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ABSTRACT

Understanding how small and fragmented populations may be affected by inbreeding is of great importance in conservation biology. Inbreeding could lead to severe fitness consequences both at the individual and population level, and may increase the risk of extinction of small populations. When inbreeding lowers an individual's fitness through for example reduced survival or reproduction, it is called inbreeding depression. In this study, inbreeding and inbreeding depression were investigated in a cyclic water vole (*Arvicola amphibious*) population on 13 different islands off the coast of Northern Norway. Little is known about the relationship between spatio-temporal variations in inbreeding and the strength of inbreeding depression in cyclic, subdivided populations. Here, a 5-year pedigree, constructed by using SNP-genotype data from 1311 individuals, was used to estimate the inbreeding coefficient of each individual with two full ancestral generations. The mean individual inbreeding coefficient was 0.0425, and the level of inbreeding differed between years and between the islands. A significant negative relationship between individual level of inbreeding and reproductive traits was found, but there appeared to be no inbreeding depression in juvenile or adult survival. A possible explanation for the observed inbreeding depression in reproductive traits might be that inbreeding affects mostly internal physiological processes, such as those related to sperm or egg quality. In contrast, survival might be driven by such extreme external environmental factors that the survival could be more or less stochastic and independent of the level of inbreeding. The overall trend is a variation in inbreeding in different years and on different islands, and more years should be included to conclude whether also inbreeding depression varies between years. To estimate the magnitude of inbreeding depression in a cyclic, fragmented metapopulation is important and central to conservation biology, as inbreeding is expected to negatively affect the viability of fragmented populations frequently going through bottlenecks.

Key words

Inbreeding depression, inbreeding coefficient, SNP-pedigree, spatio-temporal variation, fitness, metapopulation, water vole, *Arvicola amphibious*

SAMMENDRAG

Å forstå hvordan små og isolerte populasjoner kan bli påvirket av innavl er svært viktig innen bevaringsbiologi. Innavl kan ha alvorlige konsekvenser for fitness, både på individ- og populasjonsnivå, og kan øke sjansen for utryddelse av små og isolerte populasjoner. Når innavl reduserer et individs fitness gjennom for eksempel redusert overlevelse eller reproduksjon, kalles det innavlsdepresjon. I dette studiet har jeg undersøkt innavl og innavlsdepresjon i en syklisk metapopulasjon av vånd (*Arvicola amphibious*) på 13 små øyer utenfor kysten av Nord-Norge. Det finnes lite kunnskap om forholdet mellom spatio-temporale variasjoner i innavl og styrken på innavlsdepresjon i sykliske, oppdelte populasjoner. Her har jeg brukt et 5-årig slektstre (pedigree), konstruert ved bruk av SNP-genotypedata fra 1311 individer, for å estimere innavlskoeffisienten til hvert individ med fire kjente besteforeldre. Gjennomsnittlig individuell innavlskoeffisient var 0.0425, og nivået av innavl varierte mellom ulike år og øyer. En signifikant negativ sammenheng mellom nivå av innavl og reproduksjon ble funnet, mens det ikke så ut til å være innavlsdepresjon for overlevelse hos juvenile eller voksne individer. En mulig forklaring på den observerte innavlsdepresjonen i reproduksjon, kan være at innavl i større grad påvirker indre fysiologiske prosesser, slik som prosesser relatert til sperm- eller eggkvalitet. På den andre siden kan overlevelse i større grad være påvirket av ekstreme eksterne faktorer, slik at overlevelse mer eller mindre er stokastisk og uavhengig av nivå av innavl. Den generelle trenden er en variasjon i innavl mellom ulike år og på ulike øyer, og flere år bør inkluderes for å konkludere om også innavlsdepresjon varierer mellom år. Å estimere omfanget av innavlsdepresjon i en syklisk, fragmentert metapopulasjon er viktig og sentralt i bevaringsbiologi, ettersom innavl forventes å negativt påvirke levedyktigheten av fragmenterte populasjoner som jevnlig går gjennom biologiske flaskehalser.

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Trondheim, June 2020
Ida Gabrielsen

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1 INTRODUCTION

1.1 Inbreeding

Inbreeding occurs when two individuals with one or more recent common ancestors produce offspring; such offspring will be inbred (Frankham et al., 2010). Inbreeding may have severe fitness consequences as it causes reduction of genetic variability by increasing the frequency of homozygous loci, both on the individual and population level (Keller & Waller, 2002). Inbreeding depression occurs when inbreeding lowers an individual's fitness through reduced reproduction and/or survival (Frankham, 2005). Inbreeding depression can lead to reduced population growth, and may also affect the viability of a population and increase the risk of extinction (Bozzuto et al., 2019; Saccheri et al., 1998). Because inbreeding can have negative effects on fitness, one could expect that inbreeding-avoidance mechanisms should evolve (Bengtsson, 1978). The social system for many mammals, including rodents, is based on male-biased dispersal and female philopatry (Krebs, 2013). This kind of dispersal reduces the risk of inbreeding, since dispersal of one of the sexes will reduce the possibility of mating among kin. However, small and isolated populations are particularly sensitive to the negative effects of inbreeding and have, at the same time, a higher chance of inbreeding due to small numbers of possible mates (Frankham, 1998; Frankham et al., 2010; Saccheri et al., 1998). The negative consequences inbreeding might have on a population, makes inbreeding having great importance in conservation biology (Huisman et al., 2016; Keller & Waller, 2002).

1.1.1 The overdominance and partial dominance hypotheses

Two hypotheses have been suggested to explain how increased homozygosity may lead to reduced fitness: the *overdominance hypothesis* and the *partial dominance hypothesis* (Charlesworth & Charlesworth, 1999). The overdominance hypothesis assumes that there is a heterozygote advantage, so that heterozygous individuals then will have a higher fitness compared to homozygous individuals (Charlesworth & Charlesworth, 1987). Heterozygote advantage could be exemplified by assuming that two alleles, *A* and *a*, have slightly different phenotypic effects, such that the fitness of an individual will be highest when effects of both alleles are expressed. Under inbreeding, the frequency of such beneficial heterozygous allele combinations will decrease, leading to reduced fitness (Charlesworth & Charlesworth, 1987). The partial dominance hypothesis assumes that a fitness reduction is caused by an increased expression of recessive or partially recessive deleterious alleles as the frequency of homozygous

loci increases (Charlesworth & Charlesworth, 1987). The background for the partial dominance hypothesis is that in naturally outbreeding populations, recessive deleterious alleles are at low frequencies due to a balance between their origin by mutation and their removal by natural selection (Frankham et al., 2010). Since a result of inbreeding is increased homozygosity, more deleterious alleles will be exposed to selection (Frankham et al., 2010). Overall, empirical data suggest that inbreeding depression in many cases can be explained best by the partial dominance hypothesis (Charlesworth & Charlesworth, 1999; Charlesworth & Charlesworth, 1987; Charlesworth & Willis, 2009; Fu & Ritland, 1994; Roff, 2002), implying that inbreeding depression in many cases is caused by the expression of recessive deleterious alleles (Charlesworth & Willis, 2009).

1.1.2 Purging

It has been documented that in some cases, inbreeding can reduce the frequency of recessive deleterious alleles by *purging* (Hedrick & Kalinowski, 2000; Keller & Waller, 2002). The concept of purging is explained by the fact that deleterious alleles are more exposed to selection under inbreeding, and may therefore be removed by natural selection more efficiently (Frankham et al., 2010; Keller & Waller, 2002). Purging is predicted to be most efficient for large-effect mutations, while inbreeding depression caused by several mildly deleterious mutations will, because of weaker selection, be harder to remove (Charlesworth & Willis, 2009). Even though there are studies documenting purging (Byers & Waller, 1999; Crnokrak & Barrett, 2002; López-Cortegano et al., 2016), empirical evidence is weak for both captive and wild populations (Hedrick & Garcia-Dorado, 2016). One reason for the weak empirical evidence could be that it is difficult to document inbreeding depression with enough statistical power both before and after purging (Hedrick & Garcia-Dorado, 2016). Especially for captive populations purging could be difficult to detect, because both the negative effects of inbreeding and purging appear to be lower in captive conditions (Hedrick & Garcia-Dorado, 2016). Regardless, theoretical studies show mixed results regarding the particularly importance of purging in natural populations, and suggest its importance will depend on the number and effect size of deleterious alleles, as well as level and type if inbreeding the population (Hedrick & Garcia-Dorado, 2016; Keller & Waller, 2002). Purging does not conform to the overdominance mechanism, and the absence of purging is sometimes used as a support for the overdominance mechanism (Keller & Waller, 2002).

1.1.3 The role of environmental conditions

The magnitude and specific effects of inbreeding are highly variable among species and populations (Hedrick & Kalinowski, 2000). For example, the level of genetic variability and the genetic constitution of a species or population, may be important for how the species or population will be negatively affected by inbreeding (Hendrick & Kalinowski, 2000). Furthermore, the potential negative effects of inbreeding will also depend on how the genes interact with the environment (Frankham et al., 2010; Hedrick & Kalinowski, 2000). For some inbred species and populations, survival and reproduction of inbred individuals are influenced by environmental factors (Bozzuto et al., 2019; Frankham et al., 2010; Marr et al., 2006). Armbruster and Reed (2005) reviewed literature from 34 studies on the relationship between inbreeding depression and environmental stress in eleven plant, nine invertebrate and one vertebrate species. The results from this meta-study indicated that stress or harsh environmental conditions increased the level of inbreeding depression in 76% of the cases (significant in 48% of the studies considered; Armbruster and Reed (2005)). Bozzuto et al. (2019) found that inbreeding reduced population growth rate of populations of Alpine Ibex (*Capra ibex ibex*), but that the growth rate of inbred populations was further decreased when the level of precipitation was high, implying that ecological conditions can modify the extent to which inbreeding affects population growth. Another study also showed that the magnitude of inbreeding depression in populations of seed-feeding beetles (*Callosbruchus maculatus*) was positively correlated with environmental stress (Fox & Reed, 2011). Even though the correlation between environmental stress and inbreeding depression is not consistent among all populations and species, an overall trend is that inbreeding depression tends to increase under harsher environmental conditions (Armbuster & Reed, 2005), but see e.g. Niskanen et al. (2020).

The impact of environmental conditions on inbreeding depression in a population depends on the population dynamics as well as the type of selection on the population (Fox & Reed, 2011; Keller & Waller, 2002; Yun & Agrawal, 2014). For instance, population dynamics can be density dependent and selection can be either soft or hard, and further influence how a population might be affected by inbreeding. Population density can be regarded as an environmental variable, with high population density indicating harsher environmental conditions than low density. Hard selection means that an individual will be removed if it has a trait value smaller or larger than a certain value, e.g. body size in fish during gillnet fishing, where the individuals over a certain size will be selected against, and the smaller individuals could escape. In contrast, soft selection will not remove individuals under a certain trait value,

but instead a number or percentage of the "weakest individuals", e.g. 20% of the smallest or slowest individuals in a population (Wallace, 1975). Soft selection will be stronger in populations with strong density-dependence than in populations with weak density dependence. In cases of soft selection and density-dependence, selection against inbred individuals could be stronger at high densities than at low densities (Keller & Waller, 2002; Yun & Agrawal, 2014). If selection is hard, the effect of environmental conditions on strength of inbreeding depression is expected to be independent of population density, unless a higher proportion of inbred individuals are below the selection threshold at higher population densities (Keller & Waller, 2002). Thus, one may assume that the negative effects of inbreeding will depend on environmental factors, including population densities, and type of selection. The environment and population density may vary temporally and spatially, and can affect the level of inbreeding and inbreeding depression (Keller & Waller, 2002). One could therefore expect a spatio-temporal variation in inbreeding as a consequence of spatio-temporal variation in environmental conditions and population densities.

1.1.4 The inbreeding coefficient, *F*

In any population, the inbreeding coefficient (*F*) can be calculated from a multi-generational pedigree. The concept of this coefficient was developed by Wright (1921), and indicates the probability of both alleles at a locus in an individual being identical by descent. The inbreeding coefficient ranges from 0, outbred, to 1, completely inbred individuals (Frankham et al., 2010). The individual inbreeding coefficient is calculated as

$$F = \sum(0.5)^n(1 + F_A) \quad (1)$$

with *n* being the number of individuals in a path from one parent to the common ancestor and back to the other parent, F_A being the inbreeding coefficient of the common ancestor, and the sum is over the number of paths (Frankham et al., 2010; Wright, 1922). For example, an individual resulting from brother-sister (full-sib) mating, has an inbreeding coefficient of 0.25, while it will be 0.0625 for an individual resulting from first cousin mating. To construct a pedigree, it is necessary to follow the reproduction and survival of a large proportion of individuals, and determine parent-offspring links in a population over several generations (Balloux et al., 2004). Because pedigrees constructed from observed apparent parentages could involve parentage errors, pedigrees constructed from genetic data are favorable for more precise parentage assignments (Reid et al., 2014). Estimates for individual fitness, individual and

population levels of inbreeding, and inbreeding effects on fitness can then be obtained from the pedigree (Pemberton, 2008).

1.2 Population cycles and genetic diversity in rodents

A central part of conservation biology is to understand the population dynamics and ecology in the sense of how a population fluctuates in size. Populations of many small mammals fluctuate more or less regularly (Krebs, 1996), and for voles and lemmings these fluctuations typically have a 3-5-year period (Krebs, 2013). The end of a cycle is typified by a severe crash in population size (Krebs, 2013), and the future population will be conceived from the few individuals that survive this population bottleneck. A rapid decline in population size could lead to a decrease in allelic diversity (Berthier et al., 2006), and an increased level of inbreeding (Frankham, 1998). As such, inbreeding could potentially have an important influence on the future population dynamics through fitness changes at the individual level. In contrast, in density-dependent populations the reduced number of individuals after a population bottleneck could also lead to improved environmental conditions for the remaining population (Keller & Waller, 2002).

