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# Spatiotemporal Variation and Fitness Consequences of Blood Hemoglobin Concentration in free-living House Sparrow (*Passer domesticus*) Nestlings

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## **Abstract**

Studies have suggested that variation in hemoglobin concentration among individuals can be used as an indicator of individual condition as it reflects the amount of oxygen that is supplied to the tissues. Thus, it is important to examine the factors that causes this variation, as well as the fitness consequences of the individual variation in hemoglobin concentration. In this study, different factors influencing the variation in hemoglobin concentration of 11-day-old house sparrow (*Passer domesticus*) nestlings were investigated in a metapopulation in northern Norway. The central findings in the study were: (i) variation in nestling hemoglobin concentration was related to the individuals' stage of maturation, i.e. age and body mass, (ii) there was a positive effect of precipitation on the hemoglobin concentration, which was most pronounced in nestlings of low body mass, (iii) brood number had an influence on hemoglobin concentration, where nestlings in the second brood had a significant lower hemoglobin concentration compared to the first brood, and (iv) hemoglobin concentration did not significantly explain nestling mortality from 5-day age to fledging stage. These findings demonstrate that hemoglobin concentration reflects nestling's physiological stage of development, as the concentration was found to be highly correlated to both age and body mass. Altricial birds depend on substantial parental care, and the variation in average hemoglobin concentration among broods may indicate that the parents invested differently in the broods. Alternatively, the differences among broods could be explained by variations in the food availability during the breeding season, but this was not supported by the results as hatching day did not explain the variation in hemoglobin concentration.



## Sammendrag

Studier har antydnet at variasjonen i hemoglobinkonsentrasjonen blant individer kan brukes som et mål på individuell kondisjon, da det gjenspeiler mengden oksygen som fraktes rundt i blodet. Det er derfor viktig å undersøke hvilke faktorer som påvirker denne variasjonen, samt å undersøke fitnesskonsekvensene av den individuelle variasjonen i hemoglobinkonsentrasjonen. I denne studien ble det undersøkt ulike faktorer som kan ha en påvirkning på individuell variasjon i hemoglobinkonsentrasjonen til reirunger av gråspurv (*Passer domesticus*) i en metapopulasjon lokalisert på Helgelandskysten i nord-Norge. De sentrale funnene i studiet var: (i) variasjonen i hemoglobinkonsentrasjonen til reirungene ble knyttet til individenes utviklingsstadium, målt som kroppsmasse og alder, (ii) nedbør hadde en positiv effekt på hemoglobinkonsentrasjonen, og var mest merkbart på reirunger med lav kroppsmasse, (iii) kullnummer hadde en påvirkning på hemoglobinkonsentrasjonen, hvor reirunger i det andre kullet hadde en signifikant lavere konsentrasjon sammenlignet med det første kullet, og (iv) hemoglobinkonsentrasjonen forklarte ikke reirungenes dødelighet fra fem dagers alder frem til utflygning. Funnene viser at hemoglobinkonsentrasjon kan gjenspeile reirungers grad av fysiologisk utvikling, da konsentrasjonen ble funnet å være sterkt korrelert med både alder og kroppsmasse. Altrikale fugler krever mye foreldreomsorg de første levedagene, og variasjonen i gjennomsnittlig hemoglobinkonsentrasjon mellom kullene kan indikere at foreldrene investerte ulikt mellom kullene. Alternativt kan forskjellen mellom kullene forklares av at mattilgangen var varierende gjennom hekkesesongen, men dette ble ikke støttet av resultatene da klekkedag ikke forklarte variasjon i hemoglobinkonsentrasjon.



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# Introduction

Life history theories describe adaptive patterns of how individuals distribute their available resources to optimize their fitness on average throughout life (Kozłowski 1992; Stearns 1992; Roff 2002). Each individual has a limited amount of resources available to allocate between growth, reproduction and self-maintenance (Bell & Koufopanou 1986; van Noordwijk & de Jong 1986; Roff 2002). Ecological challenges generated by variation in climate and environment, will affect this process (Réale *et al.* 2010; McFarlane *et al.* 2018). The allocation of energy, along with the influence of varying environment, leads to trade-offs between the costs paid and the benefits acquired (Stearns 1992). Natural selection favors individuals who allocate their energy investments in the most optimal manner (Tuomi *et al.* 1983). However, there might be substantial heterogeneity in individual quality, such that there are large differences between individuals in resource availability (van Noordwijk & de Jong 1986; Pettifor *et al.* 2001; Vindenes *et al.* 2008).

Quantifying variation in individual quality is important in order to understand variation in individual fitness and enable predictions of eco-evolutionary processes (Vindenes *et al.* 2008). Heterogeneity in individual quality involves variation in traits related to survival and reproduction, and may also include phenotypic traits and resource allocation trade-offs (Wilson & Nussey 2010). Hemoglobin concentration (Hb) has been suggested to reflect the individual condition and to be closely related to variation in individual fitness (e.g. Bańbura *et al.* 2007). Hemoglobin is a protein in the blood that is essential for carrying oxygen from lungs or gills to tissues and organs (Bell 1999; Philipsen & Hardison 2017). Hb is a measure of the quantity of hemoglobin per total surface of erythrocytes (red blood cells) and reflects the oxygen-carrying capacity (Campbell 1995; Kostelecka-Myrcha 2002). The amount of hemoglobin would therefore reflect an individuals' adjustments to meet the oxygen needs by revealing the aerobic

capacity (Kostelecka-Myrcha 2002). Thus, Hb might be used as an index to assess an individual's current physiological condition (Milenkaya *et al.* 2015). A high concentration would increase an individual's respiratory capacity, while a low value is associated with a low capacity of oxygen transport. The latter is related to anemia, where individuals have a low number of erythrocytes (Vieth & Lane 2014; Minias 2015). Also, in birds, Hb is proportional to erythrocyte size and amount. The individual Hb remains the same under varying physiological and environmental circumstances, as changes in erythrocytes caused by the adjustment of metabolism in organs and tissues, continue proportionally with changes in Hb (Kostelecka-Myrcha 1997).

To be able to link Hb to individual fitness as a condition-dependent trait, one might need to account for several other individual characteristics and environmental conditions that have been identified in the literature to affect Hb. In birds, these include individual age, sex (Kaliński *et al.* 2012; Kloskowski *et al.* 2017), body mass (Lill *et al.* 2013), brood number (Kaliński *et al.* 2009; Cornell *et al.* 2017) and brood size (Limiñana *et al.* 2009), as well as season of the year (Kostelecka-Myrcha 1997; Kaliński *et al.* 2012; Minias 2015) and habitat quality (Kloskowski *et al.* 2017).

In birds, the increase in concentration of cellular components of blood, including erythrocytes, begin at the embryonic stage and proceeds after hatching. This leads to an increase in Hb from hatching to fledging, as number of erythrocytes and Hb are strongly correlated (Campbell 1994). The largest increase in hemoglobin occurs between 2-11 days of age (Bush & Townsend 1971), and may result in variation in nestling Hb caused by individual growth processes and development stage (Simmons & Lill 2006). Considering life-history theory, there is a trade-off in energy allocation during the individual's ontogenetic development, for example between development of organs, thermoregulatory abilities, feather eruptions and physical growth. As

Hb reflects individuals' potential to meet their oxygen demand (Kostelecka-Myrcha 1997), the oxygen-carrying capacity could be a limiting factor for the growth (Kaliński *et al.* 2012). Thus, one can hypothesize that individuals with a higher Hb will be able to mature faster and thus have a higher fledgling success.

Altricial avian species often experience a poikilotherm stage the first days of life, due to lack of thermoregulatory abilities as they first prioritize development of their organ systems (Gotie & Kroll 1973). Effective thermoregulation requires an adequate oxygen supply in the blood (Niedojadlo *et al.* 2018). As the increase in Hb is slow the first days after hatching, the ontogeny of thermoregulation depends mainly on the nestlings' ability to achieve a suitable body mass and Hb, and maintain an adequate body temperature by external factors during the nestling development (Pereyra & Morton 2001). Altricial species require substantial initial parental investment to survive, including temperature regulation, food provisioning and protection (Arterberry 2000; Pereyra & Morton 2001; Kloskowski *et al.* 2017).

Brood size might have a negative influence on nestling Hb as larger broods might lead to increased competition for food among nestlings, and thereby a decrease in the nestlings' physiological condition (Dijkstra *et al.* 1990; Siikamäki 1998; Limiñana *et al.* 2009). Variation in Hb may also be influenced by which brood number the individuals were hatched in, as seasonal variations in breeding conditions, food availability, timing of breeding, and differences in parental investment between broods, can cause individuals from one brood to be more physiologically mature than the other brood (Naef-Daenzer *et al.* 2001; Kaliński *et al.* 2009; Cornell *et al.* 2017). These differences may also explain the variation in post-fledging survival often found between broods (Naef-Daenzer *et al.* 2001; Cornell *et al.* 2017).

