Graduate thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Karina Nybø

The effect of haemoglobin concentration on the thermal conductance and metabolic rate of house sparrows

Sex differences in how whole blood haemoglobin concentration correlates with the thermal conductance and metabolic rate at temperatures below thermoneutrality in a population of free-living house sparrows *Passer domesticus* in Norway during winter

Graduate thesis in Biology
Supervisor: Henrik Jensen
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Abstract

Endotherms (mammals and birds) use metabolic energy to keep their body temperature at a stable level. The thermoregulatory curve, first described by Scholander and Irving, shows how the metabolic rate is changing with the ambient temperature for an endotherm. For populations in the colder parts of the world, such as Norway, it is especially interesting to look at the thermoregulation in cold temperatures, as this is the environmental conditions in which these populations normally live and function. A central parameter in the thermoregulatory curve is the thermal conductance, which is the slope of the curve in temperatures below thermoneutral conditions. Hence, thermal conductance reflects how much metabolic energy the animal needs to maintain homeostasis at certain ambient temperatures.

Haemoglobin is the oxygen transporting protein in the blood of most organisms. Because oxygen is driving the metabolic process in the body, the haemoglobin concentration is an important limiting factor in how fast an organism may take up oxygen from the air, transport it out to the cells in the body, and drive the energy turnover which again is used for movement, maintenance, reproduction, and thermoregulation.

In this master thesis, the metabolic rate, and the thermoregulatory curve of a population of house sparrows (*Passer domesticus*) in Åfjord, Norway was measured and seen in relation to haemoglobin concentration. Adult birds were captured in four different locations, and blood samples were taken to measure haemoglobin concentration. The metabolic measurements were done by using indirect calorimetry.

The results indicate that there is a sex difference in the effect of haemoglobin concentration on the thermal conductance in house sparrows at temperatures below thermoneutrality, where males have steeper slopes and higher metabolic rates in cold ambient temperatures, and females have the opposite response. If haemoglobin levels indicate good condition, then an adaptation like this may reflect two different strategies for optimal energy allocation for the two sexes. Males, who benefit from being able to compete physically, might have developed a strategy similar to the *increased intake hypothesis* in life history theory, where a high metabolic rate is beneficial. Females, on the other hand, who might benefit from saving energy for egg-laying and breeding, seem to have developed a strategy similar to the *compensation hypothesis*, where a low metabolic rate gives the highest fitness.

Samandrag

Endoterme dyr (pattedyr og fuglar) brukar metabolsk energi for å halde kroppstemperaturen sin på eit stabilt nivå. Den termoregulatoriske kurva, først beskrive av Scholander og Irving, viser korleis den metabolske raten endrar seg etter kvart som temperaturen i omgjevnadane endrar seg for eit endotermt dyr. For populasjonar i kaldare område av verda, slik som i Noreg, er det spesielt relevant å sjå på termoregulering i kalde temperaturar, då dette er dei miljømessige tilhøva desse populasjonane normalt lever og fungerer i. Ein sentral parameter i den termoregulatoriske kurva er konduktans, som er stigningstalet til metabolismekurva i temperaturar som er lågare enn termonøytrale tilhøve. Slik seier den termoregulatoriske kurva noko om kor mykje metabolsk energi som trengst for å oppretthalde homeostasen når temperaturen i omgjevnadane endrar seg.

Hemoglobin er proteinet som transporterer oksygen i kroppen hos den store majoriteten av organismar. Sidan oksygen er det som driv dei metabolske prosessane i kroppen, er hemoglobinkonsentrasjonen ein viktig avgrensande faktor for kor raskt ei organisme kan ta opp oksygen frå lufta, transportere det ut til celler i kroppen, og gjere om energi som igjen kan brukast til rørsle, vedlikehald, reproduksjon, og termoregulering.

I denne masteroppgåva vert metabolsk rate og den termoregulatoriske kurva sett i samanheng med konsentrasjonen av hemoglobin i blodet hos ein populasjon av frittlevande gråsporv i Åfjord, Trøndelag, for å sjå om det var mogleg å finne nokon form for korrelasjon. Vaksne gråsporv vart fanga på fire ulike lokasjonar, og blodprøver vart tekne for å måle hemoglobinkonsentrasjon. Metabolismemålingar vart gjort ved bruk av indirekte kalorimetri.

Resultata viser at det er ein kjønnsskilnad når det kjem til korleis høgare konsentrasjonar av hemoglobin korrelerer med den termoregulatoriske kurva, med høgare stigningstal og metabolsk rate i kaldare temperaturar for hannar, og motsett for hoer, med eit lågare stigningstal og metabolsk rate i kalde temperaturar. Dersom hemoglobinkonsentrasjon reflekterer god fysisk helse, kan ein slik tilpassing tyde på to ulike strategiar for optimal energiallokering hos kjønna. Hannar, som har større behov for fysisk konkurranse, kan ha utvikla ein strategi som er i tråd med *økt matinntak-hypotesen* i livshistorieteori. Hoer, som på den andre sida kan ha større fordel av å spare energi til reproduksjon og egglegging, kan ha utvikla ein strategi som er i tråd med *kompensasjonshypotesen,* i livshistorieteori, der lav metabolisme er det mest gunstige.

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Acknowledgements

I would like to thank my supervisors, Henrik Jensen, Bernt Rønning and Ådne Messel Nafstad, for all the help and support, providing me literature, helping me with my analysis, organising everything with the field work and giving me very useful feedback, and helping me improve my writing. A special thanks to Ådne, who still gave me feedback on my text even when he was on parental leave. I want to thank NTNU and the department of biology for making this project and thesis possible, for providing access to scientific literature and a reading room with a good work environment. I would also like to thank my friends and co-students for the help and tips along the way, and most importantly for the emotional support. Here, I would specifically list Hanne Alise Heien, Katharina Buchholz, and Eva Malene Stirø. Lastly, I would like to thank my son, Eldar, for being the light of my life.

Abbreviations and units

BM: Body mass (g)

BMR: Basal metabolic rate (mLO_2/h)

C: Thermal conductance, slope of the thermoregulatory curve (mLO_2/hT)

Hb: Haemoglobin (g/dL)

IC: Intercept C, the intercept of the thermoregulatory curve. MR at 0°C T_a (mLO_2/h)

MR: Metabolic rate (mLO_2/h)

RMR: Resting metabolic rate (mLO_2/h)

T_a: ambient temperature (T, °C)

T_b: body temperature (T, °C)

T_{LC}: Lower critical temperature (T, °C)

TNZ: Thermoneutral zone, zone of thermoneutrality (Interval of temperatures, T, °C)

T_{UC}: Upper critical temperature (T, °C)

1 Introduction

1.1 Endotherm energetics and thermoregulation

All organisms need energy to grow, reproduce, survive and to maintain physiological homeostasis (e.g., McNab, 2012). The rate of which this energy is transferred in the body is called metabolic rate (MR) (Bligh & Johnson, 1973), and is the most fundamental and widely researched physiological rate (Brown et al., 2004). Endotherms, contrary to ectotherms, use metabolic energy to regulate their body temperature, leading to thermoregulation taking up a large portion of their energy budget (e.g., McNab, 2012).

The idea of a basal metabolic rate (BMR) was developed at the start of the 20th century and was defined by Adolf Magnus-Levy as the ground level of metabolism for an animal at rest, in an post-absorptive state, with minimal stress, in a healthy condition and in a thermoneutral environment (Henry, 2005). This "thermoneutral environment" (or the more commonly used terms "zone of thermoneutrality" or "thermoneutral zone", TNZ) was further specified by Max Rubner around the same time as the range of ambient temperatures where the animal do not need to use metabolic energy to keep the body temperature stable.

The lower and upper points of the TNZ were called the lower and upper critical temperatures (T_{LC} and T_{UC}), respectively (Henry, 2005). Within the TNZ, the endotherm will use physical thermoregulation such as changes in insulation, posture or peripheral circulation, and the MR will remain at a stable level. As soon as the ambient temperature goes past the critical temperatures, the animal can no longer rely on only physical thermoregulation, but must change the rate of metabolism and use chemical thermoregulation (McNab, 2012). The TNZ is specific to each species, but will also vary within species, in relation to core temperature, the insulation, and the environment (Porter & Kearney, 2009). Many experiments were conducted in the early 1900s to determine the TNZ and the upper and lower critical temperatures of humans (Hardy et al., 1938).

For endotherms to balance heat loss with heat production, the metabolic rate must be proportional to the temperature differential between the body temperature (T_b) and the ambient temperature (T_a) (McNab, 1992):

Equation 1

$$\Delta T = T_b - T_a$$

Per Fredrik Scholander and colleagues put it all together in the middle of the 20th century and developed a model showing the relationship between the metabolic rate of endotherms in relation to the ambient temperature (Scholander, 1950, Fig. 1).

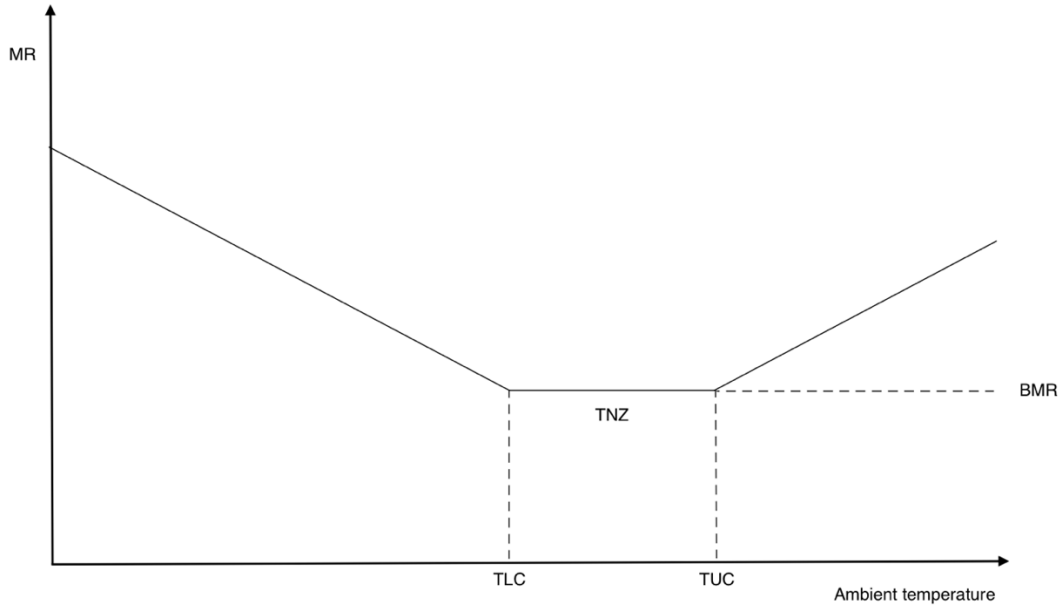

Figure 1: The Scholander-Irving curve, showing the relationship between metabolic rate (MR) and ambient temperature in an endotherm. The dotted vertical lines show the lower (T_{LC}) and the upper critical temperatures (T_{UC}). The dotted horizontal line shows the level of the basal metabolic rate (BMR). The thermoneutral zone (TNZ) is the zone of ambient temperatures where the MR is constant.

The Sholander-Irving curve (also called the thermoregulatory curve, fig. 1) shows a three-phase regression of metabolic rate (MR) on ambient temperature of an endotherm. The (flat) line between the lower and upper critical temperatures show that the MR within the thermoneutral zone is constant. Here, under optimal conditions (described by Magnus-Levy), the MR will equal BMR. On each side of the line of the TNZ, the metabolic rate is increasing as the temperature changes toward less optimal thermal conditions. When the curve of MR on T_a below T_{LC} extrapolates to MR = 0, then $T_b = T_a$, and the curve of this slope equals thermal conductance (C), which also is the inverse of insulation (McNab, 2012). This gives us the Sholander-Irving equation (Scholander, 1950):

Equation 2

$$MR = C(T_b - T_a)$$

While there are several methods of measuring metabolic rates, the most common method is measuring the rate of oxygen uptake, being the volume of O_2 per unit of body mass per unit of time (VO_2) (e.g. Arens & Cooper, 2005a; Dutenhoffer & Swanson, 1996). This method is also used in this study. Rewriting equation 2 and including VO_2 as a measure of MR (Bligh & Johnson, 1973; Tomlinson, 2016), we get:

Equation 3

$$C = \frac{VO_2}{T_b - T_a}$$

The respiratory quotient (RQ) is the ratio of CO_2 production to O_2 consumption and varies with the substrate that is being catabolized (carbohydrates, lipids, or protein):

Equation 4

$$RQ = \frac{mol\ CO_2\ out}{mol\ O_2\ in}$$

The RQ is usually 0.71 in a fasting animal, when only lipids are being catabolized (Walsberg & Wolf, 1995).

