapping generations) that loses genetic diversity or becomes inbred at the same rate as the observed population (Waples, 2002). Current N_E was estimated for the total Saimaa ringed seal population as well as for regional and temporal samples using two different approaches (III). The method based on linkage disequilibrium provides an N_E estimate for a single population sample at a single point in time (Waples, 2006; Waples & Do, 2008), whereas the temporal method is based on the extent of changes in allele frequencies between samples taken at different time points (Jorde & Ryman, 2007).

The trajectory of genetic diversity and past effective population sizes were estimated for the Saimaa, Baltic, and Ladoga subspecies using coalescent approaches (I, II). The coalescent framework was utilised to simulate the changes that have occurred in the genetic composition of each population after separation from the common ancestral population (Nordborg, 2010). As the separation time of the populations is known based on Fennoscandian geological history (Forstén & Alhonen, 1975; Ukkonen, 2002), past events could be inferred from the present-day data. Firstly, the Bayesian serial coalescent model (Excoffier *et al.*, 2000; Anderson *et al.*, 2005) was used to infer mutation and population size parameters in the Saimaa ringed seal (I). Secondly, a Markov Chain Monte Carlo method under the isolation-with-migration model (Hey & Nielsen, 2007;

Hey, 2010) was used to estimate demographic parameters for the Saimaa and Baltic populations (I). Thirdly, an approximate Bayesian computation approach (Cornuet *et al.*, 2008; 2010) was used to explore historical N_Es and to assess the best-fitting scenario for changes in N_E through time for the Saimaa, Baltic, and Ladoga subspecies (II).

2.3.3 Population differentiation and gene flow

Differences among mtDNA haplotypes of the three ringed seal subspecies were studied by constructing a haplotype network illustrating relationships and distances among haplotypes (I). Genetic differentiation among subspecies and Saimaa subsamples was estimated using *F*-statistics (I–IV), describing the distribution of genetic diversity among different levels of the sampling hierarchy (individuals, subpopulations, and total population) (Wright, 1951; see also Excoffier *et al.,* 1992). Differentiation based on both mtDNA and microsatellite variation for all sampling schemes was evaluated using F_{ST}, which measures differences in allelic frequencies among populations. For assessing mtDNA differentiation among subspecies, we also estimated Φ_{ST}, which takes differences between haplotypes into account. Genetic differences among seals originating from different populations (II) and Saimaa subpopulations (III) were examined using factorial correspondence analysis (FCA), which illustrates the distribution of genetic variation across individuals based on their microsatellite genotypes.

Spatial structuring of the Saimaa ringed seal population was also investigated using Bayesian clustering analyses (Guillot *et al.,* 2009; François & Durand, 2010; III). The analysis in general consists of two phases. First, the issue of model choice (*i.e.*, how many subpopulations are most appropriate for interpreting the data) is considered without prior information of the number of locations at which the individuals were sampled, and into which location each individual belongs. Second, the individuals in the sample are assigned probabilistically to the selected number of subpopulations on the basis of their multilocus genotypes. The basic hierarchical structure of the Saimaa population was inferred utilising only microsatellite genotypes of individuals without knowledge on sampling locations (Pritchard *et al.,* 2000; Falush *et al.,* 2003; Evanno *et al.,* 2005), and finer-scale structuring was examined by using an approach that incorporates information on the collection locations as well as the topography of the lake into the analysis (Chen *et al.,* 2007; Durand *et al.,* 2009).

The presence of an isolation-by-distance (IBD) pattern was investigated for the Saimaa population. A finding of IBD indicates that dispersal of individuals is limited and, thus, individuals found close to each other tend to be more related to each other than those with greater geographic distances (Wright, 1943). Asymmetric migration rates among Saimaa regions were estimated using the Bayesian method of Wilson & Rannala (2003), which uses multilocus genotypes of individuals for inferring recent migration rates among subpopulations. As the data included few adults (< 14%), direct assessments of dispersal of different sexes could not be made. Therefore, the relative amounts of male- and female-mediated gene flow were calculated indirectly based on F_{ST} values of maternally inherited mtDNA and biparentally inherited microsatellites (González-Suárez *et al.,* 2009).

2.3.4 Identification of individuals

A method for genetic identification of Saimaa ringed seal individuals was developed based on multiple microsatellite loci. The reliability of the method was evaluated by estimating the probability of identity (*PI*), *i.e.,* the probability that two randomly chosen individuals have identical multilocus genotypes, as well as the corresponding value for siblings (PI_{SIB}; Taberlet & Luikart, 1999; Waits *et al.,* 2001). Because the resolution of the method improves with increasing number of markers, but the probability of genotyping errors increases at the same time, the optimal number of loci was assessed by computing expected and observed mismatch distributions for the marker system (Waits & Paetkau, 2005). In addition, we examined whether the marker system developed for individual identification was adequate also for inferring parentage and kinship using full-pedigree likelihood methods utilising multilocus genotype data (Jones & Wang, 2010).

3 Results and discussion

3.1 TRAJECTORY OF GENETIC DIVERSITY IN THE SAIMAA RINGED SEAL IN RELATION TO THE BALTIC AND LADOGA SUBSPECIES

The three Fennoscandian ringed seal subspecies inhabiting the Baltic Sea and lakes Saimaa and Ladoga (Hyvärinen & Nieminen, 1990; Amano *et al.,* 2002) descend from the same ancestral population that colonised the Baltic basin after the last glacial period (Forstén & Alhonen, 1975; Ukkonen, 2002), but they currently retain very different levels of genetic diversity (Table 1; I, II, see also Palo, 2003; Palo *et al.,* 2003). The genetic diversity of the Baltic ringed seal is close that observed in Arctic ringed seals, possibly due to a large historical population size (I, II) and/or occasional incoming gene flow (Palo *et al.,* 2001; Martinez-Bakker *et al.,* 2013).

The populations of lakes Saimaa and Ladoga became isolated at roughly the same time (Donner, 1995; Saarnisto, 2011), but their genetic diversities differ considerably: the Saimaa ringed seal is genetically very uniform, whereas the Ladoga subspecies is nearly as diverse as the Baltic population (Table 1; I–III). This is most likely due to differences in their population sizes and habitats: the shallow and highly fragmented Lake Saimaa is currently inhabited by only some 300 seals (Metsähallitus, 2014), while Ladoga is deeper, more continuous, and four times larger, and maintains a population of a few thousand individuals (Sipilä *et al.,* 1996; Trukhanova *et al.,* 2013). Assuming that the diversity observed in the Baltic population today represents the original level in the lacustrine populations, the Saimaa ringed seal has lost 55% of its overall microsatellite heterozygosity, and 34% and 89% mtDNA haplotypic and nucleotide diversities, respectively (I, II). For the Ladoga subspecies, the diversity loss has been substantially milder, so that the corresponding