Earlier studies on the genetic diversity in fluctuating vole populations have found a high or constant level of genetic diversity and variability. In a study by Berthier et al. (2006), it was found that the overall genetic diversity in a cyclic water vole (*Arvicola amphibius*) population remained high in the metapopulation, despite periods of low abundance. This metapopulation had a patchy structure during periods of low density, and a continuous structure during periods of high density. An explanation for the maintenance of genetic diversity at the metapopulation level also during the low-density phases could be the random distribution of alleles to different patches during the subdivision phase. As the population size increased again, there was more gene flow, contributing to a restoration of the local genetic diversity (Berthier et al., 2006). Aars et al. (2006) found that water vole populations in patchy habitats could maintain a high genetic variability even in comparison to populations of higher density in more continuous habitats. Reasons for these findings are likely to be widespread gene flow and low variance in female reproductive success (Aars et al., 2006). The observed level of gene flow was likely to hinder loss of genetic variability due to genetic drift and inbreeding. Despite the evidence from these studies, knowledge about the variation in level of inbreeding and strength of inbreeding depression is needed in such systems.

1.3 Inbreeding in water voles (*Arvicola amphibius*) in Northern Norway

In this project, I will investigate inbreeding within a metapopulation of water voles in Northern Norway. I will use data from ca. 1300 water vole (*Arvicola amphibius*) individuals collected on 13 different islands at the coast of Helgeland (Figure 1), between spring 2016 and autumn 2019.

First, I will examine whether there is spatio-temporal variation in the level of inbreeding in the water vole metapopulation. The spatial variation refers to the variation in inbreeding between the different islands. The islands are of different sizes and the average population sizes differ among the islands, making the study system suitable for addressing spatial variation in inbreeding. It is likely that the level of inbreeding will differ between the islands, depending on their area and the average number of individuals (Billing et al., 2012; Frankham et al., 2010; Niskanen et al., 2020). The temporal variation is referring to the variation in inbreeding between years. Water vole populations are known to fluctuate in size more or less regularly between years, like many other cyclic populations of small mammals (Hansen et al., 1999). Many vole populations also show a highly fluctuating density within a year, as a large proportion of the individuals die during the winter season and the population size increases during the reproductive season in summer (Frafjord, 2016; Hansen et al., 1999). Fluctuations in density within and between years are also evident in our study system (Sommerli et al., In prep; Thorvaldsen et al., 2019), making it a suitable study system for addressing temporal variation in inbreeding. I expect the level of inbreeding to be highest when the population sizes are small, i.e. on the smallest islands and after any bottlenecks (Billing et al., 2012; Keller, 1998). However, Stenersen (2017) investigated dispersal in the same study area, and found evidence for male-biased dispersal both within and between islands, which could be a mechanism for inbreeding avoidance. Hence, dispersal may also affect the level of inbreeding.

Second, I will investigate whether inbreeding affects individual fitness (survival and reproduction) of the water voles. Several studies have reported significant negative relationships between inbreeding and fitness (Frankham, 2005; Liberg et al., 2005; Niskanen et al., 2020; Reid et al., 2003; Walling et al., 2011; Willoughby et al., 2019), while others have reported weakly negatively or non-significant relationships (Huisman et al., 2016; Keane et al., 1996; Wells et al., 2018). To my knowledge, although some studies have estimated inbreeding levels in water voles (Baker, 2015; Melis et al., 2013), no studies have so far investigated inbreeding depression in this species. The spatio-temporal variation in inbreeding and strength

of inbreeding depression in cyclic populations have previously been investigated in few wild vertebrate populations. Hence, this project will contribute with novel and important knowledge.

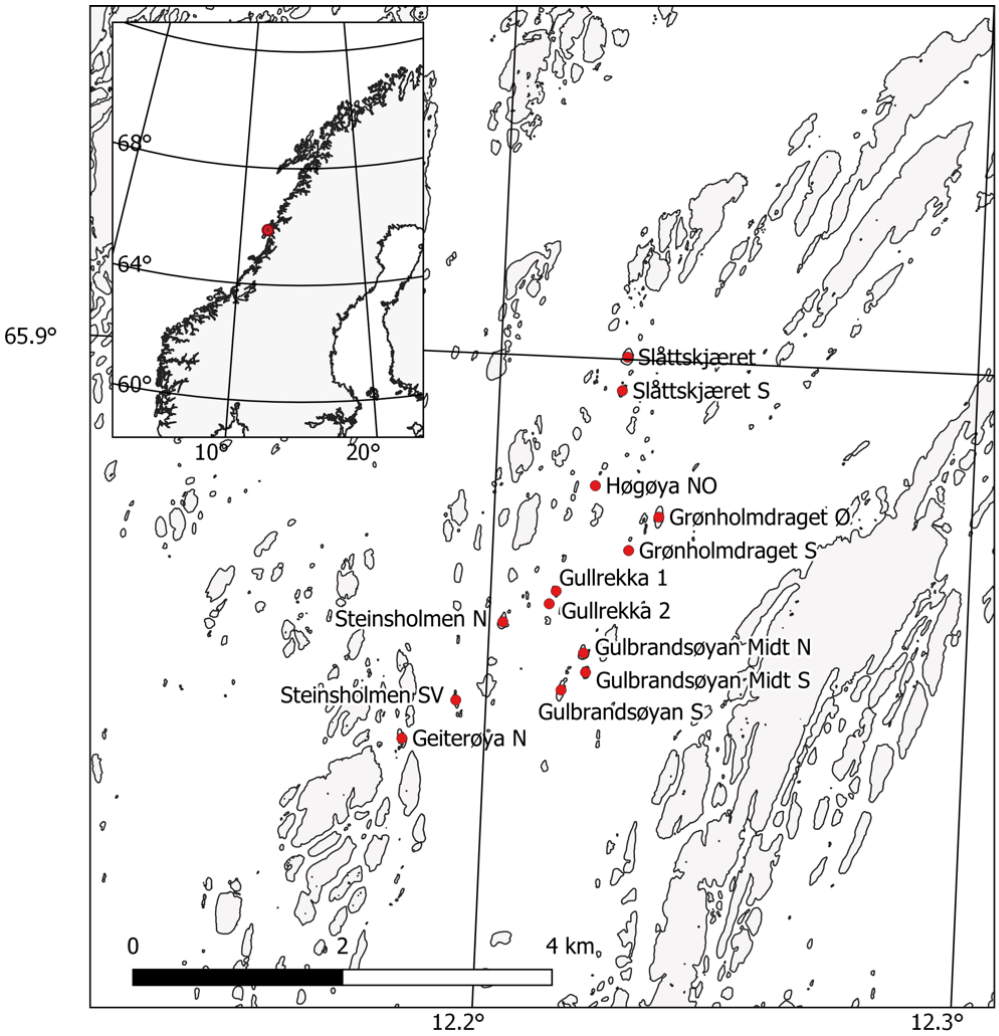

Figure 1: Map showing the Skålvær archipelago in Helgeland, Northern Norway. The study islands in the water vole study system are marked with a red dot and the name of the island.

2 MATERIALS AND METHODS

2.1 Study species

The water vole, *Arvicola amphibius* (formerly *A. terrestris*) is a rodent in the subfamily Arvicolinae, along with other voles, lemmings and muskrats (Strachan et al., 2011). The geographical distribution of the water vole covers most of European countries and a part of North Asia (Strachan et al., 2011). Adult water voles weigh between 140-350 grams, with females normally slightly smaller than males. They typically have a medium to dark brown fur color, a blunt muzzle, round body, a short tail, and short round ears hidden in the fur of the head (Strachan et al., 2011) (Figure 2). The pelage of juveniles is often glossier than that of adults until the first winter (Stoddart, 1971).

Figure 2: Water vole from Gulbrandsøyan Midt N. Photo: Ida Gabrielsen

The water voles are herbivorous, primarily feeding on aerial stems and plant leaves. The diet for the Helgeland water vole population mainly consist of different species of Poaceae (Østby, 2019) . Water voles are burrowing animals, where each vole utilizes a network of burrows with several food storage chambers, nest chambers and entrances (Strachan et al., 2011). Even though water voles are not particularly adapted to water, they are good swimmers. Swimming is used as both a dispersal and escape strategy (Strachan et al., 2011). Stenersen (2017) documented male-biased dispersal for water voles in the study system. The breeding season lasts from April to July, with most litters born in May and June (Frafjord, 2016). The data from Frafjord (2016) is collected close to the current study area (50-60 kilometers to the north), in a similar environment (i.e. on islands with composition of habitats and environmental stochasticity), making it reasonable to expect similarities between these two study (meta)populations. Gestation lasts 20-23 days (Efford, 1985), and the females produce 2-4 litters annually (Frafjord, 2016), with 5-8 pups in each (Strachan et al., 2011). Offspring normally reach sexual maturity after the first winter, but offspring born in early spring could start to breed the first autumn (Strachan et al., 2011). Water voles can survive up to three winters (Strachan et al., 2011), but in our study area they rarely survive more than one winter (Sommerli et al., In prep).

2.2 Study site

My water vole study system is located at the coast of Helgeland, northern Norway, in the Skålvær archipelago (65.885°N, 12.225°E) (Figure 1). Thirteen small islands are included in the study, none of which are inhabited by humans. The size of the islands ranges from 2053 m^2 to 12862 m^2 (mean = 7302 m^2) (Appendix 1). The subpopulations on the 13 islands in the study are part of a larger metapopulation of water voles in the archipelago.

The islands are treeless, and the habitat is mainly made up of marshes, mosses, reed beds and heaths (Appendix 2). Closest to the sea and a few meters up, all the islands have a rocky ground with no vegetation, uninhabitable for water voles. The predators in the area are all avian, including the eagle owl (*Bubo bubo*), common kestrel (*Falco tinnunculus*) and short-eared owl (*Asio flammeus*). In addition, juveniles are exposed to predation by sea gulls (mainly *Larus argentatus* and *Larus marinus*).

2.3 Sampling methods

Each island was sampled during three periods each year for four years (2016-2019); spring (beginning of April to mid-May), summer (mid- to end of July) and autumn (beginning of September to mid-October). Since the first juveniles emerge from the nest in the second half of May, only adult individuals (the individuals born in the previous year, surviving the winter) were sampled in the spring session. In the autumn session, the breeding season is over, making it possible to capture the juveniles produced since spring as well as the other individuals surviving until autumn (Sommerli et al., In prep). The spring and autumn sessions provide valuable information about the fluctuations in population size between seasons within years, and are therefore important in considering the temporal variation in inbreeding. Due to logistics and economic constraints, only four of the islands (Geiterøya N, Gulbrandsøyan Midt N, Gulbrandsøyan Midt S and Gulbrandsøyan S) were sampled during summer season.

Water voles were captured using Sherman XLF15 folding traps baited with dry grass and pieces of carrot and potato. The number of traps used on the different islands depended on the size of the island, varying between 80 and 170 traps per island. Traps were placed where latrines, tunnel openings and other signs of vole presence were observed. Using moss, soil, grass and other biota to cover the traps, they were stabilized in case of wind and protected from

overheating from the sun. Some small chunks of carrot were scattered inside, in the entrance and outside the trap (Appendix 3). Traps were checked every 1.5-3 hours.

For captured individuals, body mass was measured and sex was determined along with age and stage of reproduction. The sex and the stage of reproduction were determined by investigating the reproductive organs of sexually mature individuals. In males, testes can be felt under the scrotum and the penis tip is protruding, while in females the nipples of the mammary glands are easily observable (Stoddart, 1971). The sex of sexually immature individuals is harder to determine, and therefore there are five classes of sex: male (m), female (f), probably male (pm), probably female (pf) and unknown (u). A similar classification system was used for stage of reproduction: active, probably active, probably not active and unknown. The age was determined based on body mass and fur color. Two basic categories were used for age: individuals born during the current year (1) and individuals born earlier (2+), or unknown.

Furthermore, we took a tissue biopsy (ear puncture, 2 mm. diameter), to be used for genetic analysis (see below). A unique PIT-tag (TROVAN unique ID-100B; 11.5 x 2.12 mm) was inserted under the neck skin using an IM-200 syringe implanter. After handling, the voles were released at the same site as they were captured. The traps were cleaned, filled with new dry grass, carrots and potatoes, and replaced at the same position as earlier. If the voles were recaptured, they were scanned using a Dorset LID575 tag reader, and released at once. To ensure exhaustive trapping, the trapping was continued until the recapture rate within a period was above 70%. The high recapture rate makes the total number of individuals captured in a session a good proxy for population size (Sommerli et al., In prep).

2.4 DNA-extraction and SNP-genotyping

The DNA-extraction of the biopsy samples was performed using a DNeasy Blood & Tissue Kit (QIAGEN), mostly using the standard protocol (see Appendix 4 for details). The DNA was normalized to 32 ng/µl, and further transferred to 96-well plates. To determine the pedigree from the extracted DNA, all individuals were genotyped by using custom water vole SNP-genotyping arrays (OpenArray format) with 128 SNPs (Sommerli et al., In prep). The SNP genotyping was performed by using QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems) with OpenArray Block using standard protocols for OpenArray SNP-genotyping (Broccanello et al., 2020). In total 1578 individuals were genotyped on the 128 SNPs using this

protocol. Only individuals that scored for more than 60% of the SNPs, and only SNPs that were scored in more than 60% of the individuals were included, leaving us with a dataset consisting of 1311 individuals and 107 SNPs. Further, 9 SNPs were removed based on parent-offspring opposite homozygote errors. The final data set used to construct the pedigree included 1311 individuals that were genotyped at >60% of 98 SNPs.