BMR scales to body mass (M_b) with a relationship that can be described as:

Equation 5

$$BMR = aM_b{}^{b_p}$$

Which gives an allometric relationship where a is a constant and b_p is a scaling exponent, or slope, in a log-scale relationship (White & Kearney, 2014).

1.2 Thermoregulation in life history theory

During the second half of the 20th century, studies on animal energetics were put into a larger perspective when the theory of life histories was developed by MacArthur and Wilson (1967). The ground principle is that the energy an animal hold is limited and must be allocated among all physiological aspects of life, such as number of offspring, reproductive age, and life span, and the different species have developed different balance optima for these aspects (Stearns, 1992). This means that all species will land on a spectrum from slow to fast (Pianka, 1970). Even after accounting for body size, "slow" animals will have fewer offspring, mature later, and reproduce at slower rates, while "fast" animals will have shorter lives and more offspring (e.g., Stutchbury & Morton, 2023). As metabolic rate reflects this "pace-of-life", studies of metabolism is tightly connected to life history studies (Auer et al., 2018).

An important underlying aspect of life history theory is the aspect of fitness. This is because the underlying mechanisms for adaption and evolution is to maximise the fitness of the population, and this is what is ultimately defining the different optima we see in nature for life history traits (Stearns, 1992). There have been conducted many studies which link metabolic rate to fitness (Bech et al., 2020; Metcalfe et al., 2016; Pettersen et al., 2016; Rønning et al., 2016), and birds is the animal group which have been studied the most in this field (Ricklefs & Wikelski, 2002).

Exactly how metabolic rate is related to fitness has been a matter of scientific discussion, and two main hypotheses have been dominating. The first hypothesis is the "*increased intake hypothesis*" (McNab, 1980), and the second hypothesis is the "*compensation hypothesis*" (Cody, 1966), where logical arguments can be made for both (Arnold et al., 2021). The increased intake hypothesis predicts that a high RMR gives a fitness advantage, because the individuals with higher RMR will gain a higher growth rate early in life because they are more competitive and therefore able to win the competition for food and resources (Careau & Garland Jr, 2012; Nilsson, 2002). On the other hand, the compensation hypothesis argues that individuals with a low RMR have an advantage because their low maintenance cost makes it easier to allocate their energy to growth and reproduction (Swanson et al., 2017).

A third hypothesis which combines these two main hypotheses has been called the context dependent hypothesis, and argues that it is unlikely that only one of these hypotheses will be true in all environments, as environments are variable, especially when it comes to food availability (Burton et al., 2011). The context dependent hypothesis states that, in environments with abundant food, individuals with a high RMR will have an advantage because of the higher growth rate, larger organs, and more competitive personality, and in environments with a scarcity of food, the opposite will be true, as the high RMR individuals will suffer from their higher maintenance costs (Arnold et al., 2021).

For house sparrows, there have been evidence for both hypotheses. Evidence for the increased intake hypothesis has been found in the form of increased reproductive output positively correlating with higher concentration of thyroid hormone (T3) and higher BMR in the field (Chastel et al., 2003). Contrarily, evidence for the compensation hypothesis were found for house sparrows in Norway, with a negative effect of BMR on recruit production for females, also after controlling for body mass (Rønning et al., 2016).

1.3 Avian Oxygen transport and haemoglobin

Avian ventilation is evolved to be very efficient, with a continuous flow of air through the system, so that the blood is constantly given fresh and oxygenated air. The combination of high efficiency in the way O_2 moves across the avian respiratory surfaces and a counter current system between respiratory surfaces and the circulation system make birds have the most efficient respiratory system in the animal kingdom (Dubach, 1981; Tucker, 1968a, 1968b).

The greater physiological efficiency of the avian utilization of oxygen in the air, compared to that of mammal species, is shown to be a combination of the more efficient ventilation system, the cross-current relation in the parabronchial gas exchange, and the additional counter-current relation in the air and blood capillaries (Maina et al., 1989). This gives birds a large advantage when it comes to the ability to inhabit and remain active in various environments and makes it possible to maintain homeostasis and body temperature at very high altitudes with low oxygen levels. House sparrows may maintain activity levels and body temperature at altitudes as high as 6100 m (Tucker, 1968b). In addition to this, birds can regulate their ventilation to increase the oxygen uptake when they are in altitudes with low oxygen levels (Arens & Cooper, 2005b) and they have a high toleration for hypoxia and high CO_2 levels in the blood (Tucker, 1968b).

As animals are faced with increased thermoregulatory needs, such as colder temperatures, this must be met by an increase in the oxygen supply to cells and tissue. This may be accomplished by not only an increase in oxygen uptake, but a change in haematological variables, such as haematocrit, haemoglobin, and number of red blood cells (Niedojadlo et al., 2018).

Haemoglobin (Hb) is a protein found in a large variety of phyla and is the main respiratory protein in the blood among vertebrates (Baldwin, 1976). It is found in the red blood cells, and functions both as a transportation of oxygen from the lungs to the tissue, as well as it facilitates the transport of CO_2 from the tissue to the lungs. Because Hb works as a transport and faciliatory protein for respiratory gases, it makes it possible to transport much more oxygen in the blood than would have been possible using only diffusion (Butler, 2016). The avian red blood cell contains large amounts of haemoglobin, and is, unlike the mammalian cell, nucleated (Anderson, 2006, p. 387). There are different forms of haemoglobin in birds, and the proportions shift with age (Scanes, 2015, p. 174). Haemoglobin levels in birds vary with age, season, the process of moult (Minias, 2015), as well as habitat (Herrera-Duenas et al., 2014).

Maximum oxygen carrying capacity in the blood is directly linked to the concentration of haemoglobin in the blood, however the maximum amount of oxygen that can be carried in the

blood is also dependent on the partial pressure of oxygen (PO_2) (Butler, 2016). This relationship can be described as:

Equation 6

$$C_aO_2 = PaO_2 \times \beta$$

Where C_aO_2 is the oxygen concentration in arterial blood, PaO_2 is the partial pressure of oxygen in arterial blood and β is the capacitance coefficient for oxygen (Piiper et al., 1971), and largely proportional to the haemoglobin concentration in the blood, but also varies with PO_2 in relation to the O_2 dissociation curve (Piiper, 1982).

The haem group is the functional group of the protein, each haemoglobin molecule containing four haem groups, each with iron atoms in the ferrous state which will bind oxygen ($FO_2 \rightarrow FO_3$) when the protein is in a high-oxygen environment, such as the capillaries in proximity to the lungs (the pulmonary circulation system). Contrary, when the haemoglobin moves across tissue with low oxygen concentration, this oxygen binding property is reversible, so that the oxygen is released from the haem group ($FO_3 \rightarrow FO_2$) to the blood and absorbed in the cells in that area (Baldwin, 1976). Oxygen diffuse from the blood into the cells and is used in the aerobic respiration, crucial in all tissue (Babcock & Wikström, 1992). This reversibility with oxygen creates an equilibrium which makes it possible to predict the fraction of haem groups having oxygen bound based on the partial pressure of oxygen in the blood, forming a sigmoid curve relationship. This sigmoid relationship means that once one oxygen atom is attached to one of the haem groups, it is easier for a second oxygen atom to connect to a second haem group on the same haemoglobin molecule, known as the haem-haem interaction (Baldwin, 1976). These properties make haemoglobin a very efficient respiratory protein.

Studies have shown that birds have a high degree of flexibility when it comes to haemoglobin levels, with higher levels in years with harder physical conditions, or during breeding season (Kaliński et al., 2012). There have also been found an increase in avian Hb concentration when faced with demanding tasks, such as flying high altitudes or long migration routes (Butler, 2010; Landys-Ciannelli et al., 2002).

Whole body haemoglobin concentration reflects the oxygen carrying capacity in the blood and therefore aerobic performance (Calbet et al., 2006; Minias, 2020; Yap et al., 2019). Hence, Hb

concentration has shown to correlate positively with physical condition and overall health in a variety of mammal and bird species (Cullumbine, 1949; Minias et al., 2014; Nyholm et al., 1995; Penninx et al., 2003). Studies have revealed evidence of a positive correlation between Hb concentration and good condition, in addition to higher fitness, in several species of birds (Blums et al., 2005; Minias, 2015; Yap et al., 2019).

1.4 The study species: house sparrow *Passer domesticus*

The house sparrow is a very widely studied species in many different fields of biology, including avian energetics (Anderson, 2006; Kendeigh et al., 1977). They are known to tolerate a wide variety of temperatures, and can acclimatize to temperatures well below their TNZ (Arens & Cooper, 2005b; Hart, 1962). Although house sparrows can tolerate very cold ambient temperatures, it has a high energetic cost, as they need to extensively increase their metabolism to maintain homeostasis (Anderson, 2006; Blem, 1973). The thermoneutral zone of house sparrows range between a lower critical temperature (T_{LC}) of 20°C - 22°C and a upper critical temperature (T_{UC}) of 37°C - 38°C (Hudson & Kimzey, 1966).

Variation in house sparrow BMR and RMR is associated with circadian rhythm, seasonal variations, breeding, general health/condition, geographical location, altitude, as well as body mass, age and sex (Anderson, 2006). Mean values of haemoglobin blood concentration in house sparrows vary in the literature, with mean haemoglobin levels ranging from 13 – 17 g/dL (Baumann & Bauman, 1977; Bush & Townsend, 1971; Herrera-Duenas et al., 2014).

During nestling times, female house sparrows provide three times as much brooding as males, on average. Breeding has a high cost for females, and their body condition will decline during the nestling period (Chastel & Kersten, 2002). House sparrows lay clutches of around 3-4 eggs (Chastel & Kersten, 2002; Husby et al., 2006; Seel, 1968).

House sparrow populations stay in Norway during winter (Altwegg et al., 2000; Jensen et al., 2013; Ringsby et al., 1998). Along the coast, they often live in proximity to human settlements and are in agricultural areas typically connected to farms (Pärn et al., 2012; Ringsby et al., 2006; Skjelseth et al., 2007).

1.5 The aims of this study

House sparrows (*Passer domesticus*), as other endotherms, must use a large portion of their metabolic energy to maintain homeostasis when the ambient temperature is well below their thermoneutral zone. Therefore, I assume that an optimal level of the metabolic rate in cold temperatures have evolved in house sparrows to give the greatest fitness. For this master thesis, I aimed to look at how haemoglobin concentration in free-living house sparrows correlates with the slope and intercept of their thermoregulatory curve. In addition, I wanted to look at whether these correlations varied between the sexes. The slope, C (thermal conductance), is a measure of how fast the metabolic rate changes as the ambient temperature (T_a) starts to deviate from the bird's thermoneutral zone. The intercept (MR at 0°C) is an indicator of how much metabolic energy that is needed to maintain thermal homeostasis in ambient temperatures around 0°C. Therefore, investigating how these two parameters is affected by Hb concentration would give a good overview of the correlation between Hb concentration and the thermoregulation in house sparrows.

Hence, for this master thesis, the aims were to:

1. Investigate the correlation between haemoglobin concentration and thermoregulation in house sparrows, and
2. Look at possible sex differences in this correlation.

2 Methods

2.1 Field work

The field work was done in Lauvøya, Åfjord municipality on the coast of Trøndelag, Norway. Populations of house sparrows on the coast of Norway have been studied for many years by the department of biology, with field work and research on many different fields; from population dynamics, genetics and behaviour to physiology, morphology, and life history studies.

During field work, the populations have been carefully observed and monitored through many years, using ring marking, bird box monitoring, capture – release and field observations. In the Helgeland peninsula area in northern Norway, this has been an ongoing project for almost 30 years (Ringsby et al., 2006), while house sparrow populations in Lauvøya have been studied since 2012 (Kvalnes et al., 2017). The data used in this thesis was collected in Lauvøya and surroundings (see fig. 2) during winter season 2019, 2020, 2021 and 2022. The field work was led by Ph.D. Ådne Messel Nafstad.

Figure 2: Maps showing the location of Lauvøya. Maps are taken from kartverket (www.norgeskart.no).

The field work for this study may be divided in four steps: aviary preparation, bird capturing, registration and blood sample taking, and metabolism measuring.