Table 1. *Mitochondrial and microsatellite diversity in the three Fennoscandian ringed seal subspecies, and in the spatial and temporal subsamples of the Saimaa ringed seal population.*

	mtDNA diversity						**microsatellite diversity**						
	N	hn (uh)	a	pn	h ± SD	π ± SD	N	N_P	N_A ± SD	A_R	H_O ± SD	H_E ± SD	F_{IS}
Baltic Sea	19	16 (15)	13.77	46	0.98 ± 0.03	0.047 ± 0.038	21	17	9.00 ± 3.20	8.30	0.74 ± 0.20	0.80 ± 0.08	0.07**
Lake Ladoga	16	13 (12)	13.00	29	0.97 ± 0.04	0.015 ± 0.017	16	17	7.65 ± 2.76	7.65	0.69 ± 0.22	0.74 ± 0.16	0.06*
Lake Saimaa	215	8 (8)	4.04	10	0.65 ± 0.02	0.005 ± 0.005	172	17	3.47 ± 3.32	2.77	0.33 ± 0.21	0.36 ± 0.22	0.07***
NS	19	3 (0)	3.00	6	0.43 ± 0.12	0.004 ± 0.004	15	14	2.29 ± 1.16	2.27	0.34 ± 0.27	0.33 ± 0.24	-0.00
HV	116	4 (0)	3.06	7	0.60 ± 0.02	0.005 ± 0.005	99	15	3.24 ± 3.15	2.7	0.35 ± 0.23	0.38 ± 0.23	0.07***
KV	21	2 (0)	2.00	5	0.18 ± 0.10	0.001 ± 0.003	20	13	2.41 ± 1.66	2.32	0.37 ± 0.31	0.36 ± 0.28	-0.03
MHV	95	4 (0)	3.11	7	0.58 ± 0.03	0.005 ± 0.005	79	15	3.18 ± 2.92	2.63	0.34 ± 0.23	0.35 ± 0.24	0.02
PV	61	5 (1)	3.78	9	0.63 ± 0.03	0.006 ± 0.005	43	15	2.59 ± 1.42	2.26	0.31 ± 0.25	0.30 ± 0.23	-0.03
SS	19	5 (2)	5.00	8	0.78 ± 0.06	0.005 ± 0.004	15	14	2.47 ± 1.42	2.45	0.31 ± 0.24	0.30 ± 0.22	-0.05
yod 1980s	79	6 (0)	5.55	8	0.56 ± 0.05	0.005 ± 0.005	59	17	3.29 ± 2.62	3.21	0.35 ± 0.23	0.37 ± 0.23	0.05*
yod 1990s	54	7 (0)	7.00	9	0.61 ± 0.04	0.005 ± 0.005	48	17	3.29 ± 2.85	3.29	0.34 ± 0.22	0.37 ± 0.23	0.08*
yod 2000s	82	7 (1)	6.50	10	0.67 ± 0.04	0.005 ± 0.005	65	17	3.06 ± 2.38	2.96	0.32 ± 0.20	0.35 ± 0.22	0.09***
yob 1963-79	21	5 (0)	5.00	7	0.74 ± 0.06	0.005 ± 0.004	14	16	2.94 ± 1.75	2.94	0.38 ± 0.25	0.38 ± 0.23	-0.02
yob 1980s	67	5 (0)	3.31	8	0.49 ± 0.06	0.004 ± 0.005	54	17	3.06 ± 2.38	2.71	0.34 ± 0.23	0.37 ± 0.23	0.07**
yob 1990s	51	6 (0)	4.04	9	0.62 ± 0.04	0.005 ± 0.005	47	17	3.29 ± 2.85	2.69	0.33 ± 0.22	0.36 ± 0.23	0.07*
yob 2000s	71	7 (1)	5.07	10	0.69 ± 0.04	0.005 ± 0.005	54	17	3.06 ± 2.38	2.58	0.32 ± 0.19	0.35 ± 0.22	0.10**

NS = Northern Saimaa, HV = Haukivesi area, *KV* = Kolovesi, *MHV* = Main Haukivesi area, PV = Pihlajavesi area, SS = Southern Saimaa, yod = year of death, yob = year of birht, N = nr of samples, hn = nr of haplotypes, uh = nr of unique haplotypes, a = haplotypic richness, pn = nr of polymorphic sites, h = haplotype diversity, π = nucleotide diversity, N_P = nr of polymorphic loci, N_A = nr of alleles, A_R = allelic richness, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = fixation index, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

figures are 7%, 1%, and 68%, respectively. Consequently, the Saimaa population differs conspicuously from the other two populations (mtDNA: pairwise $\Phi_{ST} > 0.900$, $P < 0.001$; microsatellites: $F_{ST} > 0.340$, $P < 0.05$), while differentiation between the Baltic and Ladoga subspecies is clearly weaker (mtDNA: pairwise $\Phi_{ST} = 0.157$, $P < 0.001$; microsatellites: $F_{ST} = 0.041$, $P < 0.05$).

Coalescent simulations were used to assess the historical population size of the Saimaa ringed seal, *i.e.*, to find out how large the population must have been in the past to retain the level of genetic diversity observed today, and also to investigate the effects of a possible colonisation bottleneck (= founder effect) and the recent anthropogenic bottleneck (I, II). The analyses based on mtDNA and microsatellite data both suggested a historical population size of around ten thousand seals. This number clearly exceeds the estimated present-day carrying capacity of Lake Saimaa (a few thousand seals; Hyvärinen *et al.*, 1999). However, some 8,000 years ago the present-day lake was part of an enormous lake complex (Saarnisto *et al.*, 1999; Tikkanen, 2002; Oinonen *et al.*, 2014) that could have supported even such a large population (Hyvärinen *et al.*, 1999). Furthermore, the ABC analyses suggested that the colonisation bottleneck of the population during the formation of Lake Saimaa was severe and lasted for a long time, but indicated, as expected, that the recent, 20th-century bottleneck has as of yet had a negligible effect on the genetic diversity of the Saimaa ringed seal (II). In contrast, both the colonisation and anthropogenic bottlenecks in the Ladoga population were suggested to have been less severe, with only a minor effect on the level of diversity.

Hence, the Saimaa ringed seal has evidently lost most of its genetic diversity during its isolation of nearly 10,000 years (I, II, see also Palo, 2003; Palo *et al.*, 2003). For the Ladoga population, which is separated from the Baltic Sea by a river only 70 km long, the possibility of occasional gene-flow from the marine population cannot be ruled out (II).

3.2 ONGOING DIVERSITY LOSS AND EXTREMELY LOW EFFECTIVE POPULATION SIZES IN THE SAIMAA RINGED SEAL

Mammalian populations that have experienced demographic challenges exhibit > 20% lower levels of genetic diversity than do non-endangered ones (Garner *et al.,* 2005). Genetic variation in the Saimaa ringed seal (Table 1; I–III) was drastically lower than that of marine ringed seals (Palo *et al.,* 2001, 2003; Palo, 2003; Davis *et al.,* 2008; Martinez-Bakker *et al.,* 2013). Moreover, microsatellite diversity of this subspecies (Table 1; III) is among the lowest thus far reported for pinnipeds (Pastor *et al.,* 2004; Schultz *et al.,* 2009; Han *et al.,* 2010; Sanvito *et al.,* 2012).

Also mtDNA diversity of the Saimaa ringed seal was notably low (Table 1; I). Nevertheless, as many as eight haplotypes were detected in the population. This is somewhat surprising for a population that has experienced a severe bottleneck of fewer than 150 individuals (Sipilä *et al.,* 1990), as other mammalian populations that have undergone a comparable reduction in size retain only one or two mtDNA haplotypes (*e.g.*, Pichler & Baker, 2000; Randi *et al.,* 2000; Weber *et al.,* 2000). With respect to other Fennoscandian ringed seal subspecies, the Saimaa haplotypes were all located in a single clade, with very small distances among them, which was reflected in the notably low nucleotide diversity (Table 1; I). As the geological history of Lake Saimaa and, thus, the origin of its ringed seal population are reasonably well established, the high number of haplotypes could only be explained by unusually high mutation rates and/or large historical population size, as indicated by coalescent simulations (I).

