University of South Bohemia in České Budějovice
Faculty of Science

Blood glucose concentration in Barn Swallow (*Hirundo rustica*): sources of variability and association with fitness

Master thesis

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České Budějovice 2020

Master thesis

Bendová, K., 2020: Blood glucose concentration in Barn Swallow *(Hirundo rustica):* sources of variability and association with fitness. Mgr. Thesis, in English. – 57 p., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation

Glucose is an important fuel for intense activities of short duration, but in high concentration is reputed to be tissue-damaging at least in mammals. In birds, blood glucose concentration is naturally considerably higher than in other vertebrates of similar body mass. In this thesis, we focused on blood glucose concentration in the wild populations of the Barn Swallow (*Hirundo rustica*). Firstly, we investigated the level of repeatability of blood glucose concentration to find out if this trait is individual-specific and thus, it is subject to selection. Secondly, we examined which environmental and physiological variables explain variation in blood glucose level. Thirdly, we tested association of blood glucose level with fitness-related traits, namely individual body mass and lifespan.

Declaration

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Acknowledgements

V první řadě velice děkuji svému školiteli, Oldovi Tomáškovi, za trpělivost, pečlivost, obětavou pomoc a trefné připomínky při psaním práce a počítání výsledků stejně jako za veselou atmosféru v terénu. Mé poděkování patří také celému vlaštovkářskému týmu vedenému prof. Tomášem Albrechtem za veškeré zážitky během odchytů i nekonečných kontrol hnízd. Děkuji také doc. Mgr. Janu Riegertovi, Ph.D. za jeho podporu na Přírodovědecké fakultě JU v Českých Budějovicích. Přesto však největší poděkování patří mým laskavým rodičům a mému muži, kteří mě neustále podporují a bez nichž bych se nemohla věnovat tomu, co mě naplňuje.

Tato práce byla podpořena Grantovou Agentůrou České republiky, Project No. 19-22538S

Contents

1 Introduction

A universal tool of all living organisms is the ability to metabolically transform energetic resources from the environment to their benefit. The one who can use the available amount of energy most efficiently is the winner. However, not always, this rule holds.

Energy metabolism is one of the essential aspects underpinning individual phenotypes. In evolution, natural selection acts on the variability of plethora individual traits determining individual phenotypes and, consequently, fitness in a context of specific environmental conditions. Each type of environment places different demands on individuals that live there. As a consequence, all types of metabolic pathways have been fine-tuned by various selective pressures.

According to the life-history theory, the energy budget for the maintenance of homeostasis (self-maintenance), growth, reproduction, and survival is restricted (Brown *et al.*, 2004). Due to this fact, a trade-off in the allocation of energy for all fitness components is inevitable. Moreover, individuals have to deal with other constraints, such as size, temperature, and chemical composition of their bodies (Brown *et al.*, 2004). Consequently, a broad range of different metabolic strategies has evolved according to the intrinsic and extrinsic circumstances.

The role of energy metabolism is to transform energy substrates through a complex network of biochemical reactions catalysed by specific enzymes into energy utilisable by the body (Brown *et al.*, 2004). Although a vast number of enzymes and pathways have been described, there are two central processes in energy metabolism. The first one is the citric acid cycle (CAC). This pathway enables the release of energy through the oxidation of acetyl-CoA derived from lipids, carbohydrates, and proteins. This reaction also consumes water, releases carbon dioxide, and reduces the cofactor NAD^+ (nicotinamide adenine dinucleotide) and FAD (flavin adenine dinucleotide) to NADH and $FADH_2$, which is used in the second key process, the oxidative phosphorylation pathway. In this second pathway, electrons from NADH and $FADH_2$ are transferred through the respiratory electron transfer chain down to the oxygen molecule. During this process, released energy is transformed into potential energy of the proton gradient accumulated on the inner mitochondrial membrane and then stored in adenosine triphosphate (ATP), which is a key mobile energy molecule providing energy to drive many processes in living cells (Vácha, 2016).

The metabolic rate has to correspond to the actual individual needs, and its regulation is a complex task because multiple aspects have to be taken into consideration. The available fuels differ in stored quantity, energy density, speed of conversion to ATP, and water solubility. Another element is the time for which energy is needed, whether it is long-lasting low-intensity tasks or short, intense activities (Weber, 2011).

In the animal kingdom, the three main energy sources are lipids, carbohydrates, and proteins. Lipids are characterised by containing the largest amount of energy per unit mass (around 9 kcal per one gram of lipids) because they are more chemically reduced and stored dehydrated in specialised adipose tissue, thus take up less space than the other energetic resources (McCue, 2010). The next advantage is the highest amount of metabolic water produced during lipid oxidation. Although the solubility in water is low, lipids are transported quite easily by fatty acid-binding proteins (FABPs) in the cytosol and in the plasma by the solubilising action of albumin (Weber, 2011). For this reason, they are suitable for prolonged activity with low intensity where the low rate of energy supply is adequate, especially when dehydration threatens, such as long migration flight in birds (Weber, 2011). In bird's flight muscles fatty acid transporters are dramatically upregulate before migration (Guglielmo, 2010). In the course of starvation, triacylglyceride stores are mobilised and supply ß-oxidation in mitochondria (McCue, 2010). During this process, the carboxyl end of the fatty acid chain is sequentially shortened by two carbon units. Subsequently, these two-carbon units form molecules of acetyl-CoA that enter the CAC.

Carbohydrates are the second energetic resource that is typically used for short, intense activities. The reason is their ability to high rate ATP production even in anaerobic conditions and its solubility in water. However, the disadvantage of carbohydrates is the very low energy density (around 4 kcal per one gram of carbohydrates). A large space is needed to store a sufficient amount of energy, even though carbohydrates create the polymer chains as these chains are strongly hydrated. For this reason, carbohydrates are stored only in limited amounts (Weber, 2011).

The third energy source is proteins that are broken down into amino acids in a process called proteolysis. Their energetic qualities of proteins are between lipids and carbohydrates (Weber, 2011). However, amino acids do not act as a primary source of energy. They mostly serve as structural and functional units. Hence, the organisms endeavour to avoid proteins

catabolism (Weber, 2011). For example, in migrating birds only 5% of energy is derived from amino acids (Jenni-Eiermann and Jenni, 2000).

The energy metabolism efficiency varies not only between species, but also between individuals within species. These differences have to reflect the environmental condition but to some extent they are under genetic control, and thus should show significant repeatability level (González, Brokordt and Winkler, 2010). Several studies revealed that metabolic rate is heritable traits in some species (in rodents - Konarzewski, Ksiazek and Lapo, 2005; in voles - Sadowska *et al.*, 2005; in Zebra Finch - Rønning *et al.*, 2007; in wild population of Blue Tits - Nilsson, Akesson and Nilsson, 2009). This is important as traits which show significant intraspecific variation and significant repeatability levels are great substrate for evolution through selection (González, Brokordt and Winkler, 2010).

1.1 Glucose

In this thesis, we focused on the carbohydrate-type source of energy, namely glucose. Glucose is the most important monosaccharide, used as a universal energy substrate for all animal cell types (Cankaya et al., 2007). It is also a versatile biochemical precursor for organisms from bacteria to humans. For example, bacteria can manage with glucose only to build a carbon skeleton of all amino acids, membrane lipids, nucleotides in DNA and RNA, or cofactors for metabolism (Vácha, 2016). Glucose is essential source of energy of all animal cells which has to be permanently available at an adequate level in the blood (Abbas et al., 2020)

Glucose is catabolised in the cytosol in a process called glycolysis that produces two central molecules of energetic metabolism: ATP and NADH (Rigoulet et al., 2020). However, this is only the first anaerobic step of the metabolic pathway. The aerobic part is much more efficient, and it can convert one molecule of glucose into 30-32 ATP molecules in the subsequent process of oxidative phosphorylation. Other monosaccharides, such as fructose and galactose, can be converted to the intermediates of glycolysis and can be used for energy production as well.

In the case of excess, monosaccharides can bind together by glycosidic linkages and create large molecules of polysaccharides that serve as storage carbohydrates. The most crucial polysaccharide in the animal kingdom is glycogen, which is synthesised from glucose units

in a process called glycogenesis (Vácha, 2016). The next possible utilisation of redundant glucose molecules is glycerol synthesis for fat formation.

