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Master's thesis in Natural Resources Management

Supervisor: Henrik Jensen

Co-supervisor: Thor Harald Ringsby, Thomas Kvalnes, and Hamish Andrew Burnett

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Abstract

Inbreeding depression is one of the severe threats to the survival of small, isolated populations. A full understanding of the impact of inbreeding on population viability requires quantifying inbreeding depression (ID) in maternal investment since it may impact the following generation by reducing the offspring's health and survival. This study examined the impact of maternal inbreeding on clutch size and egg size to understand the consequences of inbreeding on reproductive output in an insular metapopulation of house sparrows on the coast of Helgeland in northern Norway. I used individual genomic data on 118,000 SNPs to determine the inbreeding coefficients of mothers. By analyzing runs of homozygosity, I identified homozygous sequence blocks and estimated individual inbreeding coefficients (F) by quantifying the proportion of the genome made up of runs of homozygosity (F_{ROH}). The clutch's mean egg volume, total egg volume, and clutch size were used as maternal investment indicators. Linear mixed-effects models (LME) were fitted to examine the impact of maternal inbreeding on egg volumes. Generalized linear mixed-effect models (GLMMs) were fitted to examine how individual variation in maternal inbreeding affected clutch size. The results showed no significant relationships between the inbreeding coefficient of mothers and the average or total size of eggs in a clutch, or clutch size. These results indicate that several factors may have obscured the expected negative effects of inbreeding. For example, there were increases in annual adult population sizes during my study period, pointing to potential habitat quality improvements and suggesting that environmental conditions may have obscured any negative effects of inbreeding on maternal investment. These findings highlight the need to consider various processes and factors when evaluating the effects of inbreeding and highlight the need for more studies to clarify how genetic and environmental factors interact to affect population dynamics in age-structured populations. Such in-depth knowledge can help in conservation and management actions by maintaining genetic variation and reducing any negative effects of inbreeding in wildlife populations.

Sammendrag

Innavlsdepresjon er en av de største truslene mot overlevelsen til små, isolerte bestander. For å få en fullstendig forståelse av innavlens innvirkning på populasjonens levedyktighet er det nødvendig å kvantifisere innavlsdepresjon (ID) i mors investering, siden det kan påvirke overlevelsen til den påfølgende generasjonen ved å redusere avkommets helse og overlevelse. I denne studien undersøkte jeg hvordan innavl hos mor påvirker størrelsen på unger og egg for å forstå konsekvensene av innavl på reproduksjonsevnen i en metapopulasjon av gråspurv på Helgelandskysten i Nord-Norge. Jeg brukte individuelle genomdata på 118 000 SNP-er til å bestemme innavlskoeffisienter for mødre. Ved å analysere homozygote SNPs identifiserte jeg homozygote deler av genomet og estimerte individuelle innavlskoeffisienter (F) ved å kvantifisere andelen av genomet som består av homozygote sekvenser (F_{ROH}). Gjennomsnittlig eggvolum, totalt eggvolum og kullstørrelse ble brukt som indikatorer for mors investeringer. Lineære mixed-effects-modeller (LME) ble tilpasset for å undersøke effekten av innavl hos mor på eggstørrelse. Generaliserte lineære mixed-effects-modeller (GLMM) ble tilpasset for å undersøke hvordan individuell variasjon i innavl hos mor påvirket kullstørrelse. Resultatene viste ingen signifikante sammenhenger mellom mødrenes innavlskoeffisient og den gjennomsnittlige eller totale størrelsen på eggene i et kull, eller kullstørrelsen. Disse resultatene tyder på at andre faktorer kan ha tilslørt de forventede negative effektene av innavl. Det var for eksempel en økning i den årlige størrelsen på bestandene av voksne individer i løpet av studieperioden, noe som tyder på en mulig forbedring av habitatkvaliteten og på at miljøforholdene kan ha tilslørt eventuelle negative effekter av innavl på mødrenes investeringer. Disse funnene understreker behovet for å ta hensyn til ulike prosesser og faktorer når man vurderer effekten av innavl, og understreker behovet for flere studier for å avklare hvordan genetiske og miljømessige faktorer samvirker og påvirker populasjonsdynamikken i aldersstrukturerte populasjoner. Slik inngående kunnskap kan bidra til bevarings- og forvaltningstiltak ved å opprettholde genetisk variasjon og redusere faren for innavl i ville populasjoner.

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Table of Contents

Abstract.....	i
Sammendrag.....	ii
Acknowledgment.....	iii
1. Introduction.....	1
2. Methods.....	5
2.1 Study species.....	5
2.2 Study area.....	5
2.3 Data collection.....	6
2.3.1 Egg size and clutch size measurements.....	6
2.3.2 Brood number.....	6
2.3.3 Genetic sample collection.....	6
2.3.4 Maternal effects.....	6
2.4 Estimation of inbreeding coefficient.....	7
2.5 Statistical analyses.....	7
3. Results.....	9
3.1 The effect of inbreeding on egg size.....	9
3.1.2 Effect of F on the total egg size of each brood.....	9
3.2 Effect of F on the size of the clutches.....	10
4. Discussion.....	11
5. References.....	18
6. Tables.....	25
7. Figures.....	30
8. Appendix.....	36

1. Introduction

Extinction and population decline have always been a part of natural processes for wild species and populations. However, a significant increase in extinction rates and population declines is currently observed that impacts most organisms on the planet (Díaz et al., 2019). Many populations of natural species have declined because of human activities, either directly by fragmenting or degrading natural habitats or indirectly by adversely affecting the climate and food supplies (Díaz et al., 2019).

Several processes threaten the persistence of small populations, which has been recognized within the small population paradigm framework (Caughley, 1994). This framework highlights genetic factors such as genetic drift and inbreeding depression, as well as demographic and environmental stochasticity, as significant challenges to the survival of small populations (Caughley, 1994). Small populations experience higher rates of genetic drift, which can lead to the fixation of detrimental alleles, ultimately affecting the fitness of all population members (Hedrick & Kalinowski, 2000). Small populations are also more likely to encounter bottlenecks, which can exacerbate the harmful effects of genetic drift (Hedrick & Kalinowski, 2000). Importantly, inbreeding is unavoidable in small closed populations because all the individuals become related to each other over time, and any detrimental recessive alleles are then more likely to be expressed (Frankham et al., 2002).

Inbreeding refers to mating among individuals related through common ancestors, and an individual is considered inbred if the parents share common ancestors (Frankham et al., 2002). The adverse effects of inbreeding on individual fitness, known as inbreeding depression, have been known since Darwin's time (Frankham et al., 2002), and animal and plant breeders have long been aware of the negative consequences of inbreeding between close relatives (Frankham et al., 2002). Inbreeding depression is a critical concern for animal breeders, and it has consistently gained significant attention from geneticists and livestock breeders (Leroy, 2014). Similarly, zoological gardens have always provided essential data on inbreeding depression through detailed breeding records and controlled environments. One of the most comprehensive interspecific studies on the effects of inbreeding depression was conducted on captive animals in zoological gardens by Ralls et al. (1988), who compared 38 species with different inbreeding coefficients and assessed the extent of depression. The results showed a significant increase in the mortality rate of inbred progeny across 40 captive populations within these species (Ralls et al., 1988). As a result, various conservation strategies have been implemented to ensure the survival of future generations of captive animals (Moreno Rivas et al., 2018). One such initiative is the ex-situ breeding program, which aims to protect endangered species in specialized settings like zoos (McGowan et al., 2017).

Several studies provide compelling evidence that inbreeding causes small and isolated populations to decline and eventually become extinct (Keller & Waller, 2002). For example, inbreeding resulted in increased extinction risk in fragmented butterfly (*Glanville fritillary*) meta-populations by reducing larvae survival, hatching success, and adult longevity (Saccheri et al., 1998). Furthermore, O'Grady et al. (2006) used stochastic modeling to demonstrate that inbreeding reduced population growth rates and led to shorter estimated time to extinction for 30 bird and mammal species. Similarly, studies on plants, such as pink fairies (*Clarkia pulchella*) in the United States of America and papamoscas marino (*Silene*

littorea) in Spain, have revealed a relationship between extinction probability and the degree of inbreeding (Frankham, 2005; Frankham, 2010). Thus, understanding the detrimental impacts of inbreeding in small populations is crucial for conserving and managing endangered species (Ralls et al., 2018).

Inbred individuals (offspring of related parents) usually suffer from fitness reductions due to increased homozygosity at their genetic loci (Charlesworth & Willis, 2009). Two hypotheses explain why increased homozygosity and decreased heterozygosity negatively impact fitness (Charlesworth & Charlesworth, 1999; Charlesworth & Charlesworth, 1987; Keller & Waller, 2002). The first hypothesis, known as the partial dominance hypothesis, suggests that the reduction in fitness resulting from inbreeding is caused by the increased expression of recessive or partially recessive deleterious alleles due to increased homozygosity. In other words, the effects of recessive deleterious alleles are less likely to be masked by dominant alleles (Charlesworth & Charlesworth, 1999; Charlesworth & Charlesworth, 1987). For instance, a study of blindness in the Scottish population of red-billed choughs (*Pyrrhocorax pyrrhocorax*) pointed out that blind individuals are more homozygous than healthy birds, and the blindness is caused by the expression of recessive alleles which cannot be expressed when a dominant allele is present (Trask et al., 2016). Secondly, the overdominance hypothesis states that having two different alleles at a gene (i.e., being heterozygous at the gene) is more advantageous than having two identical copies (being homozygous). Some traits that enhance fitness are more commonly found in heterozygous individuals compared to either homozygous group (Charlesworth & Charlesworth, 1999; Charlesworth & Charlesworth, 1987). This phenomenon, referred to as "heterotic balance" or heterozygote advantage, describes a situation where heterozygous individuals exhibit superior fitness compared to homozygous individuals within a given population (Singh & Kulathinal, 2017). A well-known example of this theory is sickle cell disease (Anemia) in humans. Carriers of the sickle cell allele (heterozygous individuals with one copy of the sickle cell allele and one copy of the normal allele) have a lower life expectancy but exhibit some form of resistance to malaria. In contrast, homozygotes (individuals with two copies of the sickle cell allele) rarely reach age 50; they either have no malaria protection or have a significantly higher risk of developing sickle cell disease (Aidoo et al., 2002).