When examining temporal changes in mtDNA haplotype frequencies, we detected high differentiation among the last three decades (overall and pairwise F_{ST} values ≥ 0.356, all $P < 0.001$; I). This result suggests that the population is currently so small that the effect of genetic drift is pronounced, causing haplotype frequencies to fluctuate even over such a short time span. Indeed, the total and regional effective population sizes estimated for the Saimaa ringed seal based on microsatellites were extremely low (N_E = 5 – 113; III). Comparable N_Es have been estimated for other endangered and/or bottlenecked populations of large mammals (*e.g.*, Aspi *et al.*, 2006; Ortego *et al.*, 2011; Casas-Marce *et al.*, 2013).

Furthermore, a slight, but evident decrease was observed in both mtDNA variation and individual microsatellite

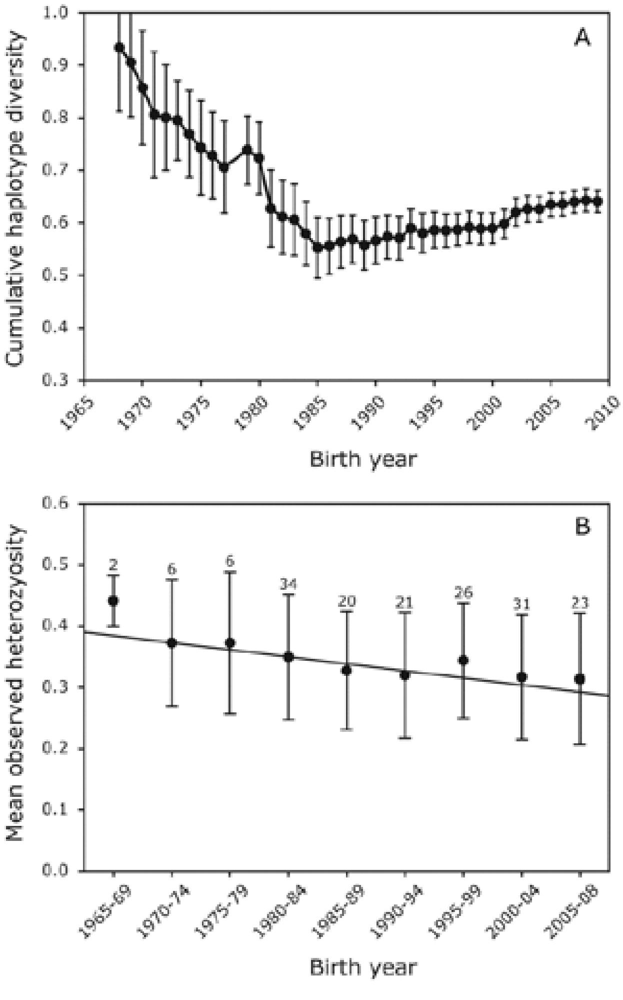


Figure 2. *Cumulative mtDNA haplotype diversity (past-to-present) in the Saimaa population (A) and observed heterozygosity of Saimaa ringed seal individuals (B) in relation to year of birth. Error bars are ±1 SD. In (B), the number of seals in each 5-year category is given above the error bar.*

heterozygosity based on the birth year of seals during the past few decades (Fig. 2AB; I, III), suggesting ongoing diversity erosion in the population. Hence, in addition to major diversity loss caused by the colonisation bottleneck and the long postglacial period of isolation (see above), the anthropogenic bottleneck in the 20th century together with subdivision of the population into small subpopulations (see below) seems to have had an effect on the genetic diversity of this landlocked subspecies.

3.3 FINE-SCALE POPULATION STRUCTURE AND LIMITED GENE FLOW WITHIN LAKE SAIMAA

Ringed seals are potentially highly mobile: Arctic ringed seals make seasonal migrations of hundreds to thousands of kilometres (Kelly *et al.,* 2010; Harwood *et al.,* 2012; Crawford *et al.,* 2012; Martinez-Bakker *et al.,* 2013), and individual Baltic ringed seals have been observed travelling comparable distances (Oksanen S., unpublished). High mobility of the species is further supported by genetic studies, which have shown near-panmixia in marine ringed seals: only weak differentiation was detected between Arctic and Baltic populations located thousands of kilometres apart (Palo *et al.,* 2001; Martinez-Bakker *et al.,* 2013). In contrast, Saimaa ringed seals are considered fairly sedentary (Kunnasranta, 2001; Koskela *et al.,* 2002; Niemi *et al.,* 2012; 2013b), although individual seals, especially juveniles, have been observed to regularly travel some tens of kilometres, a relatively long distance within the lake (Niemi *et al.,* 2012; 2013a). However, no migrations between the main basins of the lake have been detected, which has raised concerns of isolation of breeding areas.

Indeed, analyses based on both mitochondrial and nuclear markers indicated limited gene flow within the lake: differentiation among the four main basins (Fig. 3A) was statistically highly significant for both marker types (I, III). In contrast, a previous study based on eight microsatellite loci suggested only weak differentiation between the northern and southern parts of the lake (Palo *et al.*, 2003), which was evidently due to limited numbers of samples and loci in that study. Furthermore, a statistically significant isolation-by-distance pattern was detected for both markers (III). This result reflects

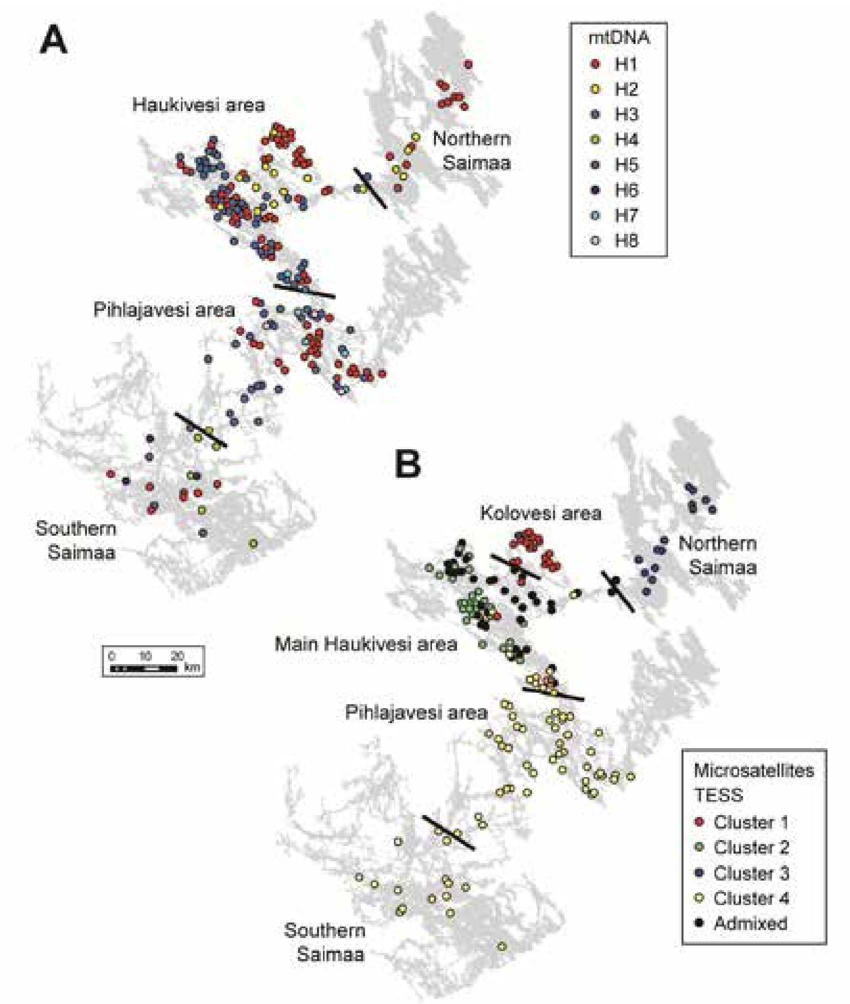


Figure 3. *Collection sites of Saimaa ringed seal specimens used in mtDNA (A) and microsatellite (B) analyses. Initial (A) and updated (B) division of the lake. Different colours denote different mtDNA haplotypes (A) and different clusters indicated by a Bayesian genotype-assignment analysis conducted using the program TESS (B).*

closer relatedness of individual seals that had been found close to each other as compared to that of individuals with a greater distance from each other, further supporting the finding of restricted gene flow within the lake.