The bloodstream transports molecules of glucose which originate in one of the three sources. The first way is through intestinal absorption, which occurs in the postprandial state (Aronoff et al., 2004). The second source is the glycogenolysis, during which glycogen is broken down into glucose. Glycogenolysis occurs especially in the liver by the action of the hormones glucagon and adrenalin (Braun and Sweazea, 2008). The third source is gluconeogenesis, during which glucose is formed from other compound classes. Gluconeogenesis is located mainly in the liver and kidneys and it is controlled by various hormones such as cortisol and insulin in mammals (Braun and Sweazea, 2008)(The Editors of Encyclopaedia Britannica, 2016). The two latter occur during starvation (Aronoff et al., 2004).

The blood glucose concentration, also called glycaemia, must be maintained in a relatively narrow range (Aronoff et al., 2004). This range is species-specific and differs considerably through the animal kingdom (Figure 1). For example, in elasmobranch fish plasma glucose level is around 3–4 mmol/L, in lampreys and Scorpaeniformes glycemia can by low 0.08 mmol/L or undetectable. In amphibians, glycaemia is very low - around 2.7 mmol/L (Polakof, Mommsen and Soengas, 2011). However, amphibian (*R. sylvatica, Pseudacris crucifer and P. triseriata*) use glucose as a cryoprotectant and then glycaemia can reach 150–300 mmol/L during winter frosts (Storey and Storey, 2017). Average glycaemia in 176 reptile species is around 5.4 mmol/L (Polakof, Mommsen and Soengas, 2011). In Amazon freshwater turtle *Podocnemis expansa* (Reptilia: Pelomedusidae) glucose level range 4.7–5.4 mmol/L (Oliveira-Júnior, Tavares-Dias and Marcon, 2009), in European Pond Turtle (*Emys orbicularis*) is 2.91 mmlo/L (Metin *et al.*, 2006), in lizard Colorado Checkered Whiptail (*Aspidoscelis neotesselata*) it is 13–22 mmol/L (Hudson *et al.*, 2020), in lizards species of genus *Gallotia* it is 6.7–10.7 mmol/L (Silvestre *et al.*, 2004). Almost all mammal species are characterized by relatively narrow range of fasting glycaemia about 5 mmol/L (Cherrington, 1999). On the other hand in birds, glycaemia can vary from 6.7 mmol/L in Mute Swan (*Cygnus olor*) to 26.9 mmol/L in Snowy Egret (*Egretta thula*) (Beuchat and Chong, 1998) but average value of 511 species is 15.6 mmol/L (Polakof, Mommsen and Soengas, 2011). In temperate passerine mean blood glucose was around 12 mmol/L, in tropical passerines mean glucose was lower around 10 mmol/L (Tomasek *et al.*, 2019). In humming birds (*Calypte anna, C. costae,* and *Archilochus colubris),* plasma glucose level

after overnight fasting (17 mmol/L) reached after feeding to 42 mmol/L (Beuchat and Chong, 1998).

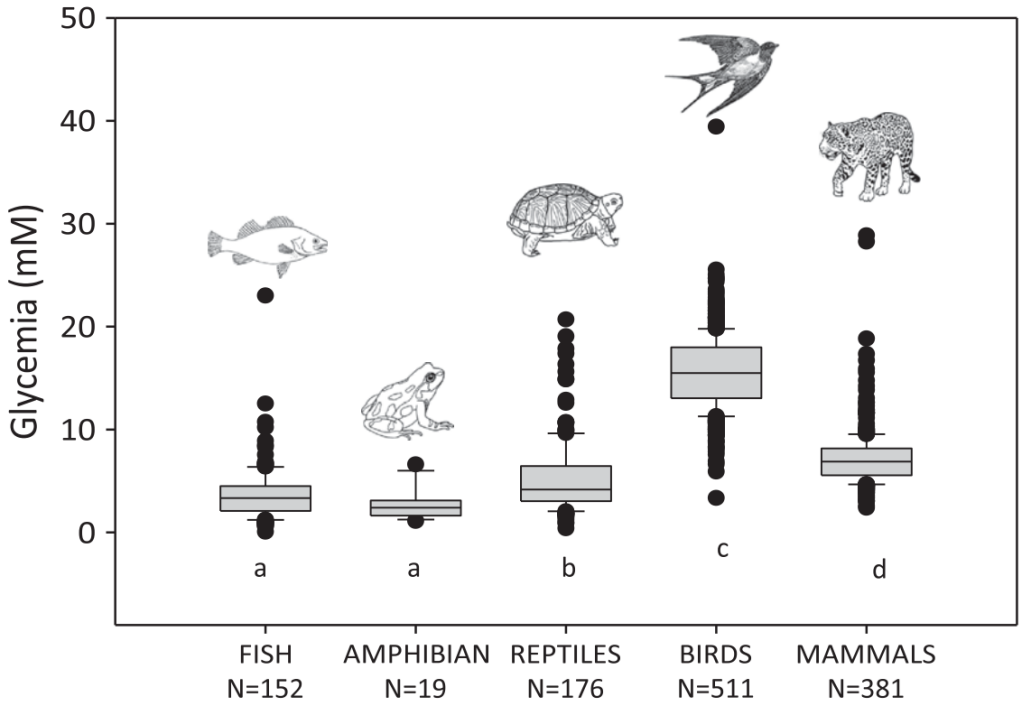

Figure 1: Glucose level of different species. Individual value – dots, median – bars. Adopted from (Polakof, Mommsen and Soengas, 2011)

Each species needs to maintain individual homeostatic blood glucose level. Regarding regulating hormones, the best known hormones produced from pancreas, insulin and its counter-regulatory hormone glucagon, regulate blood glucose level in mammals.

When the regulation is inadequate, blood glucose level can either drop to hypoglycaemic state or increase excessively to hyperglycaemic state. Both are incompatible with survival if lasting for a long time. In the former state, organism has not enough energy, whereas, in the latter, elevated blood glucose concentration in mammals causes tissue damage, such as protein glycosylation, proinflammation, elevated generation of reactive oxygen and nitrogen species (oxidative stress), apoptosis, and neuropathy (Miller, 2011; Berlanga-Acosta *et al.*, 2013). For this reason, maintenance of blood glucose level in optimal ranges is a crucial task for survival in all living organisms.

1.2 Avian glucose metabolism

Birds use glucose as rapid source of energy for intense activities of short duration. They naturally maintain considerably higher blood glucose level compared to mammals of same size. Glucose is also used for glycogen synthesis in the liver and glycolytic muscles, and for fatty acid synthesis. It is also precursor for synthesis non-essential amino acids, vitamin C and other metabolites. Glucose is also important energy substrate for nervous system (Braun and Sweazea, 2008).

Birds are highly resistant to the exogenous insulin (Braun and Sweazea, 2008; Sweazea, Braun and Sparr, 2017) and the major metabolism controlling hormone is glucagon from α-cells of pancreas (Braun and Sweazea, 2008). Glucagon concentration in avian pancreas is 8–10 times higher than in the same size pancreas of mammals. In birds, released glucagon results in increased plasma glucose (by activation glycogenolysis in the liver), triglyceride, glycerol, and free fatty acids levels (by stimulating lipolysis and suppressing hepatic lipogenesis), stimulates glycogenolysis and gluconeogenesis (Hazelwood, 1973). Glucagon is also considered to be the mediator of non-shivering thermogenesis during low temperature periods in birds (Barre and Rouanet, 1983). In birds as well as in mammals, glucagon release is inhibited by high glucose concentration (Ruffier, Simon and Rideau, 1998).

Avian pancreatic ß-cells also produces insulin but in a significantly lower dose (6–9 times) than mammals do (Ruffier, Simon and Rideau, 1998). Insulin in birds probably modulates the glomerular filtration rate and increases excretion of glucose rather than tissue absorption as in mammals (Sweazea, Braun and Sparr, 2017).

Other glucose regulating hormones are somatostatin which inhibits the release of insulin and glucagon (Sakurai *et al.*, 1974; Honey, Arimura and Weirf, 1981), pancreatic polypeptide, and triiodothyronine which stimulate effect of glucagon (Scanes and Braun, 2013)

Birds provide us with different perspectives on glucose physiology due to the noticeable differences from mammals. As mentioned above, birds have many times higher blood glucose level than any other vertebrates together with higher metabolic rates than mammals of comparable body size (Lindstedt and Calder, 1976). Birds are also characteristic due to higher body temperature than mammals which is interpreted as a result of higher metabolic rate and lower heat loss (McNab, 1966). Another unique avian traits is presence of nucleated red blood cells which exclusively rely on glucose as an energy substrate (Cox,

2013). However, high glucose concentration (hyperglycaemia) leads, at least in mammals, to oxidative stress as glucose molecules react with proteins to form oxygen species and the advanced glycation end products (Jimenez *et al.*, 2019).