Environmental conditions and food availability during nestling stage are suggested to have an impact on individual variation in nestling Hb. Several studies have found a positive correlation between Hb and food availability, food quality and habitat quality (Potti *et al.* 1999; Bańbura *et al.* 2007; Pryke *et al.* 2011). For instance, Pryke *et al.* (2011) conducted a study on Gouldian finches (*Erythrura gouldiae*) where the food quality was manipulated. Fledglings reared on low-quality diets tended to have a lower Hb compared to individuals reared on high-quality diets. Also, the survival rate was found to be lower among the fledglings on low-quality diets (Pryke *et al.* 2011). Other studies have shown that temperature and rainfall have a strong influence on nestling survival in house sparrows (Ringsby *et al.* 2002), and it is likely that the ambient weather conditions at early age influence nestling's metabolism and Hb (Kaliński *et al.* 2012). An increase in rearing temperature often results in a significantly lower Hb (Kubena *et al.* 1972; Niedojadlo *et al.* 2018). Higher temperatures, within normal range, are suggested to lower the energy requirements to regulate body temperature (Kaliński *et al.* 2012). Precipitation are hypothesized to influence Hb due to its impact on the water content in prey items (Zandt 1996) and thus individuals' physiological water balance and blood composition (Kaliński *et al.* 2012). However, studies so far have only found a non-significant negative relationship between precipitation and Hb (Kaliński *et al.* 2012). Considering annual seasonality, no correlation has been found between nestling Hb and hatching date within a year (Potti *et al.* 1999; Bańbura *et al.* 2007).

A long-term study system consisting of an insular metapopulation of house sparrows (*Passer domesticus*) in northern Norway, provided the opportunity to explore factors that influenced both spatial and temporal variation in Hb among fledglings, and furthermore how this influenced nestling mortality. In this metapopulation, previous studies have found significant spatial and temporal variation in vital rates such as survival rate of adults and fledglings (Ringsby *et al.* 1999; 2002). The aim of the present study was: 1) to investigate factors that may

influence the spatiotemporal variation in the oxygen-carrying capacity in blood, measured as Hb in house sparrow nestlings, and 2) to analyze the potential effect Hb may have on nestlings' fitness. The main hypothesis was that Hb would vary among individuals due to their age and body mass. Furthermore, the concentration was expected to be influenced by temporal and spatial variation in habitat quality as well as weather conditions (temperature and precipitation). I predicted that individuals hatched under periods of cold and rainy weather where food availability was assumed to be poor, would have a lower Hb compared to individuals hatched under more benign conditions. Furthermore, I examined if variation in Hb was related to mortality during the early life of nestlings, from 5 days of age until fledging measured at 11 days of age. The main prediction was that Hb would negatively affect mortality until fledging, since individuals with a higher Hb early in life may be better equipped for faster growth and maturation during this critical period.



# Methods

## Study species

The house sparrow is a globally distributed small passerine bird, whose length ranges from 14-16 cm and weighs from 25-35 g (Anderson 2006). The house sparrow is a sedentary species and breeds close to human settlements where they find food and shelter (Lowther & Cink 1992; Anderson 2006). Nestlings are fed with arthropods and larvae at early stage, while adults primarily feed on seeds. The adults may supplement their diet with other available food items, and the farm dwelling sparrows may feed on pellets (Anderson 2006). The house sparrows are socially monogamous, and both parents contribute to parental care (Ringsby *et al.* 2009). In the study area, the breeding season lasts from early May to mid-August (Jensen *et al.* 2003). The house sparrow reach sexually maturity after one year (Anderson 2006), and each pair can have up to three broods per season, where each brood contains five eggs on average (Husby *et al.* 2006; Pärn *et al.* 2012). The median incubation time is 11 days, and the nestlings are ready to fledge when they are around 14 days of age (Anderson 2006; Kvalnes *et al.* 2013).

## Study area

Since 1993, data on individual variation in reproduction, dispersal and survival have been systematically collected along with measurements of phenotypic traits in a metapopulation of house sparrows by annual capture, mark and recapture methods (Ringsby *et al.* 2002). The study area comprises a metapopulation of 7 insular house sparrow populations located in an archipelago at Helgeland in northern Norway (66°N 13°E, Fig. 1). The house sparrow habitat at these islands vary considerably in vegetation cover and exposure. The most important difference is the presence of dairy farms and agriculture on three islands close to the mainland (Hestmannøy, Indre Kvarøy and Nesøy). Here, the house sparrows mainly breed inside and around cattle farms where they have access to shelter and *ad libitum* access to cattle food. The

remaining four islands are far out from the mainland (Lovund, Selvær, Sleneset and Træna), where the house sparrows live in garden environments in local villages and breed primarily in artificial nest boxes. Here, they are more exposed to fluctuating weather conditions and have less access to supplementary food such as pellets, compared to the farm populations. Accordingly, the study system offers a unique opportunity to study spatial variation among habitat types, as well as temporal variation in the dynamics during the breeding season (Pärn *et al.* 2012).

### **Data collection**

During the breeding season of 2017, active nests in the study area were visited regularly to determine the date of egg-laying and hatching. Hatching day was calculated based on the evaluation of the nestlings' age at first visit after hatching. If a nest was found during egg-laying, the laying date could be predicted directly as one egg is laid each day (Anderson 2006). Otherwise, laying date was estimated based on nestling age and the median incubation period.

Fledglings (8-13 days of age) on all study islands were individually measured and ringed with a unique combination of three colored plastic leg rings and a numbered metal ring from Stavanger Museum, Norway. The marking facilitated later identification by observations using binoculars or capturing using mist nets. For the purpose of measuring nestling mortality, nestlings on Træna and Hestmannøy were also measured at the nestling stage (4-7 days of age). Body mass was measured using a Pesola spring balance with 0.1 g accuracy. To measure Hb (g/dL), blood samples (10  $\mu$ L) were collected with HemoCue cuvettes (HemoCue AB, Ängelholm, Sweden) directly from the brachial vein. These cuvettes act as reaction vessels with a content that hemolyze erythrocytes, convert hemoglobin to methemoglobin and further to hemoglobin azide (Lardi *et al.* 1998). The time it took for the hemolyze reaction to stabilize, was 4 minutes. This time was determined by experiments performed on zebra finches



(*Taeniopygia guttata*) where Hb was measured every 60 seconds (Supplementary materials, Fig. S1). When the reaction had stabilized, the cuvette was inserted into a portable photometer HemoCue Hb 201+ (HemoCue AB, Ängelholm, Sweden) to measure the Hb (Harter *et al.* 2015). The principle behind this photometric estimation of Hb is a colorimetric measurement of Hb where the erythrocytes undergo rupturing (lysis) and the amount of released hemoglobin is measured by the color intensity (Samour 2006).

### **Climate data**

We used data on daily rainfall (mm) and daily mean temperature (°C) from a weather station at the island Myken (see Fig. 1, The Norwegian Meteorological Institute 2017). The weather conditions here, 30 km northwest from the middle point of our study area, has been shown to be representative for the study area (Ringsby *et al.* 2002). To assess if the weather conditions had an impact on the nestlings' growth and Hb, the average daily temperature and daytime (0700-1900) precipitation for the first seven days after hatching was used as an index of the ambient weather conditions experienced by each brood. Henceforth referred to as temperature and precipitation. The length of the average-interval was determined by the fact that the lowest fledgling age was seven days, implying that all measured individuals had experienced the weather conditions for this interval.

### **Statistical analyzes**

Two sets of analyzes were performed: (1) analyzing factors that influenced individual variation in Hb of nestlings at fledgling stage, and (2) analyzing factors that influenced mortality of nestlings until fledging in relation to Hb measured in 5-day-old nestlings. The study included a total of 350 individuals from the seven populations that were sampled for Hb. The analyzes of individual variation in Hb comprised 336 fledglings from 109 broods (Table 1) and the mortality analyzes comprised 117 individuals from 41 broods from Træna ( $n = 79$ ) and Hestmannøy ( $n = 38$ ).