Structuring of the Saimaa ringed seal population was also examined using only data on the microsatellite genotypes of individual seals, with no presumption of spatial division of the lake (III). Bayesian clustering analyses revealed surprisingly fine-scaled structuring of the population (Fig. 3B). The analysis of the upper hierarchical population structure suggested two clusters within Lake Saimaa, one of which, quite surprisingly, almost exclusively included seals from the relatively small, labyrinthine Kolovesi basin, whereas the other represented the rest of the lake (III). A further analysis incorporating also the sampling-site locations and topography of the lake (*i.e.*, the actual dispersal routes of seals) as prior information indicated the presence of four clusters (Fig. 3B). The semi-isolation of the Kolovesi basin was confirmed and, additionally, seals from Northern Saimaa formed one cluster, as was expected in the initial division of the lake (Fig. 3A). However, individuals from the Pihlajavesi area and Southern Saimaa were suggested to belong to a single cluster. The rest of the Haukivesi area (Kolovesi excluded) mainly formed one cluster, but also seemed to represent an admixture zone. Thus, we reassessed the initial spatial division of Lake Saimaa: we retained Northern Saimaa, the Pihlajavesi area, and Southern Saimaa in their original form based on significant F_{ST} values among them, but split the Haukivesi area into two subregions (Kolovesi and Main Haukivesi; Fig. 3B). Notably, the significant heterozygote deficit found in the total population ($F_{IS} = 0.075$, $P < 0.001$) disappeared after division into the aforementioned five regions (Table 1; III).

Migration rates estimated among these five regions based on microsatellite data were very low, except for the rate from the Pihlajavesi area to Southern Saimaa, which was 20.4% and the only rate significantly different from zero (III). However, the migration rate in the opposite direction was low and did not significantly depart from zero, suggesting that the Pihlajavesi area, which is the most productive breeding area of the Saimaa ringed seal (Metsähallitus, 2014), serves as a source for Southern Saimaa. This is a particularly important finding, since identifying productive subpopulations, which may serve as sources, from less productive ones acting as sinks, is essential in designing and allocating conservation measures (Hansen, 2011).

The regional differentiation revealed by maternally inherited mtDNA was very strong (overall and pairwise F_{ST} values ≥ 0.311, all $P < 0.001$; I, III), indicating that Saimaa ringed seal females have a tendency to stay in their natal region to reproduce (I). Female philopatry has not been reported in marine ringed seals, but it has been observed in related species, such as grey seals (*Halichoerus grypus*) (Allen *et al.,* 1995; Pomeroy *et al.,* 2000) and harbour seals (*Phoca vitulina*) (Stanley *et al.,* 1996).

In contrast, spatial differentiation in biparentally inherited microsatellites was moderate (overall five-region $F_{ST} = 0.107$, pairwise F_{ST} values = 0.039 – 0.236, all $P < 0.01$; III). The difference in the level of differentiation in microsatellites and mtDNA could be due to differences in effective sizes of these markers. However, it more probably results from sex-biased dispersal, as turned out to be the case also for the Saimaa ringed seal: gene flow mediated by males was estimated to be over sevenfold compared to that by females (III). The ratio is close to values reported, for example, for harbour seals (Herreman *et al.,* 2009) and California sea lions (*Zalophus californianus*) (González-Suárez *et al.,* 2009).

3.4 IDENTIFICATION OF INDIVIDUALS AND THE UTILITY OF PLACENTAS IN GENETIC MONITORING OF THE SAIMAA POPULATION

Monitoring Saimaa ringed seals is challenging, as they not only are very few in number, but also extremely elusive animals that spend about 80% of their time submerged (Hyvärinen *et al.,* 1995; Niemi *et al.,* 2013b). We developed a method for identification of individuals from DNA samples for this genetically very uniform subspecies (IV). The marker system based on 17 microsatellite loci proved to be adequate for identifying individual Saimaa ringed seals: the probability that two randomly chosen individuals share identical genotypes at the studied loci was $PI = 4.8 \times 10^{-7}$, and the corresponding value for siblings was $PI_{\mathrm{SIB}} = 1.2 \times 10^{-3}$. This means that, in the population of around 300 seals (Metsähallitus, 2014), the expected number of genotype matches is as low as 0.0001 for unrelated individuals and 0.36 for siblings. Although the corresponding values were slightly higher for the 11-locus panel, it could be sufficient for spatially and/or temporally restricted surveys of the population. However, there is not enough power in the 17-locus marker system for conducting kinship analyses, owing to the low genetic diversity of the subspecies (III, IV).

Collecting non-invasive samples of marine mammals is often challenging, although shed skin and faeces have been successfully utilised in genetic studies (Parsons *et al.,* 2006; Valqui *et al.,* 2010; Martinez-Bakker *et al.,* 2013; Baker *et al.,* 2013). Our study using Saimaa ringed seal placentas in genetic analyses (IV) is the first to describe the use of placentas as non-invasive samples from a natural population. Postnatal consumption of the placenta (placentophagia), is routine behaviour among female mammals, but it does not occur within the order Pinnipedia (Kristal *et al.,* 2012). Hence, we found a placenta at nearly half of the inspected birth-lair sites, even though the collection was conducted two to three months after parturition.

As the pinniped placenta is composed of tissues of both the female and its offspring in close union (Stewart & Stewart, 2009), we aimed at finding the optimal sampling spot for genotyping both the mother and the pup from the placentas. The pup’s genotype was found to be reliably obtainable from the umbilical cord (IV). Thus, identification of the pup from a placenta and later encounter of the adult individual would yield information on the dispersal of seals. Additionally, genetic diversity and differentiation indices acquired using data on umbilical cord samples (*i.e.*, samples of pups) were highly correspondent with those based on the reference datasets (I, III), indicating that placentas can be used for inferring standard population-genetic parameters. However, we did not succeed in obtaining the mother's genotype separately, which is most likely due to a high level of intermingling between the maternal and foetal tissues in the areas of contact. Nevertheless, fine-tuning the sampling method together with the use of next-generation sequencing technology may enable inferring also the mother's genotype from the placenta in the near future.

4 Conclusions and challenges for conservation

This study, based on an extensive survey of neutral genetic variation of the Saimaa ringed seal, shows that the genetic diversity of this small and isolated population is extremely low and, worryingly, still declining (I, III). Microsatellite diversity in this subspecies ($H_E = 0.36$) is the lowest thus far recorded within the order Pinnipedia (III). The present effective population size estimates (N_e = 5–113, III) were remarkably low, also indicating that the population is too small to maintain the current diversity in the long term. This was further seen in high temporal differentiation at mtDNA haplotype frequencies among the past few decades, which demonstrates strikingly the strong effect of genetic drift within such a short time span (I). Although most of the original diversity of the Saimaa ringed seal undoubtedly was lost during its long isolation of nearly 10,000 years (I, II, see also Palo *et al.*, 2003), we observed evident decreases in both mtDNA variation and individual microsatellite heterozygosity even within the past few decades (Fig. 2AB, I, III). The ongoing loss of diversity suggested by our results is disconcerting given the critically endangered status of the population.