2.5 Pedigree and the inbreeding coefficient *F*

Based on the individual genotypes at each SNP locus, parentage was determined and the pedigree constructed by using the package *sequoia* (Huisman, 2017) in R (R Core Team, 2019) (see Appendix 5 for pedigree structure). Next, individual inbreeding coefficients *F*, were calculated from the pedigree using the R package *pedigree* (Coster, 2013). For further analyses, only the individuals with at least two full ancestral generations were included. The exclusion of individuals missing one or more grandparents was done to get a more precise estimate of the inbreeding coefficient, *F*, and because uncomplete pedigrees could underestimate the observed amount of inbreeding (Marshall et al., 2002; Pemberton et al., 2017). The exclusion of individuals with less than two full ancestral generations reduced the sample size to 388 individuals (Appendix 1, Appendix 6).

2.6 Analyses of spatio-temporal variation in inbreeding

To investigate the spatio-temporal variation in *F* (i.e. variation between islands and between years), linear models were fitted using the `lm` function in R. For the spatio-temporal analyzes, *F* was used as a response and island and year as predictor variables. Note that in these analyses, years represent birth years, meaning that no individuals were included in several years for the temporal variation. Due to very low sample size for some years on specific islands (Appendix 6), the interaction effect between island and year could not be included in the models. Likelihood ratio tests (LRT) were used to determine whether differences between islands or years explained any significant amount of the observed variation in inbreeding, and spatio-temporal patterns were visualized in boxplots created with the *ggplot2* package in R (Wickham, 2016).

2.7 Inbreeding depression analyses

The inbreeding depression analyses were partitioned into the effect of inbreeding on survival and the effect of inbreeding on reproduction. Since testing for the effects of *F* on different

fitness components was the main objective with this part, *F* was kept as an explanatory variable in all models. For each model that will be described, an LRT was used to exclude non-significant ($P > 0.1$) interactions and main effects. In the analyses where sex was included as a fixed factor, individuals with "pf" (probably female) and "pm" (probably male) were assumed to be females and males, respectively. The inclusion of sex reduced the sample size to 259 individuals, due to individuals with unknown sex. The recapture rate in this study is high (> 70%), so I assume that there will be little bias in the inbreeding depression analyses due to sampling effects. The number of captured individuals should be a good proxy of the total number of surviving individuals from one season to the next. The high recapture rate should also suggests that almost all offspring produced by individuals on the study islands were sampled. Note however that because all individuals were not successfully genotyped (less than 85% of the captured individuals; Appendix 1), the total number of offspring might be underestimated. However, any such bias should not be related to individual level of inbreeding.

2.7.1 Survival analyses

For the analyses of the effect of inbreeding on survival, generalized linear mixed models (GLMM) were applied using the R package *lme4* (Bates et al., 2014). In the models, a logit link function and a binomial error structure were used. Since the water vole individuals in the Helgeland metapopulation do not survive more than one winter, between-season analyses of survival were carried out: juvenile survival from autumn to spring and adult survival from spring to autumn. For each survival period, individuals surviving from one season to the next had a value of 1, while the non-surviving (i.e. non-recaptured) individuals had a value of 0. To examine the effect of inbreeding on survival, year was included as a fixed factor, and island was included as a random factor to account for any differences in islands in mean and variance of survival. To investigate if the influence of *F* on survival differed among years, I also included the interaction between *F* and year. This term was significant for the juvenile autumn-spring survival period survival, but because of very limited sample size in 2018 (the year with significant interaction with *F*: only 3 surviving and 4 non-surviving individuals) and because this interaction term was not significant ($p > 0.4$) for the spring-autumn period, results from models without the interaction term are presented. To test whether the effect of inbreeding on the probability of survival differed between the sexes, I included sex as a fixed factor, and tested for an interaction between *F* and sex. This interaction term was not significant for any of the survival periods ($p > 0.1$). Since the inclusion of sex reduced the number of individuals by more

than 100 individuals (due to unknown sex), and the main effect of sex was non-significant (LRT: $p > 0.1$), the sex as a fixed factor was excluded from the survival analyses.

Although a few individuals may reproduce in their first calendar year, most individuals reproduce in their second calendar year. Thus, survival from autumn to spring could be considered recruitment rate, i.e. the contribution to the reproducing segment of the population. Individuals born in 2019 were excluded from the survival analyses from autumn to spring because of missing survival data (i.e. no data from 2020). The survival from spring to autumn could be important for the number of litters an individual could produce, i.e. their total reproductive success. No individuals with two complete known ancestral generations were adults in 2016 (i.e. no individuals born in 2015 had two full ancestral generations), and therefore 2016 was not included in the spring-autumn analysis.

2.7.2 Reproduction analyses

In the analyses of the effect of inbreeding on reproduction, the total number of offspring surviving long enough to be captured, hereafter "number of juvenile offspring", were counted for each individual. Because there were no genotype data from autumn 2019, and the voles primarily reproduce in their second year, individuals born in 2018 and 2019 were excluded from these analyses. All the juveniles born in 2016 and 2017 were included in the reproduction analyses, also those not recaptured in their second year, because water voles may reproduce in the year of birth (Strachan et al., 2011).

In the first model, the total number of juvenile offspring was used as response variable. Because the number of juvenile offspring was not normally distributed and included a large proportion of zeros, a generalized linear mixed model with a negative binomial error structure was used. Year was included as a fixed factor, and island was included as a random factor to account for any differences in islands in mean and variance of reproduction. To test whether the effect of inbreeding on the total number of juvenile offspring differed between the sexes, I included sex as a fixed factor, and tested for an interaction between F and sex. This interaction term was significant ($p = 0.0406$), and was therefore included in the model.

In addition to the effect of inbreeding on the total number of offspring, the probability of having any juvenile offspring at all was also investigated. A GLMM with a logit link function and a binomial error structure was applied to test this. Individuals that produced at least one offspring

had a value of 1 and individuals that did not produce any offspring had a value of 0. Year was included as a fixed factor and island was included as a random factor to account for any differences in islands in mean and variance of probability of reproduction. Also here I tested whether the effect of inbreeding on the probability of having juvenile offspring differed between the sexes, and included sex as a fixed factor, and tested for an interaction between *F* and sex. This interaction term was not significant ($p > 0.1$), and was therefore not included in the model. There was however a tendency that the probability of having offspring differed between sexes ($p = 0.0716$), and sex was therefore included in the model as a main effect. For both the reproduction analyses, I also tested for an interaction between the predictor variables, *F* and year, but because this interaction term was not significant ($p > 0.1$), results from models where the interaction term was excluded are presented.

Model diagnostics were performed using the R package *DHARMa* (Hartig, 2017). Plots showing the relationship between inbreeding and fitness traits were made using the R packages *ggeffects* (Lüdecke, 2018) and *ggplot2* (Wickham, 2016).

3 RESULTS

3.1 Spatio-temporal variation in inbreeding

The population size on the different islands fluctuated considerably among years and between seasons within years during the study period, and some islands contained in general more water vole individuals than others (Figure 3, Appendix 1). The overall trend was a decline in total metapopulation size from 2016 to 2018, and an increase in population size in 2019 (Appendix 7).

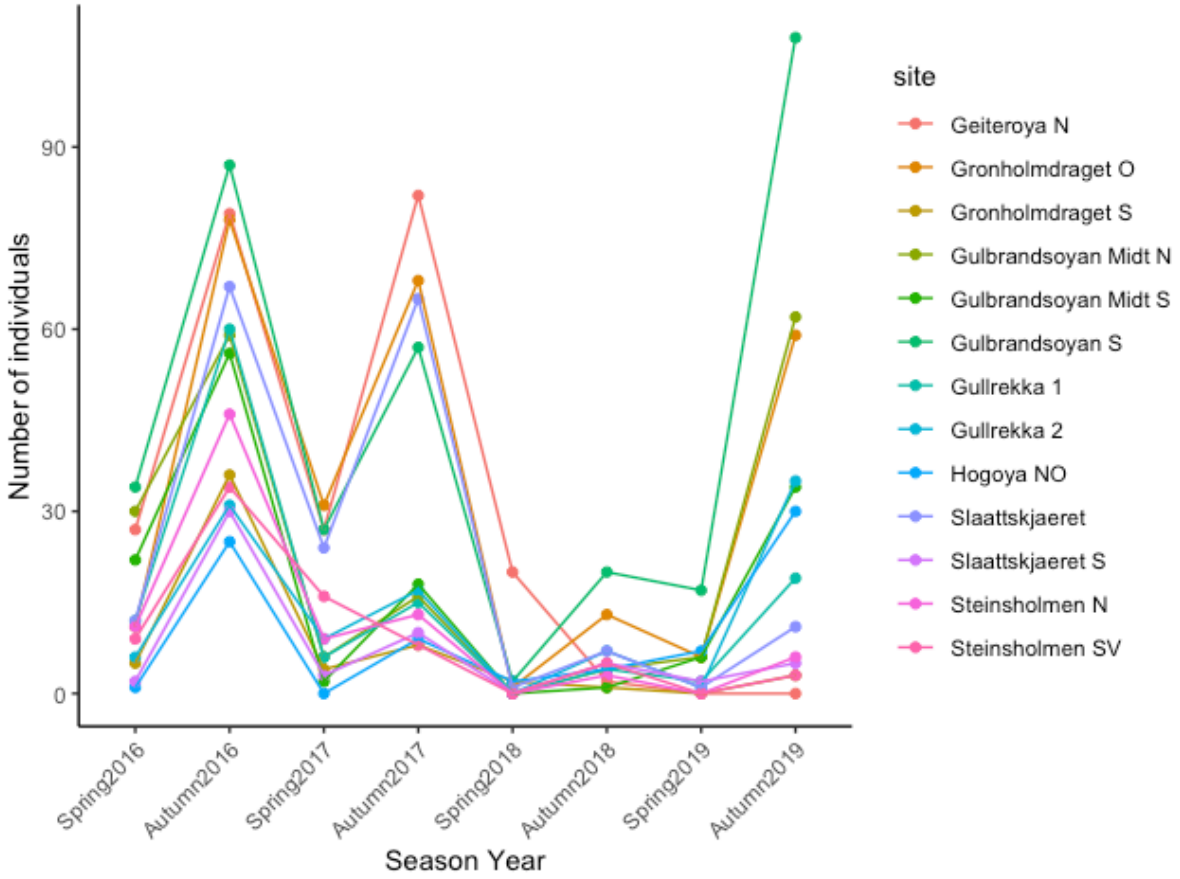

Figure 3: The total population size of water voles on each island in spring and autumn during the years 2016 to 2019. The colored dots and lines represent different islands in the study system.

The total number of individuals with two full ancestral generations was 388, and the number of such individuals varied among islands, and among years within islands (Appendix 6). The total number of individuals in the different years varied from n = 11 (2018) to n = 196 (2017), and the total number of individuals on the different islands varied from n = 1 (Steinsholmen SV) to n = 107 (Geiterøya N) (Appendix 6). Most individuals were from 2016 and 2017, where population sizes were largest, whereas sample sizes were lower in 2018 due to the population

crash. In 2019, only genotype data from the summer was available, leading to a lower number of individuals with two full ancestral generations compared to other years. For the whole metapopulation, around 35% of the individuals with two full ancestral generations had an inbreeding coefficient larger than zero (135/388 individuals), and 18% had an inbreeding coefficient larger than 0.1 (69/388 individuals) (Figure 4, Appendix 8). The individual level of inbreeding ranged from $F = 0$ to $F = 0.3125$, and the mean level of inbreeding for the whole metapopulation was $F = 0.0425$ (Standard deviation: 0.0824; median: $F = 0$).

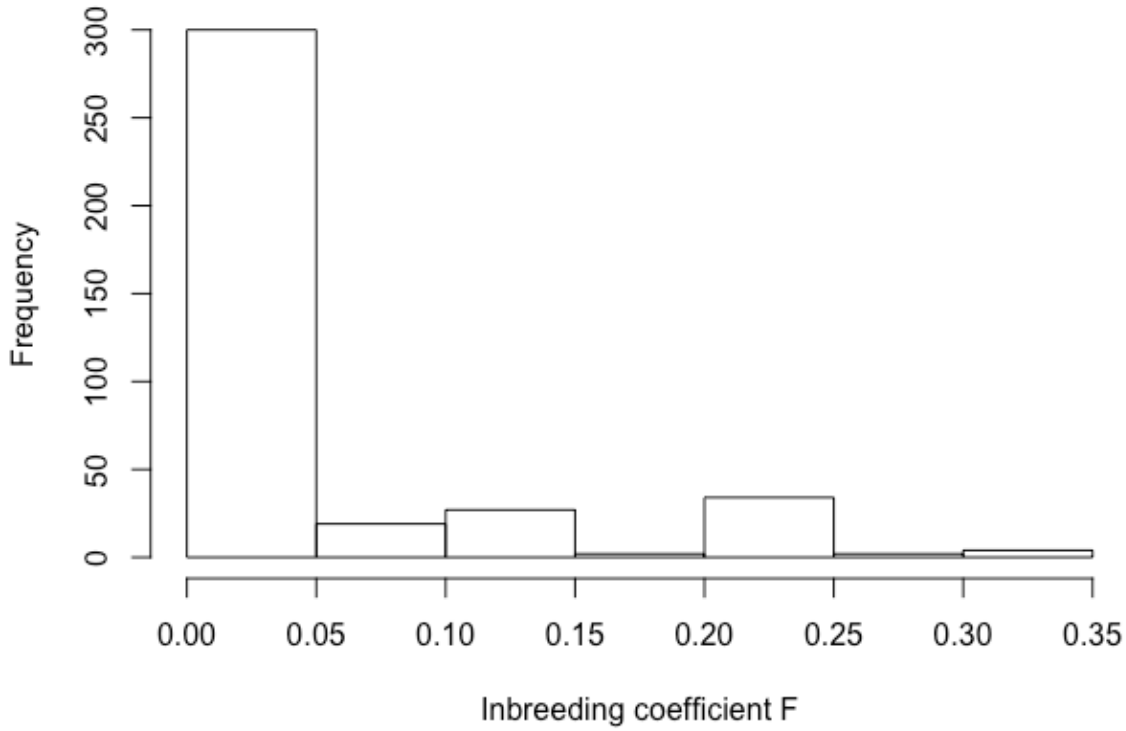

Figure 4: The frequency of different inbreeding coefficients among the water vole individuals with at least two full ancestral generations. See Appendix 8 for more detailed information about the frequency of inbreeding coefficients.