Although these facts would suggest rapid ageing in birds, the opposite is true, and they live longer than mammals of comparable size (Lindstedt and Calder, 1976; Holmes and Austad, 1995). Moreover, high blood glucose concentration does not probably lead to significant oxidative stress in bird (Braun and Sweazea, 2008; Smith *et al.*, 2011). For example, hummingbirds have the highest ever measured level of glycated haemoglobin in birds, but it is still lower than those of non-diabetic humans (Beuchat and Chong, 1998). All these facts made birds interesting model organism for research into adaptations preventing adverse effects associated with high glucose concentration.

A special mechanism is probably present in birds to avoid detrimental reaction caused by hyperglycaemia. Bird mitochondria from brain, heart, lung and kidney produce significantly lower amount of reactive oxygen species, superoxide and hydrogen peroxide when compared to the rats (Ku and Sohal, 1993; Stinefelt *et al.*, 2005). Moreover, in these tissues, Ku and Sohal (1993) detected higher level of the endogenous antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase. Another potent antioxidant is uric acid, which is present in high concentration in avian plasma (Klandorf, Probert and Iqbal, 1999; Holmes, Flückiger and Austad, 2001). When researches experimentally decreased production of uric acid by approximately 33% in birds, the result was an increase in oxidative stress and tissue damage caused reactive species (Klandorf *et al.*, 2002). Based on these studies, uric acid seems to be important antioxidant in birds that helps to manage high glucose concentration.

Blood glucose level is a labile physiological feature which quickly responds to intrinsic and environmental stimuli in minutes. Stress stimuli cause production of stress hormones, such as catecholamine (e.g. adrenalin) and later glucocorticoid (corticosterone) (Wingfield and Romero, 2011). Both of them are known to elevate blood glucose level, besides other physiological effects. The level of adrenalin increases in seconds following an exposure to a stressor. On the other hand, corticosterone is detectable in approximately three minutes following the exposure to a stressor and its effect lasts for hours (Sapolsky, Romero and Munck, 2000).

The opinions regarding corticosterone effects are not unequivocal, including the possibility that the effects are dependent on its total concentration. Corticosterone binds to two types of receptors: mineralocorticoid receptor or glucocorticoid receptor with ten folds lower affinity to glucocorticoid then the former. According to study of Selye (1950), glucocorticoid help to maintain stress reaction. According to Munck et al. (1984), glucocorticoid suppress the stress respond in order to avoid pathological consequences. Stress hormones do not always cause an increase in blood glucose concentration, but they participate in maintaining an optimal level according to current situation.

Furthermore, blood glucose level is influenced by other individual and environmental factors. One of the major individual factors is genetic background which manifests itself in variable metabolic phenotype that is difficult to ascertain. On the other hand, sex easily detectable and it can modulate blood glucose level because in general male and female have slightly different physiology, morphology and behaviour. Males and females can differ also in stress hormones metabolism which can lead to different stress-induced blood glucose concentration (Khan and Robert, 2013). In Zebra Finch, females had higher baseline corticosterone level than males (Khan and Robert, 2013). In the study comparing 30 passerine bird species the sex induced difference was significant with higher blood glucose in females (Tomasek *et al.*, 2019). However, sex did not have any significant effect in some studies (e.g. Montoya et al., 2018).

It has been shown that breeding stage is energetically demanding period in a lifecycle that in general increases metabolic rate and blood glucose level (Gayathri, Shenoy and Hegde, 2004; Pick *et al.*, 2016; Montoya *et al.*, 2018). High energy demands are placed especially during egg laying on females and then during incubation and feeding potentially on both parents.

The next individual factor is body mass, which is also indicator of physical condition (Schulte-Hostedde, Millar and Hickling, 2001). Several studies were done to examine the relationship between body mass and blood glucose level, however, with inconsistent results (Umminger, 1977; Beuchat and Chong, 1998; Braun and Sweazea, 2008; Scanes and Braun, 2013). Studies report absent or negative correlation between blood glucose level and body mass. Study comparing 30 species of passerine birds report negative correlation between these two traits (Tomasek *et al.*, 2019). Similarly, in nestlings blue tits, blood glucose was negatively associated with body mass.

The last but not least individual factor is age which affecting physiology, morphology, or behaviour. Some traits, such as these related to reproduction or haemoglobin concentration, are associated with age (Balbontín *et al.*, 2007; Kaliński *et al.*, 2016; Pazdera, 2020). Another studies focused on nestlings of Welcome Swallow revealed that blood glucose level increased linearly with age (Lill, 2011). However, only few studies investigating effect of age in wild animals is available and it is not possible to make a conclusion from these fragmented results.

All living organisms have to respond to the environment condition which can vary in minutes, days, or month. One of the most unpredictable factors is weather condition, such as temperature, precipitations, wind, and relative humidity. It is possible that to tolerate low temperature could be more energetically demanding. Vaillancourt et al. (2005) present that metabolic rate in birds is elevated due to low temperature during night or at high altitude. This suggest following studies. Vaillancourt et al. (2005) present that metabolic rate in birds is elevated due to low temperature during night or at high altitude. Blood glucose level in captive Zebra Finch was lower when ambient temperature was higher. Surrounding weather condition influences not only the adult birds, but also nestlings. Ambient temperature negatively influenced body mass of Barn Swallow nestlings. However this correlation was also dependent on total precipitations and wind speed. Moreover, fledglings were not so strongly affected by temperature (Facey *et al.*, 2020). Temperature was also negatively correlated with corticosterone level in nestlings Blue Tits (*Cyanistes caeruleus*) and Pied Flycatchers (*Ficedula hypoleuca*) but not in adults (Lobato *et al.*, 2008). These suggest that weather conditions interact with each other and depend on the life stage. Weather can also influence food availability and feeding frequency (Kaliński *et al.*, 2015; Montoya *et al.*, 2018; Facey *et al.*, 2020).

Next environmental factors are circadian rhythm. Studies, which examined association between day time and blood glucose level in birds, revealed significant changes in glycaemia. For example, blood glucose level of captive Starlings (*Sturnus* vulgarius) peaked in midday (Remage-Healey and Romero, 2000). However, Frelin (1974) found different trend of significantly lower blood glucose level between 11 and 13 o'clock in Redpolls (*Acanthis flammea*). Effect of circadian rhythm was also suggested by the study of chickens (Twiest and Smith, 1970). Twiest and Smith found out that blood glucose level in chickens was lower during the dark period, when the lights were off and higher during the light period. These study may indicate higher metabolic rate and thus higher blood glucose

level during high activity periods (Montoya *et al.*, 2018). The next study supported this suggestion, showing that the nocturnal fasting is linked with lower energy expenditure and decreased metabolic rate in the chicken (Buyse *et al.*, 1993).

Similarly, not only day time but also day length could affect physiology. However, the results are inconsistent. In study of captive Starlings, Remage-Healey and Romero (2000) found that the day length had significant effect on the blood glucose level. Birds held on short days had lower level of blood glucose compare to the birds held on long days with high blood glucose level. In contrast, Montoya *et al.* (2018) reported that blood glucose level in captive Zebra Finch was lower on longer days. Although these two studies do not correspond with each other, day length affects physiology. Nevertheless the effect could be species-specific.

Next environmental factor associated with day length is seasonal rhythm which influence living organism, especially in temperate zone where four seasons change (Jenni-Eiermann and Jenni, 1991). Both wintering and migrating birds are able to do extreme performances which are associated with physiological and behaviour changes. Changes during breeding season were discussed above. Another energy demanding period is migration and preparation for it. For example in the study of captive Garden Warbler (*Sylvia borin*), Bairlein (1983) found that blood glucose concentration was significantly higher during the autumn and spring migratory periods compared to the phase of the winter low body weight. Blood glucose can change within season as across seasons. In study of the nestling Blue Tits, blood glucose level can vary among seasons (Skwarska *et al.*, 2014).