An individual's inbreeding level can be measured by the inbreeding coefficient (F) (Wright, 1922). When an organism is inbred, it is more likely that both alleles at a specific genetic locus are derived from the same ancestral allele, inherited from a common ancestor's DNA. This condition is known as being "identical by descent" (IBD) at that particular genomic location (Hartl & Clarck, 2007). Therefore, the inbreeding coefficient of an individual expresses the probability that both alleles at a given locus are identical by descent (Frankham et al., 2002; Wright, 1922). The traditional approach for estimating an individual's inbreeding coefficient is building a pedigree that identifies parents, offspring, and other types of relatives across multiple generations (Keller, 1998; Wright, 1922). The research on inbreeding depression in natural populations has been challenging due to the difficulty of conducting long-term studies and generating ambiguous data for building parent-offspring pedigrees (Pemberton, 2008). However, emerging genomic methods make it possible to measure the level of homozygosity of cross-sectionally sampled individuals, eliminating the need for extensive long-term pedigrees (Liberg et al., 2005). Accordingly, with the advent of innovative technologies, such as large-scale high-throughput molecular genetic methods, new approaches for estimating an individual's inbreeding coefficient (F) have emerged. These methods involve assessing the degree of identity by descent at genetic markers

within the individual's genome. By examining shared genetic information, estimating the realized level of inbreeding with greater precision and efficiency is now possible. In one such procedure, genetic markers with known locations in the genome are genotyped to find identical-by-descent segments of chromosomes (IBD tracts) (Kardos et al., 2016). The inbreeding coefficient (F_{ROH}) is a statistical measure that quantifies the proportion of an individual's genome contained within identical by descent (IBD) tracts. Specific criteria can be used to determine the extent of inbreeding, such as the number of single nucleotide polymorphisms (SNPs) within a run of homozygosity (ROH), the physical length of the ROH, or the SNP density. These criteria provide a means to differentiate recent inbreeding events from more ancient ones, allowing for a more nuanced understanding of the inbreeding history of individuals or populations (Chapman & Thompson, 2003; McQuillan et al., 2012; McQuillan et al., 2008; Purcell et al., 2007).

Inbreeding often affects life-history traits more negatively than morphological traits (DeRose & Roff, 1999). In birds, the most common fitness-related consequences of inbreeding are seen in life-history traits such as components of reproductive success, hatching success, fledging success, and offspring recruitment rate (Keller & Waller, 2002). Several bird studies have demonstrated the adverse effects of inbreeding on adult survival and reproduction. For instance, a metapopulation study of house sparrows (*Passer domesticus*) in northern Norway showed substantial negative consequences of inbreeding on the production of recruits and lifetime reproductive success (Billing et al., 2012; Niskanen et al., 2020). A study on great tits (*Parus major*) in the Netherlands showed that as the inbreeding coefficient of mothers increased, both hatching failure and the number of failed clutches increased (Van Noordwijk & Scharloo, 1981). Another study on red-cockaded woodpeckers (*Picoides borealis*) in the USA found that inbreeding-related reduced hatching success was a significant cause of declining population size (Daniels & Walters, 2000).

Furthermore, the relationship between hatching failure and inbreeding depression has been documented for great reed warblers (*Acrocephalus arundinaceus*) at an isolated breeding site in Sweden (Bensch et al., 1994), in song sparrows (*Melospiza melodia*) (Keller, 1998), and several other bird species. For example, a study on the hawaiian crow (*Corvus hawaiiensis*) showed that embryo survival and chick-fledging success declined with increasing inbreeding coefficients in mothers (Hoeck et al., 2015). Inbreeding depression can be amplified by harsh environmental conditions that affect individuals' survival and mortality. For example, a study on inbred song sparrows in British Columbia, Canada, found that after a winter storm, more outbred adults survived than inbred adults, who were more negatively affected by the harsh weather (Keller et al., 1994).

Many studies have demonstrated the adaptive significance of egg and clutch size in bird species (Fox, 1994; Järvinen & Väisänen, 1983; Krist, 2011; Kvalnes et al., 2018; Lack, 1947; Maddox & Weatherhead, 2008). Egg sizes have been shown to positively affect life history traits such as nestling size, nestling growth rate, and offspring survival rates by increasing access to nutrients for chicks and contributing to larger chicks' fitness benefits (see review by Williams, 1994). Larger egg size affects the size of offspring that supports their growth and survival, and larger nestlings are more likely to receive higher levels of parental care (e.g., food provision rates) (Maddox & Weatherhead, 2008). A review by Krist (2011) found that while, in general, large early-stage propagule size means extra resources for offspring, the effect of propagule size on individual growth and fitness varied at different life stages. The effect of egg size on offspring quality has been found for several species in different taxa, such as tree sparrows (*Passer montanus*) (Pinowska et al., 2004), alpine swift (*Tachymarptis melba*) (Bize et al., 2002), zebra finches (*Taeniopygia guttata*) (Wagner & Williams, 2007), brown trout (*Salmo trutta*)

(Einum & Fleming, 1999), european starlings (*Sturnus vulgaris*) (Smith & Bruun, 1998), seed beetle (*Callosobruchus maculatus*) (Fox, 1994) and in house sparrows (Kvalnes et al., 2018).

Larger clutch sizes are associated with reduced offspring body size and survival rate in several studies due to increased competition among nestlings for parental care and food provisioning (Baltz & Thompson, 1988; Clark & Wilson, 1981; Monaghan & Nager, 1997; Perrins, 1965; Reid et al., 2000; Smith et al., 1989). Also, according to life history theory, individuals have limited access to energy resources, leading parents to allocate these resources to different life cycle aspects. One important trade-off is between the number of offspring produced (clutch size) and the size of each offspring (egg size). Generally, when parents invest in producing more offspring (larger clutch size), they tend to allocate fewer resources to each offspring (smaller egg size) (Lack, 1947). In empirical studies from wild populations, egg and clutch size are generally found to be positively related to the survival rate and fitness of offspring in birds (Krist, 2011; Kvalnes et al., 2018; Pinowska et al., 2004; Williams, 1994). However, little knowledge exists from wild populations on how inbreeding may influence egg and clutch size variation.

In this study, I use data from a long-term study of a metapopulation of house sparrows in the Helgeland archipelago in northern Norway to investigate the effects of inbreeding on egg and clutch size. The house sparrow populations have been systematically monitored since 1993 using capture-mark-recapture/-resighting methods, where nearly all individuals have been marked with a unique combination of plastic color rings and sampled for blood for genetic analyses and parent-offspring determination so that detailed pedigrees could be developed (Niskanen et al., 2020; Ringsby et al., 2002). Routine weekly nest visits during the breeding season provide reproductive data, including basic information about the onset of breeding, clutch sizes, and measurements of egg sizes. Finally, extensive Single Nucleotide Polymorphism (SNP) genotype data allow the estimation of genomic inbreeding coefficients for all sampled birds in the system (Niskanen et al., 2020).

The house sparrow study system provides a unique opportunity with a long-term data set from wild populations to address central questions regarding the effects of inbreeding on individual parental fitness during the reproductive period in the wild. By testing the following hypotheses, this study aims to determine to what extent inbreeding impacts individual fitness through effects on early reproductive stages. My predictions are:

1. Females with a higher inbreeding coefficient are expected to lay smaller eggs than females with a lower inbreeding coefficient. As discussed above, inbreeding likely affects the mother's investment in the resources put into eggs, which will be reflected in the size of the eggs. The analyses will account for clutch size, as a negative relationship between clutch size and egg size has been previously reported (Kvalnes et al., 2013).
2. Females with higher inbreeding coefficients are expected to have smaller clutch sizes than females with lower inbreeding coefficients. Given a trade-off between egg size and clutch size, more inbred mothers may invest less in offspring by reducing their clutch size.

This study's findings may significantly impact conservation efforts, particularly in managing endangered or vulnerable populations with low population sizes where inbreeding depression may be severe. The results may contribute to knowledge-based decisions regarding breeding programs and population management methods by providing information on the consequences of inbreeding on reproductive traits. These findings can enhance conservation efforts to conserve genetic variation and increase population size by assessing the influence of inbreeding on egg size and clutch size.

2. Methods

2.1 Study species

The house sparrow is one of the planet's most widely geographically distributed animals. Its native distribution extends from the British Isles through northern Scandinavia, Europe, and northern Asia, all the way to the Pacific coast, and it has been intentionally introduced to other continents such as the Americas, Africa, and Australia (Anderson, 2006).

The birds reproduce and dwell near human settlements and can be found anywhere humans give food or have enough cultivated land to catch insects (Summers-smith, 1963). House sparrows show a high level of site fidelity, where a high proportion of juveniles (around 83%) remain in the natal population (Ranke et al., 2021). Remaining in the natal population makes them the perfect species for studying breeding behaviors (Ringsby et al., 1998). The main diet of adult house sparrows consists of seed, grain, and arthropods, while nestlings are mainly fed with arthropods at early ages (Anderson, 2006).

House sparrows are socially monogamous, meaning that once they form a pair bond, they stay together throughout the breeding season, and females generally use the same nest throughout the breeding season (Anderson, 2006; Summers-smith, 1963). However, polygamy is also common in this species (Anderson, 2006).

In northern Norway, the house sparrows lay up to three clutches during the breeding season (Ringsby et al., 1998; Summers-smith, 1963). Various variables influence the clutch size, and the most common size in most populations is three to six eggs, with the first clutch typically initiated in early May and the breeding season concluding in mid-August (Anderson, 2006). In a previous study of the same population, the average clutch size was 4.60 eggs per brood (Husby et al., 2006).

The eggs in a clutch typically hatch ca. 14 days after the first egg is laid, such that the incubation period usually lasts ca. 11 days after the third to fourth egg is laid. Chicks usually fledge ca. 14 days after hatching (Kvalnes et al., 2013; Muller, 2021).