The mean inbreeding coefficient among individuals with two full ancestral generations varied among years in the study period, from 0.0595 in 2016 to 0.0162 in 2018 (Table 1, Figure 5). A likelihood ratio test (LRT) showed that year explained a significant proportion of the variance in inbreeding ($p = 0.0035$), i.e. the differences between years in the level of inbreeding were significant. Inbreeding differed significantly from zero in 2016, 2017 and 2019 (Table 1).

Table 1: Variation among years in the level of inbreeding *F* in a water vole metapopulation. The mean inbreeding coefficient with standard deviation and median inbreeding coefficient for all years are given. Bold means indicate level of inbreeding significantly different from zero ($p < 0.05$). A linear model was fitted with year as fixed factor. Parameter estimates with standard error (SE) and p-values are presented. As indicated by the p-value (*** $p < 0.001$), the intercept (2016) is significantly different from zero, and the mean in 2017 is significantly different from 2016. Note that sample sizes differed between years (Appendix 6).

	Mean *F* ± SD	**Median *F***	**Estimate ± SE**	**p**
Intercept (2016)	**0.0595** ± 0.0990	0.0000	0.0595 ± 0.0063	< 0.001 ***
2017	**0.0293** ± 0.0625	0.0000	-0.0302 ± 0.0086	0.0005***
2018	0.0162 ± 0.0372	0.0039	- 0.0433 ± 0.0253	0.0877
2019	**0.0472** ± 0.1006	0.0000	-0.0123 ± 0.0213	0.5648

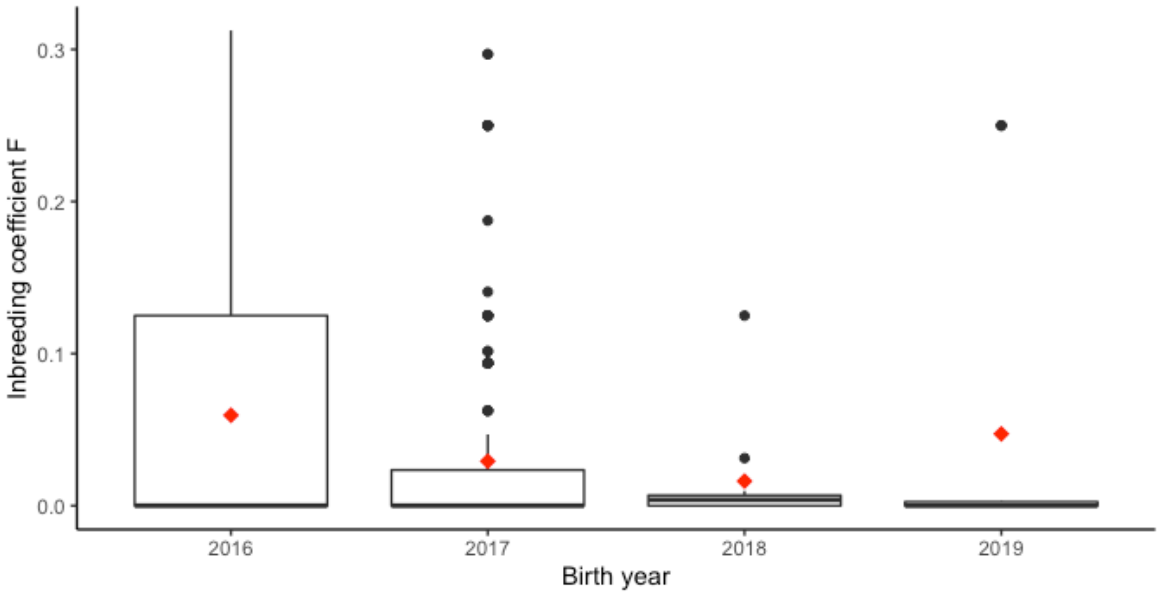

Figure 5: The level of inbreeding in water voles, indicated with the inbreeding coefficient *F*, in the different years: 2016 (n = 165), 2017 (n = 197), 2018 (n = 11) and 2019 (n = 16). From 2019 there was only genotype data from summer, meaning that no individuals from autumn 2019 are included. The red dots indicate the mean inbreeding coefficient in the given year. The black horizontal line (here: zero for all years except 2018) shows the median, and the box illustrates the interquartile range with the whiskers showing the highest and lowest values. The black points are outliers.

The mean inbreeding coefficient differed between the study islands, ranging from 0.0021 (Gullrekka 2) to 0.25 (Steinsholmen SV. Note that this island had only one individual with two full ancestral generations) (Table 2, Figure 6). An LRT showed that island explained a significant proportion of the variance in inbreeding ($p = 0.0004$), i.e. there were significant differences between islands in their level of inbreeding (Table 2). Note that when the islands with fewer than ten individuals were excluded, island differences did not explain any statistically proportion of the variance in inbreeding (Appendix 9).

Table 2: Variation among islands in the level of inbreeding *F* in a water vole metapopulation. The mean, standard deviation and median inbreeding coefficient for each island is shown. Bold means indicate level of inbreeding significantly different from zero ($p < 0.05$). A linear model was fitted with island as fixed factor. Parameter estimates with standard error (SE) and p-values are presented. As indicated by the p-value (p) (*$p < 0.5$, ** $p < 0.01$, *** $p < 0.001$), the intercept (Geiterøya) is significantly different from zero, and the mean on Grøndholmdraget Ø, Høgøya NO and Steinsholmen SV is significantly different from the intercept. Note that the sample sizes differed between islands (Appendix 6)

Island	Mean *F* ± SD	Median *F*	Estimate ± SE	p
Intercept (Geiterøya)	**0.0480** ± 0.0864	0.0000	0.0480 ± 0.0077	< 0.001 ***
Grønholmdraget Ø	0.0165 ±0.0326	0.0000	-0.0315 ± 0.0142	0.0272*
Grønholmdraget S	0.0024 ±0.0069	0.0000	-0.0455 ± 0.0293	0.1211
Gulbrandsøyan Midt N	**0.0521** ± 0.0984	0.0000	0.0041 ± 0.0134	0.7592
Gulbrandsøyan Midt S	0.0133 ± 0.0373	0.0000	-0.0347 ± 0.0253	0.1712
Gulbrandsøyan S	**0.0563** ± 0.0968	0.0000	0.0083 ± 0.0120	0.4899
Gullrekka 1	0.0151 ± 0.0367	0.0000	-0.0329 ± 0.0227	0.1484
Gullrekka 2	0.0021 ± 0.0071	0.0000	-0.0459 ± 0.0253	0.0710
Høgøya NO	**0.1377** ± 0.1014	0.1250	0.0897 ± 0.0293	0.0025**
Slåttskjæret	**0.0311** ± 0.0678	0.0000	-0.0169 ± 0.0147	0.2499
Slåttskjæret S	0.0289 ± 0.0382	0.0156	-0.0191 ± 0.0365	0.6024
Steinsholmen N	0.0491 ± 0.0917	0.0000	0.0011 ± 0.0312	0.9714
Steinsholmen SV	**0.2500** ± NA	0.2500	0.2020 ± 0.0804	0.0124*

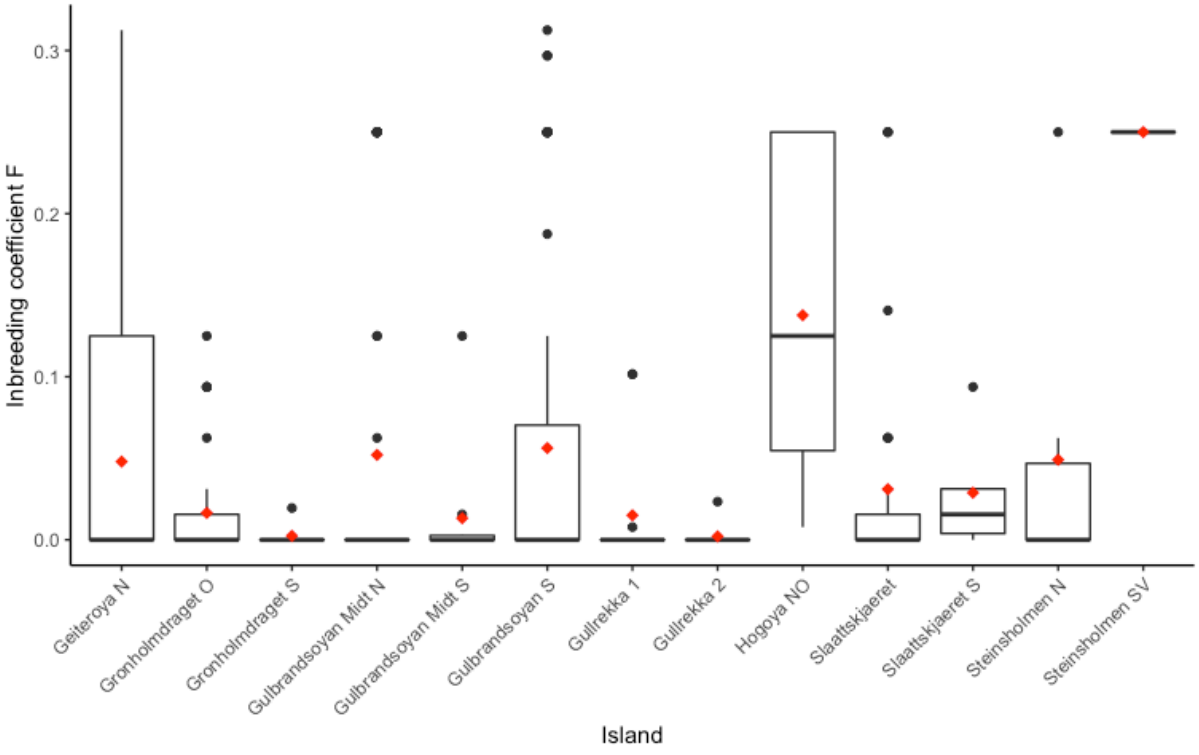

Figure 6: The level of inbreeding in water voles, indicated with the inbreeding coefficient *F*, on the different islands: Geiterøya N (n = 107), Grønholmdraget O (n = 45), Grønholmdraget S (n = 8), Gulbrandsøyan Midt N (n = 54), Gulbrandsøyan Midt S (n = 11), Gulbrandsøyan S (n = 76), Gullrekka 1 (n = 14), Gullrekka 2 (n = 11), Høgøya NO (n = 8), Slåttskjæret (n = 42), Slåttskjæret S (n = 5), Steinsholmen N (n = 7), Steinsholmen SV (n=1). The red dots indicate the mean inbreeding coefficient on the given island. The black horizontal line shows the median, and the box illustrates the interquartile range with the whiskers showing the highest and lowest values. The black points are outliers.

3.2 Inbreeding depression

3.2.1 Inbreeding effects on survival

The total number of juvenile individuals with two full ancestral generations that survived from autumn to spring was n = 50, while the number of non-surviving juveniles was n = 252. From spring to autumn the number of surviving adult individuals was n = 9 and number of non-surviving adults was n = 41 (see Appendix 10 for more details). There were no significant effects of inbreeding on survival in any of the different survival periods. Neither the juvenile survival from autumn to spring (β = -0.2481 ± 0.3619, p = 0.8944; Table 3, Figure 7), nor the adult survival from spring to autumn (β = 3.7658 ± 3.6578, p = 0.3160; Table 3, Figure 7) was significantly related to inbreeding.

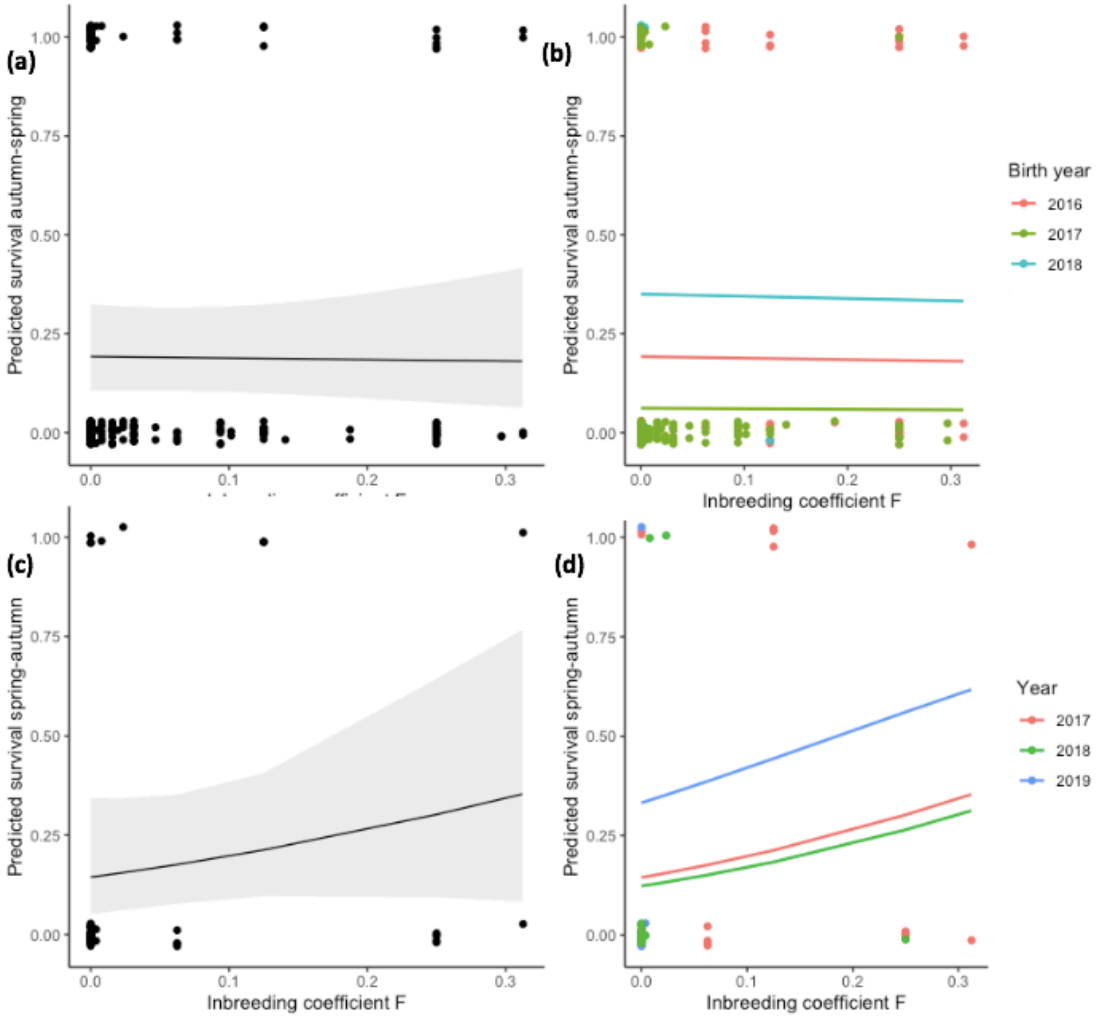

Figure 7: Relationship between survival and inbreeding coefficient *F*. Two generalized linear mixed models were fitted, where the response variable was survival (0 or 1) from autumn to spring for juveniles (panel a, b), or survival from spring to autumn for adults (panel c, d). In both models, the fixed predictor variables were inbreeding coefficient *F* and a main effect of year. Site was included as a random intercept. Both models had a binomial error structure and logit link function. The lines are predicted values from the model, and shows the partial effects of inbreeding coefficient *F* (a, c; year is held constant), and inbreeding coefficient *F* and year (b, d) on survival. The shaded area (a, c) shows the 95% confidence interval. The points are observed values. Some random noise was added to avoid overplotting (scale of jittering: height = 0.03, width = 0.00).