Relevant candidate models were constructed based on *a priori* theoretical and ecological knowledge. These were fitted by carefully defining the criteria using the dredge function in the R-package MuMIn v1.40.4 (Barton 2018). In order to evaluate the support of alternative models (fitted with maximum likelihood), Akaike's information criterion corrected for small sample sizes ( $AICc$ ) was applied (Akaike 1974; Burnham & Anderson 2002). Models with  $\Delta AICc < 2$  relative to the highest ranked model were considered to have high support in the data. These models ( $\Delta AICc < 2$ ) were then refitted with restricted maximum likelihood in order to obtain unbiased parameter estimates. To improve model convergence and make it easier to interpret the results, the explanatory variables were centered by their grand mean prior to the analyzes. To avoid multicollinearity, Pearson's product moment correlation ( $r_p$ ) was estimated between all pairs of explanatory variables to make sure that highly correlated explanatory variables ( $r_p > 0.5$ ) were not present in the same model. The software package R version 3.4.3 (R Core Team 2017) was used for all statistical analyzes. The estimated parameters and means are given with  $\pm 1$  standard error or 95 % confidence estimates (CI = [lower, upper]).

#### *Variation in blood hemoglobin concentration*

To analyze factors influencing individual Hb at fledgling stage, Hb was used as response variable in linear mixed-effects models fitted using the lme4 package (Bates *et al.* 2015) with a Gaussian error distribution. Body mass and age were both positively correlated with Hb (body mass:  $r_p = 0.525$ ,  $df = 334$ ,  $p < 0.001$ ,  $n = 336$ , age:  $r_p = 0.497$ ,  $df = 334$ ,  $p < 0.001$ ,  $n = 336$ ). These two variables were included as explanatory variables in all models to account for their effects. There was no problem with multicollinearity as body mass and age were weakly positively correlated ( $r_p = 0.286$ ,  $df = 334$ ,  $p < 0.001$ ,  $n = 336$ ). Day of hatching constituted the temporal effect and made it possible to test for seasonal effect on individual variation in Hb. Hatch day and temperature were highly correlated ( $r_p = 0.834$ ,  $df = 52$ ,  $p < 0.001$ ,  $n = 54$ ), and were therefore only included in separate models. Nestlings from the same brood shared the

same rearing environment and the same parents, and were not likely to represent independent measurements (Kaliński *et al.* 2015). Thus, brood identity nested within population was included with random intercepts in the models.

### *Nestling mortality*

The analyzes of whether Hb influenced mortality of nestlings until fledging, were based on data from nests that were visited twice; at 5 and 11 days of age. The analyzes were conducted using generalized mixed effects linear models (GLMM) in the lme4 package (Bates *et al.* 2015), fitted with a binomial error distribution and a complementary log-log link function. Mortality was the response variable, and nestlings who died between the 5 and 11 days visit, had mortality set to 1 (otherwise 0). Since the first measurements were conducted at different ages (4-7 days of age), age was included as an explanatory variable in all models to control for the effect of age. Hatch day and temperature were highly correlated ( $r_p = 0.938$ ,  $df = 23$ ,  $p < 0.001$ ,  $n = 25$ ), and were not included in the same candidate models to avoid multicollinearity. Also, body mass and Hb were correlated ( $r_p = 0.527$ ,  $df = 112$ ,  $p < 0.001$ ,  $n = 114$ ). Accordingly, the residuals from a linear regression of Hb on body mass were used as an index of relative Hb. The residuals represented the deviance in Hb from the average at a given body mass, and are not correlated with body mass. There was an uneven distribution of the number of individuals who died in the different brood numbers of the two island populations (Hestmannøy:  $n_{br1} = 7$ ,  $n_{br2} = 2$ ; Træna:  $n_{br1} = 5$ ,  $n_{br2} = 0$ ). Because all the dead individuals in the second brood were on Hestmannøy, this resulted in problems with linear separability in the models. Therefore, brood number was not included as an explanatory variable. Seasonal day of hatching may reflect the brood number as second broods were hatched later in the season. However, the timing of breeding of brood number two is known to vary a lot. If the first brood is not successfully, the onset of a second brood starts quickly (Ringsby *et al.* 2002). The models were adjusted with a random intercept for brood identity.



## Results

The Hb of house sparrow fledglings increased from 8 to 13 days of age ( $b_{age} = 0.641$ ,  $F_{(1, 334)} = 109.8$ ,  $p < 0.001$ , Supplementary materials, Fig. S2), and the average fledgling Hb was  $11.88 \pm 0.09$  g/dL ( $n = 336$ ) with a range of 6.7-15.9 g/dL. There was a significant positive difference between average Hb in nestlings and fledglings (paired t-test: mean difference = 2.896,  $t = -15.814$ ,  $df = 101$ ,  $p < 0.001$ ,  $n = 103$ , Supplementary materials, Fig. S3). The average clutch size across broods was  $5.05 \pm 0.05$  eggs ( $n = 336$ ) with a range of 2-7 and median of 5, while the mean number of fledglings in each brood across islands and broods was  $3.98 \pm 0.07$  ( $n = 336$ ) with a range of 1-6 and a median of 4. Average fledgling body mass was  $26.73 \pm 0.21$  g ( $n = 336$ ), with a range of 13.0-35.0 g. There was a positive correlation between daily precipitation and day of hatching ( $r_p = 0.457$ ,  $df = 52$ ,  $p < 0.001$ ,  $n = 54$ ). The average temperature for the nestlings' seven first days of life varied from 6.1-15.4°C, and the amount of precipitation ranged from 0.0-7.4 mm.

The best model explaining the variation in Hb, included a positive effect of body mass (Tables 2 and 3, Supplementary materials, Fig. S4), meaning that heavier individuals tended to have a higher Hb. In addition, increased precipitation was associated with increased Hb, but this association was less pronounced for heavy fledglings (Tables 2 and 3, Fig. 2). However, the CI for the interaction between body mass and precipitation marginally included zero (Table 3) and the interaction was not present in the second highly supported model (Table 2). The average Hb was also found to differ between broods, where nestlings in the second brood had a significant lower Hb compared to the first and third brood (Tables 2 and 3, Supplementary materials, Fig. S5). The highest-ranked model explaining variation in individual Hb (model 1, Table 2) had an evidence ratio (ER) twice as high as the second-ranked model (Table 2). This suggested model 1 to be considerably better than the second-ranked model. There was no

support in the data that neither hatch day or population identity influenced Hb (model 1 + hatch day:  $\Delta AICc = 2.05$ , ER = 2.75, rank = 4, and model 1 + population identity:  $\Delta AICc = 11.81$ , ER = 142.80, rank = 246). In the highest ranked model, the variance in fledgling Hb was structured, with 57.30 % due to variation between broods and 42.70 % due to differences between individuals.

Nestling mortality until fledging decreased with increased body mass (Tables 4 and 5, Fig. 3). There was no support in the data that Hb had an influence on nestling mortality (Model 1 + Hb:  $\Delta AICc = 2.12$ , ER = 2.88, rank = 7). Accordingly, there was no significant difference in mean nestling Hb between those who survived or died ( $p = 0.236$ ). There were uncertain trends indicating that mortality decreased with increased temperature (model rank 2:  $b_{temperature} = -0.546$ , CI = [-1.444, 0.352]) and hatch day (model rank 4:  $b_{hatch\ day} = -0.341$ , CI = [-1.050, 0.368]), and an uncertain trend that indicated a lower mortality in the Hestmannøy population compared to the Træna population (model rank 3:  $b_{population} = -1.632$ , CI = [-4.571, 1.306], Table 4). The variance in nestling mortality was structured, with 71.72 % due to variation between broods and 28.28 % due to individual differences.

## Discussion

Relatively few empirical studies have investigated the adaptive significance of hemoglobin in nestlings in wild bird populations. The present study confirmed that the blood concentration of hemoglobin in house sparrow nestlings in northern Norway was strongly related to body mass, and reflected the nestlings' growth process and stage of maturation, after accounting for the positive effect of age. Also, the results underpin other empirical findings in birds, as Hb was found to be affected by ambient precipitation and brood number (Table 3).

Variation in growth profile, body size, activity level and ambient environmental conditions, have resulted in varying evolutionary adaptations to meet the metabolic requirements, including adaptations in the oxygen affinity of hemoglobin (Poyart *et al.* 1992; Simmons & Lill 2006; Rafati & Ghasemian 2009). In vertebrates, young individuals are still under development, involving an ongoing process of producing blood cellular components. This implies that the blood hemoglobin content should increase with age during individuals' development (Campbell 1994). For instance, studies on both rodents and birds have shown that juveniles and nestlings have a significantly lower Hb compared to adults (Sealander 1965; Bolton *et al.* 1999; Minias 2015). The result from the present study confirms this pattern, as individual nestling Hb increased with nestling age (see Table 3, Supplementary materials, Figs. S2, S3).