2.1.1 Aviary preparation

The first thing that was done when arriving to the study location was to prepare the aviary. An old barn was used for this purpose, and the same location was used each year. All holes and possible escape routes were covered up, and windows were padded with see through plastic so that the birds should not crash into the windows. Tarp was used to cover all hard surfaces and to divide the space into smaller rooms and sections. The distance from the floor to the roof was approximately 2 m, and the size of the rooms varied between small rooms measuring approximately 10 m^2, and the largest room with approx. 50 m^2. The smaller rooms were made to keep control over groups of birds prior to respirometry measurements. Branches were put up for the birds to sit on, and lastly, *ad libitum* food and water was put inside. The goal was to make a comfortable space for the birds to be in and to reduce stress before and after metabolism measurements. Since this population of house sparrows usually live inside barns, the aviary was a familiar space to them.

2.1.2 Bird capturing

The birds were then observed and captured in different locations on Lauvøya and proximity, with four main locations: Flenstad, Rånes, Selnes and Sørdahl (see table A1 in appendix). The four main capture locations, in the data analysis listed as "farms", can be seen as sub-populations in the Lauvøya-Åfjord metapopulation. They are similar in the sense that they are part of the same metapopulation, live relatively close to each other geographically, and they share a similar environment consisting of a farm with one or several barns were the birds find food and shelter. In the analysis they are divided into these groups both to take small variations in the physical environment into account, but also the relatedness (genetic similarity) that is likely to be found within the "farms" or sub-populations.

Mist nets were used to capture the birds, both inside barns and outside in areas where there was likely to capture house sparrows. The mist nets were put up and checked regularly during the day, to make sure no birds were stuck in the net for too long before carefully taken out and put in small bags made of fabric. The birds were only in the bags for a short period of time, until they could be taken inside and registered. The registration was done inside a cabin where the field workers lived during field season, or adjacent to the aviary. The aviary was just a short walk away from the cabin. This was not only practical, but also minimised stress from transportation and handling time.

2.1.3 Registration and blood sample taking

After capture, the birds were taken inside to be registered in the database, and blood samples were taken. Because this population is being closely monitored, most birds were already ring marked and registered in the database from before. Each recapture was also registered in the database, which made it possible to follow the same individual bird over several years. If the birds were not previously ring marked, they were marked immediately after capture. The haemoglobin level of the sparrows was measured using HemoCue® Hb 201+ (HemoCue AB, Ängelholm, Sweden). A single drop of blood (25 µL) was absorbed into a microcuvette and put into the apparatus, and HemoCue® showed the Hb-values after a few minutes in *g/dL*. HemoCue® is ideal for field work, saves time because it is not necessary to send the results in to a lab, and the results are ready in only a few minutes. It has been shown to be a valid and accurate method for measuring whole blood haemoglobin concentration for a variety of bird species in the field (Harter et al., 2015; Velguth et al., 2010).

The birds were then taken to the aviary and held there before metabolism measurements. Individuals that were captured on different farms were not held in the aviary at the same time, so that all the birds in the aviary at any given time were from the same farm or subpopulation.

2.1.4 Metabolism measurements/respirometry

Prior to respirometry, the 7 birds that were going to be used for the experiment that night were kept in a smaller separate space within the aviary, to control their food intake. Food was made unavailable to the birds four hours before measuring, so that the birds were in a fasting state when measurements started (Walsberg & Wolf, 1995). The RMR equipment was logged before and after each measurement session, to control for drift in the analysing equipment. The experimental set up was in a separate room right next to the aviary, inside the same old barn as the aviary. It was important that the distance was this short to minimize stress factors prior to metabolism measurements.

The birds were then weighed in cotton bags on Ohaus® Port-O-Gram scales with a small bucket on. Birds who showed sign of bad condition or disease, or had a high mass loss since initial capture, were not used for the respirometry experiment, but were safely put back to the aviary. The decision on which birds were not fit for the experiment had to be done subjectively, as standardizing these decisions are not straightforward because mass may vary throughout the day.

iButton® loggers were attached to them using an elastic band around the right wing, over the back an around the left leg so that the logger was placed under the wing when folded (see figure 3). The iButton® loggers are small, only less than 10 mm in diameter, so they are ideal to use on small animals like house sparrows. Since this measurement was cofounding with the treatment in the chamber, being affected by the ambient temperature, this measurement had to be treated carefully (see discussion section). The birds were then ready to go through the respirometry and were placed inside small jars which functioned as respirometer chambers.

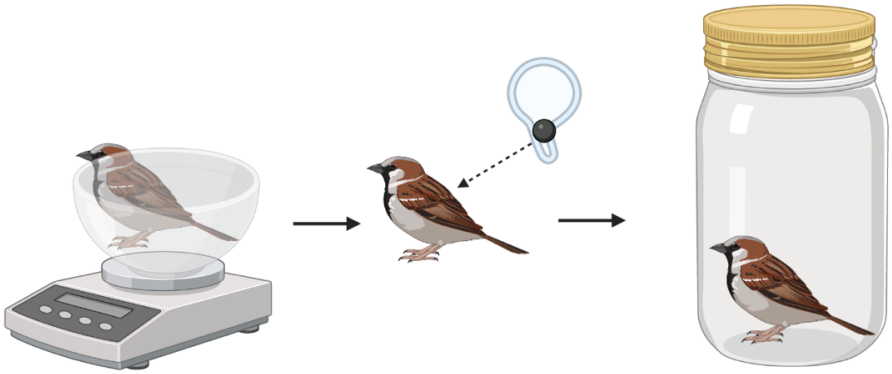

Figure 3: Preparations before a bird is going into the respirometer. Birds were weighed, iButton® was attached, and then put inside one of the chambers in the climate cabinet. The figure is made using BioRender (www.biorender.com).

Resting metabolic rates were measured using indirect calorimetry, in an open flow, push through respirometry system, based on the real-time volume of oxygen consumption (Withers, 1977). The birds were kept inside the respirometer during the night (from 7 pm to 7 am, approximately). The birds sat inside small mason jars (chambers) with air flowing in and out, and there were 8 small chambers like this inside a custom built climate cabinet (Rønning et al., 2016). The airflow through the system was carefully monitored and logged. The oxygen levels in dried air (dried with Drierite ®) were registered entering and exiting the bird-containing chambers. During the night, the temperature inside the respirometer were shifting from 28°C to 15°C and lastly to 5°C. An O_2 meter was continuously measuring the oxygen consumption (VO_2) of the birds throughout the night. For a more detailed description of the set-up, see Rønning et al. (2016).

Fig. 4 shows a model of the equipment set up, and how the air flows through the system. Note that fig. 4 only shows the path for one bird, for simplification.

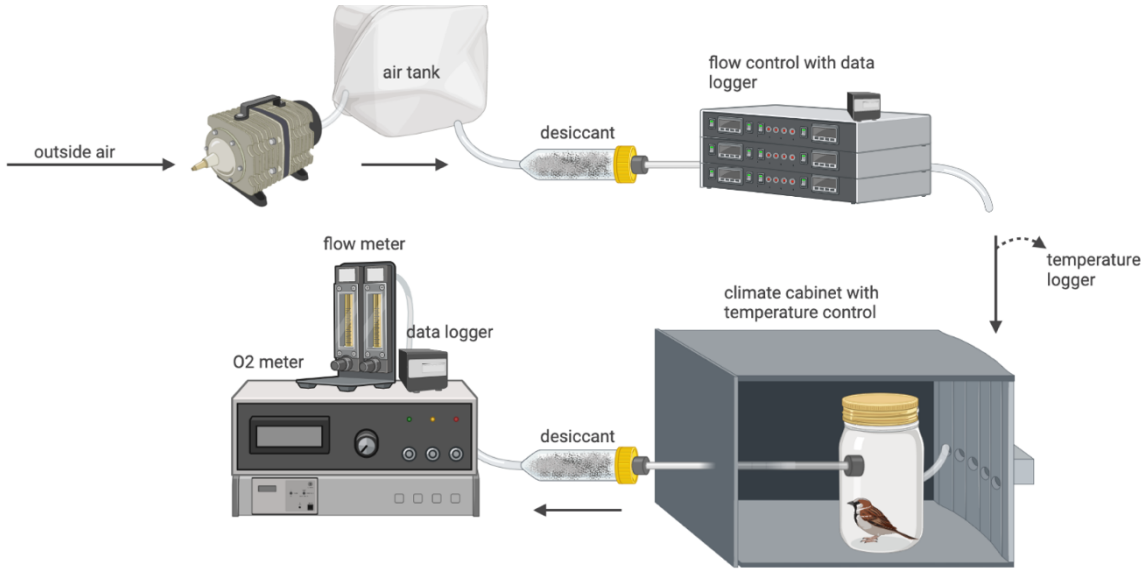

Figure 4: Schematic figure of the experimental set-up. The figure shows the path for one bird, arrows showing the direction of the air flow. The figure is made using BioRender (www.biorender.com).

2.2 Calculations

The metabolic rate was calculated using the rate of oxygen consumption (VO_2), using the equations from Withers (2001), with an RQ of 0.71 (see Nafstad et al., 2023; Rønning et al., 2016).

The temperature inside the respirometer changed from 28°C, through 15°C and ended at 5°C throughout the night. BMR was calculated using the 10 minutes lowest running average O_2 consumption (VO_2) at 28°C T_a. C and intercept C (IC) were then calculated as follows: at 15°C and 5°C T_a respectively, a value drawn from a normal distribution of the O_2 consumption was simulated, with the lowest 10 minutes running average VO_2 used as mean, and the corresponding standard error (SE) used as standard deviation, and a regression line fitted through the two points. The average regression through these, after simulating 1000 such regressions, the mean and standard deviation of the slopes and intercepts were then estimated, providing the best estimator for the slope = C and intercept = "IC" and their respective standard errors.

2.3 Statistical analyses

For the statistical analyses, R and R studio was used with R version 4.2.1. For full script, see Appendix 3.

Simple linear models were made to get an overview over possible results and correlations. At first glance there seemed to be little or no effect of haemoglobin on the C and IC, without looking at the sex interactions. When looking at interactions, using linear models, the sex-Hb interaction and sex-BM interactions were significant, so these sex interactions were included in the models.

Because of the many different variables in the models, and the non-independence within some of them (e.g., individuals from the same farm was assumed to be more genetically similar to each other), linear mixed models (LMMs) were used. Different models were tried, for example models with both ID and "chamberyear" as random effects. This did not seem to work as the models did not converge. Therefore, ID was removed as random effect in the model, and instead, to account for pseudo replication, only the first measurement by each individual was used in the models.

Since the body temperature measurements should be treated with care because of the uncertainty in the measuring method, body temperature was not included in the main models, but in complementary models, see 2.6.2.

2.6.1 Main models

Linear mixed models (LMMs) were used, with the package "lme4" (Bates et al., 2009). The main models are as follows. Random effects are coloured green. The "SD" in the models is the standard deviance for C in main model 1 and the standard deviance for intercept C in main model 2, respectively, and were included because biological errors due to stress and movement within the chambers can be expected to upward bias metabolic rate measurements.

Main model 1:

$$C = sex + Hb + BM + sex{:}Hb + sex{:}BM + BMR + SD_C + farm + chamberyear$$

Main model 2:

$$Intercept\ C = sex + Hb + BM + sex{:}Hb + sex{:}BM + BMR + SD_{IC} + farm + chamberyear$$

The fixed effects in the models were: sex, haemoglobin level (Hb), body mass (BM), BMR, standard deviance of the response variable (SD_C and SD_{IC}), and farm. Sex, Hb and BM were included as a fixed effect because it had a significant effect, and because the interaction effects with sex were included. BMR was included as a fixed effect to account for a possible correlation between BMR and C and/or IC. The standard deviance of the focal variable of each of the main models (C and Intercept C) were included in each of the models as a fixed effect. By doing this, the effect of short-term biological errors, such as movement and stress, was reduced.

Because there could be non-independence within the different catch locations, or farms, "farm" could in theory be considered a random factor, but because there were only four levels in the "farm" column (Flenstad, Rånes, Selnes, and Sørdahl), it were too few levels for it to be classified as a random factor in a LMM, so it was instead included in the models as a fixed effect.

The random factors in these models were chamber and year. They were merged into one variable, "chamberyear", in the analyses. This was done to account for the effect of taking down and setting up the equipment each year, where the chambers would be a little different each year. Another advantage was that this gave more statistical power. A disadvantage with doing this was that it was not possible to look at the variance that came from year variation only, because this variation was enclosed in the "chamberyear" variable.