Fragmentation of a population into even smaller units may further reduce its genetic diversity and increase the level of inbreeding (*i.e.,* autozygosity) of the total population (Keyghobadi 2007). This seems to be the case for the Saimaa ringed seal, as we detected unexpectedly fine-scaled differentiation among regional subpopulations (I, III). The structuring of the population is undoubtedly induced by the small population size and fragmented topography of the lake, but also by behavioural patterns of the seals. The present study demonstrated that Saimaa ringed seal females have a tendency to stay in their natal area for breeding (I), whereas males are more prone to disperse. Thus, gene flow within the lake is predominantly male-mediated, but nevertheless limited and insufficient to counteract the effect of genetic drift (III).

Despite of the low genetic diversity of this small, landlocked population, no clear signs of inbreeding depression have been detected. However, environmental changes may drive populations to extinction, unless they are able to adapt to altered conditions (Hoffmann & Sgrò, 2011). In this respect, the low neutral genetic diversity of the Saimaa ringed seal is a cause of concern, as its adaptive variation may be similarly affected (*cf.* Bollmer *et al.,* 2007; Babik *et al.,* 2009; Smith *et al.,* 2009), which could reduce its ability to respond to a warming climate and other environmental changes (Willi *et al.,* 2006; O'Corry-Crowe, 2008). Further, as small population size along with low diversity may weaken population-level resilience to both demographic and environmental stochasticity (Lacy, 1997), the significant differentiation among breeding areas raises concerns about the viability of the even smaller subpopulations and, in particular, that of the Kolovesi basin (Fig. 1B).

Genetic rescue, *i.e.*, introduction of unrelated individuals into an inbred population, is often suggested and increasingly being implemented as a conservation measure for small and isolated populations (*e.g.*, Hogg *et al.,* 2006; Bouzat *et al.,* 2009; Hedrick & Fredrickson, 2010). For safeguarding the Saimaa population, Saarnisto (2011) suggested translocations of Ladoga ringed seals to Lake Saimaa. However, as the Saimaa ringed seal is recognized as a morphologically and ecologically distinct subspecies (Hyvärinen & Nieminen, 1990; Kunnasranta, 2001), and differs also genetically from the other Fennoscandian subspecies (I, II), such actions would most likely be highly risky. Interbreeding between genetically diverged individuals may result in outbreeding depression (Tallmon *et al.,* 2004), which could compromise the adaptation of the Saimaa subspecies to its unique lacustrine habitat. In addition, the immigrants might carry diseases towards which Saimaa ringed seals are not resistant. Therefore such extreme measures should only be taken as a last resort.

Hence, along with mitigating the most acute threats posed by by-catch mortality and breeding-habitat deterioration due to warming winters (Niemi 2013; Auttila *et al.*, 2014), future conservation efforts should concentrate on preserving the current diversity of the Saimaa ringed seal and enhancing gene flow among subpopulations. The most efficient method for this would be increasing the overall population size, which would simultaneously restore both genetic diversity and gene flow. However, because rapid population growth is improbable in this slowly reproducing species, translocations of adult seals, especially females, should be considered as a short-term conservation measure. For designing such actions, this study has helped in identifying management units within the population, *i.e.*, distinguishing subpopulations that are productive and could serve as sources from ones that are semi-isolated and possibly not viable on their own in the long term. Furthermore, the non-invasive genetic sampling method based on placentas developed here can provide an efficient means not only for monitoring the population in general, but also for evaluating the success of conservation efforts.

5 References

Allen P J, Amos W, Pomeroy P P & Twiss S D (1995) Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. *Molecular Ecology*, **4**, 653–662.

Amano M, Hayano A & Miyazaki N (2002) Geographic variation in the skull of the ringed seal, *Pusa hispid*a. *Journal of Mammalogy*, **83**, 370–380.

Anderson C N K, Ramakrishnan U, Chan Y L & Hadly E A (2005) Serial SimCoal: A population genetics model for data from multiple populations and points in time. *Bioinformatics*, **21**, 1733–1734.

Aspi J, Roininen E, Ruokonen M, Kojola I & Vilà C (2006) Genetic diversity, population structure, effective population size and demographic history of the Finnish wolf population. *Molecular Ecology*, **15**, 1561–1576.

Auttila M, Niemi M, Skrzypczak T, Viljanen M & Kunnasranta M (2014) Estimating and mitigating perinatal mortality of the endangered Saimaa ringed seal (*Phoca hispida saimensis*) in a changing climate. *Annales Zoologici Fennici*, ISSN 1797-2450 (online), in press.

Babik W, Pabijan M, Arntzen J W, Cogalniceanu D, Durka W & Radwan J (2009) Long-term survival of a urodele amphibian despite depleted major histocompatibility complex variation. *Molecular Ecology*, **18**, 769–781.

Baker C, Steel D, Calambokidis J, Falcone E, González-Peral U, Barlow J, Burdin A M, Clapham P J, Ford J K B, Gabriele C M, Mattila D, Rojas-Bracho L, Straley J M, Taylor B L, Urbán J, Wade P R, Weller D, Witteveen B H & Yamaguchi M (2013) Strong maternal fidelity and natal philopatry shape genetic structure in North Pacific humpback whales. *Marine Ecology Progress Series*, **494**, 291–306.

Ballard J W O & Whitlock M C (2004) The incomplete natural history of mitochondria. *Molecular Ecology*, **13**, 729–744.

Berta A & Churchill M (2012) Pinniped taxonomy: review of currently recognized species and subspecies, and evidence used for their description. *Mammal Review*, **42**, 207–234.

Blomqvist D, Pauliny A, Larsson M & Flodin L A (2010) Trapped in the extinction vortex? Strong genetic effects in a declining vertebrate population. *BMC Evolutionary Biology*, **10**, 33.

Bollmer J L, Vargas F H & Parker P G (2007) Low MHC variation in the endangered Galápagos penguin (*Spheniscus mendiculus*). *Immunogenetics*, **59**, 593–602.

Bouzat J, Johnson J, Toepfer J, Simpson S A, Esker T L & Westemeier R L (2009) Beyond the beneficial effects of translocations as an effective tool for the genetic restoration of isolated populations. *Conservation Genetics*, **10**, 191–201.

Brøseth H, Flagstad Ø, Wärdig C, Johansson M & Ellegren H (2010) Large-scale noninvasive genetic monitoring of wolverines using scats reveals density dependent adult survival. *Biological Conservation*, **143**, 113–120.

Casas-Marce M, Soriano L, López-Bao J V & Godoy J A (2013) Genetics at the verge of extinction: insights from the Iberian lynx. *Molecular Ecology*, **22**, 5503–5515.

Charlesworth B (2009) Effective population size and patterns of molecular evolution and variation. *Nature Reviews Genetics*, **10**, 195–205.

Chen C, Durand E, Forbes F & François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Molecular Ecology Notes*, **7**, 747–756.

Chistiakov D A, Hellemans B & Volckaert F A M (2006) Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. *Aquaculture*, **255** , 1–29.

Cornuet J-M, Ravigné V & Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). *BMC Bioinformatics*, **11**, 401.