2.2 Study area

The study system includes 18 islands in northern Norway's archipelago off the Helgeland coast (66° N, 13° E; Figure 1). Research in this house sparrow metapopulation was initiated in 1993 and focuses on ecological, evolutionary, and genetic processes, often with a conservation biology focus. The islands in the study system can be categorized into farm and non-farm habitat islands. In non-farm islands, the sparrows are free-living in and around gardens and houses in local villages without farms. However, the current project includes only farm islands, where house sparrows live near barns and cowsheds of dairy farms that provide good shelter, and food is available from cattle feed. Also, the sparrow nests are distributed inside the barn under the ceiling (Pärn et al., 2012). In the present study, egg size data was collected from five farm islands (Aldra, Gjerøy, Hestmannøy, Indre Kvarøy, and Nesøy) from 2003 to 2006. In addition, data from Hestmannøy was collected from 2007 to 2009.

2.3 Data collection

During the breeding period (early May to mid-August), nest boxes distributed among houses and in gardens, as well as natural nest locations in cavities inside barns and cowsheds on the islands, were searched for new active nests weekly, and active nests with eggs and/or chicks were checked two to three times during the incubation period and two to three times during the nestling period (Jensen et al., 2007). For each nest and visit, the number of eggs and chicks was counted and recorded (Billing et al., 2012).

2.3.1 Egg size and clutch size measurements

The size of each egg within a clutch was measured by a standardized photographic method and estimated to the nearest 0.1 mm³ for all completed broods, as described in Kvalnes et al. (2013). The maximum number of recorded eggs in the nest during egg laying and incubation was used to measure the clutch size for each brood in each nest (Kvalnes et al., 2013). When I refer to egg size in this study, the value was estimated as egg volume because other egg measures like mass (i.e., weight) will change during incubation, while egg volume is defined by the eggshell, which is constant after laying (for further details, see (Kvalnes et al., 2013).

2.3.2 Brood number

Nest history and genetic maternity information were used to determine the brood number (1st, 2nd, or 3rd) for each nest and breeding season (Husby et al., 2006). Because the house sparrows have high site fidelity, the same mother can be assigned to all the broods in a given nest within a breeding season. This procedure was applied where at least one of the broods had a genetically assigned mother unless genetic data indicated otherwise (Jensen et al., 2007; Kvalnes et al., 2013).

2.3.3 Genetic sample collection

At the first capture of an individual, a small blood sample (25µl) from the brachial vein was collected for genetic parenthood analysis. Adults and fledged juveniles were captured by mist netting, while fledglings were measured and marked at around 11 days in the nest. All individuals were marked with a unique combination of three plastic color rings and a numbered aluminum ring (provided by the Norwegian bird ringing center, Stavanger Museum) for later individual identification by resighting using a telescope (Kvalnes et al., 2013).

2.3.4 Maternal effects

Genetic maternity of a given brood was determined by genotyping nestlings and putative mothers on up to 13 microsatellite loci (regions of DNA that carry short repeating sequences of genetic code) using the polymerase chain reaction (PCR) technique on DNA from the blood samples collected from the house sparrows (see, e.g., Billing et al., 2012; Jensen et al., 2003; Stubberud et al., 2017). The resultant DNA

fragments were separated by electrophoresis (a process that separates DNA fragments depending on size) using a 16-capillary ABI 3130xl Genetic Analyzer, and the alleles (different size-variants of the same microsatellite locus) were scored using GeneMapper 4.0 software. The individual microsatellite genotype data was used to determine genetic links between offspring and parents using the software Cervus 3.0 (Kalinowski et al., 2007). These parentage analyses enabled the determination of the identity of the mother of a brood.

2.4 Estimation of inbreeding coefficient

To calculate individual inbreeding coefficients of mothers, individual genotype data on ca. 118,000 autosomal SNPs not in strong linkage disequilibrium was obtained, as described in Niskanen et al. (2020). Genomic inbreeding coefficients (F_{ROH}) were then calculated based on runs of homozygosity (Niskanen et al., 2020). Briefly, PLINK (Purcell et al., 2007) was used to extract homozygous sequence blocks, and the fraction of the genome inside these homozygous sequence blocks was used to estimate F_{ROH} . I had access to data with two sizes of runs of homozygosity (RoH), one with 2.5 MB and the other with 1 MB length of homozygous sequence blocks. I used the F_{ROH} based on a 2.5 MB long RoH to examine the effects of inbreeding. In other study systems, it has previously been shown that longer RoH had more significant fitness effects (a higher number of deleterious mutations) than short RoH (Stoffel et al., 2021). Moreover, Purfield et al. (2012) found that increasing RoH size improves the accuracy of inbreeding estimation based on F_{ROH} , especially for closely related individuals.

2.5 Statistical analyses

Between 2003 and 2009, data on egg sizes and clutch sizes were collected from the five farm islands (Aldra, Gjerøy, Hestmannøy, Indre Kvarøy, and Nesøy); however, the sample sizes differed among islands, as shown in Table 1. The data set included 238 broods from 132 mothers with a known F_{ROH} . In these broods, volumes were known for 1237 eggs.

Mean and total egg volumes of broods were normally distributed, and linear mixed-effect models (with Gaussian error structures) were fitted for these response variables.

First, I tested for the impact of mothers' inbreeding level on mean egg volume using three linear mixed-effect models fitted with the lmer function in R (Bates et al., 2015). The global model included the inbreeding coefficient, brood number, clutch size, and the interaction between the inbreeding coefficient and clutch size as predictor variables. In the first alternative model, I removed the interaction effect between the inbreeding coefficient and clutch size from the global model. In the second alternative model, I did not consider the influence of the inbreeding coefficient at all, so this model only included brood number and clutch size.

Second, to investigate whether mothers' inbreeding coefficient influenced the total investment of their clutch (i.e., the total volume of eggs in a brood), I fitted two linear mixed-effect models with the lmer function in R. In both models, the response variable was the total egg volume in a brood, determined by multiplying the clutch size by the average volume of eggs in a clutch. The first model included the inbreeding coefficient and brood number as predictors, while the second model only used brood number as a predictor.

Third, I analysed whether the clutch size variation was influenced by inbreeding by fitting generalized linear mixed models (GLMMs) with a Poisson error distribution (Bolker et al., 2009). Three models were fitted using Template Model Builder (GLMM TMB, family = genpois) (Magnusson et al., 2017). First, the global model included the inbreeding coefficient of the mother, brood number, mean-centered egg volume, squared value of mean-centered egg that represents the nonlinear relationship between egg size and clutch size, the interaction between inbreeding coefficient, and brood number as predictor variables. I omitted the interaction term in the second model, while in the third model, I also omitted the inbreeding coefficient. The mean egg volume data used for these three models were mean-centered and then divided by 1000, allowing us to rescale the variable to more manageable units. Mean centering is frequently used in statistical modeling to aid model convergence among predictor variables (Shieh, 2011).

To account for the expected negative correlation between clutch size and egg size, all models examining the effect of inbreeding on egg and clutch size variation, except for total egg volume models, included quadratic and linear terms for both clutch and egg size as fixed effects (Kvalnes et al., 2013). I included island, year, and mother identity as random factors in all my models without formal hypothesis testing for their significance, as their importance for variation in female reproductive investment or output was previously documented in the house sparrow study system (Kvalnes et al., 2013; Pärn et al., 2012; Sæther et al., 1999).

To compare the performance of different models and choose the best model given the data, I utilized the Akaike Information Criterion adjusted for small sample sizes (AICc). The AICc measures relative model fit, considering the number of model parameters, and lower values indicate better fit (Bedrick & Tsai, 1994).

For each model, I used the Nakagawa R^2 estimation method for mixed models to calculate how much of the variance in the response variable was explained by the model (Nakagawa et al., 2017). This method provides two types of R^2 : 1) Marginal R^2 , which shows the amount of variance explained by fixed factors only, and 2) Conditional R^2 , which indicates the amount of variance explained by fixed and random factors (Nakagawa et al., 2017).

The statistical analyses were conducted in R version 4.2.1 (R Core Team, 2022). For the linear mixed models, I used the lme4 package (Bates et al., 2015), and for the generalized linear mixed models, I used the glmmTMB package (Magnusson et al., 2017). The tidyverse package was used for data manipulation and visualization (Wickham et al., 2019). All the estimates from the models are given ± 1 standard error of the estimates. The "car" package was used for computing the Akaike Information Criterion (AICc) and other model selection criteria (Fox et al., 2012). All plots and visualizations were created using the "ggplot2" package (Wickham et al., 2016).

3. Results

Out of 238 broods, 193 broods (81.09%) had a mother with an inbreeding coefficient greater than zero (Fig. 2). The mean F value for mothers ($N = 108$) was 0.018 ± 0.002 (mean \pm standard error). The median F value was 0.008, ranging from 0 to 0.246. The mean inbreeding coefficient of mothers varied between islands, ranging from a mean of 0.011 ± 0.002 in Nesøy to a mean of 0.036 ± 0.012 in Aldra. (Table 1). Furthermore, there was considerable variation between years within islands in the average inbreeding level of mothers (Figure 3). The overall mean egg volume for all the mothers was $2835.743 \text{ mm}^3 \pm 13.864$, whereas the mean clutch size was 5.226 ± 0.053 . The average egg volume was similar across islands, whereas there was some variation between islands in average clutch size (Table 2). Some of this spatial variation could be due to differences in the number of mothers and broods between islands; the island Hestmannøy had data on 88 mothers, whereas data was only available for three mothers on Aldra, and after 2006, only data from Hestmannøy was available (Figure 4).

3.1 The effect of inbreeding on egg size

3.1.1 The effect of F on the average egg volume

Evaluating the candidate models testing for an effect of mothers' inbreeding level on egg size revealed that the model that received the highest support in the data according to AICc did not include the effect of inbreeding and deviated with $\Delta\text{AICc} > 2$ (Table 3a). Thus, the highest-ranked model had a substantially higher AICc weight than the second. Also, there was no evidence for a relationship between the inbreeding coefficient F of mothers and the average egg volume ($\beta = -181.30$, $CI[-1237.655, 866.969]$) in the model that accounted for both the linear and nonlinear effect of clutch size and the declining egg volumes in brood 2 and 3, respectively (Table 4, Figure 5). This final model had a marginal R^2 of 0.134, suggesting that 13.4% of the variation in mean egg volume in the study area was explained by fixed effects only.