3.2.2 Inbreeding effect on reproduction

The total number of individuals with two full ancestral generations and known sex that produced offspring was 69, while the number of non-reproducing individuals was 190 (Appendix 11). There was a statistically significant negative effect of inbreeding on reproduction, measured by total number of juvenile offspring ($\beta = -5.4869 \pm 2.0844$, $p = 0.0036$; Table 3, Figure 8). The analysis also showed a significant difference between male and female in the effect of *F* (interaction term LRT: $p = 0.0406$), suggesting that males contributed most to the negative relationship between inbreeding and total juvenile reproduction ($\beta_{male} = -16.1185 \pm 7.3865$, $\beta_{female} = -3.2816 \pm 7.7352$, Figure 9). Furthermore, there was a statistically significant negative effect of inbreeding on the probability of producing offspring ($\beta = -5.4136 \pm 2.2492$, $p = 0.0099$; Table 3, Figure 8). There was no significant interaction between inbreeding *F* and year for neither the effect of inbreeding on total number of juvenile offspring nor the probability of producing offspring (interaction term LRT: $p > 0.7$). For both reproduction measures, there was still a significant negative effect of inbreeding when sex was excluded from the model (Appendix 12).

Table 3: The effect of inbreeding (*F*) on different fitness components in a water vole population in Northern Norway. The different fitness components are: juvenile survival from autumn to spring, adult survival from spring to autumn, total number of juvenile offspring produced, and probability of producing (juvenile) offspring. The parameter estimate for the effect of *F* and its standard error (SE) are given, and the significance of the effect was tested using a likelihood ratio test (chi-square (χ^2) and p-value (p) are given. * $p < 0.05$, ** $p < 0.01$).

Fitness component	**Parameter estimate**	**SE**	**χ^2**	**p**
Juvenile survival autumn-spring [o]	-0.2481	1.8674	0.0176	0.8944
Adults survival spring-autumn [o]	3.7658	3.6578	1.0053	0.3160
Number offspring [+]	-5.4869	2.0844	11.2310	0.0036**
Probability offspring [o]	-5.4136	2.2492	6.6555	0.0099**

[o] Generalized linear mixed model (GLMM) with a binomial error structure
[+] Negative binomial generalized linear mixed model

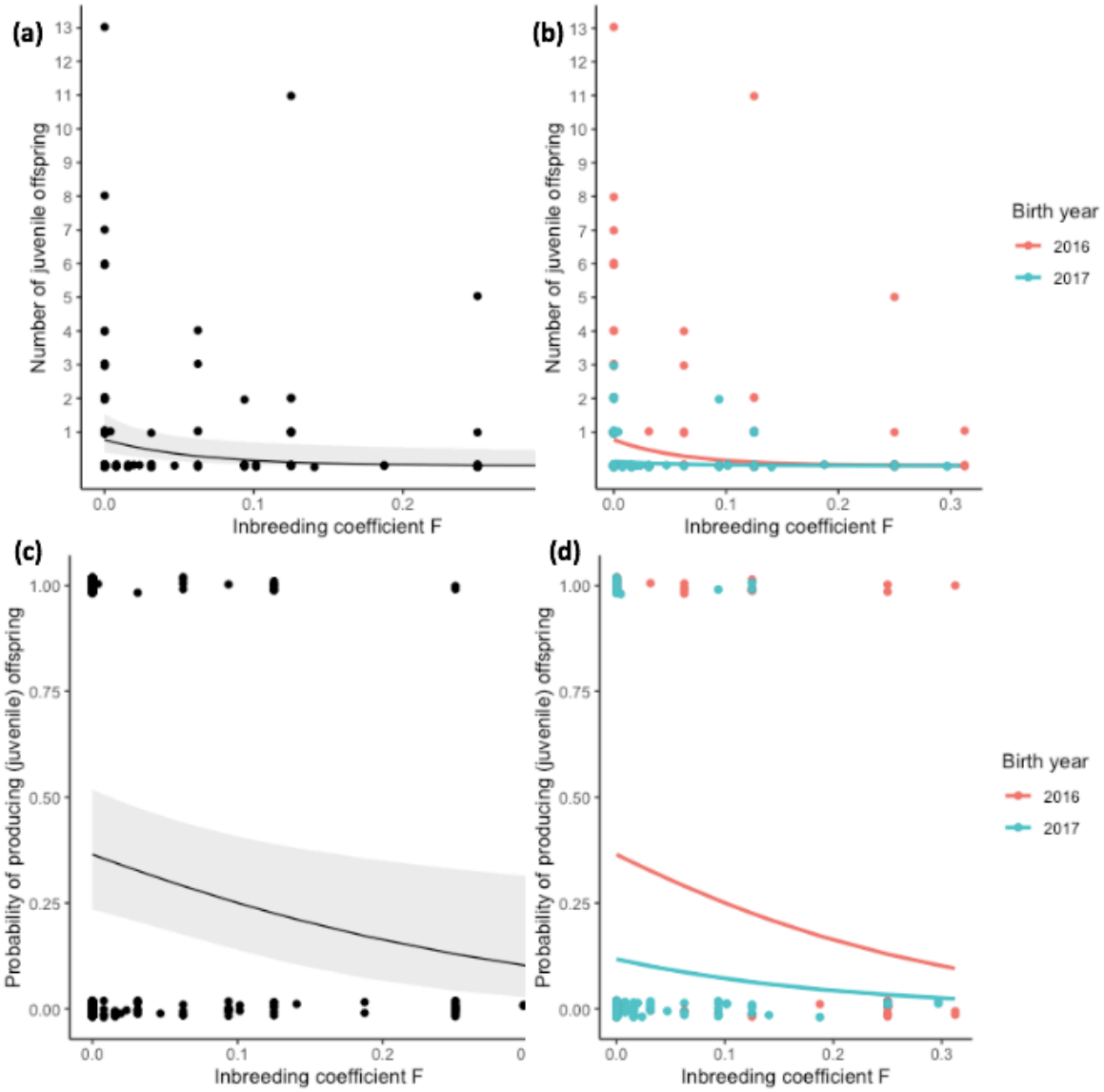

Figure 8: Relationship between reproduction and the inbreeding coefficient *F*. Two generalized linear mixed models were fitted, where the response variable was total number of juvenile offspring produced (negative binomial error structure; panel a, b), or the probability of producing juvenile offspring (binomial error structure and logit link function; panel c, d). In both models, the fixed predictor variables were inbreeding coefficient *F*, and main effects of sex and year. In the model of total number of offspring, the interaction between *F* and sex was also included. Site was included as a random intercept. The lines are predicted values from the models, and show the partial effects of inbreeding coefficient *F* (a, c), and inbreeding coefficient *F* and year (b, d) on reproduction, when other predictors are held constant. The shaded area shows the 95% confidence interval (a, c). The points are observed values. A small random noise was added to the points to avoid overplotting (scale of jittering: height = 0.03, width = 0.00).

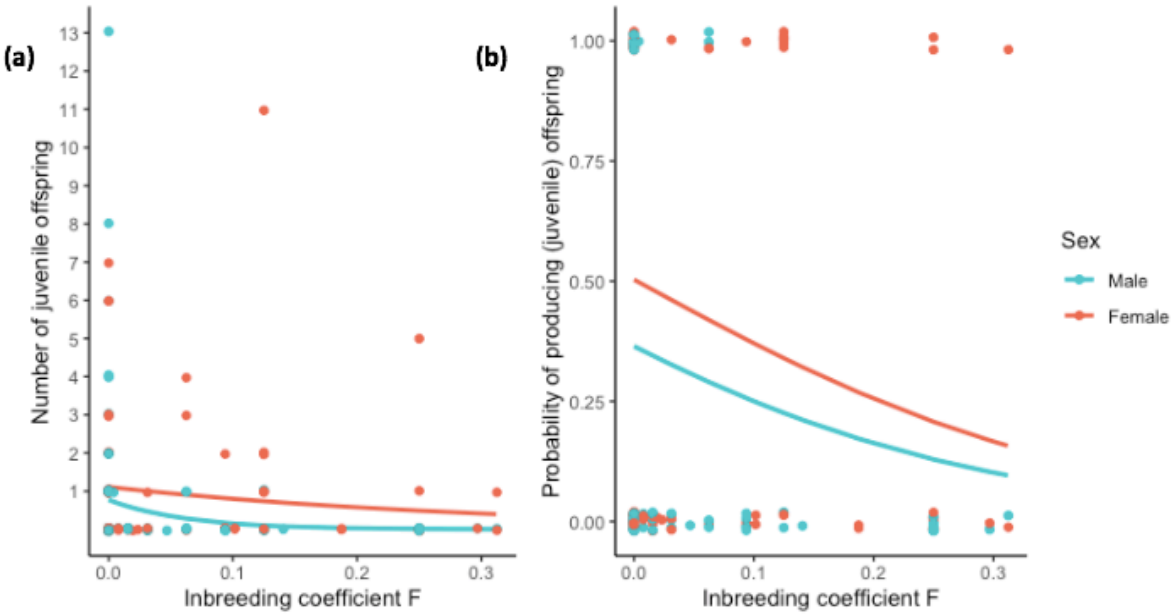

Figure 9: Relationship between reproduction and the inbreeding coefficient *F* for the two sexes. Two generalized linear mixed models were fitted, where the response variable was total number of juvenile offspring produced (negative binomial error structure; panel a), or the probability of producing juvenile offspring (binomial error structure and logit link function; panel b). In both models, the fixed predictor variables were inbreeding coefficient *F*, and main effects of sex and year. In the model of total number of offspring, the interaction between *F* and sex was included. Site was included as a random intercept. The lines are predicted values from the models, and show the partial effects of inbreeding coefficient *F* and sex when other predictors are held constant. The points are observed values. Some random noise was added to the points to avoid overplotting (scale of jittering: height = 0.03, width = 0.00). See Appendix 11 for distribution of offspring among inbred and non-inbred males and females.

4 DISCUSSION

This study shows that the level of inbreeding differed among years and among islands (Table 1, Table 2, Figure 5, Figure 6) in a metapopulation of water voles on 13 islands off the coast of Northern Norway. There appeared to be no negative effects of inbreeding on the probability of survival, neither for juvenile survival from autumn to spring, nor adult survival from spring to autumn (Table 3, Figure 7). Furthermore, there was evidence for inbreeding depression in reproductive traits, as there was found a significantly negative relationship between inbreeding and the total number of juvenile offspring produced, and inbreeding and the probability of producing juvenile offspring (Table 3, Figure 8). There was also evidence for males being more negatively affected by inbreeding than females in the total number of produced offspring (Figure 9).

4.1 Spatio-temporal variation in inbreeding

In small and isolated populations, inbreeding is unavoidable because of a decreased number of potential mates, and thus an increase in matings between close relatives (Frankham, 1998; Frankham et al., 2010; Saccheri et al., 1998). This seems to be the case also in the Helgeland water vole population: around 17% (67/388) of the individuals with at least two full ancestral generations were found to be offspring from matings between first cousins or closer relatives ($F \geq 0.125$). Also, because pedigrees which goes back only two generations, as in the current study, might miss relationships from several previous generations, it is likely that the true proportion of inbred individuals is higher (Marshall et al., 2002; Pemberton et al., 2017). It seems reasonable to assume that there is inbreeding in the metapopulation of water voles on Helgeland.

It was somewhat surprising to find such a large number of individuals with two full ancestral generations in 2016 (Appendix 6), but this is probably due to the assignment of "dummy parents" using *Seqioua* in the pedigree construction. Dummy parents are assigned when clusters of full-siblings and/or half-siblings have unsampled parents (Huisman, 2017). Of the 165 individuals born in 2016, 28 of them were assigned one or two dummy parents, and 149 of the individuals had one or more dummy grandparents (Appendix 5). Note however that no individuals included in the analyses in this study were assigned both dummy parents and dummy grandparents, i.e. no individuals had two ancestral "dummy generations". In this study, 98 SNPs were used to construct the pedigree. When using down to about 100 independent

SNPs, Huisman (2017) found parentage assignments to be highly accurate. The number of SNPs used in this is study is approximately the minimum number of what Huisman (2017) suggested, making it reasonable to assume that the parentage assignments are reliable. In contrast, Huisman (2017) recommended at least 200 independent SNPs to achieve subsequent sibling clustering with high accuracy, so when parental genotypes are missing in my data, this could have led to some relatedness errors in the pedigree.