Cornuet J-M, Santos F, Beaumont M A, Robert C P, Marin J-M, Balding D J, Guillemaud T & Estoup A (2008) Inferring population history with DIY ABC: a user-friendly approach to approximate Bayesian computation. *Bioinformatics*, **24**, 2713–2719.

Crawford J, Frost K, Quakenbush L & Whiting A (2012) Different habitat use strategies by subadult and adult ringed seals (*Phoca hispida*) in the Bering and Chukchi seas. *Polar Biology*, **35**, 241–255.

Davis C S, Stirling I, Strobeck C & Coltman D W (2008) Population structure of ice-breeding seals. *Molecular Ecology*, **17**, 3078–3094.

Davoli F, Schmidt K, Kowalczyk R & Randi E (2013) Hair snaring and molecular genetic identification for reconstructing the spatial structure of Eurasian lynx populations. *Mammalian Biology*, **78**, 118–126.

De Barba M, Waits L P, Garton E O, Genovesi P, Randi E, Mustoni A & Groff C (2010) The power of genetic monitoring for studying demography, ecology and genetics of a reintroduced brown bear population. *Molecular Ecology*, **19**, 3938–3951.

Donner J (1995) *The Quaternary History of Scandinavia*. Cambridge University Press, Cambridge.

Durand E, Jay F, Gaggiotti O E & François O (2009) Spatial inference of admixture proportions and secondary contact zones. *Molecular Biology and Evolution*, **26**, 1963–1973.

Evanno G, Regnaut S & Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Excoffier L, Novembre J & Schneider S (2000) SIMCOAL: a general coalescent program for the simulation of molecular data in interconnected populations with arbitrary demography. *Journal of Heredity*, **91**, 506–509.

Excoffier L, Smouse P E & Quattro J M (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Falush D, Stephens M & Pritchard J K (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Fedy B C, Martin K, Ritland C & Young J (2008) Genetic and ecological data provide incongruent interpretations of population structure and dispersal in naturally subdivided populations of white-tailed ptarmigan (*Lagopus leucura*). *Molecular Ecology*, **17**, 1905–1917.

Ferchaud A-L, Lyet A, Cheylan M, Arnal V, Baron J P, Montgelard C & Ursenbacher S (2011) High genetic differentiation among French populations of the Orsini's viper (*Vipera ursinii ursinii*) based on mitochondrial and microsatellite data: implications for conservation management. *Journal of Heredity*, **102**, 67–78.

Foote A D, Hofreiter M & Morin P A (2012) Ancient DNA from marine mammals: studying long-lived species over ecological and evolutionary timescales. *Annals of Anatomy*, **194**, 112–120.

Ford M J, Hanson M B, Hempelmann J A, Ayres K L, Emmons C K, Schorr G S, Baird R W, Balcomb K C, Wasser S K & Parsons K M (2011) Inferred paternity and male reproductive success in a killer whale (*Orcinus orca*) population. *Journal of Heredity*, **102**, 537–553.

Forstén A & Alhonen P (1975) The subfossil seals of Finland and their relation to the history of the Baltic Sea. *Boreas*, **4**, 143–155.

Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131–140.

François O & Durand E (2010) Spatially explicit Bayesian clustering models in population genetics. *Molecular Ecology Resources*, **10**, 773–84.

Garner A Rachlow J L & Hicks J F (2005) Patterns of genetic diversity and its loss in mammalian populations. *Conservation Biology*, **19**, 1215–1221.

González-Suárez M, Flatz R, Aurioles-Gamboa D, Hedrick P W & Gerber L R (2009) Isolation by distance among California sea lion populations in Mexico: redefining management stocks. *Molecular Ecology*, **18**, 1088–1099.

Gottelli D, Sillero-Zubiri C, Marino J, Funk S M & Wang J (2012) Genetic structure and patterns of gene flow among populations of the endangered Ethiopian wolf. *Animal Conservation*, **16**, 234–247.

Greenwood P J (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Guillot G, Leblois R, Coulon A & Frantz A C (2009) Statistical methods in spatial genetics. *Molecular Ecology*, **18**, 4734–4756.

Han J-B, Sun F-Y, Gao X-G, He C B, Wang P L, Ma Z Q & Wang Z H (2010) Low microsatellite variation in spotted seal (*Phoca largha*) shows a decrease in population size in the Liadong Gulf colony. *Annales Zoologici Fennici*, **47**, 15–27.

Hansen A (2011) Contribution of source-sink theory to protected area science. In: *Sources, Sinks, and Sustainability across Landscapes* (eds Liu J, Hull V, Morzillo A & Wiens J), pp. 5918–5933. Cambridge University Press, Cambridge.

Harwood L A, Smith T G & Auld J C (2012) Fall migration of ringed seals (*Phoca hispida*) through the Beaufort and Chukchi Seas, 2001 – 02. *Arctic*, **65**, 35–44.

Hedrick P W & Fredrickson R (2010) Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. *Conservation Genetics*, **11**, 615–626.

Herreman J K, Blundell G M, McDonald D B & Ben-David M (2009) Asymmetrical male-mediated gene flow between harbor seal (*Phoca vitulina*) populations in Alaska. *Canadian Journal of Zoology*, **87**, 498–507.

Hey J (2010) Isolation with migration models for more than two populations. *Molecular Biology and Evolution*, **27**, 905–920.

Hey J & Nielsen R (2007) Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proceedings of the National Academy of Sciences of the USA*, **104**, 2785–2790.

Hoeck P E A, Bollmer J L, Parker P G & Keller L F (2010) Differentiation with drift: a spatio-temporal genetic analysis of Galapagos mockingbird populations (*Mimus* spp.). *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **365**, 1127–1138.

Hoffmann A A & Sgrò C M (2011) Climate change and evolutionary adaptation. *Nature*, **470**, 479–485.

Hogg J T, Forbes S H, Steele B M & Luikart G (2006) Genetic rescue of an insular population of large mammals. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 1491–1499.

Hyvärinen H & Nieminen M (1990) Differentiation of the ringed seal in the Baltic Sea, Lake Ladoga and Lake Saimaa. *Finnish Game Research*, **47**, 21–27.

Hyvärinen H, Hämäläinen E & Kunnasranta M (1995) Diving behavior of the Saimaa ringed seal (*Phoca hispida saimensis* Nordq.). *Marine Mammal Science*, **11**, 324–334.

Hyvärinen H, Sipilä T, Koskela J & Kunnasranta M (1999) The Saimaa ringed seal. In: *Saimaa, a Living Lake* (ed Kuusisto E), pp. 126–136. Tammi, Helsinki.

Jackson J A, Laikre L, Baker C S & Kendall K C (2011) Guidelines for collecting and maintaining archives for genetic monitoring. *Conservation Genetics Resources*, **4**, 527–536.

Jansson E, Harmoinen J, Ruokonen M & Aspi J (2014) Living on the edge: reconstructing the genetic history of the Finnish wolf population. *BMC Evolutionary Biology*, **14**, 64.

Jones O R & Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, **10**, 551–555.

Jorde P E & Ryman N (2007) Unbiased estimator for genetic drift and effective population size. *Genetics*, **177**, 927–935.

Kalinowski S T (2004) Counting alleles with rarefaction: private alleles and hierarchial sampling designs. *Conservation Genetics*, **5**, 539–543.

Kekkonen J, Wikström M & Brommer J E (2012) Heterozygosity in an isolated population of a large mammal founded by four individuals is predicted by an individual-based genetic model. *PLoS ONE*, **7**, e43482.

Keller L F & Waller D M (2002) Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, **17**, 230–241.