2.6.2 Complementary models

Two complementary models were made, looking at the effect of body temperature on the thermal conductance and the IC. The same format was used as for the main models, LMMs with "farm" and standard deviances as fixed effects and "chamberyear" as random effect. BMR was not used in these models, because the focus was on the difference in change in body temperature between the two sexes when ambient temperature decreased. At 28 °C, it was assumed that birds were at BMR level.

A new subset of the main dataset was made. Because the goal this time was to look at the temperature measured by the ibut at 5 °C, it was important to remove the data points that were clearly incorrect. For example, some iButtons had fallen off, and some showed very extreme values which were probably wrong. To figure out where to cut off the data and find the threshold for where a value was "too extreme", a histogram was made of the frequency of the measured change in skin temperature from BMR-levels to 5 °C. Since this change in skin temperature was expected to follow a normal distribution, the cut-offs were made at each end of the bell-curve from the histogram. Values outside this were considered to be extreme values due to measurement error (see Appendix 2). The change in body temperature (/skin temperature) from thermoneutral levels were noted as ΔT_b.

Complementary model 1:

$$C = sex + \Delta T_b + sex{:}\,\Delta T_b + SD_C + farm + chamberyear$$

Complementary model 2:

$$Intercept\ C = sex + \Delta T_b + sex{:}\,\Delta T_b + SD_{IC} + farm + chamberyear$$

As for the main models, LMMs were used for the complementary models, also with the "lme4" package and REML=True, and SD as fixed effect.

3 Results

Haemoglobin concentration and body mass were significantly different between male and female house sparrows, while BMR, C and IC were not (see table A2 in appendix).

3.1 Main models

The main models showed a significant interaction effect between sex and haemoglobin concentration on the thermoregulatory slope, C (95% CI [-1.09, -0.0952], t = -2.310). Equivalently, a significant interaction effect between sex and haemoglobin concentration for the metabolic rate at cold temperatures, "intercept C" was observed (95% CI [0.716, 16.50], t = 2.115).

Body mass also had an interaction effect with sex. For metabolic rate at cold temperatures, "intercept C", the interaction effect with sex was significant (male and body mass interaction with 95% CI [1.48, 12.55], t = 2.456). The same pattern was found in the thermoregulatory slope, or conductance C, with a significant sex and body mass interaction (male and body mass interaction with 95% CI [-0.838, -0.138], t = -2.707). See figures A13 and A14 in appendix 2.

No effect of Hb concertation on BMR was found (95% CI [-1.63, 1.08], t = -0.398). BMR was strongly affected by body mass (95% CI [2.53, 4.49], t = 6.961), but not by sex (95% CI [-30.31, 74.60], t = 0.818).

The significant sex-Hb interactions on the thermoregulatory curve (C and IC) show that the correlations between haemoglobin concentration and thermal conductance are different (have different slopes) in males and females. Figures 5 and 6 show the effect of haemoglobin concentration (standardised to the grand mean) on C and IC, respectively, for males (blue) and females (red).

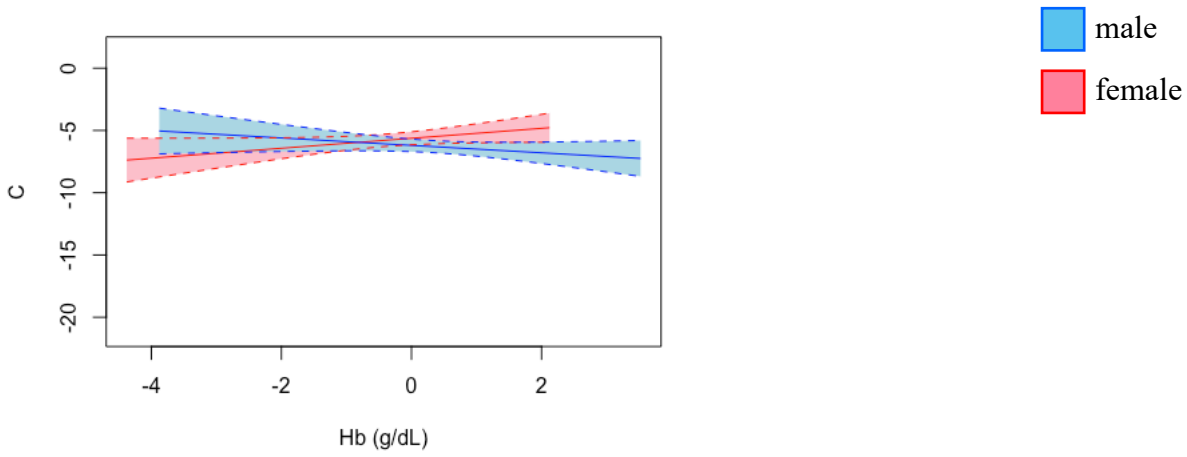

Fig 6: The effect of haemoglobin levels (x-axis) on the thermal conductance (y-axis) in male (blue area) and female (red area) house sparrows. [Hb] (g/dL) is standardised to the grand mean. Polygon area and dashed lines show 95% confidence intervals.

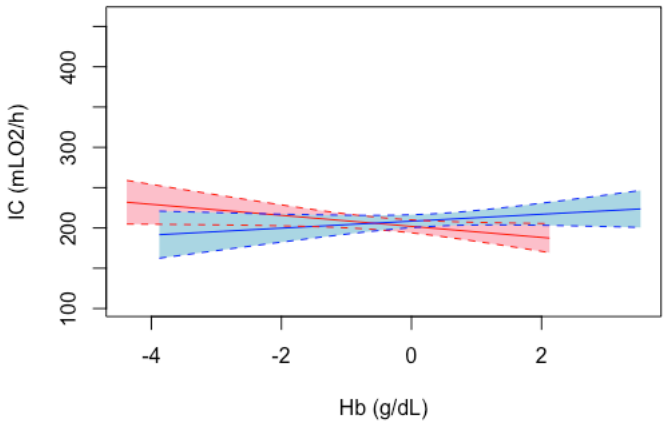

Fig 7: The effect of haemoglobin levels (x-axis; here standardised to the mean) on the metabolic rate at 0 degrees Celsius (y-axis) in male (blue area) and female (red area) house sparrows. Polygon area and dashed lines show 95% confidence intervals.

Figures 6 and 7 show that male and female house sparrows have opposite correlations between haemoglobin concentration and the thermoregulatory curve parameters (C and IC). For males, there is a negative correlation between [Hb] and C, which means steeper thermoregulatory curve with higher Hb concentration. This is also reflected in the positive correlation between [Hb] and IC, which means higher metabolic rates at 0°C with higher haemoglobin concentration.

For females, the opposite result was found. There was a positive correlation between [Hb] and C, reflecting a shallower thermoregulatory slope, and equivalently, a negative correlation between [Hb] and IC, reflecting lower metabolic rates at ambient temperatures of 0°C with higher haemoglobin concentrations.

Figure 8 gives a descriptive view of male and female house sparrows metabolic responses to cold ambient temperatures for individuals with high haemoglobin levels. Note that this is only a descriptive figure, which is not based on the real data. Therefore, the steepness of the slopes (C), intercept (IC) and the distance between the slopes are not accurately describing the actual mean slopes and intercepts.

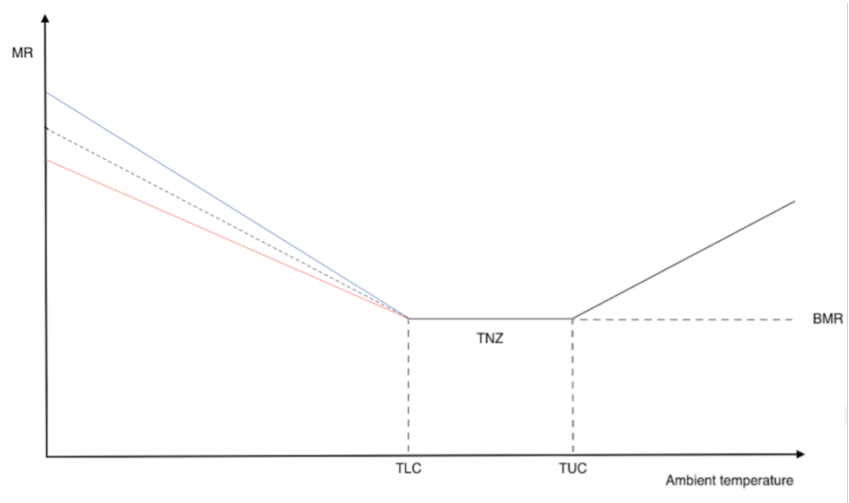

Fig. 8: Model graph showing how the Sholander-Irving curve below thermoneutrality would look for individuals with high Hb-levels, contrary to individuals with average Hb-levels (dotted line). The blue line representing the thermoregulatory curve for males and the red line for females.

3.2 Complementary models

The complementary models showed no correlation between degree of change in body temperature (from thermoneutral levels to ambient temperatures of 5°C, ΔT_b) and thermoregulatory curve (C and IC). There was no significant sex interaction with ΔT_b on neither C (95% CI [-0.078, 0.13], t = 0.482) nor on IC (95% CI [-1.88, 1.31], t = -0.340).

4 Discussion

4.1 Main findings

In this thesis, the goal was to look at how haemoglobin concentration correlated with the parameters of the thermoregulatory curve in house sparrows. The results of the analyses revealed an opposite response for males and females in this correlation. Males with higher haemoglobin levels had higher metabolic rates at colder ambient temperatures, while females with higher Hb concentrations had shallower thermoregulatory curves at ambient temperatures below thermoneutral conditions. This correlation was linear for both sexes, so the opposite, equivalent result could be observed for individuals with lower-than-average Hb-levels: shallower slopes for males and steeper slopes for females.

There was no correlation between Hb concentration and BMR in none of the sexes, meaning that the effect from [Hb] on metabolic rates only becomes visible when the temperature gets below thermoneutral conditions. The effect of body mass on thermal conductance (C) and metabolic rate at cold temperatures (IC) showed the same results as [Hb] did on these parameters, with a sex difference in the correlation equivalent to what was observed with [Hb]. Therefore, figure 8 could just as well have been a model graph showing the thermoregulatory curve for individuals with higher-than-average body mass.

From the complementary models, there was no correlation between change in body temperature and thermoregulation. One way to look at this result is that the variation in the thermoregulatory slopes (C) and intercepts (IC) that was observed was not explained by the ability (or lack of ability) to keep a stable body temperature (or maintain homeostasis). There was found no significance in the interaction effect with sex, indicating no sex difference in how change in body temperature was related to the slope and intercept of the thermoregulatory curve. However, as discussed in 4.2.1, the way of measuring body temperature could have been done differently to get more accurate results, which in turn could have given different results.

The lowest temperature in the respirometry was 5°C, which is close to the actual ambient temperatures in the study area during the time of year that the measurements were done (see https://tinyurl.com/yrnolauvoya for weather statistics). Therefore, the metabolic rates of the birds in the respiratory chambers at 5°C is closer to the actual daily metabolic rates for house sparrows during winter than the RMRs measured within the TNZ. This is an important point, because the respirometry was not done under extreme conditions but rather conditions that were true to the daily life of the sparrows. The energy expenditure the birds showed in the climate cabinet was very similar to what they would experience on a regular night, at least temperature and energy wise. Since the temperatures outside often get much colder than this during winter, it is logical to assume that the metabolic capacity of house sparrows at 5°C is well below their maximum metabolic (or aerobic) capacity.

4.2 Implications

4.2.1 Haemoglobin and fitness

In a review of over 120 studies, Minias (2015), argues that haemoglobin concentration can be used as an indication of condition in birds, as haemoglobin concentrations were positively correlated with other commonly used indications of condition in birds, such as body mass, diet quality and fat loads. Some studies also show a correlation with fitness related traits, such as survival (Krams et al., 2013). In a comparative analysis by Yap et al. (2019), they argue that birds with higher Hb concentration might have higher fitness in terms of reproductive success (Velmala et al., 2015). A large scale study stretching over 15 years, it was found that relative body condition was linked to fitness both when it came to survival, as well as reproductive output in three species of female ducks (Blums et al., 2005).