3.1.2 Effect of F on the total egg size of each brood

When analyzing the variance in total egg volume of each brood, the model with the highest support, according to AICc, did not include the effect of mothers' inbreeding (Table 3b). Also, the first-ranked model received a higher AICc weight than the second-ranked model (Table 3b), and the second-best model deviated by 1.83 in AICc. However, there was no evidence of a relationship between mothers' inbreeding level and the total egg volume broods in the second-ranked model ($\beta = 3064.9$, $CI[-7802.451, 13777.355]$, Table 5). There was, however, a decrease in egg volume from the first and third brood (see Table 5, Figure 6). This final model had a marginal R^2 of 0.009, suggesting that only 0.9% of the variation in total egg volume in the study area was explained by fixed effects only.

3.2 Effect of F on the size of the clutches

When analyzing the variance in clutch size of each brood, the model with the highest support, according to AICc, did not include the effect of the inbreeding level of mothers (Table 3c). According to the second-best model, which included mothers' inbreeding level, there was no significant relationship between the inbreeding level and the clutch size of each brood ($\beta = 0.329$, $CI[-0.324, 0.982]$) (Table 6, Figure 7). This final model had a marginal R^2 of 0.047, suggesting that 4.7% of the variation in clutch size in the study area was explained by fixed effects only.

4. Discussion

There was no evidence of inbreeding depression in egg size and clutch size, indicators of maternal investment, in five island populations of house sparrows off the coast of northern Norway. However, this was in contrast with the prediction that inbreeding in mothers would have a negative impact on their reproductive investment. Several studies have found that inbred females lay smaller clutches, including butterflies (Saccheri et al., 1996), rainbow trout (*Oncorhynchus mykiss*) (Su et al., 1996), kakapo parrot (*Strigops habroptilus*) (White et al., 2015), japanese quails (*Coturnix japonica*) (Sittmann et al., 1966), and a study conducted on white leghorn chickens (*Gallus gallus domesticus*) (Sewalem et al., 1999).

Although there have not been many studies on the adverse effects of inbreeding on egg size, a study of house sparrows in North America found that both clutch and egg size were significantly positively related to heterozygosity, which may be a proxy for the inbreeding level where lower heterozygosity indicates more inbred individuals (Wetzel et al., 2011).

Considering that maternal investment positively affects other fitness-related life history traits (Williams, 1994), my study's findings are contradictory to the findings of several other studies in the wild that found a negative effect of inbreeding on fitness traits (Bensch et al., 1994; Billing et al., 2012; Crnokrak & Roff, 1999; Daniels & Walters, 2000; Hedrick & Kalinowski, 2000; Hemmings et al., 2012; Ihle et al., 2017; Jensen et al., 2007; Keller & Waller, 2002; Liberg et al., 2005; Niskanen et al., 2020; Spottiswoode & Møller, 2004; Van Noordwijk & Scharloo, 1981).

Even though this study did not document any significant effects of inbreeding depression on the egg size and clutch sizes of the house sparrows, other studies in the same system showed that inbreeding could have detrimental impacts on the production of recruiting offspring (Billing et al., 2012; Niskanen et al., 2020) and recruitment rate of fledgling house sparrows (Jensen et al., 2007).

The finding that there was no evidence of inbreeding of mothers affecting their investment in the size of eggs and clutch size is surprising given the negative effect of parental inbreeding levels on the production of recruits previously documented in the study system (Billing et al., 2012; Niskanen et al., 2020). Recruit production includes three stages: propagule production, survival of eggs and nestlings to fledging, and recruitment of fledglings into the adult population, which all depend on several factors, including maternal and environmental factors (Gaillard et al., 2008). Maternal investment in egg size has been found to positively affect the first stage of recruit production; producing heavier/larger eggs leads to more recruits (Gaillard et al., 2008). One would expect egg size and clutch size to follow the same pattern as annual recruit production if inbreeding were impacting the overall reproductive success; however, it does not necessarily imply the absence of negative effects of inbreeding on other fitness traits of house sparrows because the negative effect of inbreeding on single life history traits (e.g., number of eggs) may be difficult to detect (Szulkin et al., 2007).

One possible point of research will be to see if inbreeding impacts the composition of eggs. When maternal hormones are transported into the eggs, they contribute to embryonic composition and can impact embryo development, eventually affecting the offspring's phenotype and growth (Ruuskanen, 2015). Egg weight is essential for determining egg composition and nutritional value, as heavier eggs have more yolk and albumin, the key nourishment suppliers for the growing chick (Chen et al., 2016).

As a result, egg weight can impact chick quality, an essential factor in determining survival, development, and health (Ulmer-Franco et al., 2010). A study on rainbow trout found that inbred females had lower total egg mass (egg number multiplied by egg weight) than outbred females, indicating lower maternal investment (Kincaid, 1983). On the other hand, some studies suggested that egg composition is more important than egg size (Nager et al., 2000; Reed et al., 2009).

The Niskanen et al. (2020) and Jensen et al. (2007), and Billing et al. (2012) findings may also be explained by the length of time that parents spend incubating their eggs. Parental incubation is critical for offspring survival, and studies have shown that longer incubation periods improve the chances of successful hatching (Deeming, 2002). This has been observed in various birds such as peregrine falcons (*Falco peregrinus*) (Burnham, 1983), whooping cranes (*Grus americana*) (Smith et al., 2011), and brown kiwis (*Apteryx australis*) (Robertson et al., 2006). Given the high energy cost of incubation (Tinbergen, 2002) and the potential for inbred parents to have poor body conditions (Knaepkens et al., 2002), maternal inbreeding is likely to influence the incubation duration. Notably, previous studies have shown that inbreeding depression significantly affects the attentiveness of incubation in zebra finches (Pooley et al., 2014). In the study by Hoeck et al. (2015) on the hawaiian crow, there was a negative correlation between the inbreeding level and parental incubation time, which may affect the offspring's survival and fitness.

A study by Pilakuota et al. (2015) showed that in addition to being crucial for offspring's survival and development in beetles, parental care could indirectly protect against the harmful consequences of inbreeding depression by providing offspring the extra resources they need to cope with environmental challenges. Providing maternal care significantly increases the survival rate and extends the lifespan of inbred offspring compared to their outbred counterparts; the care provided by parents not only has a positive effect while offspring are dependent but also carries over into their independent adult lives (Pilakouta et al., 2015). Accordingly, another explanation for lower recruit production of inbred parents previously found in my study system (Billing et al., 2012; Niskanen et al., 2020) might be deleterious effects of inbreeding on parental behavior, as providing food and care for offspring is energetic and costly for parents (Deeming, 2002); so if inbreeding negatively affect parental energy, it can affect the survival of nestling till fledgling and post-fledging stages. The ability of fledglings to survive and recruit is closely tied to their body mass at the late nestling stage, which has been positively associated with parents' food provisioning (Magrath, 1991; Schwagmeyer & Mock, 2008). Given that bird's offspring rely on their parent's care due to their inability to forage independently, begging behavior serves as a means for young birds to express their needs, including hunger, and elicit increased parental care (Budden & Wright, 2001). Research on canaries (*Serinus canaria*) suggests that the genes responsible for low begging levels may be recessive, with individuals possessing two copies of the recessive gene exhibiting minimal begging behavior (Fresneau et al., 2020). Begging behavior consumes energy and raises the metabolic rate in offspring (Bachman & Chappell, 1998). Thus, if offspring become weakened due to the detrimental effects of inbreeding, it may impact their ability to exhibit effective begging behavior.

Despite the importance of parental care in reducing the negative consequences of inbreeding depression, studies on the direct influence of inbreeding on parental behavior and the subsequent implications on offspring survival are scarce. As a result, there is a pressing need for additional research to investigate

this relationship in greater depth. Future studies should try to fill this knowledge gap by examining the effects of inbreeding on parental behavior and the implications for offspring survival and lifespan.

In my study, I did not investigate whether inbred mothers produced inbred offspring. However, it is essential to consider that if inbred parents produce inbred offspring, the negative effects of inbreeding on their fitness traits could potentially explain the disparity in results observed between this study and previous studies in the same study system.

Overall, producing offspring and ensuring their survival requires high energy investments from parents, so if inbreeding negatively affects parents, it can impact the number of recruits they can produce.

Another possible explanation for the disparity of the results can be the differences in sample sizes, islands included, and years covered by my study and the other studies on inbreeding depression in the same house sparrow metapopulation. For example, the Niskanen et al. (2020) study had access to an extensive dataset that included information on the inbreeding levels, survival, and recruit production of 3,116 individuals (90% of the adult house sparrow population). Also, the data were from a longer period (from 1998 to 2013) than the current study. The mean was higher, and the range of F_{ROH} was wider in Niskanen et al. (2020); F_{ROH} ranged from 0.000 to 0.363. Similar differences in the inbreeding range (0.000 to 0.344) and data set duration (1993-2004) can be seen in the research of Jensen et al. (2007). Also, in the study of Billing et al. (2012), data set duration (1998-2008) and inbreeding range (0.06-0.10) showed differences.

There could also be reasons related to environmental conditions that reduced or removed any negative impacts of inbreeding on egg size and clutch size in my study.

During the breeding season, the maternal investment may be limited by higher energy requirements for thermoregulation or food scarcity due to cold weather (Fox & Czesak, 2000; Järvinen & Väisänen, 1983; Nager et al., 2000; Nager & Van Noordwijk, 1992; Ojanen, 1983; Williams, 1996). Accordingly, Kvalnes et al. (2013) reported that the weather conditions two weeks before producing eggs affected the eggs' size positively and the size of the clutch in the same house sparrow study system. Thus, the largest eggs were found in the middle-temperature range (Kvalnes et al., 2013). The impact of weather on the size of eggs and clutches has not been considered in this study. Accordingly, the lack of relationship between the maternal level of inbreeding and egg size in the present study might have been concealed because the pre-laying weather conditions or other unknown environmental factors were not included as explanative factors.