In the last mentioned study, they reported that also the breeding site can influence blood glucose level. They compared urban parkland with forest site where the blood glucose concentration of the nestling Blue Tits were significantly lower than in parkland each season. They assign this finding to smaller amount of stressful factors in the forest and higher quality of this site (Skwarska *et al.*, 2014).

To conclude, glucose is an indicator of ability to mobilize energy in stress situations and seems to be one of the important aspects for individual fitness. Despite the indisputable importance of glucose, there are only few short-term studies observing only small number of individuals. In this these we analysed blood glucose level in wild populations of the Barn Swallow comprising hundreds individuals across their life.

1.3 Barn Swallow (*H. rustica*)

The Barn Swallow is a medium-sized songbird species, which is well-known owing to its wide distribution, large abundance, and synanthropic behaviour. For these reasons, as well as for its calm and patient nature, the Barn Swallow has become a popular model species in ecological and evolutionary research.

The Barn Swallow is the most abundant species from the family *Hirundinidae*. It breeds throughout Eurasia and North America. Its wintering grounds locates in Central and South America, southern Spain, Morocco, Egypt, sub-Saharan Africa, the Middle East, India, Indochina, Malaysia, northern Australia, and Micronesia (Figure 2) (Brown and Brown, 2020).

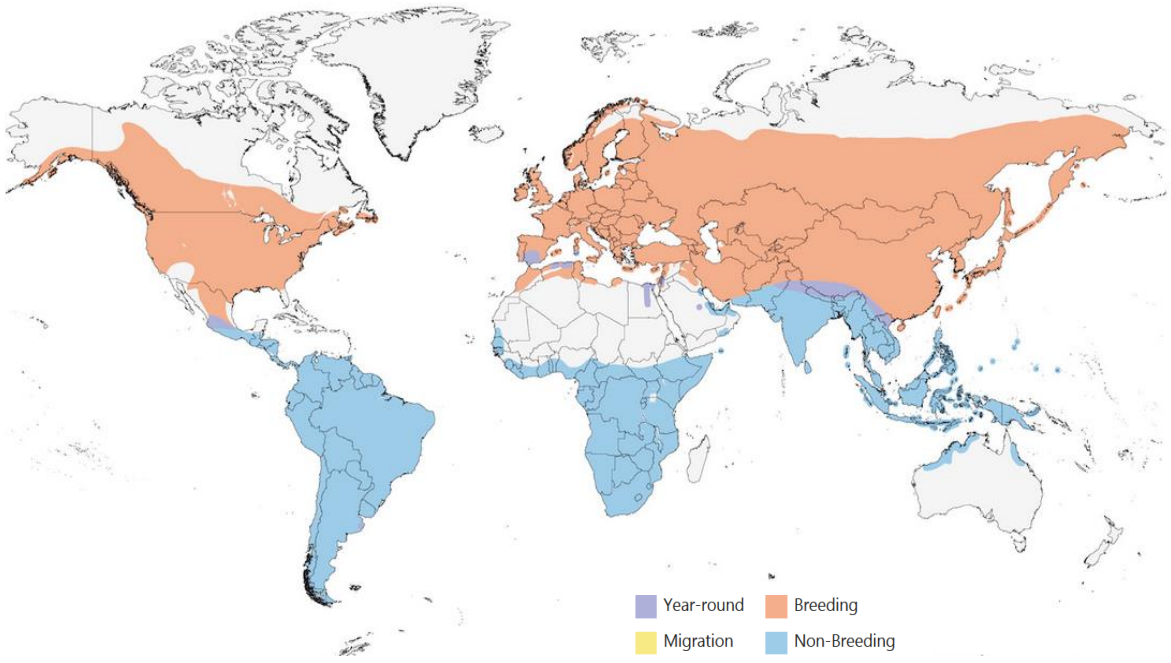

Figure 2: Distribution of the Barn Swallow. Adopted from (Brown and Brown, 2020).

Six closely related subspecies of the Barn Swallow is distinguished: *H. r. savignii*, *H. r. transitiva*, *H. r. rustica*, *H. r. gutturalis*, *H. r. erythrogaster*, and *H. r. tytleri* (del Hoyo and Elliott, 2014). They differ from each other in the coloration of the throat and ventral plumage, geographical distribution (Figure 3), and phylogenetic analyses. This thesis concerns subspecies *H. r. rustica*.

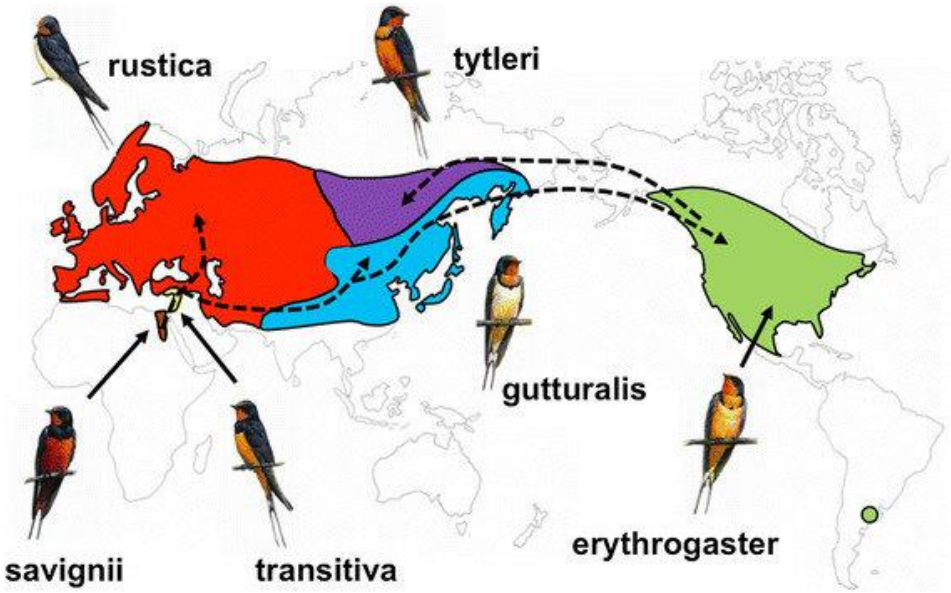

Figure 3: Distribution of six subspecies of the Barn Swallow. Adopted from (Scordato and Safran, 2014).

Most of the subspecies yearly migrate from south to north and back. Barn Swallows are diurnal migrants. This fact allows them to forage on aerial insects as they fly (Brown and Brown, 2020). Birds arrive from wintering grounds during April and May.

This insectivorous bird forages in open areas, such as fields, meadows, wetlands, often nearby to the water body. Insect matter accounts for 99.8 % of stomach content (Barrentine, 1980). Despite the origin nest site was in mountains and riparian areas with several caves and rock protrusions, or large tree hollows, nowadays nests are built primarily in rural structures in farms with livestock, but also in the suburbs, in cities, and along highways (Brown and Brown, 2020). According to A. P. Møller, the Barn Swallow lives with humans for more than 2000 years (Møller, 1994). For this reason, they are a great model organism, which is not disturbed by the presence of people as much as other passerines.

Nesting and breeding site fidelity is characteristic of the Barn Swallow. While natal philopatry is quite low, only 0–4 % of yearling birds return to their birth area, adult fidelity to the breeding site is high (Balbontín *et al.*, 2007; Petrželková *et al.*, 2015; Pap *et al.*, 2019) and less than one percent of individuals change the breeding location during life (Møller and DeLope, 1999). Due to this characteristic, it is possible to study the same individuals repeatedly for many seasons through their live. For both, more returnees are males (Brown and Brown, 2020; our observation). Based on our observations, the birds even return to the same nest or at least close to it.

Barn Swallows live solitary or in groups. Although they form socially monogamous pairs, they are genetically polygamous in reality since the extra-pair paternities are common. For example, in our population in South Bohemia, 51.2% of nests were found to contain extra-pair paternity. Michálková *et al.* also found out that age is the major predictor of male and female promiscuity (Michálková *et al.*, 2019).

Birds reused the old nest or built the new one. Both sexes cooperate with collecting the mud for the cup-shaped construction, which is finally lined with soft grass, feathers, and horsehairs before the egg-laying. Males guard their females during nest-building and egg-laying. The clutch is usually comprised of 3 to 6 eggs. The only female incubates the eggs for around 14 days. Both parents feed hatched nestlings for at least the next 21 days. Although the fledgelings can fly in about twenty days, they remain in the nest (or nearby) for the next several days. Second or even third brood in the season is not rare, and it is more probable in older females than in first-years (Brown and Brown, 2020).