An initial objective of this project was to investigate the spatio-temporal variation in inbreeding. On the question of temporal variation, this study found a significant variation in inbreeding between years in the study period (Table 1, Figure 5). The level of inbreeding, estimated as mean *F*, was highest when the population size was highest, in 2016. When a population is founded by a small number of individuals or a population has gone through a bottleneck, and there is low immigration, it is likely to see an increased level of inbreeding (Frankham et al., 2010; Keller & Waller, 2002). For example, a subpopulation of house sparrow (*Passer domesticus*) on the island Aldra on Helgeland, which was founded by four individuals, showed an increased level of inbreeding after the founder event (Billing et al., 2012), and a population of song sparrow (*Melospiza melodia*) on Mandarte Island showed a strong increase in mean inbreeding level after a bottleneck in 1989 (Keller, 1998). In my study, the population crashed in 2018, and I expect an increased level of inbreeding following the crash. Unfortunately, there is only genetic data available until summer 2019. There was indeed a small increase in mean level of inbreeding in 2019 compared to 2018, but due to limited data there is difficult to conclude whether there is an increased level of inbreeding after a bottleneck. It would be necessary to have genetic data from more years that covers more cycles to see if the increased level of inbreeding after a bottleneck is an actual trend.

The results from this study showed a spatial variation, with a significant effect of island on inbreeding (Table 2, Figure 6). However, when excluding the five islands with the smallest sample sizes from the analyses, the effect disappeared (Appendix 9). There does not seem to be a trend with the smallest islands being islands with higher levels of inbreeding, so any spatial differences rather seem to be independent of island size and population size (Appendix 13). Some of the smallest islands with small population sizes, like Høgøya NO and Steinsholmen SV, show high levels of inbreeding compared to other islands. However, these results must be interpreted with caution because of the low number of individuals with two full ancestral generations, especially since this low number does not constitute a large proportion of the

individuals in the population. Larger sample sizes of individuals with two full ancestral generations on each island, or another measure of inbreeding (e.g. heterozygosity, see below), are needed to investigate the relationship between population size, island size and inbreeding properly.

4.2 Inbreeding depression analyses

This study investigated the effect of inbreeding in traits related to fitness. Inbreeding depression is predicted to have greatest impact on fitness related traits, compared to morphological traits, because these traits are likely to be under stronger directional selection (DeRose & Roff, 1999; Keller & Waller, 2002). In this study, it was not detected any evidence for inbreeding affecting survival, but a significant negative relationship between inbreeding and reproduction was found (Table 3, Figure 7, Figure 8). Previous studies focusing on the relationship between pedigree-based inbreeding coefficients and fitness in mammals and birds show mixed results. Several studies have found a clearly negative relationship (Keller et al., 2008; Liberg et al., 2005; Norén et al., 2016; Reid et al., 2003; Walling et al., 2011; Willoughby et al., 2019), while other have found non-significant or weakly negative relationships (Huisman et al., 2016; Keane et al., 1996; Wells et al., 2018). One reason for why analyses using pedigree-based inbreeding coefficients can produce non-significant relationships between inbreeding and fitness might be due to a lower power of pedigrees to detect inbreeding depression, compared to genomic data. The lower power could be because pedigree based inbreeding coefficients are not estimated with a very high accuracy (Kardos et al., 2015). Another possibility is that inbreeding effects on fitness vary among species or populations (Hedrick & Kalinowski, 2000), as for example different mean levels of inbreeding could be needed to have an impact on fitness. The variation in effects of inbreeding in different populations may also be due to the number of lethal equivalents in the population (Lacy et al., 1996), due to environmental factors or due to the traits investigated (Hedrick & Kalinowski, 2000)

4.2.1 Survival

When considering the inbreeding effect on juvenile survival from autumn to spring and the adult survival from spring to autumn, there were no significant effects (Table 3, Figure 7). Previous studies in some other species have also failed to find a significant effect of inbreeding on survival. For example, Wells et al. (2018) found no evidence for inbreeding affecting survival neither to nutritional independence nor beyond nutritional independence (i.e. no effect

on longevity) in a wild population of banded mongooses (*Mungos mungo*), and Keane et al. (1996) found no evidence for inbreeding affecting adult survival in dwarf mongooses (*Helogale parvula*). In this study, we were not able to determine survival of the individuals from birth to their emerge from their nests as juveniles. Inbreeding depression could often affect fitness in early life stages (Keller & Waller, 2002), so we cannot exclude the possibility that inbreeding affects survival in earlier stages of development, for example *in utero* or before emergence from their burrows (Wells et al., 2018). Still, the evidence from this study suggests that inbreeding is not important in explaining variation in neither juvenile nor adult survival.

Even though individual survival might be affected by inbreeding at earlier life stages than measured in this study, other mechanisms could also be possible explanations for these findings. One hypothesis is that external factors, like predators, food limitations and/or harsh climate conditions, could outweigh the negative effects of inbreeding. If the relative importance of inbreeding is small compared to other factors, it will be hard to find any significant relationship between level of inbreeding and individuals survival. The mortality rate for both juvenile and adult survival was relatively high (Appendix 10), so stochasticity might be more important for survival than inbreeding.

4.2.2 Reproduction

The results of this study show that the total number of offspring and the probability of having offspring were significantly affected by inbreeding (Table 3, Figure 8). These results are in accordance with the expectations, and correspond with several previous studies finding a negative relationship between inbreeding and reproductive success (Billing et al., 2012; Huisman et al., 2016; Marr et al., 2006; Niskanen et al., 2020; Willoughby et al., 2019). For example, Willoughby et al. (2019) found a significant negative relationship between an individual's pedigree-estimated inbreeding coefficient and both its lifetime number of juvenile offspring and lifetime reproductive success in banner-tailed kangaroo rats (*Dipodomys spectabilis*). A significant negative relationship between level of inbreeding and reproductive success has also been found in populations of house sparrow in Northern Norway (Billing et al., 2012; Niskanen et al., 2020).

The lowered reproduction due to inbreeding could be caused by some internal mechanisms, where inbred individuals have more problems with producing offspring, compared to outbred or less inbred individuals. In males it could be mechanisms related to male-male competition

(Huisman et al., 2016) or sperm quality, and in females it could be related to egg-production (de Boer et al., 2018). In this study, there was a significant interaction effect between inbreeding and sex on the total number of juvenile offspring produced, with males being more negatively affected by inbreeding than females (Figure 9). Also Huisman et al. (2016) found males to be more negatively affected by inbreeding than females in lifetime breeding success in a population of red deer (*Cervus elaphus*) in Scotland, and suggested a polygynous breeding system with strong male-male competition as a possible explanation. In a water vole population located around 50-60 km north of our study site, there was evidence for a polygynous mating system (Frafjord, 2016). A possible explanation for the observed sex differences then might be due to a polygynous mating system, with a higher variance in total number of offspring in males compared to females. However, based on the limited sample size for both sexes with a larger number of offspring (Appendix 11), my current data set does not allow me to conclude whether this could be the case. In a comparative study by Fitzpatrick and Evans (2009), the relationship between inbreeding and sperm quality in 20 mammal species was investigated. Heterozygosity was used as a measure of level of inbreeding, and it was found a negative correlation between abnormal sperm and heterozygosity, and a positive correlation between sperm motility and heterozygosity for the endangered species included in the study. It remains to be seen if reduced sperm quality is a consequence of inbreeding also for the water vole males in the current study population, but based on the results from the study, this could be a possible explanation. The potential internal mechanisms for inbreeding depression in reproductive traits could be tested for. Since internal mechanisms for reproduction should be less affected by environmental conditions, one could test if the observed trend is independent of year and island. The different years and islands will represent different levels of competition and environmental conditions, which are factors that could be affecting inbred individuals to a larger extent than non-inbred individuals (Armbruster & Reed, 2005)

An important discussion point for the reproduction analyses in the current study is the consequences of using a dataset including all juvenile offspring with two full ancestral generations and known sex, and not only adult individuals present in the spring of their second living year, i.e. when they are most likely to reproduce. This choice was made because of the possibility for having overlapping generations within a summer season in the water vole study system (Strachan et al., 2011, see Appendix 11). The consequence is that in addition to the mating success and production of a fertilized zygote for each individual itself, these fitness measures potentially included a survival component from juvenile stage to sexual maturity

(because individuals first captured as recruited offspring were also counted), and the number of offspring (i.e. zygotes) surviving to the stage where they can emerge from their nests and be captured. Because the analyses of juvenile survival showed that inbreeding had no significant effect, my results suggest that the negative effect of inbreeding that I found on reproduction is probably caused by the other components (i.e. mating success, number of zygotes produced, and the survival of zygotes to juvenile stage).

4.2.3 Environmental factors

In the current study, only two to three years of data were included in each of the inbreeding depression analyses, making it difficult to analyze temporal variation in the strength of inbreeding depression on the fitness components included. Regardless, the data used in this study did not show any significant pattern of inbreeding depression differing between years (except from juvenile survival from autumn to spring which was excluded from results due to very low sample size, see methods), but more data from more years is needed to determine whether this apparent consistency is real. For example, the population crash in 2018 could allow for detection of variation in inbreeding depression under different population densities (Yun & Agrawal, 2014). Inbreeding depression is often expected to vary with different environmental conditions (Armbruster & Reed, 2005; Keller & Waller, 2002), but there are mixed results about the actual effects. Armbruster and Reed (2005) found inbreeding depression to increase under environmental stress in 76% of the cases (46% significant), but they also found decreased inbreeding depression under environmental stress in 24% of the cases. Niskanen et al. (2020) found a constant level of inbreeding depression across space and time, despite different population sizes, inbreeding levels and environmental conditions, in a metapopulation of house sparrow in Northern Norway. The results of Niskanen et al. (2020)'s study challenge the idea that inbreeding depression in general is dependent on ecological variables. Because of data from relatively few years and ecological data was not included in my study, one should not make a strong conclusion regarding whether inbreeding depression in fitness related traits is related to ecological conditions in this metapopulation of water voles.

4.2.4 Purging

The population in this study is fluctuating in size both within and between years, and has probably gone through several bottlenecks. Purging could potentially mask negative effects of inbreeding (Keller & Waller, 2002). One could speculate whether purging could be relevant for

the non-significant evidence for inbreeding depression in survival, i.e. whether deleterious recessive alleles may have been purged during bottlenecks (Bouzat, 2010; Facon et al., 2011; Lacy et al., 1996). For example, Bouzat (2010) suggests that cyclic populations that regularly undergo bottlenecks or population crashes could be suffering less from inbreeding due to a history of purging. However, Saccheri et al. (1998) found that in small populations of a butterfly species (*Melitaea cinxia*) on Åland, the selection against deleterious recessive alleles was probably inefficient due to strong genetic drift. Both mildly and hardly deleterious recessive alleles can contribute to inbreeding depression, and purging is most likely to remove large-effect mutations, since milder deleterious mutations are harder to remove due to weaker selection (Keller & Waller, 2002). Deleterious recessive alleles may have been purged during the bottlenecks in this study (Bouzat, 2010; Facon et al., 2011; Lacy et al., 1996), but the non-significant effect of inbreeding on survival could also be that the probability of survival is stochastically determined instead of genetically determined. In my study, there is evidence for inbreeding depression in reproductive fitness, making it reasonable to assume that any potential purging is not sufficient on this fitness component. For small and isolated populations suffering from inbreeding depression, one would expect that dispersal could be necessary for maintenance of genetic variation and for potentially introducing new beneficial alleles. On the other side, if a population is able to purge hardly recessive deleterious mutations, dispersal could counteract the purging process by introducing new deleterious mutations (Hedrick & Garcia-Dorado, 2016; Keller & Waller, 2002). However, the current study did not allow for detection of purging, so these speculations must be regarded as such.

4.2.5 The role of dispersal

If dispersal is not causing an introduction of new deleterious mutations into a population, it might be a mechanism to avoid inbreeding, and to limit the negative consequences of inbreeding. In our study population, Stenersen (2017) found evidence for a relatively low level of between-island dispersal, mainly male-biased dispersal, making it reasonable to assume at least some level of gene flow. Other studies on fluctuating water vole populations have found widespread gene flow and high genetic variability despite periods of low abundance (Berthier et al., 2006; Aars et al., 2006). In the study of Berthier et al. (2006) they suggested the subdivision of the population, with alleles randomly distributed to different patches, as an explanation for the maintenance of genetic diversity during the periods of low abundance, followed by gene flow between the populations under the increasing population size phase. The observed gene flow was likely to hinder loss of genetic variability due to genetic drift and

inbreeding. In another metapopulation of water voles off the coast of Northern Norway, it was found that there was a high level of genetic differentiation between the island subpopulations (Melis et al., 2013). In Melis et al. (2013)'s study, the islands were in general somewhat larger (2350 – 35 000 m^2), and the distances between islands were approximately similar (distances inside each locality were ranging from 0.1 to 5.93 km) compared to my study population. Around 1% of the individuals were identified as immigrants in Melis et al. (2013)'s study, and this was similar to the level of dispersal Stenersen (2017) found in our study population. The relatively low dispersal rate could make us expect opposite results of what was found in the study of Berthier et al. (2006) and Aars et al. (2006). Both the mentioned study population of Melis et al. (2013) and the water vole metapopulation of this study have a patchy habitat structure, consisting of subpopulations on different islands, i.e. dispersal between islands will involve swimming. Dispersal is likely to involve both costs and benefits, as dispersing individuals may fail to survive, and at the same time would reduce the likelihood of mating with close relatives is they survive (Bengtsson, 1978). Therefore, it might be more expedient to take the risk of mating with a relative instead of not to mate at all. In this study, we have no basis to say how the level of observed gene flow is contributing to the genetic variation among subpopulations, and for example the relationship between level of inbreeding and immigrants should be considered in another study.