4.2.2 Sex difference

When the findings in this study is put into the context of fitness, it is interesting to look at the observed sex difference in the response of haemoglobin concentration on the thermoregulatory curve. High [Hb]-individuals are likely to have a good condition, and to have a higher fitness, than the average in the population. However, males and females with high haemoglobin levels have the completely opposite thermoregulatory response in cold temperatures. If both the males

and the females with high haemoglobin concentrations have higher fitness, then this opposite response indicates that the two sexes have two different optimal strategies. Hence, the response that was observed in males show evidence for the increased intake hypothesis, while the response that was observed in females show evidence of the compensation hypothesis. This was further backed up by the fact that the correlation pattern between body mass and thermoregulation was the same as for the Hb-thermoregulation correlation pattern (figure A13 and A14 in appendix 2). Body mass is typically used as an indicator of good condition and has been linked to fitness in house sparrows (Bonneaud et al., 2004; Jensen et al., 2004; Moreno-Rueda, 2015).

4.2.3 The context dependent hypothesis

Because the results show evidence of both hypotheses, the collective result is supporting the context-dependent hypothesis, where "context" also can be in the form of sex. According to this hypothesis, the optimal metabolic rates will depend on the environment, and especially the access to food (Burton et al., 2011). High-fitness males have higher metabolic rates in cold ambient temperatures, and their thermoregulatory slope is steeper, meaning that they can increase their metabolic rate faster. The context-dependent hypothesis states that it is beneficial to have a high RMR in food abundant environments, as individuals with higher metabolic rates are more likely to have the competitive advantage. Being risk taking individuals, they are then able to collect more food, which has a positive effect on their growth rate. This again gives them larger organs and can become larger animals with higher fitness (Burton et al., 2011).

In this study, males had significantly higher body mass than females (see table A2 in appendix 1), which is corresponding to what other studies have found (Chastel et al., 2003; Johnston & Selander, 1973). However, not all studies have found a significant sexual dimorphism in the size of house sparrows (Chappell et al., 1999). House sparrows have shown to have different personality traits, some being more "risk takers" than others (Bókony et al., 2012). A possible explanation for the evidence of the increased-intake hypothesis in males can therefore be that males have higher body mass and/or possibly more competitive personalities, as the competition for mating might reward competitive males. These large, "risk taking" males would then have higher fitness, and higher metabolic rates, which would make sense in the perspective of the increased-intake hypothesis.

Females showed a negative correlation between Hb concentration and metabolic rate in cold temperatures, as well as thermal conductance. The context-dependent hypothesis states that in environments with a scarcity of food, it is beneficial to have a low metabolic rate, because this gives lower maintenance requirements (Burton et al., 2011). Individuals with low maintenance costs, hence low metabolic rates, can save energy and have more energy to spare to grow and reproduce. The findings for female house sparrows indicate an underlying mechanism similar to this hypothesis. However, it is likely to assume that it is not the scarcity of food that makes it beneficial for the females in this study to have a low metabolic rate at cold temperatures. If that was the case, one would expect to see a similar strategy in males. Also, the studied house sparrow populations were living inside barns where the availability of food in the form of grain was abundant, so there is little evidence of food scarcity, even though the experiments were done during winter.

Female house sparrows have a very high cost of reproduction, which requires almost half of the energy budget during breeding season (Krementz & Ankney, 1986). A compensation hypothesis for metabolic rate can maybe be explained by this high energy cost. The metabolism measurements were done pre-breeding season, in February-March. It is possible that in addition to the cost of maintaining homeostasis in the cold winter temperatures this time of the year, it is especially beneficial for the females to be able to save energy prior to breeding season.

4.3 Comparison to other studies

There has not been done much research on how haemoglobin concentration affects the thermal conductance and the metabolic rate at cold ambient temperatures, neither in house sparrows nor in other endotherm species. However, some literature exists. In a study of arctic tern chicks, no correlation was found with haemoglobin concentration, neither with BMR nor with (cold induced) maximum oxygen uptake (Bech & Klaassen, 1996). It was discussed that the reason for this might be that chicks were transitioning to a different kind of haemoglobin, and that the results found in days year old chicks might not correspond to the results found in adult birds.

Research on how iron-deficiency affects thermoregulation in humans showed a reduced ability to keep a stable body temperature as the ambient temperature got colder (Beard et al., 1990; Martinez-Torres et al., 1984). Similar findings were observed in rats (Dillmann et al.,

1980; Dillmann et al., 1979). In this study, there was no evidence of a negative correlation between Hb concentration in the blood and body temperature at cold ambient temperatures. However, this measure was not a direct measure of body temperature and was cofounded with the temperature in the chamber, and body temperature was not included in the main models.

Several studies have found evidence for either the increased intake or the compensation hypothesis in one of the sexes and not both (Blackmer et al., 2005; Jimeno et al., 2020; Rønning et al., 2016; Schimpf et al., 2012). However, to the authors knowledge, no other studies have found support for both hypothesis for the same species, with one hypothesis for males and one for females.

4.4 Methods used

For the methods used in this project, some limitations exist, as further elaborated in this section. Including the effect of body temperature and the effect of year in the analysis could make the results even clearer and bring some new information about the effects that was shown in the results.

4.4.1 Body temperature

iButton® loggers have been proven to be a highly accurate way of measuring skin temperature on humans (Hasselberg et al., 2013; Lichtenbelt et al., 2006). The device has also been used in a variety of studies on different groups of animals, as a measure of body temperature, by surgically implanting the device into the intraperitoneal cavity (Lovegrove, 2009). It has, however, not been used as much to measure skin temperature on birds or other animals. The role of skin temperature and insulation in thermoregulation, as well as the relationship between core body temperature and skin temperature, are complex and non-straightforward matters that in themselves need more research (Romanovsky, 2014; Taylor et al., 2014).

iButton® loggers were attached under the wing of the birds and would track the change in skin temperature. It was assumed that skin and body temperature would follow each other (in this case, declining) in a proportional matter, so that the T_{sk} between birds would be comparable in the same way as if T_b was measured per se. This assumption could be met if the skin and body

temperature had the same (linear, or log-linear) relationship for all birds (with the same slope or scaling exponent). However, it is possible that individual variation in physiological factors such as fat loads, size (surface-to-volume ratio), body mass, degree of insulation (feathers, down), and difference in the ability to allocate heat may violate this assumption.

The measurements from the iButton® loggers were also affected by the ambient temperatures, resulting in a cofounding effect between the iButton® loggers and the treatment in the chamber.

Even though the loggers were attached as carefully as possible, some stress was observed on some of the birds that might have been from the possible discomfort of the elastic bands and loggers on them. In respirometry, it is crucial to minimise the stress as much as possible, as the goal is to measure the lowest metabolic rates. Other ways of measuring body temperature should be considered for similar research in the future, or to simply not measure skin/body temperature in the respirometry.

Suggestions for how to measure body temperature has been presented by Lovegrove (2009). By modifying the iButton® loggers, the loggers could be implanted in animals weighing 20-30 g, which is within the range of an adult house sparrow. This method was successfully used in a study of a passerine bird (*Plocepasser mahali*) to measure T_b (Smit et al., 2013). However, as this is an invasive surgery, a thorough ethical assessment and training needs to be done in beforehand, and considerations should be done to decide if this is something that is possible to accomplish in a safe and sterile way in the field.

4.4.2 Effect of year and season

Both Hb concentrations and (resting) metabolic rates are plastic traits that can vary with season and change in energetic demands (Dawson & Marsh, 1989; Kaliński et al., 2012; Landys-Ciannelli et al., 2002; Swanson, 2010). Different years would naturally be associated with different environmental factors such as, the access of food, changes in the weather, difference in the ambient temperature, random changes in population structure, and the presence of parasites or disease.

For the analysis, the effect of year was included, but it was merged with chamber to account for the effect of taking down and putting up the equipment each year. Therefore, the effect of year, season, or weather per se was not accessible. However, having too many random factors

in an LMM can be problematic, as the model might not converge. Therefore, including weather, year or season into the models might explain more, but also decrease the statistical power.

As the experiments were done the same (pre-breeding, winter) season each year, it was not necessary to include season as a factor. For further research, it could have been interesting to see how metabolic rates, haemoglobin levels and the observed sex difference in this correlation might change during a year. For example, the same experiments could have been done post-breeding, summer/autumn. This would give a broader overview on how this dynamic is maintained, or changes, with season, and give a deeper understanding on the physiology and energetics of house sparrows and how they interact with the environment.

Conclusion

Understanding the physiology and functioning of wild animals, the limitations of their energy expenditure, and how they interact with the environment is crucial when wanting to understand how climate change affects wildlife.

In this thesis, I have looked at how haemoglobin concertation in male and female house sparrows correlate to the slope and the intercept of their thermoregulatory curve in temperatures below thermoneutral conditions. The results show a significant sex difference in this correlation. For the males, a positive correlation was found between haemoglobin level and both the steepness of the thermoregulatory slope and the metabolic rate at 0°C. For females, the opposite correlation was found. In the discussion part I contextualised this into life history theory and introduced the context dependent hypothesis as a possible explanation for the sex difference in the response of Hb on thermal conductance in this population of house sparrows.

More research on thermoregulation and metabolism for several species is needed to grasp the nature of eco-physiological processes. These processes are the foundation for understanding how species will adapt to changes in temperature and environment, how they will meet new energy demands, and what consequences this will have for the ecological dynamics in nature.

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Appendix

Appendix 1: Tables

Table A1 shows the population estimate and the number of captured birds each year on each of the four farms (capture locations). Table A2 shows the means and standard deviances of BMR, Hb, C, IC and BM, and the p-values from T-tests comparing male and female means of each trait.

TABLE A1: NUMBER OF BIRDS ESTIMATED AND CAPTURED ON THE DIFFERENT FARMS OVER THE COURSE OF THE YEARS OF THIS STUDY

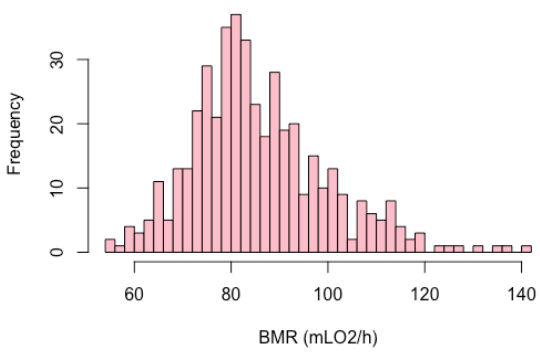
	2019		**2020**		**2021**		**2022**	
Farm	**Pop. estimate**	**Capt.**	**Pop. estimate**	**Capt.**	**Pop. estimate**	**Capt.**	**Pop. estimate**	**Capt.**
Sørdahl	36	33	33	26	58	48	71	68
Selnes	14	11	18	17	20	20	18	18
Flenstad	24	21	17	15	21	17	37	30
Rånes	30	24	22	19	23	22	53	49
SUM	**104**	**89**	**90**	**77**	**122**	**107**	**179**	**165**

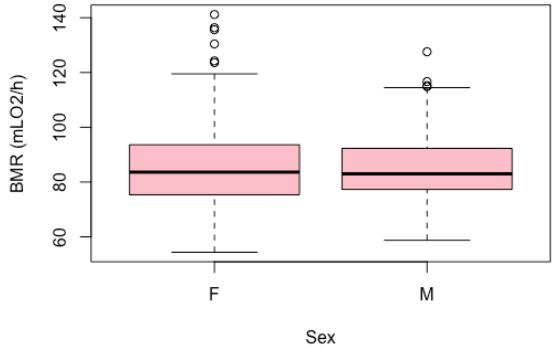
TABLE A2: MEAN VALUES (± SD) OF KEY PARAMETERS AND P-VALUE FROM WELCH TWO-SAMPLE T-TEST OF SEX DIFFERENCE IN MEAN

	mean females	*mean males*	*grand mean*	*p (T-test)*
BMR (mLO2/h)	85.90 ± 15.38	85.60 ± 12.48	85.74 ± 13.92	0.6276
Hb (g/dL)	17.50 ± 1.18	18.22 ± 1.16	17.88 ± 1.22	<0.0001***
C (mLO2/hT)	-5.72 ± 3.58	-6.25 ± 3.66	-6.00 ± 3.63	0.1296
IC (mLO2/h)	203.62 ± 55.31	208.84 ± 58.36	206.35 ± 56.92	0.3426
BM (g)	29.31 ± 1.62	30.15 ± 1.79	29.75 ± 1.76	<0.0001***

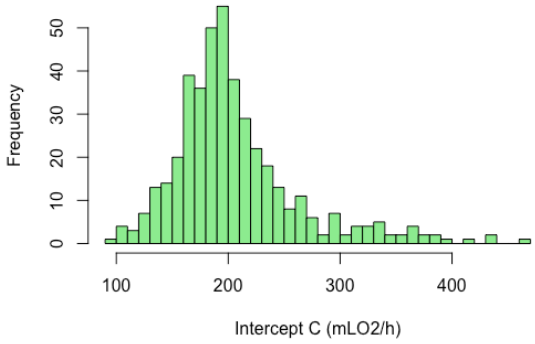
Appendix 2: Figures

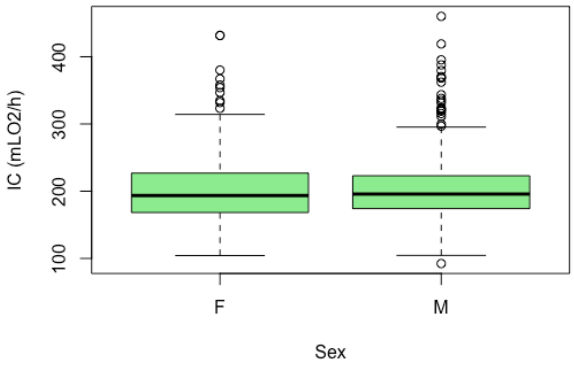
Figures A1-A10 shows distribution plots and boxplots for key parameters in the dataset. Figures A11 and A12 shows distributions of ΔT_b between 28°C and 5°C, before (A11) and after (A12) cut-off of the dataset for the complementary models. Figures A13 and A14 shows the effect of body mass on the thermoregulatory slope (C, $mLO_2h^{-1}T^{-1}$) and the metabolic rate at 0°C (Intercept C, mLO_2h^{-1}).