Kelly B, Badajos O, Kunnasranta M, Moran J R, Martinez-Bakker M, Wartzok D & Boveng P (2010) Seasonal home ranges and fidelity to breeding sites among ringed seals. *Polar Biology*, **33**, 1095–1109.

Keyghobadi N (2007) The genetic implications of habitat fragmentation for animals. *Canadian Journal of Zoology*, **85**, 1049–1064.

Kokko H, Helle E, Lindström J, Ranta E, Sipilä T & Courchamp F (1999) Backcasting population sizes of ringed and grey seals in the Baltic and Lake Saimaa during the 20th century. *Annales Zoologici Fennici*, **36**, 65–73.

Kopatz A, Eiken H, Hagen S, Ruokonen M, Esparza-Salas R, Schregel J, Kojola I, Smith M E, Wartiainen I & Aspholm P E (2012) Connectivity and population subdivision at the fringe of a large brown bear (*Ursus arctos*) population in North Western Europe. *Conservation Genetics*, **13**, 681–692.

Koskela J T, Kunnasranta M, Hämäläinen E & Hyvärinen H (2002) Movements and use of haul-out sites of radio-tagged Saimaa ringed seal (*Phoca hispida saimensis* Nordq.) during the open-water season. *Annales Zoologici Fennici*, **39**, 59–67.

Kovacs K M, Aguilar A, Aurioles D, Burkanov V, Campagna C, Gales N, Gelatt T, Goldsworthy S D, Goodman S J, Hofmeyr G J G, Härkönen T, Lowry L, Lydersen C, Schipper J, Sipilä T, Southwell C, Stuart S, Thompson D & Trillmich F (2012) Global threats to pinnipeds. *Marine Mammal Science*, **28**, 414–436.

Kristal M B, DiPirro J M & Thompson A C (2012) Placentophagia in humans and nonhuman mammals: causes and consequences. *Ecology of Food and Nutrition*, **51**, 177–197.

Kunnasranta M (2001) Behavioural biology of two ringed seal (*Phoca hispida*) subspecies in the large European lakes Saimaa and Ladoga. PhD thesis, University of Joensuu.

Lacy R C (1997) Importance of genetic variation to the viability of mammalian populations. *Journal of Mammalogy*, **78**, 320–335.

Latch E K, Boarman W I, Walde A & Fleischer R C (2011) Fine-scale analysis reveals cryptic landscape genetic structure in desert tortoises. *PLoS ONE*, **6**, e27794.

Liao W & Reed D H (2009) Inbreeding-environment interactions increase extinction risk. *Animal Conservation*, **12**, 54–61.

Louy D, Habel J C, Schmitt T, Assmann T, Meyer M & Müller P (2007) Strongly diverging population genetic patterns of three skipper species: the role of habitat fragmentation and dispersal ability. *Conservation Genetics*, **8**, 671–681.

Madsen T, Stille B & Shine R (1996) Inbreeding depression in an isolated population of adders *Vipera berus*. *Biological Conservation*, **75**, 113–118.

Martinez-Bakker M E, Sell S K, Swanson B J, Kelly B P & Tallmon D A (2013) Combined genetic and telemetry data reveal high rates of gene flow, migration, and long-distance dispersal potential in Arctic ringed seals (*Pusa hispida*). *PLoS ONE*, **8**, e77125.

Mattila A L K, Duplouy A, Kirjokangas M, Lehtonen R, Rastas P & Hanski I (2012) High genetic load in an old isolated butterfly population. *Proceedings of the National Academy of Sciences of the USA*, **109**, E2496–E2505.

McNeely J A, Miller K R, Reid W V, Mittermeier R A & Werner T B (1990) *Conserving the World's Biological Diversity*. IUCN Gland, Switzerland.

Metsähallitus, Natural Heritage Services (2014) Saimaannorppa (Saimaa ringed seal, in Finnish). http://www.metsa.fi/sivustot/metsa/fi/Luonnonsuojelu/Lajitjaluontotyypit/Uhanalaisetelaimet/Saimaannorppa/Sivut/Saimaannorppa.aspx. Accessed 7 June 2014.

Niemi M (2013) Behavioural ecology of the Saimaa ringed seal - implications for conservation. PhD thesis, University of Eastern Finland.

Niemi M, Auttila M, Viljanen M & Kunnasranta M (2012) Movement data and their application for assessing the current distribution and conservation needs of the endangered Saimaa ringed seal. *Endangered Species Research*, **19**, 99–108.

Niemi M, Auttila M, Viljanen M & Kunnasranta M (2013a) Home range, survival, and dispersal of endangered Saimaa ringed seal pups: implications for conservation. *Marine Mammal Science*, **29**, 1–13.

Niemi M, Auttila M, Valtonen A, Viljanen M & Kunnasranta M (2013b) Haulout patterns of Saimaa ringed seals and their response to boat traffic during the moulting season. *Endangered Species Research*, **22**, 115–124.

Nordborg M (2010) Coalescent theory. In: *Handbook of Statistical Genetics* (eds Balding D J, Bishop M & Cannings C), pp. 179–212. John Wiley & sons, LTD, Chicester.

Oinonen M, Pesonen P, Alenius T, Heyde V, Holmqvist-Saukkonen E, Kivimäki S, Nygrén T, Sundell T & Onkamo P (2014) Event reconstruction through Bayesian chronology: massive mid-Holocene lake burst triggered a large-scale ecological and cultural change. *Holocene*, in press.

Ortego J, Yannic G, Shafer A B A, Mainguy J, Festa-Bianchet M, Coltman D W & Cote S D (2011) Temporal dynamics of genetic variability in a mountain goat (*Oreamnos americanus*) population. *Molecular Ecology*, **20**, 1601–1611.

O'Corry-Crowe G (2008) Climate change and the molecular ecology of Arctic marine mammals. *Ecological Applications*, **18**, S56–S76.

Palo J U (2003) Genetic diversity and phylogeography of landlocked seals. PhD thesis, University of Helsinki.

Palo J U, Hyvärinen H, Helle E, Mäkinen H S & Väinölä R (2003) Postglacial loss of microsatellite variation in the landlocked Lake Saimaa ringed seal. *Conservation Genetics*, **4**, 117–128.

Palo J U, Mäkinen H S, Helle E, Stenman O & Väinölä R (2001) Microsatellite variation in ringed seals (*Phoca hispida*): genetic structure and history of the Baltic Sea population. *Heredity*, **86**, 609–617.

Palsbøll P J, Bérubé M & Allendorf F W (2007) Identification of management units using population genetic data. *Trends in Ecology & Evolution*, **22**, 11–16.

Palstra F P & Fraser D J (2012) Effective/census population size ratio estimation: a compendium and appraisal. *Ecology and Evolution*, **2**, 2357–2365.

Palstra F P & Ruzzante D E (2008) Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? *Molecular Ecology*, **17**, 3428–3447.

Parsons K M, Durban J W, Claridge D E, Herzing D L, Balcomb K C & Noble L R (2006) Population genetic structure of coastal bottlenose dolphins (*Tursiops truncatus*) in the northern Bahamas. *Marine Mammal Science*, **22**, 276–298.

Pastor T, Garza J C, Allen P, Amos W & Aguilar A (2004) Low genetic variability in the highly endangered Mediterranean monk seal. *Journal of Heredity*, **95**, 291–300.

Pichler F B & Baker C S (2000) Loss of genetic diversity in the endemic Hector's dolphin due to fisheries-related mortality. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 97–102.

Pomeroy P P, Twiss S D & Redman P (2000) Philopatry, site fidelity and local kin associations within grey seal breeding colonies. *Ethology*, **106**, 899–919.