The present study revealed a positive correlation between nestling body mass and blood concentration of hemoglobin (Table 3, Supplementary Materials, Fig. S4). This is consistent with previous studies, and is caused by physical growth and physiological development (Bolton *et al.* 1999; Simmons & Lill 2006; Lill *et al.* 2013; Minias 2015). When several vertebrates reach adult size, the relation between body mass and Hb becomes the opposite: smaller individuals tend to have higher hemoglobin values and a larger oxygen demand per unit body weight (Sealander 1965; Poyart *et al.* 1992; Kostelecka-Myrcha *et al.* 1993, Kostelecka-Myrcha

& Chołostiakow-Gromek 2001). This can be related to the fact that the oxygen affinity of hemoglobin is lower in animals with a lower weight (Schmidt-Nielsen & Larimer 1958). Exceptions from this trend might be associated with seasonal variation, altitude or special cases of physiological adaptations (Sealander 1965).

Insects are important for nestlings, as their water content is necessary for nestling development (Zandt 1996). Heavy precipitation might reduce the prey availability (Barnett & Facey 2016), thus reducing the water that the nestlings acquire. A water shortage might result in a hemoconcentration with an increased concentration of blood cells and associated hemoglobin concentration (Puerta *et al.* 1995). Smaller individuals are more prone to dry out faster, supporting the findings where the positive effect of precipitation was most pronounced for nestlings of low body mass (Fig. 2).

The present results indicated that brood number influenced the individual variation in nestling Hb (Table 3). Kaliński *et al.* (2009) studied nestling Hb in facultatively double-brooded great tits (*Parus major*), and found that the second brood had a statistically significant higher Hb compared to the first brood. This variation might be explained by the fact that the food provisioning was higher in the second part of the breeding season (Kaliński *et al.* 2009). From the results in the present study, and given that the variation in nestling Hb is affected by food availability, we thus expect preferred insect prey availability to be reduced during the time of the second brood. As the abundance of the prey that house sparrow nestlings primarily are fed on usually increases throughout spring and summer (Anderson 2006), the observed variation in Hb could be caused by annual environmental stochasticity, as suggested by Kaliński *et al.* (2009). Nestling Hb might be influenced by variation in weather condition, nutrient supply and access to food among years (Bańbura *et al.* 2007; Kaliński *et al.* 2009; Cornell *et al.* 2017), so the study should be carried out several years to achieve more accurate results.



House sparrows are multiple breeders and need to allocate their available resources into investments in current and future broods (Trivers 1972; Anderson 2006). A possible explanation for the reduction in Hb in nestlings from first brood to the second brood, can be a biased parental investment among broods. In general, future opportunities for investing in reproduction have been found to be negatively influenced by the costs of earlier investment (Dijkstra *et al.* 1990; Stearns 1992; Nilsson & Svensson 1996; Gross 2005). For instance, manipulated brood enlargements have been shown to have a negative influence on nestling growth and survival, as the limited resources available needs to be shared between additional individuals, and the parents' investment exceeds what is optimal for them (Dijkstra *et al.* 1990). Parents can manage to increase the provisioning rate to compensate for an increased brood size, but this might cause a reduction in the parents' survival or future reproductive success (Dijkstra 1990; Nilsson & Svensson 1996). Hence, if the parents in the present study invested much in the first brood, as reflected by the nestlings' higher Hb, then the resources available for investment in the second brood were reduced. There was a minority of house sparrows that had a third brood (7.02 %, Supplementary materials, Fig. S5), and these parents may potentially be of high-quality, provisioning a high investment in their offspring, as evidenced by the increased Hb in nestlings of the third brood.

Furthermore, as an increase in brood size have been suggested to increase the within-brood competition for food and resources (Mock & Parker 1998), one could expect the brood size to have an influence on individuals' Hb as it is strongly related to body mass (Limiñana *et al.* 2009). However, the present study did not find a significant relationship between nestling Hb and brood size. This may indicate that the parents managed to compensate by increasing the provisioning rate of food per nestling (Minias 2015), indicating surplus of food in the habitat, and that the parents managed to adjust the brood size to what they managed to provide (Pettifor *et al.* 2001).

Habitat quality and variation in the diet of nestlings have been reported to cause variations in nestling Hb, where nestling Hb increases in association with higher food abundance (Potti *et al.* 1999; Bańbura *et al.* 2007; Kaliński *et al.* 2009, 2012; Pryke *et al.* 2011). Cattle breeding and agriculture might facilitate a nutrient rich environment, hereunder the natural fertilization of the farmland, which is assumed to result in better living conditions for arthropods on the farm-islands in the present study area. The possible differences in food availability was suggested to cause inter-population variations in nestlings Hb, but this was not supported by the data. Studies that found food availability to cause variation in individual Hb, showed a significant difference in the amount of food present (Bańbura *et al.* 2007). For instance, blue tit (*Cyanistes caeruleus*) nestlings in woodland with a food abundance 2-5 times higher than parkland, had a significantly higher Hb compared to the parkland nestlings (Bańbura *et al.* 2007). In the present study, the differences in food availability on the island populations might not be large enough, or as differentiated as the wood- and parkland, to affect Hb significantly. Another plausible explanation for the result is that the parents, who supported the nestlings, were likely to have adjusted to the available food resources in their habitat and compensated by increasing their feeding capacities to maintain nestling growth and to achieve breeding success (Siikamäki 1998; Minias 2015).

Increasing temperatures have been found to decrease oxygen affinity as a result of the exothermic properties of hemoglobin oxygenation (the binding of oxygen releases heat) (Nikinmaa 2011). For instance, studies conducted by Jensen *et al.* (2016) found that variation in the atmospheric temperature produced shifts in the oxygen-binding capacity of hemoglobin in deer mouse populations. The tendency is the same in avian species, where an increase in rearing temperature has been reported to lower nestling Hb, along with hematocrit and erythrocyte counts, as less energy is then required to adjust the body temperature (Kubena *et al.* 1972; Niedojadlo *et al.* 2018). However, the present study did not find any effect of

temperature on nestling Hb. There was a daily increase in temperature throughout the breeding season, but the average ambient temperature might not differ enough to have had an influence on nestling Hb (6.1-15.4°C). For comparison, Kubena *et al.* (1972) discovered an increased Hb for broiler chicks reared at 21.1°C compared to chicks reared at 32.2°C, and Niedojadlo *et al.* (2018) found the same tendency in zebra finches reared at 12°C and 32°C. The temperature differences in these studies was more extreme for the nestlings, specially the latter on passerine birds. Also, these studies were conducted using climate chambers with constant temperatures. These nestlings did not experience the daily temperature fluctuations faced by the free-living nestlings in the present study. Further, the measured temperature used in the present study was from the air outside the nest, and nestlings may have experienced other temperatures due to isolation in the nest and parental warmth. This may explain why the present study did not find any relationship between temperature and nestling Hb.

In consistence with previous studies of both mammals and birds (Lindstedt & Boyce 1985; Magrath 1991), the present study revealed that nestling mortality was reduced with increasing body mass (Table 5, Fig. 3). A result that also has been found in a previous study in the same metapopulation (Ringsby *et al.* 1998). Considering the variation in nestling body mass, a size-dependent hierarchy and a rank order may have been formed in the nest, such that larger nestlings (higher body mass) would have a competitive advantage (Ringsby *et al.* 1998; Nilsson & Gårdmark 2001). In such situations, there will be a skewed distribution of resources, and smaller individuals will acquire less food and nutrition and thus be more vulnerable to die. This effect may be the causing agent in the present study, but was not tested due to lack of ranking of the nestlings within nests.

House sparrow nestlings typically initially invest in accelerated growth and gaining body mass (Anderson 2006), a strategy that promotes juvenile survival but may cause a reduction in the

longevity (Pereyra & Morton 2001; Metcalfe & Monaghan 2003; Reichert *et al.* 2015). The investment in early growth entails a trade-off between costs and benefits in the short- and long-term (Reichert *et al.* 2015). Fledging is a critical step in nestlings' life history, and to reduce post-fledging mortality, nestlings should be as developmentally mature as possible before they fledge (Cornell *et al.* 2017). In the present study, fledglings at 12 days of age had about 78 % of average adult Hb (Supplementary materials, Fig. S2; Bush & Townsend 1971). As Hb reveals individual aerobic capacity and physiological condition (Campbell 1995; Kostelecka-Myrcha 1997), I expected a negative relationship between Hb and nestling mortality until fledging. However, this was not supported by the data as Hb did not explain variation in nestling mortality.

Theory and empirical knowledge implies the development of blood hemoglobin to be slowest in the first days of life (Pereyra & Morton 2001), which could explain the findings in the present study. The development of hemoglobin was still in progress when the measurements were conducted on the 5-day-old nestlings, and the concentration was therefore about equal for the nestlings as they were at the same developmental stage (Supplementary materials, Fig. S2). Using a reaction norm of individual growth, allows comparing variation within and between nestlings regarding the relative investment in body mass versus Hb, and examining the consequences this will have for the individual fitness. A longitudinal sampling of the same individuals would make it possible to detect if individuals with higher Hb have a higher probability to survive until recruitment in the next years breeding population.