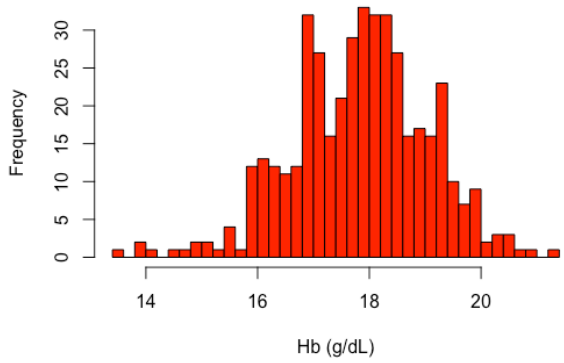


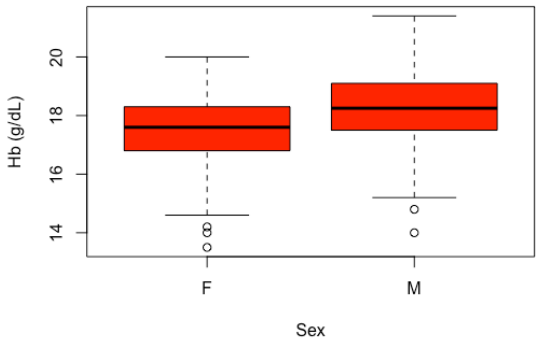
Figures A1, A2: Distribuiton of BMR



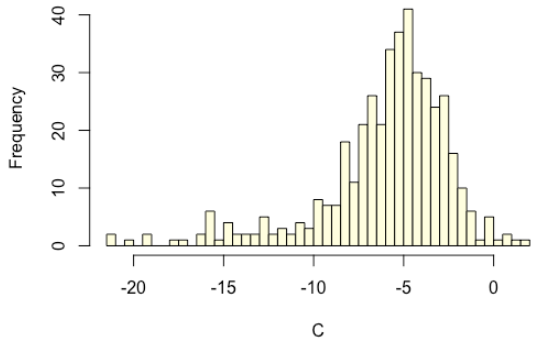


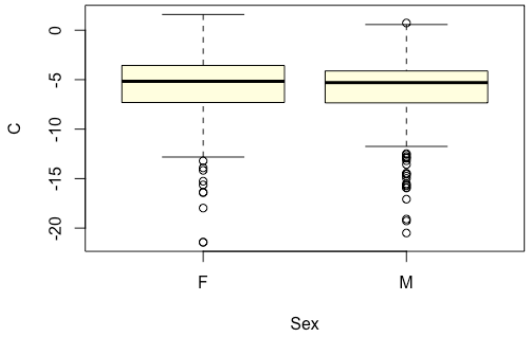
Figures A3, A4: Distribution of IC (mLO_2/h)



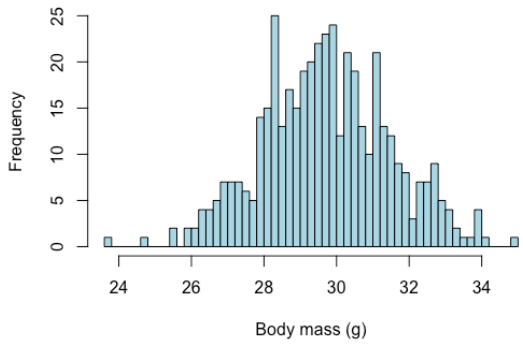


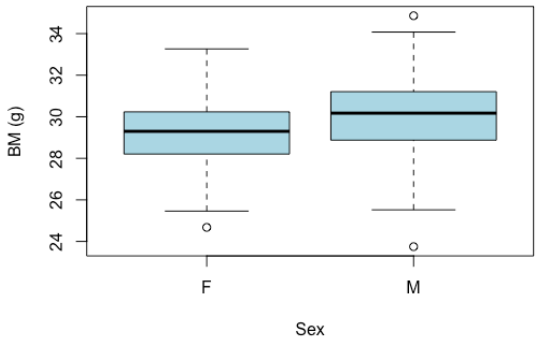
Figures A5, A6: Distribution of Hb (g/dL)



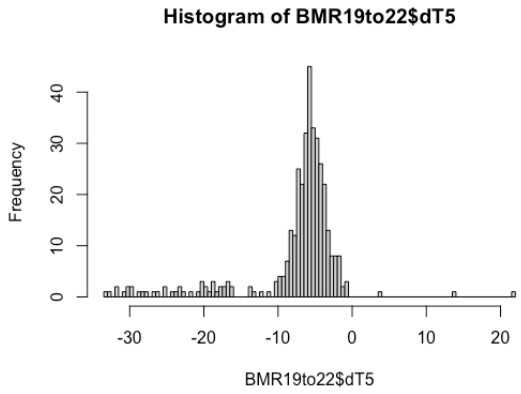


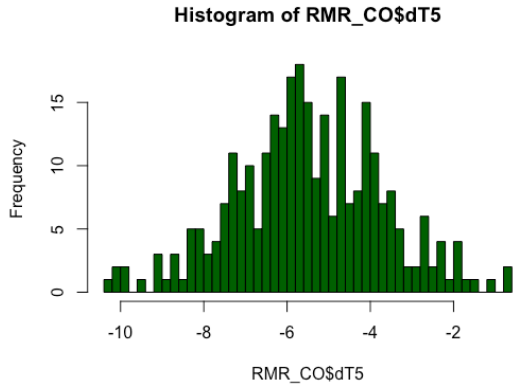
Figures A7, A8: Distribution of C ($mLO_2h^{-1}T^{-1}$)



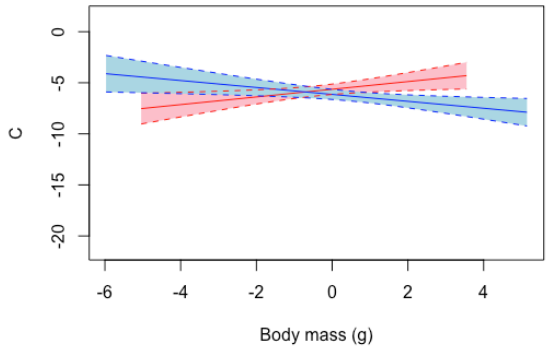


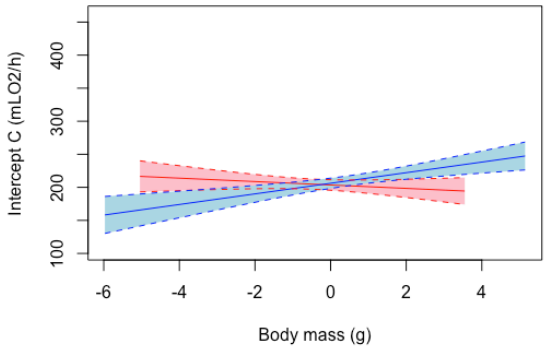
Figures A9, A10: Distribution of BM (g)





Figures A11, A12: Distribution of dT in original (A11) and new (A12) dataset





Figures A13, A14: The effect of body mass on C and IC for males (blue) and females (red). Body mass is standardised to the grand mean.