The weather strongly affects the life of Swallows. Adverse weather conditions can cause juvenile as well as adult mortality. Prolonged rain together with low temperature results in a shortage of flying insects which is indispensable for swallows nutrition. In addition, small nestlings are unable to maintain a sufficient body temperature while their parents are foraging. The opposite extremes, swelter and droughts have the same effects. Furthermore, when the puddles dry out, the source of building material for the nest disappears. Nevertheless, to some extent, birds are able to survive the unfavourable period, and they tolerate starvation lasting several days. This fact is the next advantage of studying this species.

2 Objectives

The main aims of this thesis are following:

- Analysis of variability in blood glucose concentration in free living Barn Swallows
- Repeatability of individual glycaemia during season and across seasons
- Identification of the sources of variability in blood glucose concentration
- Examining of the connection between blood glucose level and survival or life expectancy

3 Methods

3.1 Study sites

My study was conducted within a large long-term research project focused on the free living Barn Swallow population, which is underway since 2009. The research was carried out on the several localities around the town Třeboň in the Czech Republic, namely Hamr Farm in Lužnice, Fish ponds Šaloun in Lomnice nad Lužnicí, and cow farm in Břilice. Between 2013 and 2016, the research also took place in the horse stable Obora in Třeboň. Finally, in the year 2019 cow farm in Stará Hlína was added to the list of our field sites.

All these places are quite isolated from the other potential nesting sites. For this reason, and because of high breeding-site fidelity, the dispersal between our study populations and the surrounding populations are rare (see also Saino *et al.*, 1999; Schaub and Von Hirschheydt, 2009; Pap *et al.*, 2019).

In our field sites, birds breed in colonies. Only a few solitary pairs breed partly isolated, but the distance is no more than 100 m from the main colony (Michálková et al., 2019). In the vast majority, birds prefer the barns with livestock, where the number of nest reaches tens. The most extreme nest density is in the sheepfold in Fish ponds Šaloun, where around 40 nests are found on approximately 50 m^2. Lower density is in Hamr Farm, where milking parlour and stable with bulls accommodate 21 and 25 nests, respectively. In the other livestock premises, there are usually around 10 nests.

3.2 Field data collection

In each locality, we conducted at least three capture days during the breeding season from April to July. For this reason, almost all the adult birds from the given locality were captured within the season. In their nesting site, adult Barn Swallows of both sexes were captured using the mist nets at the daybreak. The birds were placed in paper bags of appropriate size with the note about capture time and they were transported to the improvised field laboratory, where measurements and sampling took place. The birds were weighed to the nearest 0.1 g on the digital scales (Pesola, MS500, the resolution of 0-500 g) and we

determined individuals' sex by visual examination of the presence of a brood patch (present only in females) or the length of the tail feather (males have longer tail feathers on average).

A blood sample (~100 µl) was taken from the jugular vein with a heparinized insulin syringe at the predefined time, so-called blood sampling latency (see below). Blood sampling latency is the time from the moment the bird hits the net (initial stressor) to the moment when the blood collection is done.

To test changes in physiology under stress conditions, we carried out three consecutive blood collections in the same individual in the years 2013, 2014, and 2015. The first sample was done within 3 minutes from the bird hitting the net, which is considered to reflect basal glucose level and corticosterone level (Remage-Healey and Romero, 2000). Subsequently, the bird was returned to the bag and the second blood sample was collected 15 minutes from the initial capture. In the year 2014, we also collected the third blood sample 30 minutes following the capture.

In 2016–2020, blood samples were also taken 45 and 60 minutes after the capture. The reason was to assess the stress-induced dynamics of the blood glucose level beyond the usually studied period of 30 minutes from the capture.

Immediately after the blood sampling, we measured blood glucose concentration using FreeStyle Freedom Lite portable glucometers (Abbott Diabetes Care, Alameda, USA) with a linear range of 1.1–27.8 mmol/L, which has been shown to be reliable devices (Breuner, Delehanty and Boonstra, 2013; Tomasek *et al.*, 2019). The measurements were carried out in duplicates on two different glucometers. If the difference between the two devices was higher than 1.0 mmol/L, a new duplicate measurement was taken. Only a drop of collected blood was used for the determination of glucose level. A drop of blood was stored in 95% ethanol for later genetic analysis. The rest of the blood sample was stored in liquid nitrogen for biochemical analyses not included in this work.

Birds were tagged with the ornithological aluminium rings with a unique number (Czech Bird Ringing Station of National Museum Praha, Czech Republic), and three colour plastic rings. These four rings together created a unique combination allowing the identification of individuals in the field using binoculars or camera. This information was used for the determination of social parents of each nest during the breeding season.

We were able to determine the age of most individuals because our localities are carefully monitored since 2009. In the individuals ringed as nestlings, the exact age was known. If the individual was captured for the first time as an adult and the locality was intensely monitored in the year preceding this first capture, we considered the individual to be one year old. This is possible owing to the high breeding site fidelity, as the migration of adult birds between breeding colonies is extremely rare (Møller and DeLope, 1999; Balbontín *et al.*, 2007; Petrželková *et al.*, 2015; Michálková *et al.*, 2019; Pap *et al.*, 2019). Similarly, we considered individuals to be dead when they did not return to the breeding colony in the following year.

During the breeding season from April to September, all breeding attempts were observed. All the nests within the locality were checked every third day. The active nests were controlled every day from the time when birds started to line the nest with feathers or horsehairs, throughout eggs laying, until the time when the incubation began. Every new egg was numbered and, upon clutch completion, the whole clutch was photographed. Everyday controls started three days before the expected hatching. Nestlings were weighed the first three days and marked by cutting a specific tip of one claw. When they were 9 days old, we weighted them, ringed them, and collected blood samples. The nests were controlled unless fledging flew out. Parents of almost every nest were determined by observing the nest from a hiding place and by taking photos. All birds were treated in accordance with the Animal Protection Law of the Czech Republic No. 246/1992 Sb.

In around half of our samples, we knew the current breeding stage of the individual. In other cases we termed it as unknown. We defined eight categories: mating, pre-breeding, incubation, nestlings, fledglings, post-breeding, non-breeding, and unknown (for more details see Table 1).

Table 1: Breeding stages definition.

Breeding stage	Specification	
	Start	End
pre-breeding	more than 7 days before the initiation of the first clutch	7 days before the initiation of the first clutch
mating	7 days before clutch initiation	clutch initiation
incubation	clutch initiation	hatching
nestlings	hatching	20 days after hatching
fledglings	21 days after hatching day	28 days after hatching day
post-breeding	29 days after hatching day	7 days before initiation of the following clutch
non-breeding	no breeding attempt within the season	
unknown	uncertain or missing data	

3.3 Other data sources

Meteorological data were obtained from two sources, which were consistent with each other. The first source was the Czech Hydrometeorological Institute, which publishes historical meteorological data of average daily temperature, total rainfall, and relative humidity (ČHMÚ, 2020). Hourly temperature data were purchased from OpenWeatherMap (London, UK).

3.4 Statistical analysis

3.4.1 Data

In this thesis, I used the blood glucose data that were collected during breeding seasons in the years 2013–2020. I have been participating in the data collection since 2019.

3.4.2 Programmes and R packages

The data processing and statistical analysis was carried out using the R 3.1.6 software (R Core Team, 2017). During data preparation, following packages were used: *data.table* (Dowle *et al.*, 2020), *suncalc* (Thieurmel B., Elmarhraoui A., 2019), *chron* (James D., Hornik K., 2020), *lubridate* (Spinu *et al.*, 2020). We also used packages *effects* (Fox *et al.*, 2020) and *ggplot2* (Wickham H., 2016) for plotting the graphs. Data were analysed using

linear mixed-effects models with Gaussian distribution of the residuals implemented in the *lme4* (Bates *et al.*, 2020) package. For all analyses, we set the significance level at 0.05.