In the analyzes of nestling mortality, only 14 nestlings out of 117 were recorded as dead. This may be insufficient to provide statistical power in the analyzes of mortality (Cohen 1992). During the data collection, the fieldworkers decided whether it was safe to take blood samples from the nestlings for measurements of Hb, and avoided taking samples from those in poorest

condition. This might have caused a bias in the data set towards nestlings in better condition, culminating in a lower effect size that was more difficult to detect.

In conclusion, the present study suggests that variation in nestling Hb was related to differences in age, body mass and amount of precipitation, along with which brood number the nestlings were hatched in. It is likely that precipitation affected hemoglobin indirectly through the influence it had on the food availability. Brood number may also have a similar indirect effect, as the variation in Hb could display differences in parental investment and food availability at the different brood numbers. All this was reflected in nestlings' body mass, which were highly positively correlated with Hb. Possibly due to lack of statistical power, the study did not confirm that Hb could be used as an indicator of individual condition, as there was no significant relationship between Hb and nestling mortality. However, Hb measured at an early stage might have important fitness consequences later in life.



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*Scientia potentia est.*





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## Tables

**Table 1:** Overview of the insular populations in a house sparrow (*Passer domesticus*) metapopulation located in northern Norway and their sample sizes,  $n$ . Total number of fledglings in the metapopulation is 336.

Island	$n$
Hestmannøy	56
Indre Kvarøy	45
Lovund	28
Nesøy	13
Selvær	21
Sleneaset	71
Træna	102

**Table 2:** Ranking of models explaining variation in fledgling hemoglobin concentration (g/dL) in a house sparrow (*Passer domesticus*) metapopulation located in northern Norway. Only models with  $\Delta AICc < 2$  compared to be highest ranked model are shown. Linear mixed models are fitted with brood identity and island identity as random intercepts.  $\Delta AICc$  is the difference in  $AICc$  value from the top ranked model, *weight* is the Akaike weight and *ER* is the evidence ratio for each model compared to the top model. Interactions are shown with symbol “×”. The best model is shown in bold.

Rank	Model Parameters	$\Delta AICc$	<i>weight</i>	<i>ER</i>
<b>1</b>	<b>Age + Brood number + Body mass + Precipitation + Body mass × Precipitation</b>	<b>0.00</b>	<b>0.088</b>	<b>1.00</b>
2	Age + Brood number + Body mass + Precipitation	1.47	0.042	2.10

**Table 3:** Parameter estimates and 95 % confidence intervals for the estimate of each explanatory variable in the most parsimonious model (Table 2) describing variation in fledgling hemoglobin concentration (g/dL) in a house sparrow (*Passer domesticus*) metapopulation in northern Norway. Interactions are shown with symbol “×”.

Variable	Estimate	95 % Confidence interval	
		Lower limit	Upper limit
Intercept	12.080	11.788	12.371
Clutch 2	-0.564	-1.025	-0.103
Clutch 3	1.085	-0.050	2.221
Age	0.482	0.317	0.648
Body mass	0.185	0.151	0.220
Precipitation	0.136	-0.005	0.278
Body mass × Precipitation	-0.021	-0.042	0.001

**Table 4:** Ranking of models explaining nestling mortality until fledging (from  $5.77 \pm 0.07$  to  $10.32 \pm 0.07$  days of age) in a house sparrow (*Passer domesticus*) metapopulation located in northern Norway. Only models with  $\Delta AICc < 2$  compared to the highest-ranked model are shown. Generalized linear models are fitted with brood identity as random intercepts.  $\Delta AICc$  is the difference in  $AICc$  value from the top ranked model, *weight* is the Akaike weight and *ER* is the evidence ratio for each model compared to the top model. The best model is shown in bold.

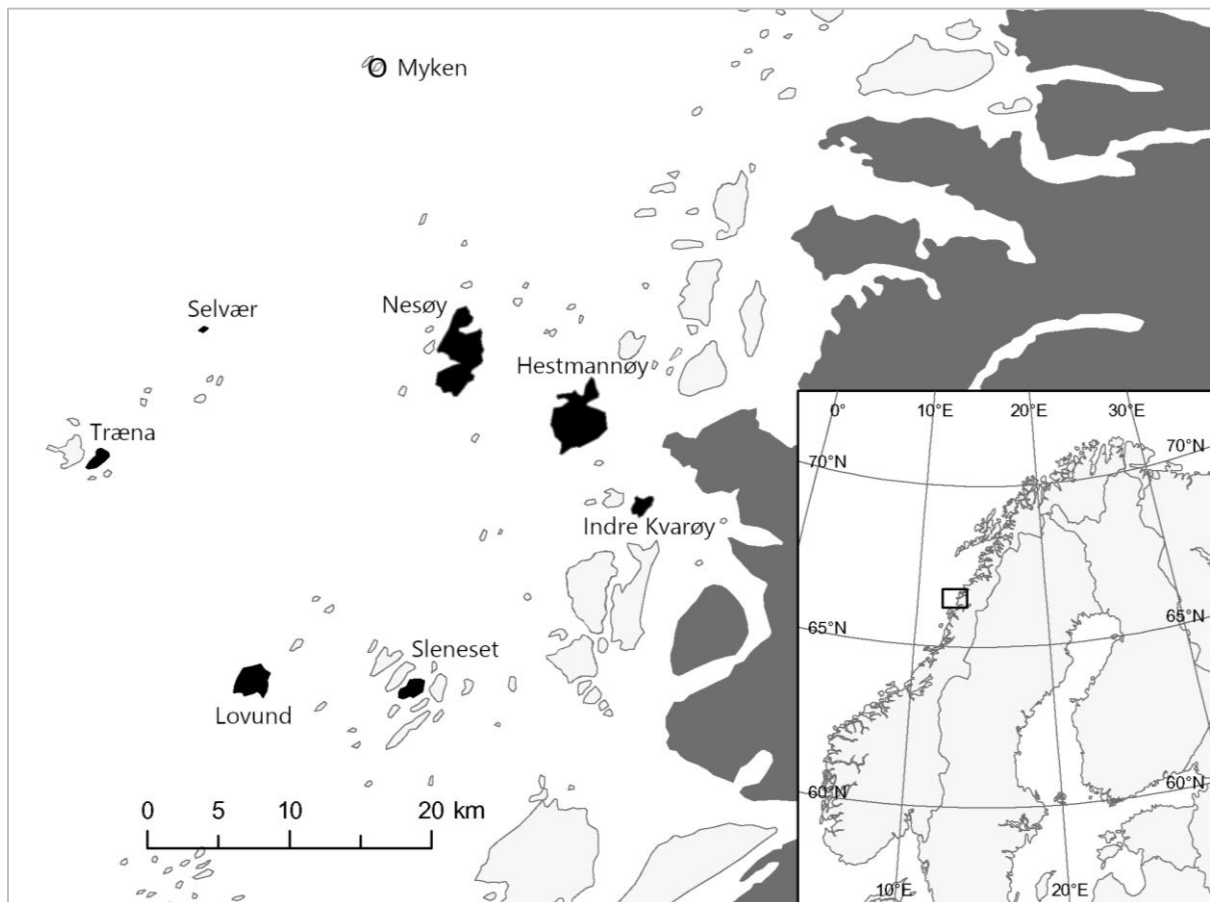
Rank	Model Parameters	$\Delta AICc$	<i>weight</i>	<i>ER</i>
<b>1</b>	<b>Age + Body mass</b>	<b>0.00</b>	<b>0.075</b>	<b>1.00</b>
2	Age + Body mass + Population + Temperature	0.82	0.050	1.50
3	Age + Body mass + Population	0.85	0.049	1.53
4	Age + Body mass + Hatch day + Island	1.95	0.028	2.68

**Table 5:** Parameter estimates and 95 % confidence intervals for the estimate for each explanatory variable in the most parsimonious model (Table 4) describing nestling mortality until fledgling (from  $5.77 \pm 0.07$  to  $10.32 \pm 0.07$  days of age) in a house sparrow (*Passer domesticus*) metapopulation in northern Norway.