Appendix 3: R script

```r
#set working directory
setwd("~/Desktop/Masterprosjekt")

#read and view
BMR19to22 <- read.csv("~/Desktop/Masterprosjekt/RMR_winter19202122_Karina20.09.2022.csv")
View(BMR19to22)#(also include hemo)
head(BMR19to22)

#check unique individuals and farms
length(unique(BMR19to22$ID)) #315
unique(BMR19to22$farm) #4 unique farms

#making new coloumn ChamberYear (for the GLMMs)
BMR19to22$ChamberYear <- paste(BMR19to22$Chamber,BMR19to22$Year)

#looking at the data
?hist
hist(BMR19to22$hemo, breaks = 50, main = NULL, col = "red", xlab = "Hb (g/dL)") #looking at distribution
hist(BMR19to22$hemo)
mean(BMR19to22$BMR28) #mean BMR 85.68535
mean(BMR19to22$hemo, na.rm = T) #mean hemo 17.87765
boxplot(BMR19to22$Mass_BMR28~BMR19to22$Sex, xlab = "Sex", ylab = "BM (g)", col = "lightblue") #looks like no sex differences in BMR
boxplot(BMR19to22$hemo~BMR19to22$Sex, xlab = "Sex", ylab = "Hb (g/dL)", col = "red")
#looks like higher hemo values for M
boxplot(BMR19to22$Intercept_C~BMR19to22$Sex, xlab = "Sex", ylab = "Intercept C", col="lightyellow") #looks like no sex diff. in IntC
boxplot(BMR19to22$C~BMR19to22$Sex, xlab = "Sex", ylab = "C", col="lightblue")

#Making GLMMs
#Response: BMR, C, IntC (and as f.e. when not response)
#Fixed effects: Sex, hemo, (Sex*hemo), mass, (SD_C), farm
#Random effects: ID, ChamberYear
```

```r
library(lme4)

#mod_BMR1 is without any of the C-related parameters.
#REML = True, should it be false?

mod_BMR1 <- lmer(BMR28~Sex*hemo+Mass_BMR28+farm+SE_BMR28+(1|ID)+(1|ChamberYear),data=BMR19to22)
summary(mod_BMR1)


mod_BMR2 <- lmer(BMR28~Sex*hemo+Mass_BMR28+farm+C+(1|ID)+(1|ChamberYear),data=BMR19to22)
summary(mod_BMR2)

mod_BMR3 <- lmer(BMR28~Sex*hemo+Mass_BMR28+farm+C+SD_C+(1|ID)+(1|ChamberYear),data=BMR19to22)
summary(mod_BMR3)

mod_BMR4 <- lmer(BMR28~Sex*hemo+Mass_BMR28+farm+C+SE_BMR28+(1|ID)+(1|ChamberYear),data=BMR19to22)
summary(mod_BMR4)

mod_C1 <- lmer(C~Sex*hemo+Mass_BMR28+farm+(1|ID)+(1|ChamberYear),data=BMR19to22)
summary(mod_C1)

mod_C2 <- lmer(C~Sex*hemo+Mass_BMR28+farm+BMR28+(1|ID)+(1|ChamberYear),data=BMR19to22)
summary(mod_C2)

mod_C3 <- lmer(C~Sex*hemo+Mass_BMR28+farm+BMR28+SD_C+(1|ID)+(1|ChamberYear),data=BMR19to22)
summary(mod_C3)

 mod_IntC1 <- lmer(Intercept_C~Sex*hemo+Mass_BMR28+farm+BMR28+(1|ID)+(1|ChamberYear),data=BMR19to22)
summary(mod_IntC1)

mod_IntC2 <- lmer(Intercept_C~Sex*hemo+Mass_BMR28+farm+BMR28+C+SD_C+(1|ID)+(1|ChamberYear),data=BMR19to22)
summary(mod_IntC2)
```

```r

mod_IntC3 <- lmer(Intercept_C~Sex*hemo+Mass_BMR28+farm+BMR28+(1|ID)+(1|ChamberYear)+(1|C),data=BMR19to22)
summary(mod_IntC3)

mod_IntC4 <- lmer(Intercept_C~Sex*hemo+Mass_BMR28+farm+BMR28+SD_Intercept_C+(1|ID)+(1|ChamberYear),data=BMR19to22)
summary(mod_IntC4)

#Confidence intervals

confint(mod_BMR4)
###
#Master meeting 07.10.: looking at plots on the sex difference in C and Intercept C (the interaction between hemo and sex)
###

#dTb5 and sex effects on C:
temp<-subset(BMR19to22,subset=Ibut_lost==0&dT5>-15) #change in body temperature at 5 deg, removing those who lost the ibut
plot(C~dT5,data=temp) #C to change in body temp at 5 deg
summary(lm(C~dT5*Sex,data=temp))
#we see a negative effect on dT5 on C as expected(?) (more stable body temp gives more energy used on keeping it stable), but even stronger eff. of sex on C and an interaction between dT5 and sex (different response).



#Intercept C and effect of hemo and sex:
plot(Intercept_C~hemo, data=BMR19to22, col=factor(Sex))
#want to plot the different sex responses of hemo on Intercept_C
abline(lm(Intercept_C~hemo+Mass_BMR28+BMR28+SD_Intercept_C,data=BMR19to22[BMR19to22$Sex=="F",])) #slope for female (worked?)
abline(lm(Intercept_C~hemo+Mass_BMR28+BMR28+SD_Intercept_C,data=BMR19to22[BMR19to22$Sex=="M",]),col=2) #slope for male (this didn't work)

###

#Want to look at the different sex responses of hemo to Intercept_C (and C).

#making simple lms to look at summary
summary(lm(Intercept_C~factor(Sex)*hemo, data = BMR19to22))
summary(lm(C~factor(Sex)*hemo, data = BMR19to22))
#Sign. interaction effects of sex on both Intercept C and C which means F and M has different response. The slope for F is sign. negative for hemo on C, while the slope for M has no sign. effect of hemo on C. On intercept C, the estimate is very significant for F, but the estimates here must be intercept?

summary(lm(C~-1+factor(Sex)*hemo, data=BMR19to22)) #can also look at the values without reference level.

###

#plotting the sex effects of hemo on C and Intercept C
plot(Intercept_C~hemo, data=BMR19to22, col=factor(Sex))
plot(C~hemo, data=BMR19to22, col=factor(Sex))
?lines() #want to make a plot with slopes for both m and f

library(ggplot2)

#make data frame from the data

dfBMR <- data.frame(BMR19to22)
head(dfBMR)

#plot with ggplot. se=standard error bars. fullrange: extended lines.

ggplot(dfBMR, aes(x=hemo, y=C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)


ggplot(dfBMR, aes(x=hemo, y=Intercept_C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE,
```

```
###

#lms that take body mass into account

summary(lm(Intercept_C~factor(Sex)+hemo+Mass_BMR28, data = BMR19to22))
#sign. eff. of mass on IntC
summary(lm(C~factor(Sex)+hemo+Mass_BMR28, data = BMR19to22))
#no eff. of mass on C, meaning the effect we see of mass on InterceptC is really the effect of mass on BMR?

summary(lm(Intercept_C~factor(Sex)*hemo+Mass_BMR28, data = BMR19to22))
summary(lm(Intercept_C~factor(Sex)*Mass_BMR28+hemo, data = BMR19to22))

summary(lm(C~factor(Sex)*Mass_BMR28+hemo, data = BMR19to22))
summary(lm(C~factor(Sex)*hemo+Mass_BMR28, data = BMR19to22))

#sign. eff. of sex-mass interaction and sex-hemo int. on C and IntC

ggplot(dfBMR, aes(x=Mass_BMR28, y=C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)
ggplot(dfBMR, aes(x=Mass_BMR28, y=Intercept_C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)

#we see the exact same effect of sex on C and Intercept C when looking at the mass as when we look at hemo!

#hemo and mass

summary(lm(hemo~Mass_BMR28*factor(Sex), data = BMR19to22))
#no eff of mass on hemo (with and without sex)

ggplot(dfBMR, aes(x=Mass_BMR28, y=hemo, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)
#same for F and M


boxplot(hemo~Sex, data=BMR19to22)
boxplot(Mass_BMR28~Sex, data=BMR19to22)
boxplot(BMR28~Sex, data = BMR19to22)

#M have higher hemo and mass, but hemo and mass are independent and no inter

#glmms with hemo as response
summary(lmer(hemo~Sex+Mass_BMR28+farm+BMR28+(1|ID)+(1|ChamberYear),data=BMR19to22))
confint(lmer(hemo~Sex+Mass_BMR28+farm+BMR28+(1|ID)+(1|ChamberYear),data=BMR19to22))
#sex most significant factor, also here the interaction mass sex is nonsign to the effect on hemo

###

#effect of mass and BMR on C and Intercept C

summary(lm(C~factor(Sex)*Mass_BMR28, data = BMR19to22))
#BMR most important effect on C
summary(lm(Intercept_C~factor(Sex)+BMR28+Mass_BMR28, data = BMR19to22))
#mass most important effect on Intercept_C

ggplot(dfBMR, aes(x=Mass_BMR28, y=C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)
ggplot(dfBMR, aes(x=Mass_BMR28, y=Intercept_C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)

#the effect of mass on C and Intercept C follows same pattern as the effect of hemo on C and Intercept C, with contradicting response in F and M.

ggplot(dfBMR, aes(x=BMR28, y=C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)
ggplot(dfBMR, aes(x=BMR28, y=Intercept_C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)
```

```
#Individuals with higher BMR gives less steep C, but does not change intercept_C. This response is similar in males and females (no sign. interaction)

##

#Are M and F with high hemo and mass just as good at keeping their Tb stable, even though F have lower C and Intercept C?

##



#dT5 and dT15 should be as small as possible --> better thermoregulation.
#using "temp" which has removed some extreme values
ggplot(temp, aes(x=dT5, y=C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)
#we see expected response in F, but opppsite in M

summary(lm(formula = Intercept_C ~ dT5*Sex, data = temp))
#sign sex interaction, but no sign eff of dT5 alone (female response?)
summary(lm(formula = C ~ dT5*Sex, data = temp))
#both dt5 and interaction is sign.

ggplot(temp, aes(x=dT5, y=Intercept_C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)
#looks like positive slope for F, but not sign. different from 0. Male slope is sign. different from F and 0.

#want to look at dT15 too to see if we see the same.

temp<-subset(BMR19to22,subset=Ibut_lost==0&dT5>-15&dT15>-7)

summary(lm(formula = Intercept_C ~ dT15*Sex, data = temp))
summary(lm(formula = C ~ dT15*Sex, data = temp))
```

```
#sign eff of both dT15 and sex interaction on both C and Int C

ggplot(temp, aes(x=dT15, y=Intercept_C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)

ggplot(temp, aes(x=dT15, y=C, color=Sex)) + geom_point(shape=16) + geom_smooth(method=lm, se=FALSE, fullrange=TRUE)


summary(lm(formula = C ~ dT15*Sex+BMR28, data = temp))
#even though BMR has shown to have an eff on C on previous models, here has no sign. effect. (?)

### dT and hemo, mass and BMR ###

#subset males and females
females<- subset(temp, subset = Sex=="F")
head(females)
males<- subset(temp, subset = Sex=="M")
head(males)

mean(females$dT5)
mean(males$dT5)
t.test((females$dT5),(males$dT5))
#mean dT5 not different in males and females

var(females$dT5)
var(males$dT5)
#similar var

summary(lm(dT5 ~ BMR28+Mass_BMR28+hemo, data = females))
#small effect of BMR on dT5, but no eff of mass
summary(lm(dT5 ~ BMR28*Mass_BMR28+hemo, data = males))
#effect of BMR and mass on dT5 in males + interaction (what does the interaction mean? Different slope for different masses?)
```

```
###

## GLMMs with all sex interactions ##

# Intercept C:


mod_s <- lmer(Intercept_C~Sex*hemo+Sex*Mass_BMR28+Sex*dT5+BMR28+SD_Intercept_C+(1|ID)+(1|ChamberYear),data=BMR19to22
)
summary(mod_s)

confint(mod_s)
#sign. negative sex (male) interaction with mass and sign. pos. eff. of male on C.
#strong (negative) eff. of male on Intercept C. Sign. (pos) eff of interaction male and mass on Intercept C.

################# M A I N   M O D E L S ############## Changed from containing (1|ID) to only taking the first
measure using [!dup. This bc random eff was not converging. Also removed sex:dT5, this can be looked at as follow-up
questions as they do not "answer" the main questions.

# Intercept C:
mod_s2 <- lmer(Intercept_C~Sex*hemo+Sex*Mass_BMR28+BMR28+SD_Intercept_C+farm+(1|ChamberYear),data
=BMR19to22[!duplicated(BMR19to22),])
summary(mod_s2)
confint(mod_s2) #sexM:hemo and sexM:mass pos eff within CI

# C:
mod_s3 <- lmer(C~Sex*hemo+Sex*Mass_BMR28+BMR28+SD_C+farm+(1|ChamberYear),data=BMR19to22 [!duplicated(BMR19to22),])
summary(mod_s3)
confint(mod_s3) #negative eff of sexM:mass. Hemo has pos. eff. M pos eff.
                #Also neg eff of hemo:sexM!
    #    changed on meeting: removed sex:dT5 (fe) and ID (re) and added !dup



# analyse M and F separately:

# males

mod_m_IntC <- lmer(C~hemo+Mass_BMR28+BMR28+SD_C+farm+(1|ChamberYear),data=males[!duplicated(males$ID),]) #C (changed
on meeting)
length(unique(males$ID))
nrow(males)

summary(mod_m_IntC)
confint(mod_m_IntC)
#hemo pos eff within 95% CI. (Farm Sordahl negative effect.)

mod_m_C <- lmer(C~hemo+Mass_BMR28+dT5+BMR28+SD_C+farm+(1|ID)+(1|ChamberYear),data=males)
summary(mod_m_C)
confint(mod_m_C)
#hemo neg eff within 95% CI. dT5 pos eff. (Farm Sordal pos eff.)

#(tendency to pos eff of mass on IntC)

# females

mod_f_IntC <- lmer(Intercept_C~hemo+Mass_BMR28+dT5+BMR28+SD_Intercept_C+farm+(1|ID)+(1|ChamberYear),data=females)
summary(mod_f_IntC)
confint(mod_f_IntC)
#tendency to negative eff of hemo but not within 95% CI. (pos eff of Selnes)

mod_f_C <- lmer(C~hemo+Mass_BMR28+dT5+BMR28+SD_C+farm+(1|ID)+(1|ChamberYear),data=females)
summary(mod_f_C)
confint(mod_f_C)
#(only neg eff of Selnes)
```

```
303
304
305 ############### F O L L O W - U P   M O D E L S ########## USE CO DS
306
307 mod_dT5_C <- lmer(C~Sex*dT5+SD_C+farm+(1|ChamberYear),data=BMR19to22 [!duplicated(BMR19to22),])
308 summary(mod_dT5_C)
309 confint(mod_dT5_C)
310
311 mod_dT5_IntC <- lmer(Intercept_C~Sex*dT5+SD_Intercept_C+farm+(1|ChamberYear),data=BMR19to22 [!duplicated(BMR19to22
    ),])
312 confint(mod_dT5_IntC)
313 summary(mod_dT5_IntC)
314 # I don´t find the effect with GLMMs that I did with LMs, the confidence intervals completely overlap with 0 when it
    comes to the response from dT5 in males and females on C.
315
316 # (try with males and females separate data, although it is best to use all the data. )
317
318 mod_dT5_C_m <- lmer(C~dT5+SD_C+farm+(1|ChamberYear),data=males [!duplicated(males),])
319 summary(mod_dT5_C_m)
320 confint(mod_dT5_C_m)
321
322 # CIs show pos eff of dT5 in C on males. Different from using the all data. Can the difference come from that all
    data has some extreme values. Should remove extreme values.
323
324 mod_dT5_C_f <- lmer(C~dT5+SD_C+farm+(1|ChamberYear),data=females [!duplicated(females),])
325 summary(mod_dT5_C_f)
326 confint(mod_dT5_C_f)
327
328 #no eff of dT5 on C in females.
329
330 #  #  IntC with males and females data (extreme values removed)# #
331
332 mod_dT5_IntC_m <- lmer(Intercept_C~dT5+SD_Intercept_C+farm+(1|ChamberYear),data=males [!duplicated(males),])
```

```
332 mod_dT5_IntC_m <- lmer(Intercept_C~dT5+SD_Intercept_C+farm+(1|ChamberYear),data=males [!duplicated(males),])
333 confint(mod_dT5_IntC_m)
334
335 #males: CI overlap with 0 on dT5 but most of CI is on negative side
336
337 mod_dT5_IntC_f <- lmer(Intercept_C~dT5+SD_Intercept_C+farm+(1|ChamberYear),data=females [!duplicated(females),])
338 confint(mod_dT5_IntC_f)
339
340 #females: CI shows eff of dT5 on IntC completely overlap with 0.
341
342 ###### Cutoff for dT5 values in dataset, modify dataset.
343
344 hist(BMR19to22$dT5, breaks = 100)
345 ?hist
346
347 # seems like a good idea to remove all values < - 11 and > 0 only based on the statistics.
348
349 #       Q: a 10 deg drop in skin temperature seems like a lot. Should the              cutoff be stricter?
350
351 RMR_CO<-subset(BMR19to22,subset=Ibut_lost==0&dT5>-11&dT5<0)
352 View(RMR_CO)
353 hist(RMR_CO$dT5, breaks = 50, col = "darkgreen")
354
355 ######### Follow-up Models with cutoff dataset ####### USE THESE
356
357 mod_dT5_C <- lmer(C~Sex*dT5+SD_C+farm+(1|ChamberYear),data=RMR_CO [!duplicated(RMR_CO),])
358 summary(mod_dT5_C)
359 confint(mod_dT5_C) #CI overlap with 0 but sexM:dT5 most on pos side
360
361 mod_dT5_IntC <- lmer(Intercept_C~Sex*dT5+SD_Intercept_C+farm+(1|ChamberYear),data=RMR_CO [!duplicated(RMR_CO),])
362 confint(mod_dT5_IntC)
363
364 malesCO<-subset(RMR_CO,subset=Sex=="M")
```

```r
femalesCO<-subset(RMR_CO,subset=Sex=="F")

# males

mod_dT5_C_m2 <- lmer(C~dT5+SD_C+farm+(1|ChamberYear),data=malesCO [!duplicated(malesCO),])
confint(mod_dT5_C_m2) # CI overlap with 0 but most on pos side
summary(mod_dT5_C_m2) # positive estimate but sign. too low

mod_dT5_IntC_m2 <- lmer(Intercept_C~dT5+SD_Intercept_C+farm+(1|ChamberYear),data=malesCO [!duplicated(malesCO),])
confint(mod_dT5_IntC_m2)
summary(mod_dT5_IntC_m2)

#females

mod_dT5_C_f2 <- lmer(C~dT5+SD_C+farm+(1|ChamberYear),data=femalesCO [!duplicated(femalesCO),])
confint(mod_dT5_C_f2)

mod_dT5_IntC_f2 <- lmer(Intercept_C~dT5+SD_Intercept_C+farm+(1|ChamberYear),data=femalesCO [!duplicated(femalesCO),]
)
confint(mod_dT5_IntC_f2)

######## Main models with cutoff dataset ######### NOT TO BE USED ####

#NOTE: I will not be using this. I will use the BMR19to22 dataset for my main models.


# Intercept C:
mod_s2_co <- lmer(Intercept_C~Sex*hemo+Sex*Mass_BMR28+BMR28+SD_Intercept_C+farm+(1|ChamberYear),data
=RMR_CO[!duplicated(RMR_CO),])
summary(mod_s2_co)
confint(mod_s2_co) #SexM:hemo and SexM:mass pos eff within CI

# C:

mod_s3_co <- lmer(C~Sex*hemo+Sex*Mass_BMR28+BMR28+SD_C+farm+(1|ChamberYear),data=RMR_CO [!duplicated(RMR_CO),])
summary(mod_s3_co)
confint(mod_s3_co) #sexM:hemo and sexM:mass neg eff within CI
#                   (+hemo pos eff in itself!)

confint(mod_s2) #same result with and without cutoff data on both C and intC.




#### to the next meeting (15.12.22):
#1)Sentrere hemo (og masse og BMR).
#2)Lage figurar med predict.



############change the names of main models

mmod_C <- mod_s3
mmod_IntC <- mod_s2

confint(mmod_C)    #SexM:hemo        -1.09103648  -0.09525652
#                   SexM:Mass_BMR28  -0.83809721  -0.13841313

confint(mmod_IntC) #SexM:hemo          0.7155876   16.4951215
#                   SexM:Mass_BMR28    1.4769300   12.5447404

############ center the predictions on the mean


# make a matrix with all the predictor variables I want to center
# or should I just take one vector (coloumn) at a time? Ok lets try that