Numerous studies found that the magnitude of inbreeding depends on the environmental condition of the populations (Fox & Reed, 2011). A more challenging environment can exacerbate any negative consequences of inbreeding on fitness traits (Armbruster & Reed, 2005; Fox & Reed, 2011; Frankham et al., 2002). The lack of inbreeding depression on maternal investment in this study might be due to a possible improvement of habitat quality, potentially mitigating the negative effects of inbreeding on reproduction. Previous research has demonstrated the detrimental impact of inbreeding on reproduction in various wild populations (Fang & Li, 2023; Keller & Waller, 2002; Sittmann et al., 1966; Van Noordwijk & Scharloo, 1981). Interestingly, the recorded increase in island population sizes within the house sparrow study system from 2004 to 2012 (Baalsrud et al., 2014) suggests favorable ecological and

weather conditions (Saatoglu et al., 2021). It is well-established that good quality habitat positively affects egg size and clutch size in avian species by increasing food availability (Christians, 2002).

Consequently, any improvement in the abundance and quality of food resources may mask any negative impacts of inbreeding on egg and clutch sizes. Although research explicitly investigating the effect of food availability on inbreeding depression for egg size and clutch size is currently lacking, studies conducted on cactus finches (*Geospiza scandens*) and medium ground finches (*Geospiza fortis*) have revealed that inbreeding depression has a substantial impact on the survival and production of individuals only when food is scarce. The results showed more breeding failure for inbred parents in low-quality habitats with less food availability (Keller et al., 2002), which can be a sign of smaller egg size since the hatching success of small eggs is lower than medium and large eggs (King'ori, 2011). The hypothesized enhanced food supply and better habitat quality in my study may have led to the failure to detect the negative effects of inbreeding on egg and clutch sizes. This hypothesis gains support from the higher mean clutch size observed in this study (5.22) compared to a previous estimate in the same house sparrow study system (4.6) obtained by Husby et al. (2006). The larger average clutch size suggests a suitable habitat and favorable temperature conditions before egg laying for house sparrows, as Kvalnes et al. (2013) indicated.

Another factor that may affect inbreeding depression is the population's demographic characteristics, i.e., population size (Angeloni et al., 2011). While the island population sizes have fluctuated over the years, there was an overall increase during our study period (see Figure 2 in Baalsrud et al., 2014). An increase in the population size increases the number of available partners, automatically providing more opportunities to avoid inbreeding (Billing et al., 2012). A recent investigation by Ghimire (2022) on the impact of inbreeding on recruitment probability in the same study system showed that, although the researchers were unable to identify the impact of inbreeding depression on juvenile recruitment in the period from 2003 to 2013, inbreeding depression had a substantial influence on recruitment probability in the period from 1994 to 2002. This suggests that inbreeding depression influenced certain features in the past but was not expressed in some traits during the more recent research period. Consequently, the increase in population sizes in the house sparrow populations during the period of the present study (2003-2009) might have contributed to reducing any negative effects of inbreeding depression in the population, as more unrelated individuals were present for reproduction that may have reduced the individual inbreeding levels, which in turn could have reduced the power to detect negative inbreeding effects as the most inbred individuals that potentially drive any inbreeding depression were lacking from the data set. Furthermore, Ranke et al. (2021) documented that an increase in the mean population size of farm islands resulted in a higher influx of immigrant recruits. In other words, farm islands with larger population sizes tend to attract more individuals to join (Ranke et al., 2021). Additionally, studies on the house sparrows' dispersal patterns revealed yearly variations, with different numbers of birds emigrating and immigrating between subpopulations and islands (Saatoglu et al., 2021). Notably, Hestmannøy, characterized by low dispersal rates, experienced an increase in population size and a potential influx of immigrants, which may have contributed to the absence of detectable harmful effects of inbreeding depression on egg size and the population's low inbreeding coefficient (Bertorelle et al., 2022; Saatoglu et al., 2021).

According to Baalsrud et al. (2014), the population size of each island varies, with some islands having larger populations than others. Furthermore, there is also variability in population size between farms within each island at Gjørøy and Hestmannøy. This local disparity in population size is believed to influence the degree of inbreeding and subsequent inbreeding depression observed within the population

(Jensen et al., 2007). As was done in this study, the island was included as a random factor in the mixed models, thereby accounting for any differences in means and variances of egg size and clutch size between islands.

Large and small populations can experience different levels of inbreeding for several reasons. While inbreeding can be more frequent in small populations due to the greater likelihood of relatives mating, inbreeding depression can also be less severe in small populations due to purging. Purging could be more successful in small, inbred populations since there is more chance for selection against homozygotes for harmful genes (Charlesworth & Charlesworth, 1999). Recessive harmful alleles, on the other hand, are often at low frequencies in large populations and thus rarely arise in a homozygous state. Due to their rarity, natural selection is less successful in removing these harmful alleles from large populations. These alleles are not fixed because of the reduced impact of genetic drift and more efficient selection in big populations (Wang et al., 1999). As a result, these deleterious alleles remain concealed in the population, making natural selection harder to eliminate them. Despite the expectation that there may be more inbreeding depression in a larger population and less opportunity for purging like Hestmannøy, there was a lack of detectable effect, which could be partly due to the fact that inbreeding is so rare that the effects are difficult to observe.

Although the size of local island populations is unlikely to cause significant changes in the selection against inbred individuals, previous studies have indicated that inbreeding has a more significant impact on the average fitness of smaller populations. This suggests that although the strength of inbreeding depression may remain constant, its influence on population fitness varies depending on population size (Niskanen et al., 2020).

I found that the brood number is a crucial factor affecting egg and clutch size, suggesting that in birds with multiple broods, this should be included in the statistical models when studying the relationship between egg and clutch size and other reproductive parameters (Kvalnes et al., 2013). Such findings have also been documented for many avian species (Christians, 2002). The present study showed that being born in a later brood could negatively affect the investment offspring receive from mothers through egg size; the eggs belonging to the second and third broods were significantly smaller than eggs in the first brood (Table 4).

Empirical studies show that variation in egg size is influenced by the mother's body size, which is often found to positively impact egg size (Järvinen & Väisänen, 1983). Also, the mother's age can affect the maternal investment; reproductive investment per egg is predicted to remain constant, increase, or decrease with age, according to theoretical studies (Roff, 2002). In a study of house sparrows in the same metapopulation, Kvalnes et al. (2013) found that there was a positive relationship between the mother's body mass and the size of the eggs, while the trade-off between the egg size and the clutch size was present. Also, the finding of this study verified that the mother id significantly affects both clutch sizes and egg size.

Also, as I did in my study, the spatio-temporal effects of island and year should be considered while analyzing the egg and clutch size variation. Previous studies have reported significant variation in the reproductive rates of house sparrows across islands and years in my study metapopulation (Sæther et al., 1999). Island habitat type has been identified as an essential factor affecting the timing of breeding start

and the yearly dispersal rate of the house sparrows (Pärn et al., 2012). Additionally, there are significant differences in the average hatch day of the first clutch, fledgling size, number of fledglings per starting clutch, and fledgling mortality rate between years and islands (Ringsby et al., 2002). Similarly, a part of the variation in egg size is explained by the differences between years (Kvalnes et al., 2013).

Despite high capture and recapture rates observed in all the study islands (Ranke et al., 2021), some nests were inaccessible and hence lacked any information, and there was a considerable number of broods with egg size and clutch size data that had unknown mothers and hence no information on the mothers' inbreeding coefficient. The exclusion of these broods could have introduced bias into the study if the excluded data differed systematically from the included data in ways that could impact the analyses. For example, if the data excluded from the analysis had a higher mean or variance in inbreeding coefficient or egg and clutch size, this could potentially affect the relationships between these parameters. To minimize the impact of excluding this data, assessing whether the excluded data significantly deviates from the included data regarding critical variables such as inbreeding coefficient, egg size, clutch size, or any other relevant factors is crucial. However, the mean egg volume for each nest in the two datasets does not differ significantly. The initial dataset, consisting of 515 nests, had a mean egg volume of 2839.929 ± 10.423 (mean \pm standard error). After excluding certain data points, the revised dataset of 238 nests showed a mean egg volume of 2535.743 ± 13.864 . The T-test conducted to compare the mean egg volume before and after excluding data resulted in a p-value of 0.809, indicating no significant difference in the mean egg volume between the two datasets. There was marginal evidence suggesting a significant difference between the two datasets regarding the clutch size. The mean clutch size for the initial dataset of 515 nests was 5.095 ± 0.041 (mean \pm standard error), while the other dataset of 283 nests had a mean clutch size of 5.226 ± 0.053 . The T-test performed to compare the mean clutch size values yielded a p-value of 0.050, suggesting a possible significant difference in mean clutch size between the datasets. However, it is essential to note that further investigation or larger sample size is recommended to draw more conclusive results.

Overall, these findings highlight the importance of careful analysis and consideration of sample sizes when examining egg volume and clutch size, such as in this study.

Nevertheless, there are indications of systematic differences; it might be necessary to account for this potential bias in the analysis or consider alternative strategies to include or address the missing data. Ultimately, it is crucial to acknowledge and discuss the potential impact of missing data on the analysis and take appropriate steps to address potential biases.

In this study, I used the F_{ROH} based on a 2.5 MB long RoH to model the effect of inbreeding. Longer ROH is often the consequence of more recent parental relatedness, whereas shorter ROH is typically the product of earlier haplotype relatedness (Kristensen et al., 2010). To comprehensively understand the effect of inbreeding on the egg and clutch size, I investigated the F_{ROH} on a 1 MB threshold to determine if the result obtained using individual levels of inbreeding over longer time spans differed by considering a shorter ROH-threshold. The mean F_{ROH} with a 1 MB threshold for the same mothers (N = 238) was 0.028 ± 0.002 (mean \pm standard error). The median F value was 0.017, ranging from 0 to 0.263. For the F_{ROH} based on 2.5 MB, the mean F value of the mothers was 0.018 ± 0.002 . The median F value was 0.008, ranging from 0 to 0.246. This result shows that considering F_{ROH} on 1MB, all the

individuals have an inbreeding coefficient greater than zero (Figure A1). However, by analyzing all the models using F_{ROH} on a 1 MB, the result did not show any evidence of the effect of inbreeding on the mean egg volume ($\beta = -217.414$, $CI[-1220.477, 777.781]$), total egg volume ($\beta = 3540.2$, $CI[-6849.929, 13790.353]$) and the clutch size ($\beta = 0.355$, $CI[-0.282, 0.994]$).