3.4.3 Repeatability

We first tested individual repeatability of blood glucose level to assess whether and to what extent it is an individual-specific trait. This is important as the less the trait is individual-specific, the less scope is there for natural selection on that trait. Specifically, we tested the individual repeatability during the season and individual's life. To assess the effect of capture day and year, we also estimated repeatability of the glucose level at these two levels fitted as random effects. We only used data of the first samples and analysed them with the package *rptR* (Stoffel, Nakagawa, Schielzeth, 2019). We tested these four types of repeatability in four separated steps. First, to calculate individual repeatability in blood glucose level within a season we grouped data by special predictor ring-year, which included information about ring number and simultaneously information about the year. Second, to calculate repeatability of individual's blood glucose level across years over its lifetime we grouped data only by the ring number. Third and fourth, to calculate repeatability of blood glucose level in capture day and capture year in we grouped data by capture day and capture year respectively.

3.4.4 Potential predictors

Firstly, we carried out exploratory data analysis of the environmental variables to find out potential errors, to optimize handling of missing data, and to reveal correlated pairs of variables. Reciprocal dependence was verified visually using the *pairs* function (R Core Team and contributors, 2019). To select environmental predictors, we used only a subset with the first blood samples in a sequence.

One of the main goals of my thesis was the exploration of the sources of variation in blood glucose level in free-living Barn Swallows. Based on the other studies (e.g. Lill, 2011; Montoya *et al.*, 2018; McGraw *et al.*, 2020), we assumed that following variables could significantly influence the blood glucose level: sex, body mass, age, breeding stage, capture

time (as the time from sunrise), blood sampling latency, Julian day (ordinal day of the year), sample order (first, second or third on that day) and weather conditions such as temperature, rainfall and relative humidity. These predictors were included in the models as fixed effects. We further assumed that blood glucose level is to some extent an individual-specific trait and hence we included individual identity as a random effect. Furthermore, date and year were also included as random effects to assess and control for the autocorrelation related to these factors.

The next step was to select the best variables concerning weather conditions for our final model. We used function *dredge* from the package *MuMIn* (Bartoń, 2020), which compares candidate models based on the Akaike information criterion with small sample correction (AICc). We adjusted sample selection to avoid cross correlation in the model. We used following variables as fixed variables: sex, capture age (first, second, and third order polynomial), time from sunrise (first, second, and third order polynomial), and blood sampling latency (first, second, third, and fourth order polynomial). These fixed variables were present in all candidate models and have not been tested. Polynomials were used to test potential non-linear trends and were selected based on previous knowledge or exploratory data plotting. For example, it is necessary to fit at least third order polynomial of age, because if only a second order polynomial is fitted and interpreted it may indicate presence of senescence only as a mathematical artefact of quadratic function in cases, where there is initial trait increase in early adulthood, followed by plateauing (but not senescence) in late life (Bouwhuis et al., 2009).

The selection of the weather variables best explaining blood glucose concentrations was carried out in two steps because only a limited number of variables were allowed for the dredge function within one round. In the first step we tested following variables: current temperature (first and second order polynomial), long-term average temperature calculated over one, two, three, and five days prior to the day of the capture (first and second order polynomial), current relative humidity, long-term average relative humidity calculated over one, two, three, and five days prior to the day of the capture (linear effects only), Julian day (first, and second order polynomial), and interactions between the current and long-term temperature and humidity.

In the second step we tested variables giving the intensity of rainfall: total precipitation for one, two, three, and five days before capture (first, and second order polynomial), and Julian day. The candidate models in this step also included the variables selected in the first step.

In the final step of variables selection, we used weather variables included in the highest ranked model (according to AICc) containing the tested weather variables together with all the other hypothetical predictors. Importantly, to examine age-related changes in blood glucose level, we tested the effect of chronological age by fitting it among fixed predictors. Nevertheless, even in mixed-effect models with individual identity as a random effect, the effect of age is estimated at the population level and includes the effect of selective survival (i.e. non-random with respect to the response variable) if it is present in the population (Van de Pol & Verhulst, 2006). To control for the possible effect of selective survival, we fitted lifespan (calculated as the difference between the last year of capture and birth year) as another fixed factor. This combination of lifespan and chronological age can separate the within-individual effect of age from the effect of selective survival (Van De Pol and Verhulst, 2006). Moreover, we tested the effect of the biological age, which was calculated as the number of years until death, which is commonly used to test the individual variation in ageing trajectories (Froy *et al.*, 2019). We defined *dredge* function to select either chronological or biological age in one candidate model. Age and years until death were never fitted together in one model.

In this final step of predictor selection, we used *dredge* function. In subset, we used above mentioned variables.

3.4.5 Final model

Continuous variables in the data set were centred using the function *scale* from the package *base* (R Core Team and contributors worldwide, 2019).

To calculate variation explained by our final linear mixed-effects model, we used function *r.squaredGLMM* from the package *MuMIn* (Bartoń, 2020). Using this function we determined marginal and conditional coefficient of determination (R^2). The first is interpreted as the variance explained by the fixed effects, whereas the latter represents the variance explained by the entire model, including both fixed and random effects (Bartoń, 2020).

3.4.6 Body mass

Body mass was only measured in some years. For this reason, its effect was tested only in the subset of individuals. The effect of body mass was tested by adding body mass as a predictor to a model with otherwise the same structure as a final model above.

3.4.7 Blood sample order

In the foregoing analyses, we used only subset of the first blood samples per individual and a day. However, in some seasons we carried out second or even third blood sample in one individual to follow the stress dynamics. Blood sampling is a stressful intervention, which cause physiological changes such as pain due to the puncture or a removal of a certain volume of blood (Remage-Healey and Romero, 2000, 2001). For this reason, we tested the effect of a repeated blood sample in sequence, while controlling the sampling latency. Body mass was included in this model as a predictor because it was available from those years with repeated blood sampling.

4 Results

Overall we have analysed 2286 blood glucose measurements of 715 individuals (300 females and 415 males) over the course of seven breeding seasons. Number of ringed individuals per season varied from 122 individuals in 2013 to 362 individuals in 2019. Median of blood glucose concentration in this dataset was 11.2 mmol/L, CI_{95} = [9.1, 14.3] mmol/L. However, the individual values ranged from 6.0 to 23.3 mmol/L. In general, females showed higher blood glucose level (median 11.5 mmol/L, CI_{95} = [9.3, 15] mmol/L) than males (median 10.8 mmol/L, CI_{95} = [8.9, 13.6] mmol/L) (Figure 4).

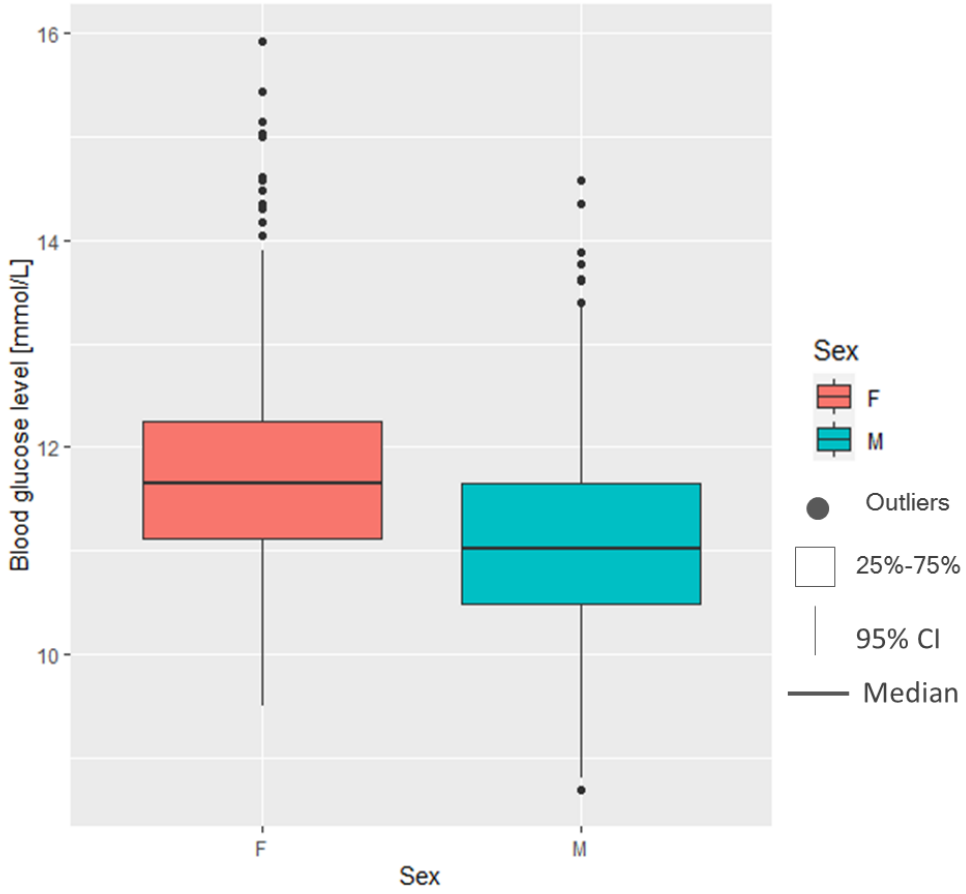

Figure 4: Variation in blood glucose level between females and males in the Barn Swallow. The difference was significant in the main model (t_{1695} = –2.48; p = 0.013; see Table 5). Box and whiskers plot of predicted blood glucose level and sample order. Blood glucose level data are predicted data from model (Table S4).