```

```r
# the predictor variables I want to center are: hemo, mass, BMR.

newdfBMR <- as.data.frame(BMR19to22)

newdfBMR$hemo <- newdfBMR$hemo - mean(newdfBMR$hemo, na.rm = T)

newdfBMR$Mass_BMR28 <- newdfBMR$Mass_BMR28 - mean(newdfBMR$Mass_BMR28)

newdfBMR$BMR28 <- newdfBMR$BMR28 - mean(newdfBMR$BMR28)

hist(newdfBMR$BMR28)

#now hemo, BMR and mass is centered in the newdfBMR df. Nothing exept this is different from BMR19to22 and newdfBMR.


#making my main models again but using the centered predictor values, so using the newdfBMR df as my data.

########## MAIN MODELS USING CENTERED PREDICTOR VALUES ########

M_C <- lmer(C ~ Sex * hemo + Sex * Mass_BMR28 + BMR28 + SD_C + farm + (1 |  ChamberYear), data =
newdfBMR[!duplicated(newdfBMR), ])

summary(M_C)
confint(M_C) #exactly the same as the model without cpv

M_IntC <- lmer(Intercept_C ~ Sex * hemo + Sex * Mass_BMR28 + BMR28 + SD_Intercept_C + farm + (1 |  ChamberYear),
data = newdfBMR[!duplicated(newdfBMR), ])

summary(M_IntC)
confint(M_IntC)

################################
#### predictor models plots  ####
################################

# C

dfnarm <- subset(newdfBMR, !(is.na(hemo)))
View(dfnarm) #df with nas removed from hemo

fdf <- subset(dfnarm, Sex == "F") #subsets
mdf <- subset(dfnarm, Sex == "M")

mod_f <- lm(C~hemo, data = fdf)
mod_m <- lm(C~hemo, data = mdf) #lm

# predicts + interval
newx <- seq(min(fdf$hemo),max(fdf$hemo),length.out=100)
preds_c <- data.frame(cbind(newx,predict(mod_f, newdata = data.frame(hemo=newx),
                                         interval = 'confidence')))

head(preds_int)

newxm <- seq(min(mdf$hemo),max(mdf$hemo),length.out=100)
predsm_c <- data.frame(cbind(newxm,predict(mod_m, newdata = data.frame(hemo=newxm),
                                           interval = 'confidence')))


# plot
plot(C ~ hemo, data = dfnarm, type = 'n', xlab = "Haemoglobin (g/dL)", ylab = "C (enhet)")

# add fill
polygon(c(rev(newx), newx), c(rev(preds_c[ ,4]), preds_c[ ,3]), col = 'pink', border = NA)
polygon(c(rev(newxm), newxm), c(rev(predsm_c[ ,4]), predsm_c[ ,3]), col = 'lightblue', border = NA)

# model
```

```r
lines(preds_c$fit~preds_c$newx,col="red")
lines(predsm_c$fit~predsm_c$newxm, col="blue")

# intervals
lines(newx, preds_c[ ,3], lty = 'dashed', col = 'red')
lines(newx, preds_c[ ,4], lty = 'dashed', col = 'red')


# intervals
lines(newxm, predsm_c[ ,3], lty = 'dashed', col = 'blue')
lines(newxm, predsm_c[ ,4], lty = 'dashed', col = 'blue')


####################


# Intercept C

mod_f_int <- lm(C~hemo, data = fdf)
mod_m_int <- lm(C~hemo, data = mdf) #lm


# predicts + interval
newx <- seq(min(fdf$hemo),max(fdf$hemo),length.out=100)
preds_int <- data.frame(cbind(newx,predict(mod_f_int, newdata = data.frame(hemo=newx),
                 interval = 'confidence')))

head(preds_int)

newxm <- seq(min(mdf$hemo),max(mdf$hemo),length.out=100)
predsm_int <- data.frame(cbind(newxm,predict(mod_m_int, newdata = data.frame(hemo=newxm),
                  interval = 'confidence')))

```

```r

# plot
plot(C ~ hemo, data = dfnarm, type = 'n', xlab = "Hb (g/dL)", ylab = "C")

# add fill
polygon(c(rev(newx), newx), c(rev(preds_int[ ,4]), preds_int[ ,3]), col = 'pink', border = NA)
polygon(c(rev(newxm), newxm), c(rev(predsm_int[ ,4]), predsm_int[ ,3]), col = 'lightblue', border = NA)

# model
lines(preds_int$fit~preds_int$newx,col="red")
lines(predsm_int$fit~predsm_int$newxm, col="blue")

# intervals
lines(newx, preds_int[ ,3], lty = 'dashed', col = 'red')
lines(newx, preds_int[ ,4], lty = 'dashed', col = 'red')


# intervals
lines(newxm, predsm_int[ ,3], lty = 'dashed', col = 'blue')
lines(newxm, predsm_int[ ,4], lty = 'dashed', col = 'blue')

### SLUTT #################

levels(BMR19to22$farm)
as.factor(BMR19to22$farm)

dfnarm2 <- subset(BMR19to22, !(is.na(C)))
mean(dfnarm2$Mass_BMR28) #17.87765
sd(dfnarm2$Mass_BMR28) #1.222587

with(dfnarm2, tapply(Mass_BMR28, Sex, sd))

#F        M
```

```r
#17.49757 18.22105

#Ok so, f mean is 0.379 lower than grand mean, m mean is 0.343 higher than gm.

with(dfnarm2, t.test(Mass_BMR28, Sex))
t.test((females$dT5),(males$dT5))

temp<-subset(BMR19to22,subset=Ibut_lost==0&dT5>-15)

females2 <- subset(BMR19to22, subset = Sex=="F")
View(females2)

males2 <- subset(BMR19to22, subset = Sex=="M")

t.test(females2$Mass_BMR28, males2$Mass_BMR28)

mod_s3 <- lmer(BMR28~Sex*hemo+Sex*Mass_BMR28+Intercept_C+farm+(1|ChamberYear),data=BMR19to22[!duplicated(BMR19to22),])
summary(mod_s3)
confint(mod_s3)


#### Body mass ##### interaction model

mod_f_BM <- lm(Intercept_C~Mass_BMR28, data = fdf)
mod_m_BM <- lm(Intercept_C~Mass_BMR28, data = mdf) #lm

# predicts + interval
newx_BM <- seq(min(fdf$Mass_BMR28),max(fdf$Mass_BMR28),length.out=100)
preds_c_BM <- data.frame(cbind(newx_BM,predict(mod_f_BM, newdata = data.frame(Mass_BMR28=newx_BM),
                                        interval = 'confidence')))

head(preds_int)
```

```r
head(preds_int)

newxm_BM <- seq(min(mdf$Mass_BMR28),max(mdf$Mass_BMR28),length.out=100)
predsm_c_BM <- data.frame(cbind(newxm_BM,predict(mod_m_BM, newdata = data.frame(Mass_BMR28=newxm_BM),
                                          interval = 'confidence')))


# plot
plot(Intercept_C ~ Mass_BMR28, data = dfnarm, type = 'n', xlab = "Body mass (g)", ylab = "Intercept C (mLO2/h)")

# add fill
polygon(c(rev(newx_BM), newx_BM), c(rev(preds_c_BM[ ,4]), preds_c_BM[ ,3]), col = 'pink', border = NA)
polygon(c(rev(newxm_BM), newxm_BM), c(rev(predsm_c_BM[ ,4]), predsm_c_BM[ ,3]), col = 'lightblue', border = NA)

# model
lines(preds_c_BM$fit~preds_c_BM$newx,col="red")
lines(predsm_c_BM$fit~predsm_c_BM$newxm, col="blue")

# intervals
lines(newx_BM, preds_c_BM[ ,3], lty = 'dashed', col = 'red')
lines(newx_BM, preds_c_BM[ ,4], lty = 'dashed', col = 'red')


# intervals
lines(newxm_BM, predsm_c_BM[ ,3], lty = 'dashed', col = 'blue')
lines(newxm_BM, predsm_c_BM[ ,4], lty = 'dashed', col = 'blue')
```

NTNU

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