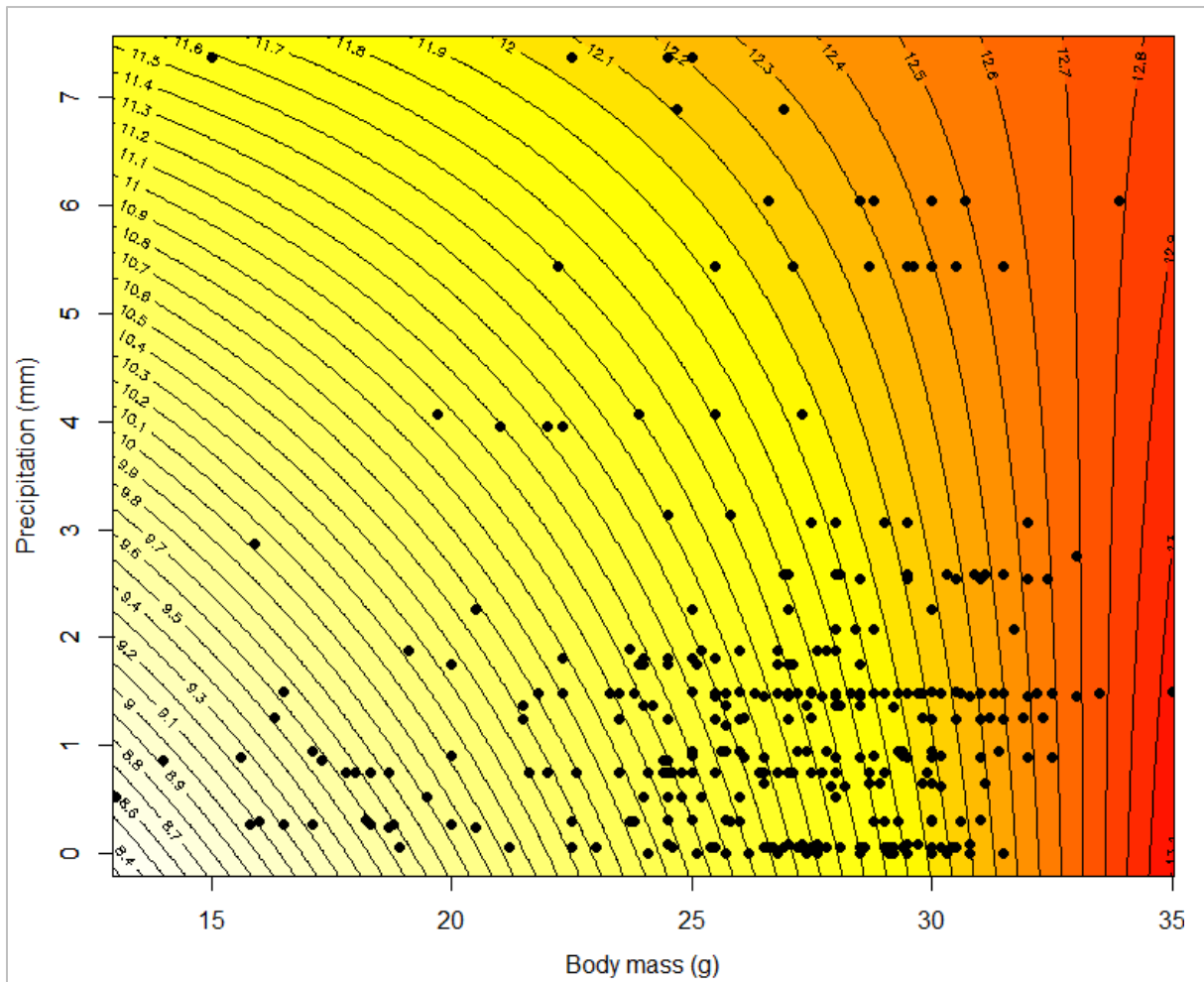
Variable	Estimate	95 % Confidence interval	
		Lower limit	Upper limit
Intercept	-4.554	-7.717	-1.391
Age	-2.569	-4.547	-0.591
Body mass	-0.313	-0.604	-0.021



## Figures

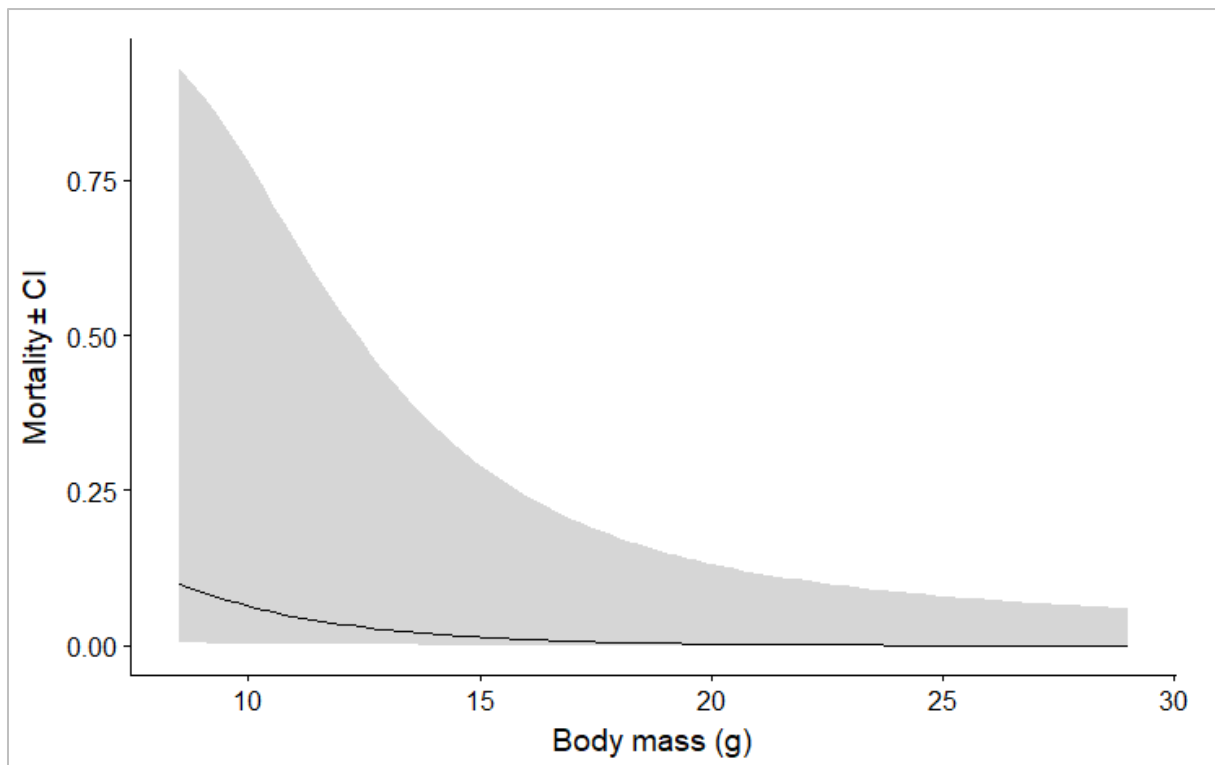


**Fig. 1:** The study area located in an archipelago on the coast of Helgeland, northern Norway ( $66^{\circ}\text{N}$   $13^{\circ}\text{E}$ ). The islands used in the study are colored black, and the mainland is grey shaded. The weather station located at Myken is indicated by a solid circle.



**Fig. 2:** The effect of the interaction between body mass (g) and precipitation (mm) on fledgling hemoglobin concentration (g/dL) in a house sparrow (*Passer domesticus*) metapopulation in northern Norway,  $n = 336$ . Fledgling age was kept at mean value, and brood number was set to “2”. The effect of body mass on fledgling hemoglobin concentration depends on the amount of precipitation during the first seven days of life. Both body mass and precipitation have a positive effect on the hemoglobin concentration, while the positive effect of precipitation is limited in fledglings with a higher body mass. The red-colored area shows individuals with a high hemoglobin concentration, and the yellow area indicates individuals with a lower hemoglobin concentration. The observed data is indicated by solid circles.