1.4 Repeatability and variation explained by sampling date and year

We found moderate individual repeatability of blood glucose level within season and across years over its life (r = 0.37 and 0.28 respectively). In contrast, repeatability of blood glucose level within capture days and within season was quite low (r = 0.10 and 0.05 respectively).

1.5 Selecting weather variables

First, we wanted to find the environmental variables that best explained the blood glucose data. In the first step, we selected variables of temperature and relative humidity (Table 2). In the second step, we selected variables of precipitation in the context of temperature and relative humidity variables selected in the previous step (Table 3). Along with weather variables, we were also testing Julian day, which may be a major predictor of weather. The following predictors, assumed to be associated with variation in blood glucose level, were used as a background in all the candidate models: sex, capture age (first, second, and third order polynomial), day time (first, second, and third order polynomial), and blood sampling latency (first, second, third, and fourth order polynomial).

Table 2: Candidate models of blood glucose as a function of temperature and relative humidity with ΔAICc < 2.

	Selected variables with their coefficients	df	AICc	ΔAICc	logLik	weight
1	0.165*JulDay - 0.047 * JulDay^2 - 0.136 * TempDay	19	4732.49	0.00	-2347.03	0.022
2	0.145 * JulDay - 0.125 * TempDay	18	4732.79	0.30	-2348.21	0.019
3	**0.160 * JulDay - 0.046 * JulDay^2 + 0.034 * RVDay - 0.121 * TempDay**	**20**	**4733.71**	**1.22**	**-2346.62**	**0.012**
4	0.170 * JulDay - 0.053 * JulDay^2 - 0.141 * TempDay + 0.017 * TempDay ^ 2	20	4733.84	1.35	-2346.68	0.011
5	0.141 * JulDay + 0.037 * RVDay - 0.109 * TempDay	19	4733.89	1.40	-2347.73	0.011
6	-0.021 * HrTemp + 0.025 * HrTemp^2 + 0.170 * JulDay - 0.055 * JulDay^2 - 0.121 * TempDay	21	4734.14	1.65	-2345.81	0.010
7	0.163 * JulDay - 0.051 * JulDay^2 - 0.144 * TempDay – 0.021 * RV5day	20	4734.29	1.80	-2346.91	0.009
8	-0.024 * HrTemp + 0.167 * JulDay - 0.048 * JulDay^2 - 0.118 * TempDay	20	4734.35	1.86	-2346.94	0.009
9	-0.012 * HrHum + 0.165 * JulDay - 0.047 * JulDay^2 - 0.139 * TempDay	20	4734.396	1.91	-2346.96	0.008
10	0.166 * JulDay - 0.050 * JulDay^2 + 0.031 * RVDay - 0.142 * TempDay - 0.014 * RV3day	20	4734.407	1.92	-2346.97	0.008
11	0.169 * JulDay - 0.050 * JulDay^2 - 0.135 * TempDay - 0.031 * RVDay:TempDay	21	4734.466	1.98	-2345.98	0.008

Df – degree of freedom, **AICc –** Akaike information criterion with small sample correlation, Δ**AICc –** AICc difference from the best supported model, logLi weight k – log-likelihood, **weight** – weight of the model, **HrHum** – current relative humidity at hour of capture, **HrTemp** – current temperature at hour of capture, **JulDay** – Julian day, **RVDay** – average relative humidity day before capture, **TempDay** - average temperature day before capture, **RV3day** – average relative humidity three days before

capture, **RV5day** – average relative humidity five days before capture, **RVDay:TempDay** – interaction between average relative humidity day before capture and average temperature day before capture.

Based on the first model set, we selected Julian day (first, and second order polynomial), and both the average temperature and relative humidity of the day preceding the day of capture. These variables entered the second set of candidate models along with the focal precipitation variables. Julian day (first, and second order polynomial), average temperature of the day preceding the day of capture, and total precipitation over the last two days preceding the day of the capture were selected in the second step. Relative humidity was excluded from further analyses as it did not occur in any of the supported models together with rainfall and because rainfall received higher support based on AICc.

Table 3: Candidate models of blood glucose as a function of precipitation with ΔAICc<2.

	Selected variables with their coefficients	df	AICc	ΔAICc	logLik	weight
1	0.165 * JulDay - 0.047 * JulDay^2 - 0.136 * TempDay	19	4732.49	0.00	-2347.03	0.058
2	0.145 * JulDay - 0.125 * TempDay	18	4732.793	0.30	-2348.21	0.050
3	**0.039 * Rain2Day + 0.154 * JulDay-0.049 * JulDay^2 - 0.131 * TempDay**	**20**	**4732.986**	**0.50**	**-2346.26**	**0.045**
4	0.111 * Rain2Day - 0.025 * Rain2Day^2 + 0.130 * JulDay - 0.116 * TempDay	20	4733.261	0.77	-2346.40	0.039
5	0.031 * Rain5Day - 0.031 * Rain5Day ^{2}+ 0.153 * JulDay - 0.130 * TempDay	20	4733.279	0.79	-2346.41	0.039
6	0.103 * Rain2Day - 0.022 * Rain2Day ^{2}+ 0.147 * JulDay - 0.043 * JulDay ^{2}- 0.126 * TempDay	21	4733.366	0.88	-2345.43	0.037
7	0.037 * Rain2Day + 0.135 * JulDay - 0.120 * TempDay	19	4733.496	1.01	-2347.54	0.035
8	0.160 * JulDay - 0.046 * JulDay ^{2} + 0.034 * RVDay - 0.121 * TempDay	20	4733.706	1.22	-2346.62	0.031
9	-0.030 * Rain5Day + 0.172 * JulDay - 0.048 * JulDay ^{2}-0.143 * TempDay	20	4733.711	1.22	-2346.62	0.031
10	0.020 * Rain5Day - 0.026 * Rain5Day ^{2}+ 0.169 * JulDay - 0.039 * JulDay ^{2} - 0.140 * TempDay	21	4733.759	1.27	-2345.62	0.031
11	0.141 * JulDay + 0.037 * RVDay - 0.109 * TempDay	19	4733.891	1.40	-2347.73	0.029
12	-0.028 * Rain5Day + 0.152 * JulDay - 0.132 * TempDay	19	4734.112	1.62	-2347.84	0.026
13	0.020 * Rain5Day - 0.029 * Rain5Day ^{2} + 0.151 * JulDay + 0.039 * RVDay - 0.115 * TempDay	21	4734.327	1.84	-2345.91	0.023
14	0.164 * JulDay - 0.047 * JulDay ^{2} + 0.008 * RainDay -0.137 * TempDay	20	4734.456	1.97	-2346.99	0.022
15	0.009 * Rain3Day + 0.161 * JulDay - 0.047 * JulDay ^{2}-0.134 * TempDay	20	4734.459	1.97	-2346.99	0.022
16	0.169 * JulDay - 0.050 * JulDay ^{2} + 0.031 * RVDay -0.135 * TempDay - 0.031 * RVDay:TempDay	21	4734.466	1.98	-2345.98	0.021

JulDay – Julian day, **TempDay** - average temperature day before capture, **RainDay** – total precipitation for 1 day before capture, **Rain2Day** – total precipitation for 2 days before capture, **Rain3Day** – total precipitation for 3 days before capture, **Rain5Day** – total precipitation for 5 day before capture, **RVDay** – average relative humidity day before capture, **RVDay:TempDay** – interaction between average relative humidity day before capture and average temperature day before capture.