Pritchard J K, Stephens M & Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Randi E, Lucchini V, Christensen M F, Mucci N, Funk S M, Dolf G & Loeschcke V (2000) Mitochondrial DNA variability in Italian and East European wolves: detecting the consequences of small population size and hybridization. *Conservation Biology*, **14**, 464–473.

Rassi P, Hyvärinen E, Juslén A & Mannerkoski I (eds) (2010) *The Red List of Finnish Species 2010*. Ympäristöministeriö ja Suomen ympäristökeskus, Helsinki.

Reed D H (2010) Albatrosses, eagles and newts, Oh My!: exceptions to the prevailing paradigm concerning genetic diversity and population viability? *Animal Conservation*, **13**, 448–457.

Reed D H & Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology*, **17**, 230–237.

Reeves R R (1998) Distribution, abundance and biology of ringed seals (*Phoca hispida*): an overview. In: *Ringed Seals in the North Atlantic* (eds Heide-Jørgensen M P & Lydersen C), pp. 9–45. North Atlantic Marine Mammal Commission (NAMMCO) Scientific Publications, Tromsø.

Runemark A, Hey J, Hansson B & Svensson E I (2012) Vicariance divergence and gene flow among islet populations of an endemic lizard. *Molecular Ecology*, **21**, 117–129.

Saarnisto M (2011) Challenging the early Holocene isolation dating of the Saimaa and Ladoga ringed seals. In: *Times, Things & Places* (eds Harjula J, Helamaa M & Haarala J), pp.

28–39. J.-P. Taavitsainen Festschrift Committee, Turku & Helsinki.

Saarnisto M, Simola H & Kuusisto E (1999) Prehistory. In: *Saimaa, a Living Lake* (ed Kuusisto E), pp. 11–20. Tammi, Helsinki.

Sanvito S, Dueñes Meza A, Schramm Y, Hernández P C, Garrigos Y E & Galimberti F (2012) Isolation and cross-species amplification of novel microsatellite loci in a charismatic marine mammal species, the northern elephant seal (*Mirounga angustirostris*). *Conservation Genetics Resources*, **5**, 93–96.

Schlötterer C (2000) Evolutionary dynamics of microsatellite DNA. *Chromosoma*, **109**, 365–371.

Schultz J K, Baker J D, Toonen R J & Bowen B W (2009) Extremely low genetic diversity in the endangered Hawaiian monk seal (*Monachus schauinslandi*). *The Journal of Heredity*, **100**, 25–33.

Schwartz M K, Luikart G & Waples R S (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution*, **22**, 25–33.

Segelbacher G, Strand T M, Quintela M, Axelsson T, Jansman H A, Koelewijn H P & Höglund J (2014) Analyses of historical and current populations of black grouse in Central Europe reveal strong effects of genetic drift and loss of genetic diversity. *Conservation Genetics*, DOI 10.1007/s10592–014–0610–3.

Sipilä T (2003) Conservation biology of Saimaa ringed seal (*Phoca hispida saimensis*) with reference to other European seal populations. PhD thesis, University of Helsinki.

Sipilä T, Helle E & Hyvärinen H (1990) Distribution, population size and reproductivity of the Saimaa ringed seal (*Phoca hispida saimensis* Nordq.) in Finland, 1980-1984. *Finnish Game Research*, **47**, 3–10.

Sipilä T, Medvedev N V & Hyvärinen H (1996) The Ladoga seal (*Phoca hispida ladogensis* Nordq.). *Hydrobiologia*, **322**, 193–198.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.

Smith T G (1973) Population dynamics of the ringed seal in the Canadian Eastern Arctic. *Bulletin of the Fisheries Research Board of Canada*, **181**, 1–55.

Smith S, Belov K & Hughes J (2009) MHC screening for marsupial conservation: extremely low levels of class II diversity indicate population vulnerability for an endangered Australian marsupial. *Conservation Genetics*, **11**, 269–278.

Spielman D, Brook B W & Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the USA*, **101**, 15261–15264.

Stanley H F, Casey S, Carnahan J M, Goodman S, Harwood J & Wayne R K (1996) Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). *Molecular Biology and Evolution*, **13**, 368–382.

Stewart R E A & Stewart B S (2009) Female reproductive systems. In: *Encyclopedia of Marine Mammals* (eds Perrin W F, Wursig B & Thewissen J G M), pp. 423–428. Academic Press, Canada.

Swanson B J, Kelly B P, Maddox C K & Moran J R (2006) Shed skin as a source of DNA for genotyping seals. *Molecular Ecology*, **6**, 1006–1009.

Taberlet P & Luikart G (1999) Non-invasive genetic sampling and individual identification. *Biological Journal of the Linnean Society*, **68**, 41–55.

Tallmon D A, Luikart G & Waples R S (2004) The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology & Evolution*, **19**, 489–496.

Tikkanen M (2002) Long-term changes in lake and river systems in Finland. *Fennia*, **180**, 31–42.

Trukhanova I S, Gurarie E & Sagitov R A (2013) Distribution of hauled-out Ladoga ringed seals (*Pusa hispida ladogensis*) in spring 2012. *Arctic*, **66**, 417–428.

Ukkonen P (2002) The early history of seals in the northern Baltic. *Annales Zoologici Fennici*, **39**, 187–207.

Valqui J, Hartl G B & Zachos F E (2010) Non-invasive genetic analysis reveals high levels of mtDNA variability in the endangered South-American marine otter (*Lontra felina*). *Conservation Genetics*, **11**, 2067–2072.

Waits L P & Paetkau D (2005) Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. *Journal of Wildlife Management*, **69**, 1419–1433.

Waits L P, Luikart G & Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology*, **10**, 249–256.

Waples R S (2002) Definition and estimation of effective population size in the conservation of endangered species. In: *Population Viability Analysis* (eds Beissinger S R & McCullough D R), pp. 147–168. University of Chicago Press, Chicago.

Waples R S (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conservation Genetics*, **7**, 167–184.

Waples R S & Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources*, **8**, 753–756.

Weber D S, Stewart B S, Garza J C & Lehman N (2000) An empirical genetic assessment of the severity of the northern elephant seal population bottleneck. *Current Biology*, **10**, 1287–1290.

Welch A, Wiley A, James H (2012) Ancient DNA reveals genetic stability despite demographic decline: 3,000 years of population history in the endemic Hawaiian petrel. *Molecular Biology and Evolution*, **29**, 3729–3740.

Willi Y, Van Buskirk J & Hoffmann A A (2006) Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 433–458.

Wilson G A & Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, **163**, 1177–1191.

Wright S (1943) Isolation by distance. *Genetics*, **28**, 114–138.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–354.

MIA VALTONEN

Conservation genetics of the Saimaa ringed seal

– insights into the history of a critically endangered population

A critically endangered subspecies of the ringed seal has remained isolated in Lake Saimaa in Finland since the last glacial period, *i.e.*, for nearly 10,000 years. The small population of ~300 seals is currently threatened by anthropogenic factors, such as high by-catch mortality and climate change. This thesis examines changes in genetic diversity and population structure of the Saimaa ringed seal, and provides new information for conservation.

UNIVERSITY OF
EASTERN FINLAND

PUBLICATIONS OF THE UNIVERSITY OF EASTERN FINLAND
Dissertations in Forestry and Natural Sciences

ISBN 978-952-61-1582-5 (PRINTED)
ISSNL 1798-5668
ISSN 1798-5668
ISBN 978-952-61-1583-2 (PDF)
ISSNL 1798-5668
ISSN 1798-5676