Moreover, the absence or deficit of data from islands or years with high inbreeding maternal coefficients (see, e.g., Niskanen et al., 2020) may explain why the present study did not find a negative relationship between inbreeding depression and egg and clutch size. Currently, the only data available after 2006 is from Hestmannøy, which is a large population with a relatively low mean inbreeding coefficient (0.017 ± 0.002) (mean \pm standard error), whereas the available data from the study islands with higher levels of inbreeding was low (Table 1). As a result, despite large overall sample sizes, the statistical power needed to document strong evidence for any negative impact of inbreeding may have been relatively low. Increasing the sample sizes, especially for mothers with higher inbreeding coefficients, might reveal different patterns than those documented in my study.

A deeper understanding of how ecological and evolutionary processes interact and influence each other, including the causes and consequences of inbreeding in small populations, is critical for conservation management since such knowledge is essential to minimize the risk of extinction. My current research project, however, may be unable to properly document such trends since it relies on restricted information with a restricted variance in the inbreeding coefficient. As a result, more extensive studies are needed to understand better the role of inbreeding in fragmented populations and their influence on population survival. Also, as the importance of egg weight mentioned above, for further research focusing on inbreeding effects on maternal investment, I recommend measuring the weight of eggs, which includes the weight of the eggshell, yolk, and albumen, in addition to clutch size. The study's results may have been influenced by the favorable environmental conditions in which it appears the study was conducted. To examine the generality of my findings, it would be necessary to replicate the study in different or more challenging environmental conditions that are less favorable to the group of individuals being examined. The best strategy to investigate the influence of inbreeding on egg size and clutch size would be to conduct experimental research that allows us to control for other factors and potentially reveal casual relationships that cannot be verified in correlational studies like the present study.

Understanding the possible impacts of inbreeding on the mother's and offspring's behavior may illuminate the mechanisms causing the reduced recruitment probability and lower recruit production of inbred birds in the same study system. Information on any negative effects of inbreeding on maternal investment can help conservation efforts by offering insights into the possible dangers of inbreeding in small populations and directing management techniques to avoid such risks.

5. References

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6. Tables

Table 1. Overview of the number of mothers, broods, and the mean (± 1 standard error), median, and range of mothers' inbreeding coefficients across five farm islands.

island	Years with data	No. mothers	No. broods	Mean F \pm SE	Median F	Range F
Aldra	2003-2006	3	3	0.036 \pm 0.012	0.027	0.020-0.061
Gjerøy	2003-2006	18	36	0.022 \pm 0.008	0.007	0-0.246
Hestmannøy	2003-2009	88	163	0.017 \pm 0.002	0.008	0-0.109
Indre Kvarøy	2003-2006	10	13	0.024 \pm 0.011	0.012	0-0.157
Nesøy	2003-2006	13	23	0.011 \pm 0.002	0.008	0-0.044

Table 2. Overview of egg volume and clutch size characteristics across five farm islands in a house sparrow metapopulation in northern Norway during the breeding seasons of 2003-2009. Information on the number of broods and the number of eggs in these broods is also shown.

Island	Number of broods	Number of eggs	Mean egg volume \pm SE	Mean clutch size \pm SE
Aldra	3	14	2.855 \pm 193.7	4.67 \pm 0.333
Gjerøy	36	186	2.864 \pm 37.9	5.22 \pm 0.120
Hestmannøy	163	842	2.839 \pm 16.8	5.25 \pm 0.065
Indre Kvarøy	13	63	2.811 \pm 52.9	4.92 \pm 0.348
Nesøy	23	122	2.784 \pm 39.5	5.30 \pm 0.117

Table 3. AICc ranking table for all models fitted to test for any effects of inbreeding on variation in mean egg size, total egg volume in a brood, and clutch size in an insular metapopulation of house sparrows off the coast of northern Norway during the breeding seasons of 2003-2009. The best models based on AICc describing variation in egg size and clutch size are shown in bold. In all the models mother's identity, year, and island were included as random factors. Other explanatory variables included are inbreeding coefficient F (F_{ROH}), CS=clutch size, BN=brood number, TEV=total egg volume, and MES=mean centered egg size). The AICc score relative to the highest-ranked model ($\Delta AICc$), Akaike weight (AICc wt), and evidence ratio (ER) are listed for each model.

Rank	Model terms	AICc	$\Delta AICc$	AICc wt	ER	
Response variable: mean egg volume within the brood						
(a)	1	CS +CS² + BN	3187.656	0.00	0.614	1.00
	2	F + CS + CS ² + BN	3189.720	2.063	0.219	0.356
	3	F * CS +CS ² + BN	3190.270	2.613	0.166	0.270
Response variable: total egg volume within the brood						
(b)	1	BN	4400.292	0.00	0.713	1.000
	2	F + BN	4402.121	1.829	0.286	0.400
Response variable: clutch size						
	1	MES + MES²+BN	587.935	0.000	0.55	1.000
(c)	2	F + MES + MES ² +BN	589.123	1.187	0.306	0.552
	3	F * MES + MES ² + BN	590.709	2.774	0.138	0.249

Table 4. Parameter estimates from the best model that included the mother's inbreeding coefficient F (i.e., the one ranked second in the AICc comparison of linear mixed effect models) explaining variation in the average egg volume laid in first, second, and third broods in an insular metapopulation of house sparrows in an archipelago located off the coast of northern Norway during the breeding seasons of 2003-2009. The model included the mother's identity, year, and island as random factors.

parameter	Estimates \pm SE	Confidence intervals	
		2.5%	97.5%
F	-181.30 \pm 529.66	-1237.655	866.969
Clutch size	135.22 \pm 103.85	-69.232	341.949
Clutch size ²	-13.56 \pm 10.05	-33.628	6.2435
2 nd Brood	-78.14 \pm 21.96	-121.449	-33.885
3 rd Brood	-141.89 \pm 37.50	-216.900	-67.958
Random variances			
σ^2 Mother	27256.5		
σ^2 Year	635.1		
σ^2 island	0		

Table 5. All parameter estimates from the models ranked second in the AICc comparison of linear mixed effect model using the inbreeding coefficient to explain variation in the total egg volume laid in the nests in an insular metapopulation of house sparrows in an archipelago located off the coast of northern Norway during the breeding season of 2003-2009. The fixed effects included brood number, and the model included mother, year, and island as random factors.

parameter	Estimates \pm SE	95% Confidence intervals	
		lower	upper
F	3064.9 \pm 5442.9	-7802.451	13777.355
2 nd Brood	175.6 \pm 328	-469.876	823.488
3 rd Brood	-1181.1 \pm 565.3	-2296.066	-60.217
Random variance			
σ^2 mother	775660		
σ^2 year	0		
σ^2 island	0		

Table 6. All parameter estimates from the models ranked second in the AICc comparison of the GLMM model using the inbreeding coefficient to explain variation in the size of the clutch laid in the nests in an insular metapopulation of house sparrows in an archipelago located off the coast of northern Norway during the breeding season of 2003-2009. The fixed effects also included mean-centered egg volume and brood number. The model also included mother, year, and island as random factors.

parameter	Estimates±SE	95% Confidence intervals	
		lower	upper
F	0.329±0.333	-0.324	0.982
Mean-centered egg volume	-0.064±0.048	-0.158	0.030
Mean-Centered Egg Size Squared	-0.239±0.147	-0.528	0.049
2 nd Brood	0.037±0.019	-0.000	0.076
3 rd Brood	-0.035±0.035	-0.104	0.034
Random variance			
σ^2 mother	0.002		
σ^2 year	0.000		
σ^2 island	0.000		