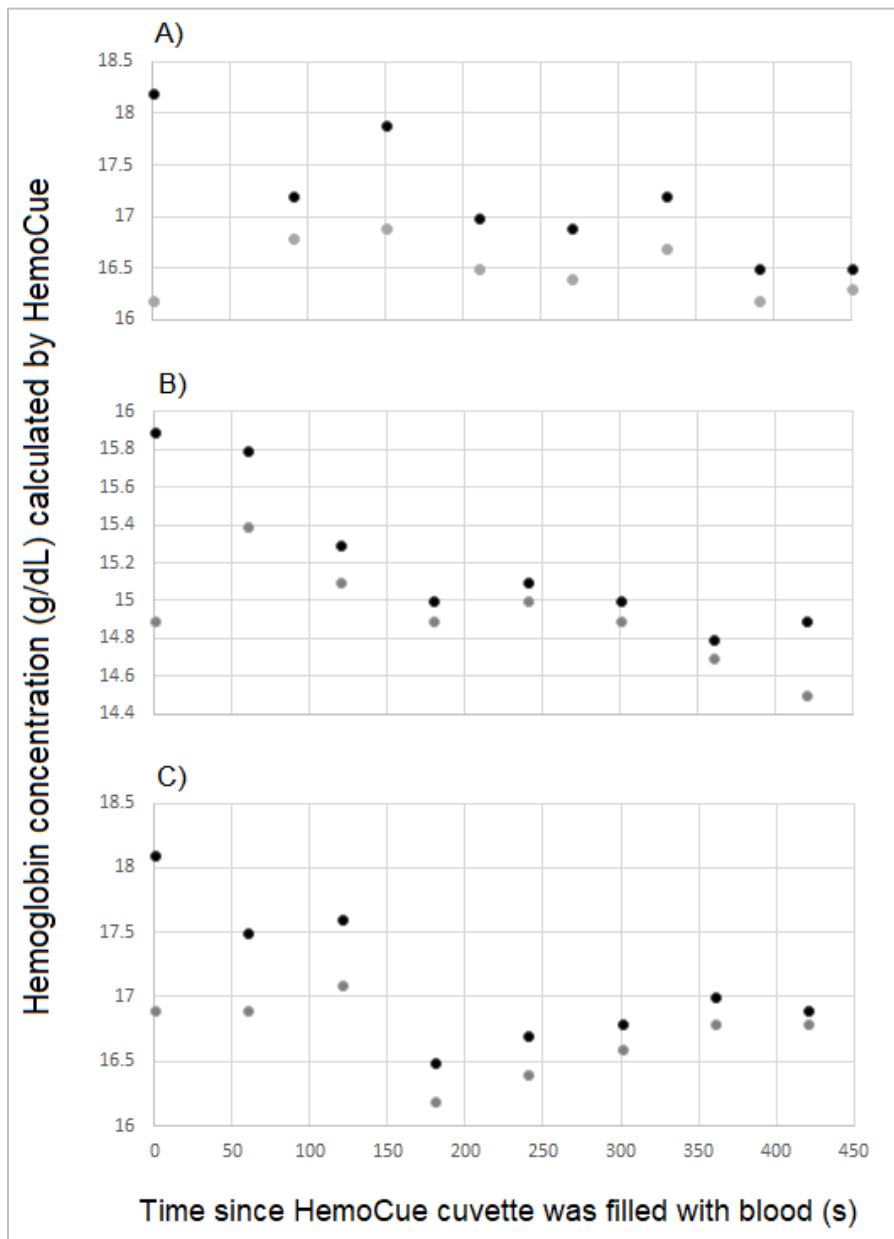




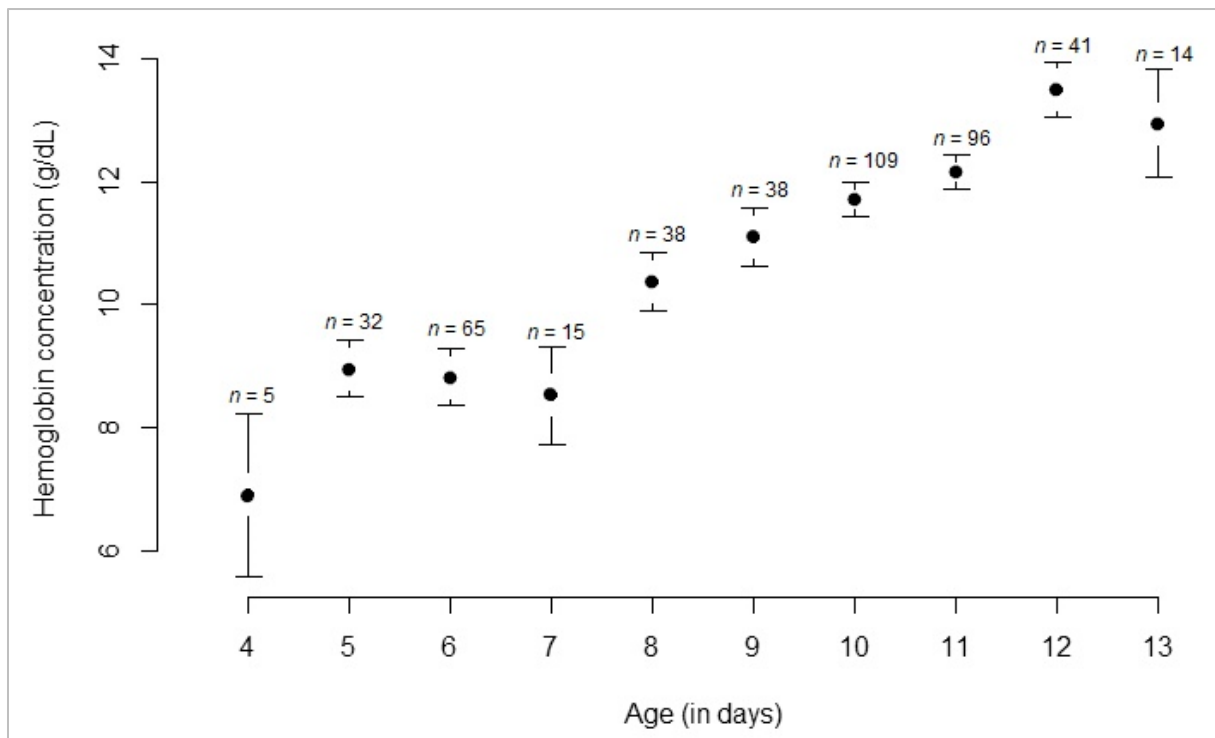
**Fig. 3:** The predicted influence of body mass (g) on nestling mortality from age 6 days until fledging in a house sparrow (*Passer domesticus*) metapopulation in northern Norway. The predictions are presented with a shaded 95 % confidence interval (CI). Nestlings with a higher body mass tend to have a lower probability of dying.