4.3 Limitations and further studies

Firstly, a limitation with this study is the reduction of individuals (from 1311 to 388 individuals) when including only individuals with two full ancestral generations. In this study, I have used a pedigree based estimator of *F*. Since the use of pedigrees, in addition to a reduced sample size due to low pedigree completeness, could include assignment errors and therefore an underestimation of inbreeding (Kardos et al., 2016; Reid et al., 2014), it would have been favorable to use another measure of inbreeding. To look into this possibility, I calculated the individual standardized multilocus heterozygosity (sMLH; Stoffel et al., 2016) for the individuals genotyped for more than 60% of the SNPs, using the R package *InbreedR* (Stoffel et al., 2016). I found a negative correlation between this heterozygosity estimate and the pedigree-based *F* for the individuals with four assigned grandparents (Appendix 14). Other studies have also found a correlation between sMLH and *F*, with heterozygosity decreasing with increased inbreeding (Jensen et al., 2007; Ólafsdóttir & Kristjánsson, 2008; Wells et al., 2018). Since such a correlation between sMLH and *F* was detected, a natural next step would

have been to run all the analyses performed in this study by using sMLH in addition to *F*. Then all the genotyped individuals would have been included, and the inbreeding depression analyses could have been performed with a greater power. Because most of the individuals present on the 13 islands over the four years has sMLH information, it would then also be possible to investigate e.g. whether there was a relationship between population size and sMLH (inbreeding). In addition, it would be possible to investigate whether population size could explain inbreeding depression, i.e. an interaction between sMLH and population size in the effect on fitness. Due to time limitations, these analyses were not possible to perform, but are recommended for further studies.

Secondly, an initial goal with this study was to see if there was any spatio-temporal variation in inbreeding. Due to missing genotype data from autumn 2019, fewer years than intended were included. An inclusion of more years would be preferable both for the power and depth of the pedigree (Kardos et al., 2015) and for the analyses of temporal variation. In this study system, years could represent different environmental conditions and different population densities. There are still unanswered questions about the relationship between inbreeding, inbreeding depression and external factors in this water vole metapopulation, so the inclusion of more years will be important for such understanding.

Lastly, several fitness components could have been included if more data was available. For example, in this study, I investigated the effect of inbreeding on reproduction for all juveniles, which entailed that a survival component from juvenile to sexual maturity was included in these analyses. If more available data, one could have focused on the effect of inbreeding on reproduction of individuals at certain age classes, and their production of offspring and mating success. To develop a full picture of the effect of inbreeding, additional studies focusing on a broader specter of traits could be needed.

5 CONCLUSION

The purpose of the current study was firstly to investigate the level of inbreeding and spatio-temporal variation in inbreeding in a cyclic metapopulation of water voles off the coast of Northern Norway. The results indicated that around 35% of the population had an inbreeding coefficient larger than zero, and around 17% had an inbreeding coefficient which indicated mating between first cousins or closer relatives ($F \geq 0.125$). The level of inbreeding varied between different years as well as between the different islands, indicating spatio-temporal variations in inbreeding. By using another measurement of inbreeding, e.g. heterozygosity, where a higher proportion of the individuals would have been included, a further study could investigate such spatio-temporal patterns with higher reliability and e.g. whether the level of inbreeding depends on population size. Also, more years should be included to determine how the level of inbreeding changes during and after a population bottleneck.

The second aim of this study was to investigate whether there was inbreeding depression in the population. The results showed a significantly negative effect of inbreeding on reproduction, but not on survival. Furthermore, males were found to suffer more from inbreeding depression in one of the reproductive traits (total number of juvenile offspring produced), compared to females. Knowledge about how inbreeding depression affects a population could be important for future studies investigating whether inbreeding depression impacts the population fluctuations seen in the Helgeland metapopulation of water voles.

Even though the water vole is not a threatened species in Scandinavia, knowledge about the level of inbreeding and the consequences of inbreeding depression from this study suggest that inbreeding is a relevant process affecting population viability in the system, and could also be important for future conservation management of cyclic, small and fragmented populations.

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7 APPENDIX

Appendix 1: (a) The island size (area m^2) and the population size of water voles on 13 different islands on the Helgeland archipelago in Northern Norway from spring 2016 to autumn 2019. The population size was determined based on the number of captured individuals on each island in the given season. (b) The total number of sampled individuals born each year with the respective number of genotyped individuals in brackets. *only genotype data from summer.

(a)

Island	**Area (m^2)**	**Population size**							
		Spring 2016	Autumn 2016	Spring 2017	Autumn 2017	Spring 2018	Autumn 2018	Spring 2019	Autumn 2019
Geiterøya N	9 692	**27**	79	**27**	83	**20**	2	**0**	0
Grønholmdraget Ø	12 862	**11**	78	**31**	69	**1**	13	**6**	59
Grønholmdraget S	2 564	**5**	36	**4**	8	**2**	1	**0**	3
Gulbrandsøyan Midt N	9 226	**30**	59	**6**	16	**0**	4	**6**	62
Gulbrandsøyan Midt S	6 568	**22**	56	**2**	18	**0**	1	**6**	34
Gulbrandsøyan S	10 980	**34**	87	**27**	27	**2**	20	**17**	108
Gullrekka 1	4 070	**12**	60	**6**	15	**0**	4	**2**	19
Gullrekka 2	2053	**6**	31	**9**	17	**1**	7	**1**	35
Høgøya NO	4 333	**1**	25	**0**	12	**2**	4	**7**	30
Slåttskjæret	12 524	**12**	67	**24**	66	**1**	7	**1**	11
Slåttskjæret S	5 357	**2**	30	**3**	10	**0**	5	**2**	5
Steinsholmen N	9 717	**11**	46	**9**	13	**0**	3	**0**	6
Steinsholmen SV	4 975	**9**	34	**16**	9	**0**	5	**0**	3

(b)

Island/ Birth year	**2015**	**2016**	**2017**	**2018**	**2019**
Geiterøya	26 [25]	93 [82]	130 [108]	10 [7]	0 [NA]
Grønholmdraget S	11 [11]	79 [77]	56 [51]	17 [9]	54 [NA]
Grønholmdraget Ø	6 [5]	34 [20]	9 [6]	1 [1]	3 [NA]
Gulbrandsøyan Midt N	36 [34]	93 [66]	21 [16]	6 [4]	79 [36]*
Gulbrandsøyan Midt S	21 [20]	127 [96]	22 [19]	9 [8]	60 [17]*
Gulbrandsøyan S	36 [34]	99 [83]	71 [57]	35 [29]	94 [NA]
Gullrekka 1	12 [12]	54 [39]	11 [8]	7 [3]	16 [NA]
Gullrekka 2	10 [6]	29 [17]	12 [11]	7 [5]	34 [NA]
Høgøya NO	4 [4]	21 [13]	10 [8]	9 [6]	26 [NA]
Slåttskjæret	15 [15]	64 [52]	63 [47]	7 [6]	11 [NA]
Slåttskjæret S	2 [2]	31 [23]	8 [6]	6 [5]	5 [NA]
Steinsholmen N	11 [11]	41 [34]	13 [11]	3 [1]	6 [NA]
Steinsholmen SV	7 [6]	37 [22]	6 [5]	5 [3]	3 [NA]

Appendix 2: Above: the habitat on Gulbrandsøyan S. The yellow mark showed in the right corner is a numbered trap-flag indicating a trap site. Below: the habitat on Steinsholmen S consists of more heaths and trenches. The yellow marks are trap-flags. Photo: Ida Gabrielsen.

Appendix 3: Sherman XLF15 folding traps baited with dry grass and pieces of carrot and potato. The trap was covered with moss as protection against wind and overheating. All traps were marked with a number matching the number of the trap-flag. Photo: Ida Gabrielsen.

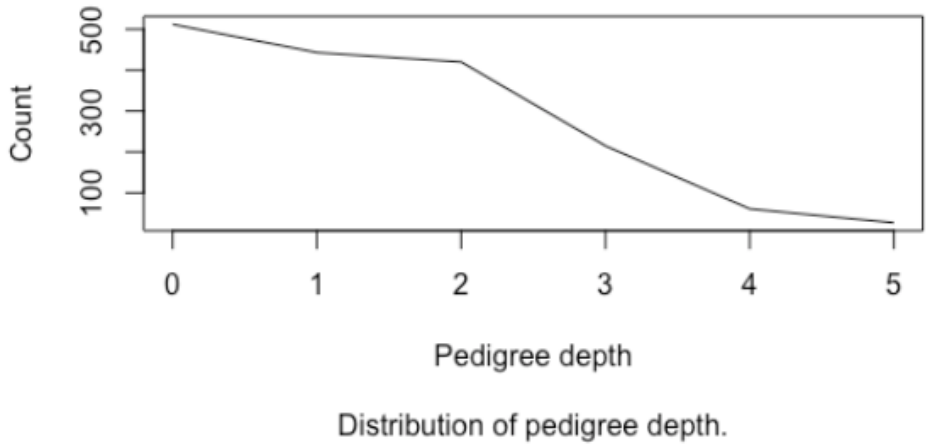
Appendix 4:

The DNA-extraction of the biopsy samples was performed using a DNeasy Blood & Tissue Kit (QIAGEN), mostly using the standard protocol. First, 180 µl Buffer ATL was added into a 1.5 ml microcentrifuge tube, after which the biopsy was added. Proteinase K (20 µl) was then added into the tube. The tube was mixed by vortexing, and incubated at 56 °C until completely lysed, around 2 hours. The tubes with its content were vortexed after incubation. Next, 200 µl Buffer AL and 200 µl ethanol (96-100%) was added, and mixed by vortexing. The mixture was pipetted into a DNeasy Mini spin column placed in a 2 ml collection tube, before it was centrifuged at ≥ 6000 x g (8000 rpm) for 2 minutes. The flow-through and the collection tube were discarded. The spin column was then placed in a new 2 ml collection tube, and 500 µl Buffer AW1 was added, before it was centrifuged for 2 minutes at ≥ 6000 x g (8000 rpm). The spin column was then placed in a new 2 ml collection tube, and 500 µl Buffer AW2 was added, before it was centrifuged for 6 minutes at 20 000 x g (14 000 rpm). Spin columns were transferred to new 1.5 ml centrifuge tubes. DNA was eluted by adding 50 µl Buffer AE to the center of the spin column membrane, incubating it for 1 minute in room temperature (15-25 °C) and centrifuged for 2 minutes at ≥ 6000 x g. The last step was repeated for increased DNA yield; the flow-through was pipetted back to the spin column membrane, and centrifuged for 2 minutes at ≥ 6000 x g.

Appendix 5: Pedigree structure

The table shows the pedigree structure for the total number of genotyped individuals, and the figure is showing the distribution of pedigree depth.

Pedigree structure	Number of individuals/generations
Two known parents	1103
Only known mother	33
Only known father	30
Total maternities	1136
Total paternities	1133
Total fullsibs	864
Number of dummy parents included	375
Mean pedigree depth (full generations)	1.17
Mean pedigree depth (max generations)	2.50



Distribution of pedigree depth.

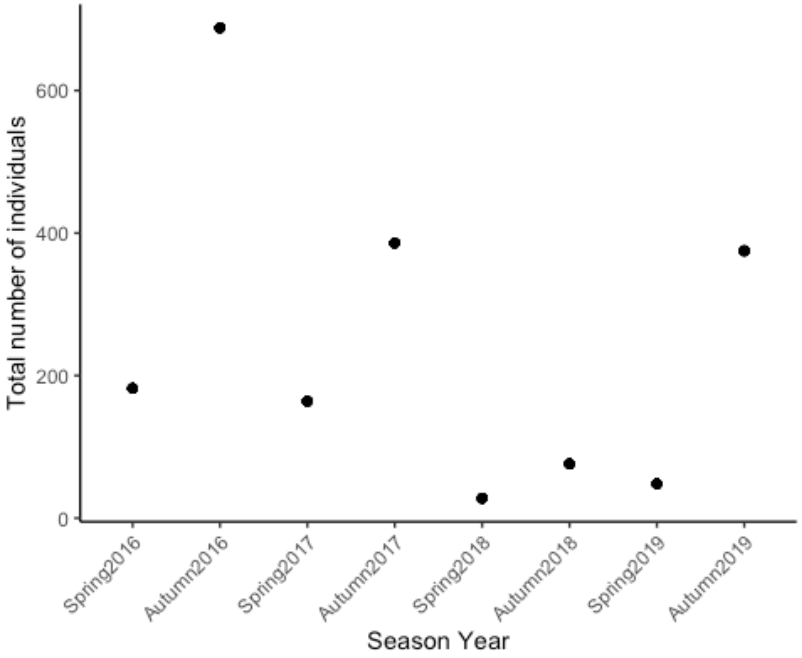
The table below shows the number of individuals with dummy parents and dummy grandparents in each of the years for individuals with two full ancestral generations. A relatively high number of dummy parents was included, but note that no dummy parents were assigned new dummy parents (i.e. no individuals with two generations of dummy parents) were included in the analyses.

	2016	2017	2018	2019
Number of individuals with dummy parents	28	79	9	3
Number of different dummy parents	23	57	10	1
Number of individuals with dummy grandparents	149	123	8	16
Number of different dummy grandparents	45	43	8	4

Appendix 6: The number of individuals captured on each island found to have at least two full ancestral generations. The years in the table are the birth years of the captured individuals. The total number of individuals was reduced from 1311 to 388 individuals, and the total number of genotyped individuals on each island each year is shown in brackets.