7. Figures

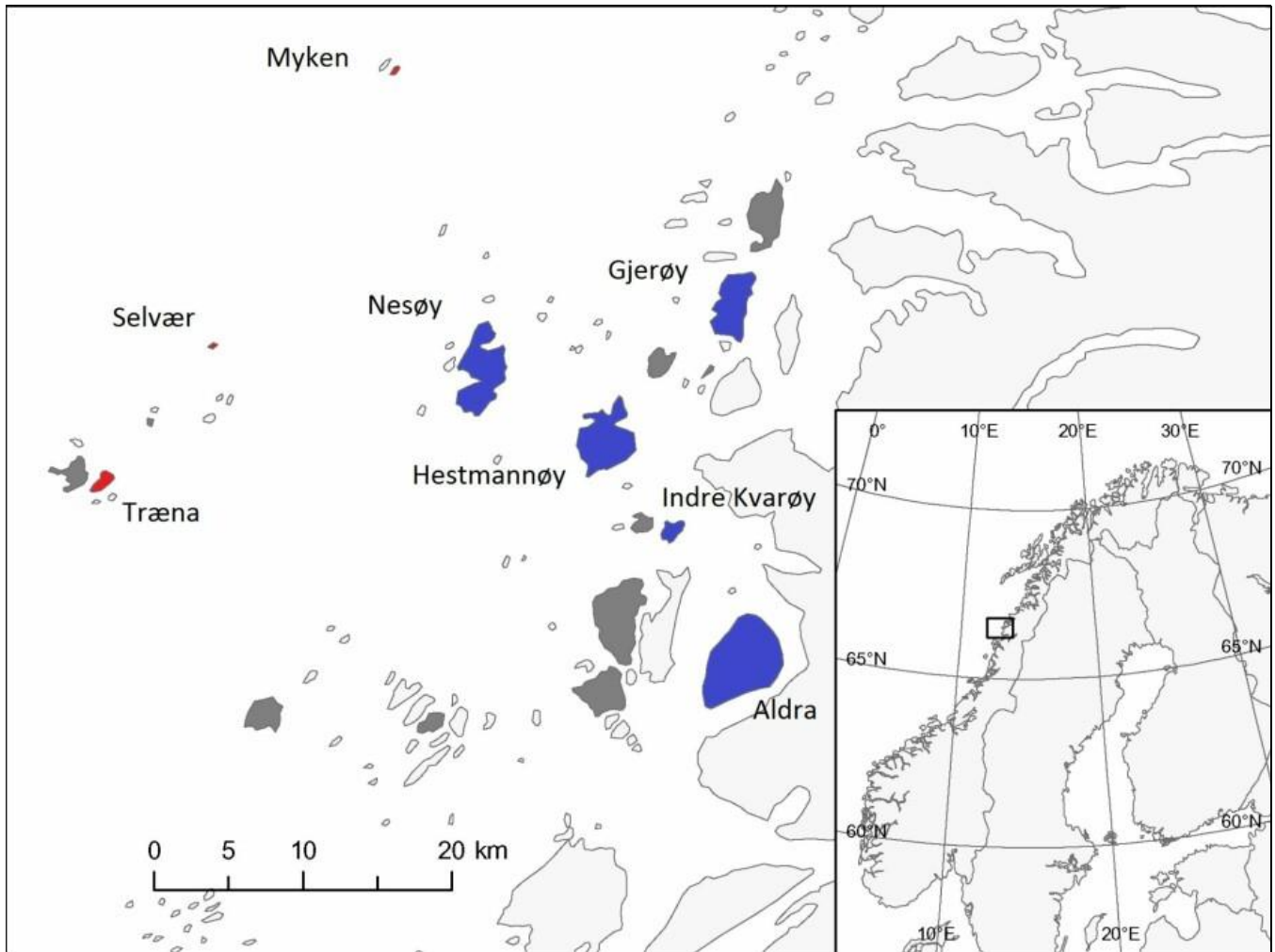


Figure 1. Map of the house sparrow study metapopulation showing the five study islands in blue and its location within Norway in the insert map. The five islands where sampling took place during the breeding seasons from 2003 to 2009 were Aldra, Gjerøy, Hestmannøy, Indre Kvarøy, and Nesøy. “The map was modified from Fig. 1 in Jensen et al. (2007).”

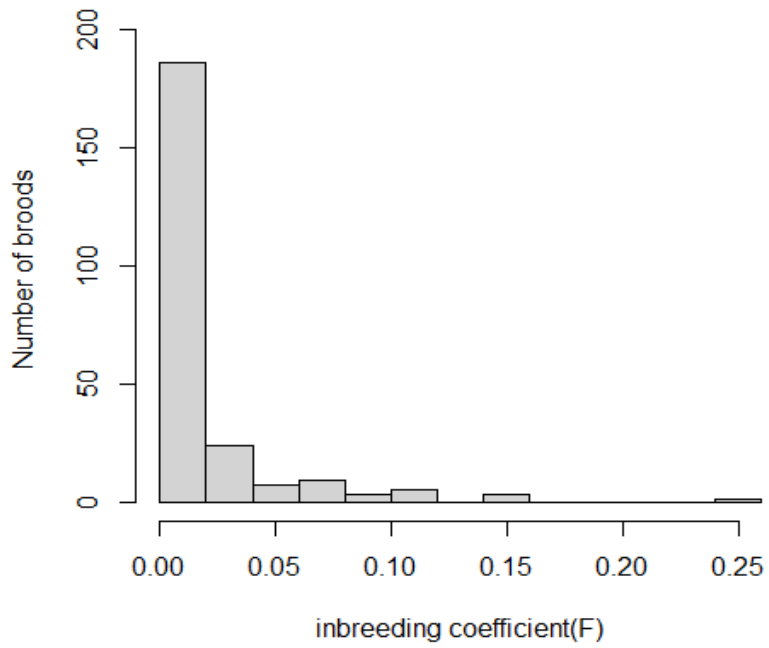


Figure 2. The inbreeding coefficient of the broods in a metapopulation of house sparrows across five islands in an archipelago located off the coast of northern Norway in the breeding seasons from 2003 to 2009.

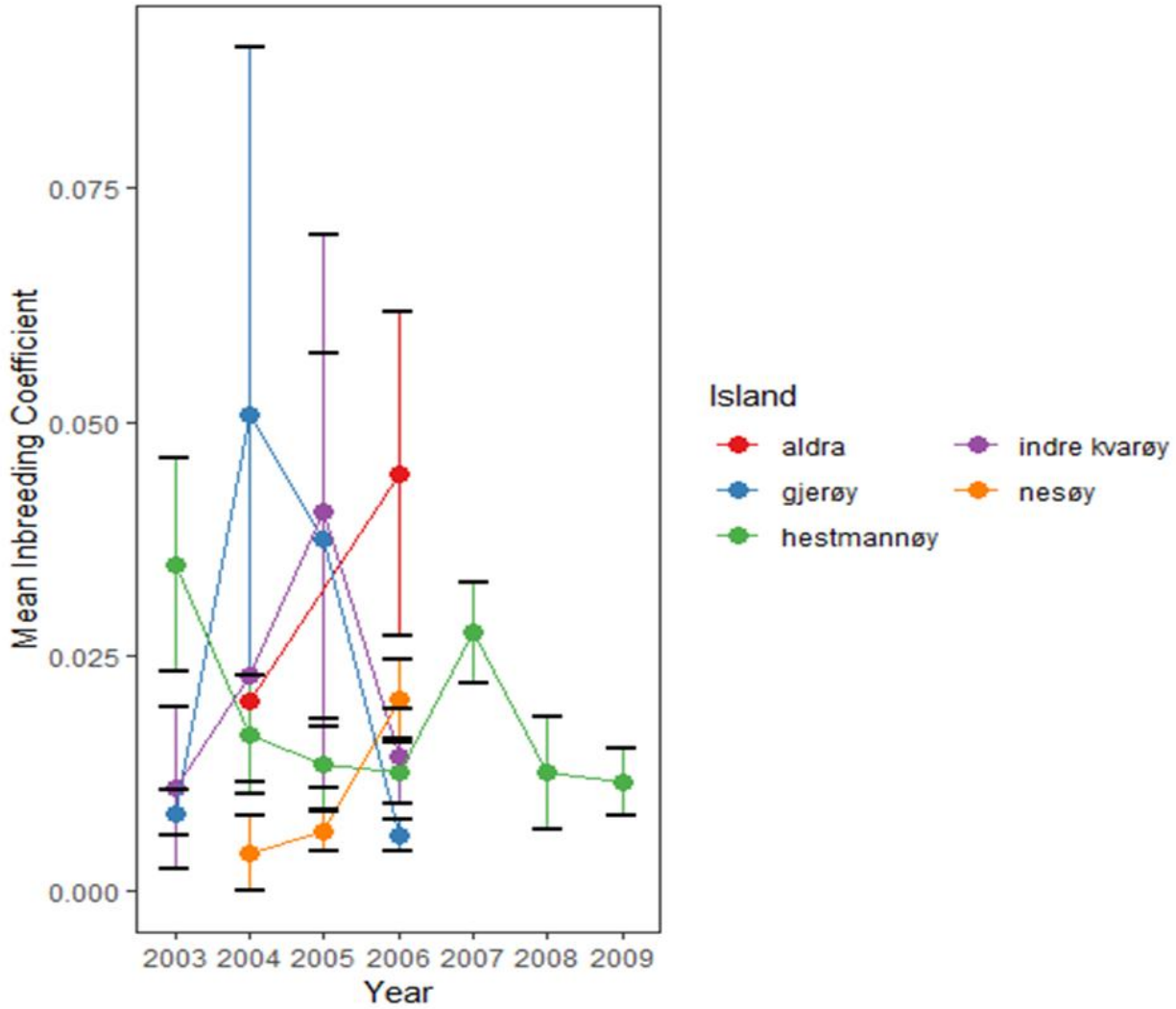


Figure 3. The mean inbreeding coefficient of the mothers of broods (± 1 standard error) for each year in a metapopulation of house sparrows across five islands in an archipelago located off the coast of northern Norway in the breeding seasons from 2003 to 2009. The points represent the mean inbreeding coefficients, and the lines represent ± 1 standard error of the mean.