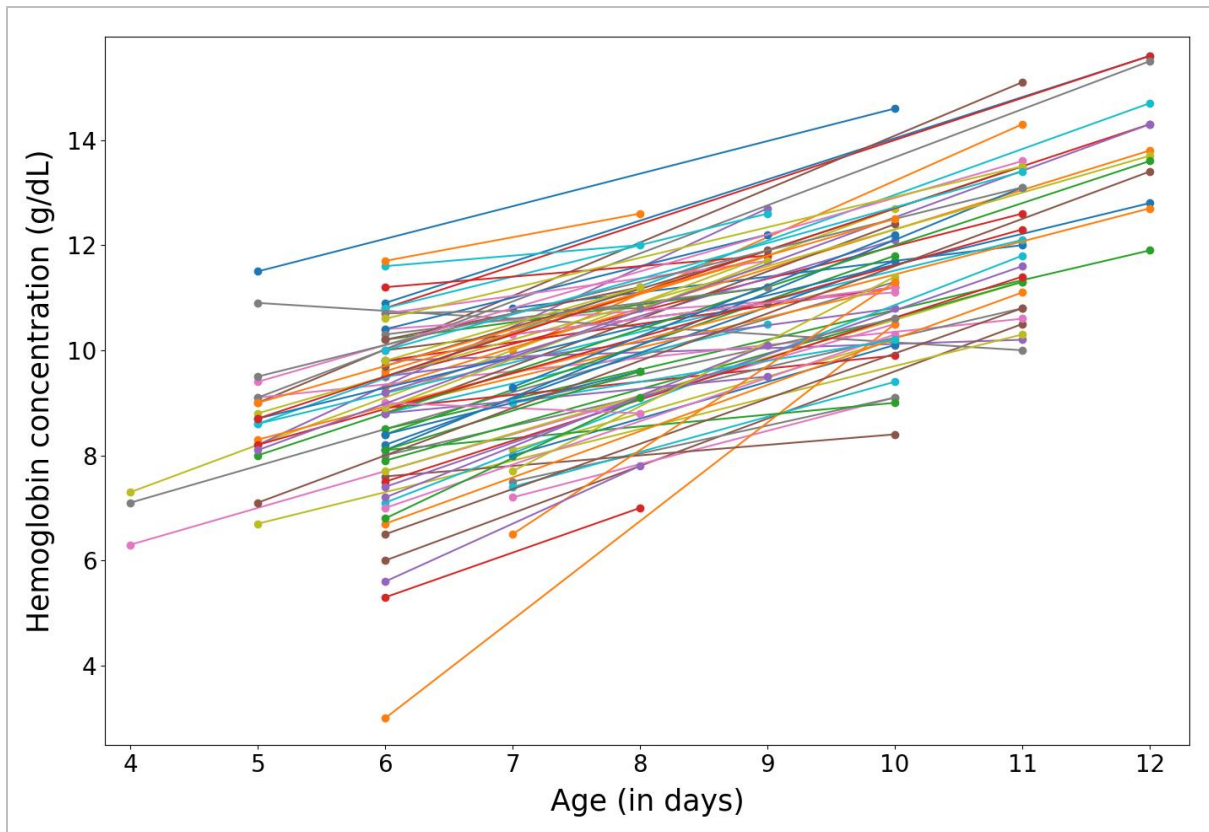
## Supplementary materials



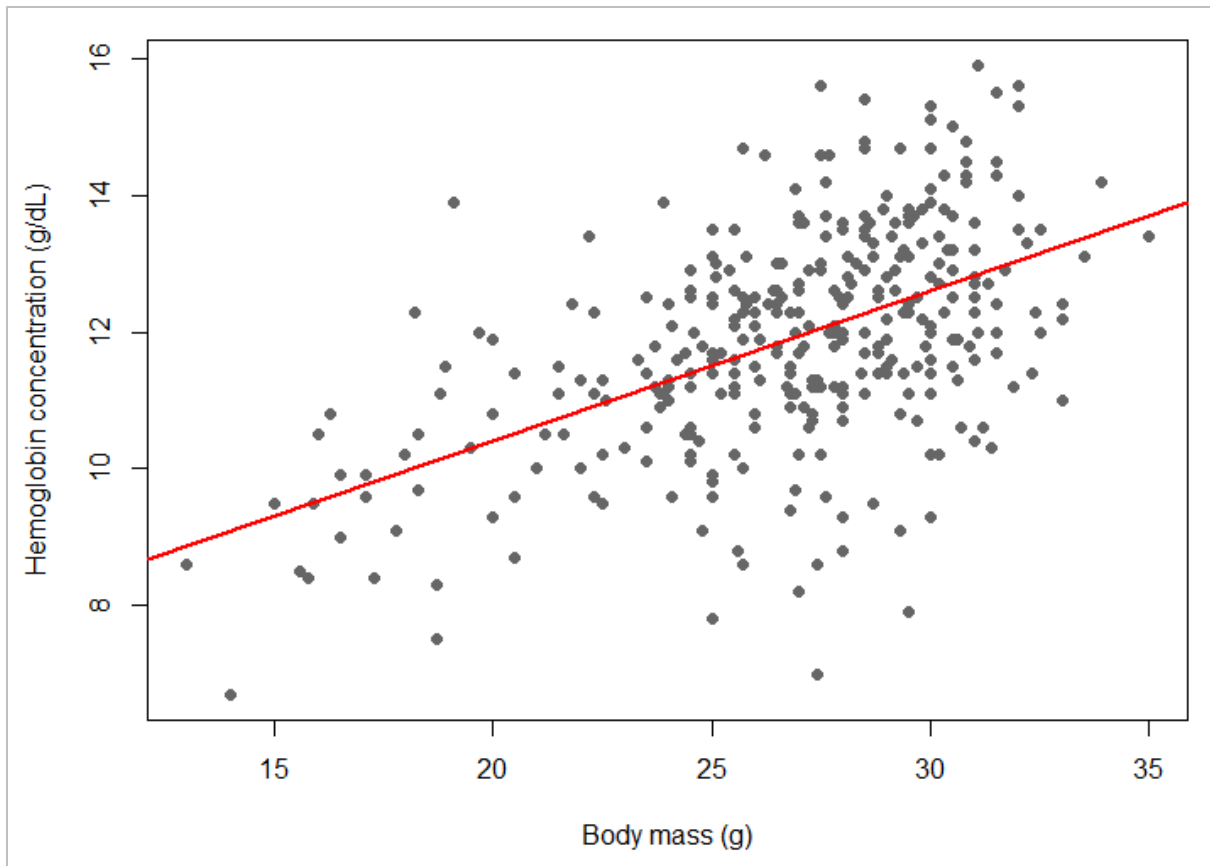
**Fig. S1:** Relative changes in the estimated hemoglobin concentrations (g/dL) from the HemoCue Hb 201+ photometer per time after filling the cuvettes with 10  $\mu$ L blood from a (A) male zebra finch (*Taeniopygia guttata*) at ambient temperature 22.2°C, (B) male zebra finch at ambient temperature 22.2°C, (C) female zebra finch at ambient temperature 24.2°C. The black dots represent the first measurement and belonging time series, and the grey dots indicates the second measurement. The reaction in the cuvette stabilized after about 240 seconds.



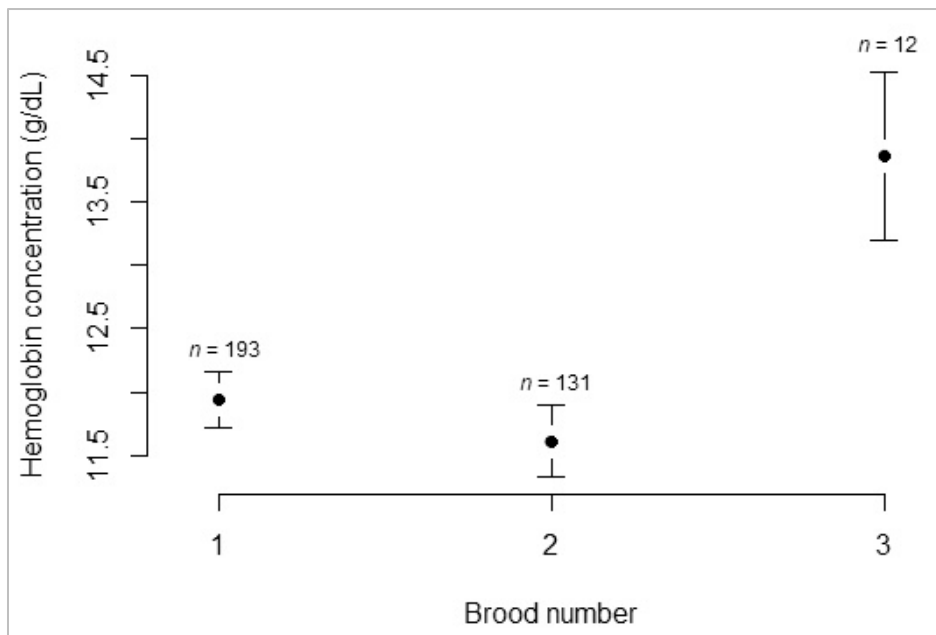
**Fig. S2:** Observations of house sparrow (*Passer domesticus*) nestlings' hemoglobin concentration (g/dL) at different developmental stages given the age in days,  $n = 350$ . Mean hemoglobin concentration for each age category: 4 = 6.7, 5 = 8.8, 6 = 8.8, 7 = 8.55, 8 = 10.3, 9 = 11.0, 10 = 11.7, 11 = 12.2, 12 = 13.3, 13 = 12.9. The means are indicated with circles along with their 95 % confidence intervals (CI) indicated by horizontal lines. The number of individuals in each age category are shown at the top of each category. 103 individuals are sampled twice: both at 5 (4-7) days of age and 11 (8-13) days of age.



**Fig. S3:** The individual development of house sparrow (*Passer domesticus*) nestlings' hemoglobin concentration (g/dL),  $n = 103$ . The first measurement on hemoglobin concentration is conducted at age 4-7 days, and the second measurement is conducted at age 8-12 days. The measured concentrations are marked with solid circles, and each line represents the different nestlings' development. There is a significant difference between the mean hemoglobin concentration in each measurement (paired t-test: mean difference = 2.896,  $t = -15.814$ ,  $df = 101$ ,  $p < 0.001$ ,  $n = 103$ ). Generally, the figure shows a positive trend where individual hemoglobin concentration increases from the first to the second measurement. This indicates a positive development in the nestlings.



**Fig. S4:** A simple linear regression between fledgling body mass and hemoglobin concentration (g/dL) in a house sparrow (*Passer domesticus*) metapopulation,  $n = 336$ . Each circle represents observed fledgling ( $10.32 \pm 0.07$  days of age) hemoglobin concentrations. The regression line is marked with a red-colored straight line. Body mass has a significant positive effect on fledgling hemoglobin concentration ( $b_{body\ mass} = 0.220$ ,  $F_{(1, 334)} = 127.4$ ,  $p < 0.001$ ).



**Fig. S5:** The observed average fledgling hemoglobin concentration (g/dL) in different brood numbers in a house sparrow (*Passer domesticus*) metapopulation. The mean hemoglobin concentration for each brood number category are indicated with circles along with their 95 % confidence intervals indicated by horizontal lines. The means in each group are: 1 =  $11.94 \pm 0.11$ , 2 =  $11.61 \pm 0.14$ , 3 =  $13.86 \pm 0.30$  g/dL. The number of individuals in each brood number category are shown at the top of each category, with the following number of broods in each category: 1 = 57, 2 = 48, 3 = 4. Total number of individuals sampled are 336.