Island / Birth year	2016	2017	2018	2019
Geiterøya N	**30**	72	**5**	0
Grønholmdraget Ø	**14**	31	**0**	0
Grønholmdraget S	**7**	1	**0**	0
Gulbrandsøyan Midt N	**30**	10	**0**	14
Gulbrandsøyan Midt S	**5**	3	**1**	2
Gulbrandsøyan S	**37**	36	**3**	0
Gullrekka 1	**7**	7	**0**	0
Gullrekka 2	**1**	10	**0**	0
Høgøya NO	**7**	1	**0**	0
Slåttskjæret	**23**	18	**0**	0
Slåttskjæret S	**1**	2	**2**	0
Steinsholmen N	**2**	5	**0**	0
Steinsholmen SV	**1**	0	**0**	0

Appendix 7: The total number of individuals captured in the different seasons illustrated with a black dot. The figure is visualizing the differences in population size within year, with the spring season consisting of fewer individuals compared to the autumn season. In addition, the figure is showing the fluctuations in population size between years.



Appendix 8: Histogram showing the frequency of the inbreeding coefficients. Around 35% of the individuals had an inbreeding coefficient larger than zero (135/388 individuals), and 18% had an inbreeding coefficient larger than 0.1 (69/388 individuals).

Inbreeding coefficient	**0**	**[0.004-0.5]**	**0.0625**	**0.0937**	**0.1016**	**0.1250**	**0.1406**	**0.1875**	**0.2500**	**0.2969**	**0.3125**
Number of individuals	253	47	9	10	2	24	1	2	34	2	4

Appendix 9: Variation between islands in the level of inbreeding *F* in a water vole metapopulation. Here, the islands with less than ten individuals are excluded. An LRT showed that island did not explain a significant proportion of the variance in inbreeding ($p = 0.0684$), i.e. there was not a significant effect of island on the level of inbreeding. The mean, standard deviation and median inbreeding coefficient for each island is shown. Bold means indicate level of inbreeding significantly different from zero ($p < 0.05$). A linear model was fitted with island as fixed factor. Parameter estimates with standard error (SE) and p-values are presented. As indicated by the p-value (p) (*$p < 0.5$, ** $p < 0.01$, *** $p < 0.001$), the intercept (Geiterøya) is significantly different from zero, and the mean on Grøndholmdraget Ø is significantly different from the intercept.

Island	**Mean *F* ± SD**	**Median *F***	**Estimate ± SE**	**p**
Intercept (Geiterøya)	**0.0480** ± 0.0864	0.0000	0.0480 ± 0.0080	< 0.001 ***
Grønholmdraget Ø	0.0165 ± 0.0326	0.0000	-0.0315 ± 0.0147	0.0328*
Gulbrandsøyan Midt N	**0.0521** ± 0.0984	0.0000	0.0041 ± 0.0138	0.7669
Gulbrandsøyan Midt S	0.0133 ± 0.0373	0.0000	-0.0347 ± 0.0262	0.1858
Gulbrandsøyan S	**0.0563** ± 0.0968	0.0000	0.0083 ± 0.0124	0.5043
Gullrekka 1	0.0151 ± 0.0367	0.0000	-0.0329 ± 0.0235	0.1622
Gullrekka 2	0.0021 ± 0.0071	0.0000	-0.0459 ± 0.0262	0.0808
Slåttskjæret	**0.0311** ± 0.0885	0.0000	-0.0080 ± 0.0151	0.5958

Appendix 10: Overview of the number of surviving individuals between different survival periods. The assumed non-surviving individuals have a value of 0 and the surviving individuals have a value of 1. Individuals with missing survival data in the given season are excluded from the overview.

Autumn-spring: Juvenile survival data from autumn to spring shown for the whole metapopulation and for the sample size with at least two full ancestral generations (the individuals used in the inbreeding depression analyses). The number total number of surviving individuals and the number of surviving individuals in each year are shown in (a) and (b), respectively.

(a)

	Total population		Sample size with at least two full ancestral generations	
Survival	**Number**	**Proportion**	**Number**	**Proportion**
0	833	76.35%	251	83.39%
1	258	23.65%	50	16.61%

(b)

		Total population		Sample size with at least two full ancestral generations	
Year	**Survival**	**Number**	**Proportion**	**Number**	**Proportion**
2016	0	454	72.87%	102	76.12%
	1	169	27.13%	32	23.88%
2017	0	323	91.24%	145	90.62%
	1	31	8.76%	15	9.38%
2018	0	56	49.12%	4	57.14%
	1	58	50.88%	3	42.86%

Spring-autumn: Adult survival data from spring to autumn shown for the whole metapopulation and for the sample size with at least two full ancestral generations (the individuals used in the inbreeding depression analyses). The number total number of surviving individuals and the number of surviving individuals in each year are shown in (c) and (d), respectively. No individuals born in 2015 have two full ancestral generations, therefore there is no survival information spring-autumn in 2016.

(c)

	Total population		Sample size with at least two full ancestral generations	
Survival	**Number**	**Proportion**	**Number**	**Proportion**
0	185	71.71%	41	82.00%
1	73	28.29%	9	18.00%

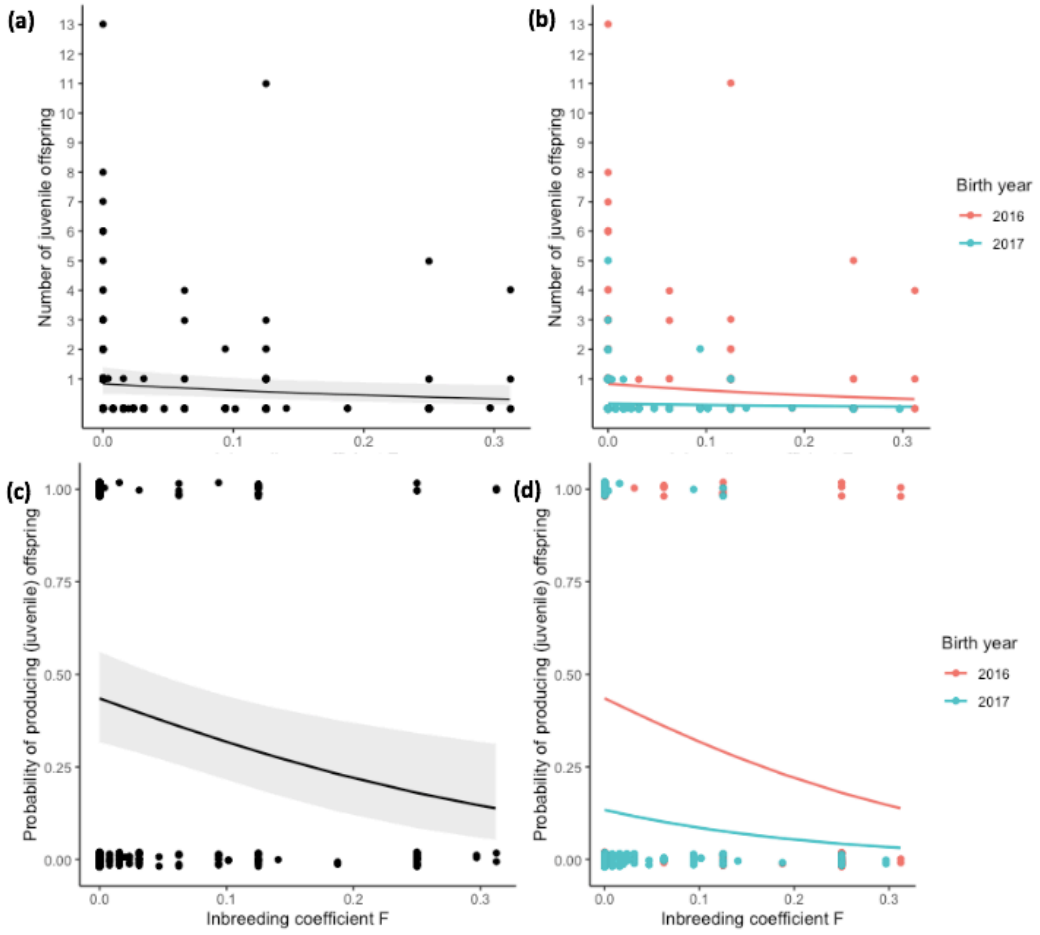
(d)

		Total population		Sample size with at least two full ancestral generations	
Year	**Survival**	**Number**	**Proportion**	**Number**	**Proportion**
2017	0	124	73.37%	26	81.25%
	1	45	26.63%	6	18.75%
2018	0	29	93.55%	13	86.67%
	1	2	6.45%	2	13.33%
2019	0	32	55.17%	2	66.67%
	1	26	44.83%	1	33.33%

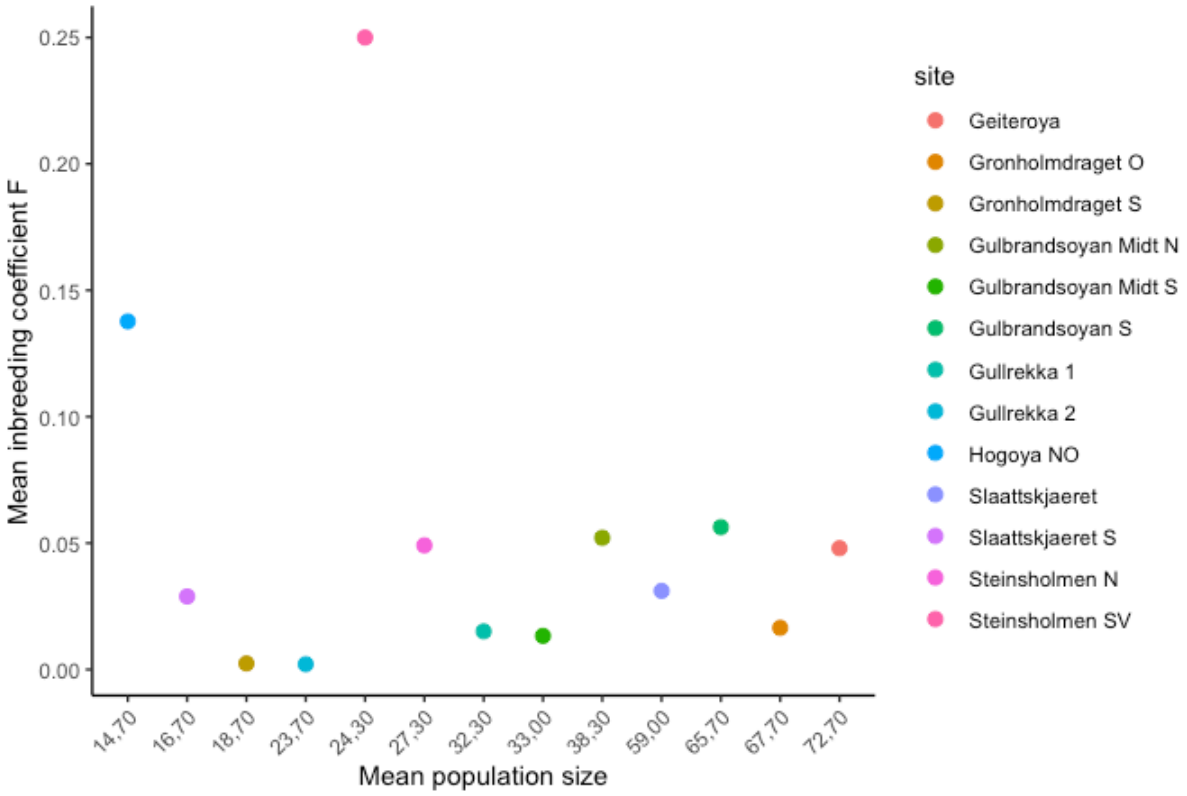
Appendix 11: In total 259 individuals with two full ancestral generations had known sex. The table shows the distribution of males and females with inbreeding coefficient equal to and larger than 0, as well as the number of offspring. Note also that the total number of reproducing individuals was larger than the number of individuals surviving from autumn to spring (Appendix 10), indicating some individuals reproducing in the year of birth.

		Total number offspring			
Inbreeding	**Sex**	**0**	**1**	**2-5**	**6-13**
$F = 0$	Male	75	18	7	3
	Female	49	16	5	3
$F > 0$	Male	32	4	0	0
	Female	34	6	6	1

Appendix 12: There was a significant effect of inbreeding on both the total number of juvenile offspring ($\beta = -3.1147$, $p = 0.0292$) and the probability of having offspring ($\beta = -5.0245$, $p = 0.0035$) when only year was included as a fixed factor and site was included as a random factor. The figure is showing the relationship between reproduction and the inbreeding coefficient *F*. Two generalized linear mixed models were fitted, where the response variable was total number of juvenile offspring produced (negative binomial error structure; panel a, b), or the probability of producing juvenile offspring (binomial error structure and logit link function; panel c, d). In both models, the fixed predictor variables were inbreeding coefficient *F*, and main effect of year. Site was included as a random intercept. Sex was not included in the models. The lines are predicted values from the models, and show the partial effects of inbreeding coefficient *F* (a, c), and inbreeding coefficient *F* and year (b, d) on reproduction, when other predictors are held constant. The shaded area shows the 95% confidence interval (a, c). The points are observed values. A small random noise was added to the points to avoid overplotting (scale of jittering: height = 0.03, width = 0.00).



Appendix 13: The relationship between the mean population size (based on population size from 2016 to 2018) and mean level of inbreeding on the respective island with this population size. The islands with fewer than ten individuals with two full ancestral generations were Grønholmdraget S (mean $F = 0.0024$), Høgøya NO (mean $F = 0.1377$), Slåttskjæret S (mean $F = 0.0289$), Steinsholmen N (mean $F = 0.0489$) and Steinsholmen SV (mean $F = 0.2500$).



Appendix 14: The individual standardized multilocus heterozygosity (sMLH), hereafter called heterozygosity (*H*), was calculated using the R package *inbreedR* (Stoffel et al., 2016). The heterozygosity and the inbreeding coefficient *F* were found to be significantly negatively correlated, and the best fitting line was given by $H = 1.0151 - 1.0155 * F$ ($p < 0.001$).