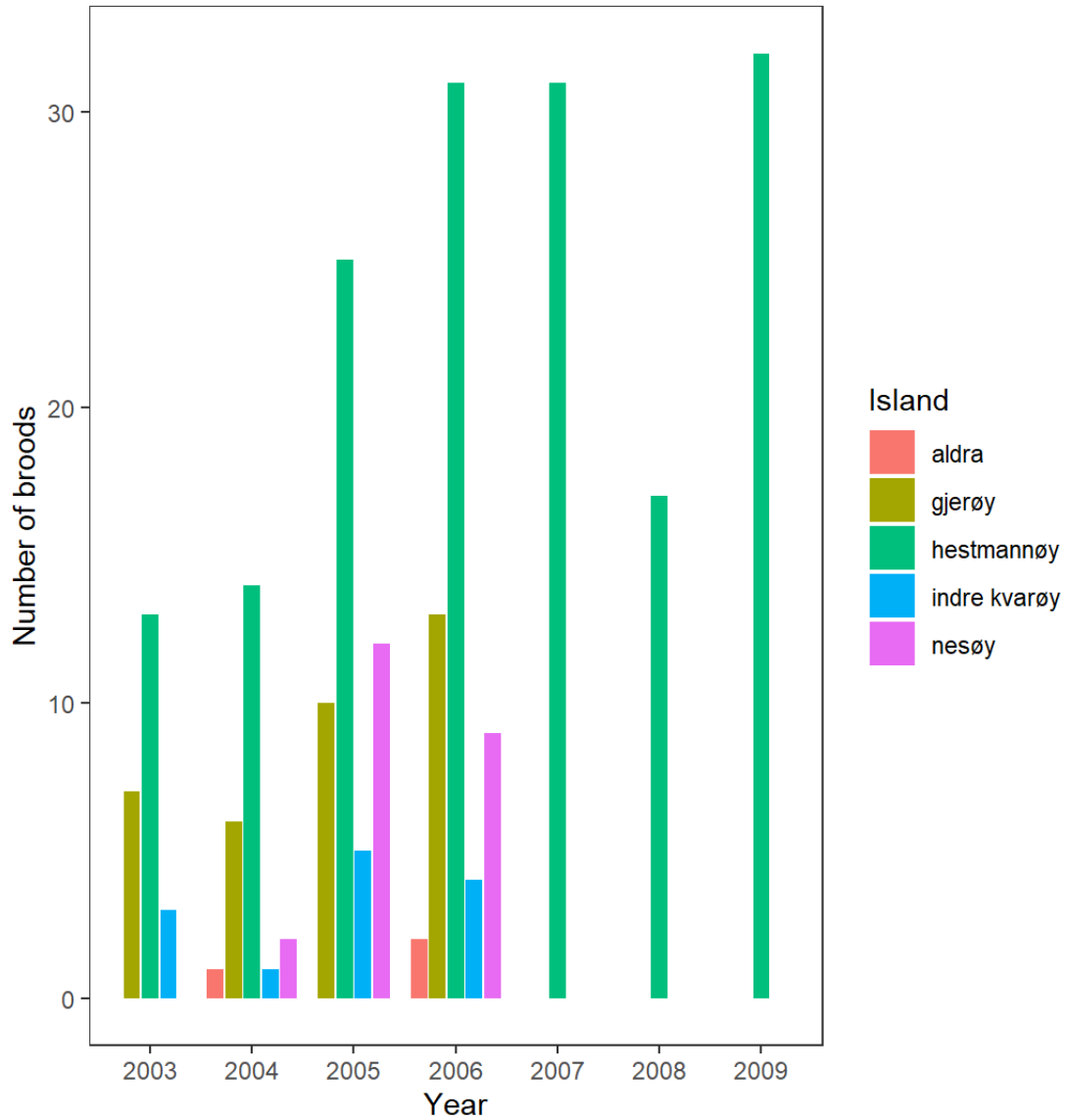


Figure 4. The number of broods per island and year in a metapopulation of house sparrows across five islands in an archipelago located off the coast of northern Norway in the breeding seasons from 2003 to 2009.

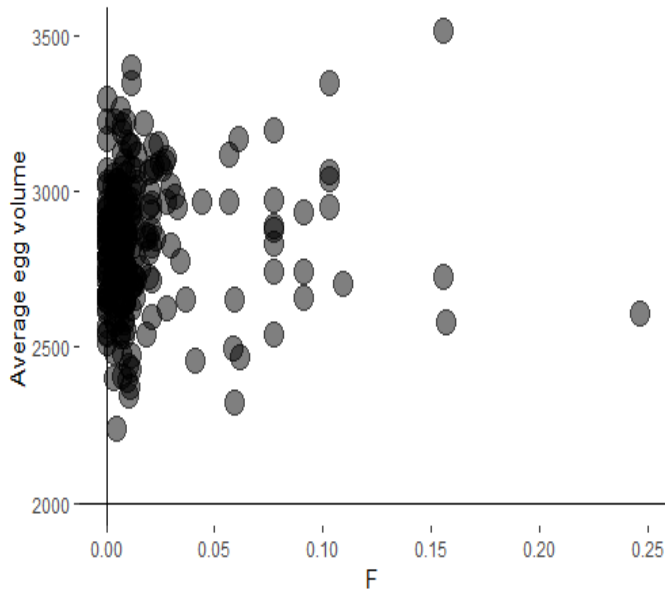


Figure 5. The relationship between the inbreeding coefficient of mothers and the average egg volume of their broods on five islands during the breeding seasons of 2003-2009 in a metapopulation of house sparrows in an archipelago located off the coast of northern Norway.

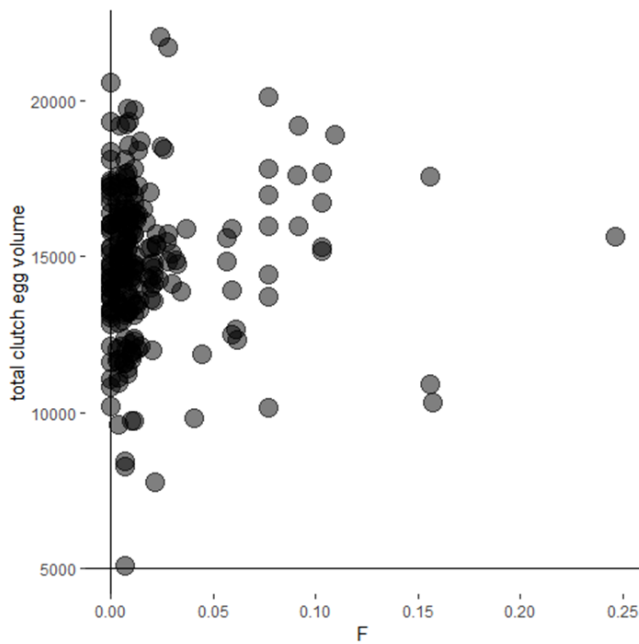


Figure 6. The relationship between the inbreeding coefficient of mothers and the total egg volume of their broods on five islands during the breeding seasons of 2003-2009 in a metapopulation of house sparrows in an archipelago located off the coast of northern Norway.

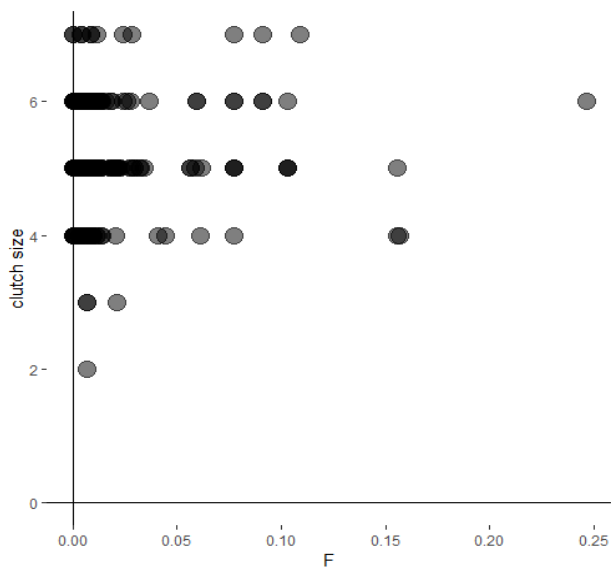


Figure 7. The relationship between the inbreeding coefficient of mothers and clutch size that they laid in their broods on five islands during the breeding seasons of 2003-2009 in a metapopulation of house sparrows in an archipelago located off the coast of northern Norway.

8. Appendix

Table A1. Parameter estimates from the models ranked first in the AICc comparison of the linear mixed effect model, explaining variation in the average egg volume laid in the nests in an insular metapopulation of house sparrows in an archipelago located off the coast of northern Norway during the breeding season of 2003-2009. The fixed effects included clutch size, quadratic clutch size, and brood number. The model also included mother, year, and island as random factors.

parameter	Estimates±SE	95%Confidence intervals	
		lower	upper
Clutch size	136.92±103.83	-67.526	343.620
Clutch size ²	-13.74±10.05	-33.803	6.055
2 nd Brood	-78.23±21.98	-121.586	-33.934
3 rd Brood	-141.149±37.52	-216.539	-67.543
Random variance			
σ^2 mother	27200.3		
σ^2 year	603.6		
σ^2 island	0.00		

Table A2. Parameter estimates from the models ranked first in the AICc comparison of the linear mixed effect model, explaining variation in the total egg volume laid in the nests in an insular metapopulation of house sparrows in an archipelago located off the coast of northern Norway during the breeding season of 2003-2009. The fixed effect included the Brood number, and the model also included mother, year, and island as random factors.

parameter	Estimates±SE	95% Confidence intervals	
		lower	upper
2 nd Brood	181.4±327.5	-463.273	828.619
3 rd Brood	-1201.6±564.3	-2313.922	-84.247
Random variance			
σ^2 mother	810539		
σ^2 year	0		
σ^2 island	0		

Table A3. Parameter estimates from the models ranked first in the AICc comparison of the GLMM model, explaining variation in the clutch size laid in the nests in an insular metapopulation of house sparrows in an archipelago located off the coast of northern Norway during the breeding season of 2003-2009. The fixed effects included mean-centered egg volume and brood number, and the model also included mother, year, and island as random factors.

parameter	Estimates±SE	95% Confidence intervals	
		lower	upper
Mean-centered egg size	-0.062±0.048	-0.157	0.032
Mean-Centered Egg Size Squared	-0.217 ±0.145	-0.502	0.067
2 nd Brood	0.038 ±0.019	-0.000	0.077
3 rd Brood	-0.037±0.035	-0.107	0.031
Random variance			
σ^2 mother	0.003		
σ^2 year	0.000		
σ^2 island	0.000		

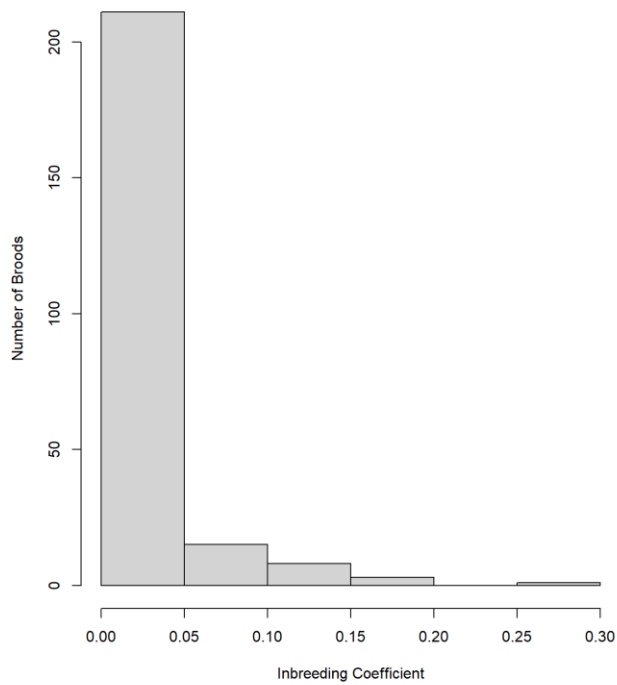


Figure A1. The inbreeding coefficient of the broods based on F_{ROH} on a 1MB scale (short runs of homozygosity) in a metapopulation of house sparrows across five islands in an archipelago located off the coast of northern Norway in the breeding seasons from 2003 to 2009.



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