1.6 Predictors of blood glucose concentration

In the final step, we tested all hypothetical predictors of blood glucose concentration that are mentioned in the Methods section 3.4.4, including the environmental variables selected in the previous steps (Table 4). Body mass was excluded in this model due to its smaller sample size (see below).

Table 4: Final model selection. Shown are candidate models with Δ AICc < 2.

	Selected variables with their coefficients	df	AICc	ΔAICc	logLik	weight
1	**0.268 * BSL – 0.394 * BSL ^{2} + 0.092 * BSL ^{3}+ BrSt + BrSt:Sex + 0.135 * Age – 0.050 * Age ^{2} + 0.181 * JulDay – 0.165 * TempDay + 0.168 * TFS – 0.038 * TFS ^{2}**	**29**	**3306.36**	**0.00**	**-1623.48**	**0.036**
2	0.265 * BSL – 0.399 * BSL ^{2} + 0.094 * BSL ^{3} + BrSt + BrSt:Sex + 0.135 * Age – 0.050 * Age ^{2} + 0.178 * JulDay – 0.163 * TempDay + 0.176 * TFS – 0.074 * TFS ^{2} + 0.007 * TFS ^{3}	30	3306.92	0.56	-1622.70	0.027
3	0.160 * BSL – 0.421 * BSL ^{2} + 0.171 * BSL ^{3} - 0.019 * BSL ^{4} + BrSt + BrSt:Sex + 0.136 * Age – 0.050 * Age ^{2} + 0.181 * JulDay – 0.166 * TempDay + 0.164*TFS – 0.038 * TFS ^{2}	30	3307.13	0.76	-1622.81	0.025
4	0.153 * BSL – 0.428 * BSL ^{2} + 0.176 * BSL ^{3} - 0.019 * BSL ^{3} + BrSt + BrSt:Sex + 0.135 * Age – 0.050 * Age ^{2} + 0.178 * JulDay – 0.164 * TempDay + 0.172 * TFS – 0.075 * TFS ^{2} + 0.008 * TFS ^{3}	31	3307.58	1.22	-1621.98	0.020
5	0.266 * BSL – 0.392 * BSL ^{2} + 0.092 * BSL ^{3} + BrSt + BrSt:Sex + 0.122 * Age – 0.005 * Age ^{2} – 0.011 * Age ^{3} + 0.181 * JulDay – 0.165 * TempDay + 0.169 * TFS – 0.038 * TFS ^{2}	30	3307.81	1.44	-1623.15	0.018
6	0.271 * BSL – 0.396 * BSL ^{2}+ 0.092 * BSL ^{3} + BrSt + BrSt:Sex + 0.136 * Age – 0.050 * Age ^{2} + 0.024 * Rain2Day + 0.174 * JulDay –0.160 * TempDay + 0.167 * TFS – 0.038 *TFS ^{2}	30	3308.03	1.66	-1623.26	0.016
7	0.267 * BSL – 0.392 * BSL ^{2} + 0.092 * BSL ^{3} + BrSt + BrSt:Sex + 0.118 * Age – 0.050 * Age ^{2} + 0.180 * JulDay + 0.028 * Lifespan – 0.164 * TempDay + 0.168 * TFS – 0.038 * TFS ^{2}	30	3308.23	1.86	-1623.36	0.014
8	0.266 * BSL – 0.393 * BSL ^{2} + 0.092 * BSL ^{3} + BrSt + BrSt:Sex + 0.136 * Age – 0.051 * Age ^{2} + 0.186 * JulDay - 0.016 * JulDay ^ 2 – 0.167 * TempDay + 0.168 * TFS – 0.038 * TFS ^{2}	30	3308.23	1.87	-1623.36	0.014

BLS – blood sampling latency, **Age** – age at capture time, **JulDay** – Julian day, **TFS** – day time (day time), **HrTemp** – current temperature at hour of capture, **TempDay** - average temperature day before capture, **Rain2Day** – total precipitations in two days before capture.

We chose the best supported model as a final model (Table 5). The final model included the following fixed effect predictors: sex, breeding stage, interaction between sex and breeding stage, age (first, and second order polynomial), time of day (first, and second order polynomial), blood sampling latency (first, second, and third order polynomial), Julian day, and the average temperature of the day preceding the day of capture. We used ring number, capture date, and capture year as variables with random effect. Effect of lifespan was not significant in any candidate model, which indicates that survival is not related to blood glucose concentration.

Table 5: Final model of blood glucose level.

	Estimate	SE	df	t value	p
(Intercept)	0.761	0.381	8.9	2.00	0.077
Sex Males	**–0.498**	**0.201**	**1694.0**	**–2.48**	**0.013**
BreedingStage - Mating	–0.277	0.261	1719.0	–1.06	0.289
BreedingStage - Incubation	**0.807**	**0.191**	**1748.0**	**4.23**	**< 0.001**
BreedingStage - Nestlings	–0.097	0.206	1728.0	–0.47	0.637
BreedingStage - Fledglings	**–0.813**	**0.324**	**1605.0**	**–2.51**	**0.012**
BreedingStage - Post_breeding	0.628	0.739	1708.0	0.85	0.396
BreedingStage - Non_breeding	–0.067	0.226	1641.0	–0.30	0.766
BreedingStage - Unknown	0.192	0.249	1715.0	0.77	0.440
Capture Age	**0.175**	**0.060**	**1720.0**	**2.91**	**0.004**
Capture Age ^{2}	**–0.063**	**0.019**	**1701.0**	**–3.38**	**0.001**
Day time (hours)	**0.144**	**0.031**	**1703.0**	**4.63**	**< 0.001**
Day time (hours) ^{2}	**–0.017**	**0.008**	**1494.0**	**–2.11**	**0.035**
Blood Sampling Latency	–0.012	0.009	1530.0	–1.45	0.147
Blood Sampling Latency ^{2}	**–0.004**	**0.001**	**1192.0**	**–8.16**	**< 0.001**
Blood Sampling Latency ^{3}	**1.4×10^{-4}**	**1.2×10^{-5}**	**1632.0**	**6.70**	**< 0.001**
Julian day	**0.012**	**0.003**	**95.4**	**4.09**	**< 0.001**
Temperature 1day before capture	**–0.057**	**0.015**	**96.3**	**–3.67**	**< 0.001**
SexM:BreedingStage - Mating	0.228	0.332	1656.0	0.69	0.492
SexM:BreedingStage - Incubation	**–0.777**	**0.238**	**1672.0**	**–3.27**	**0.001**
SexM:BreedingStage - Nestlings	–0.055	0.248	1642.0	–0.22	0.826
SexM:BreedingStage - Fledglings	**1.029**	**0.394**	**1526.0**	**2.62**	**0.009**
SexM:BreedingStage - Post_breeding	–1.212	0.859	1660.0	–1.41	0.158
SexM:BreedingStage - Non_breeding	0.130	0.267	1661.0	0.49	0.626
SexM:BreedingStage - Unknown	–0.221	0.270	1725.0	–0.82	0.412

According to the best supported model, female Barn Swallows had significantly higher blood glucose level than males (Table 5, Figure 1). Glucose was further explained by breeding stage and its interaction with sex. To avoid multiple post-hoc tests comparing all categories with each other, we considered pre-breeding stage as a reference level and compared all the other levels to this stage. Specifically, females had higher glucose concentration during incubation and lower during feeding fledglings compare to the pre-breeding stage. To tested effect of breeding stage in males we run this final model on reparameterized data in which males were coded as a reference level of sex. In males, none of the breeding stages was significantly different compared to the pre-breeding stage (Table 6, Figure 5).

Table 6: Effects of breeding stage in males. The effects were obtained from the reparametrized final model, with male coded as a reference level of sex.

	Estimate	SE	df	t value	p
BreedingStage - Mating	-0.048	0.214	1617.0	-0.23	0.822
BreedingStage - Incubation	0.029	0.163	1641.0	0.18	0.860
BreedingStage - Nestlings	-0.151	0.173	1657.0	-0.88	0.382
BreedingStage - Fledglings	0.216	0.255	1550.0	0.85	0.396
BreedingStage - Post_breeding	-0.584	0.481	1589.0	-1.22	0.225
BreedingStage - Non_breeding	0.065	0.153	1739.0	0.42	0.672
BreedingStage - Unknown	-0.029	0.210	1679.0	-0.14	0.891

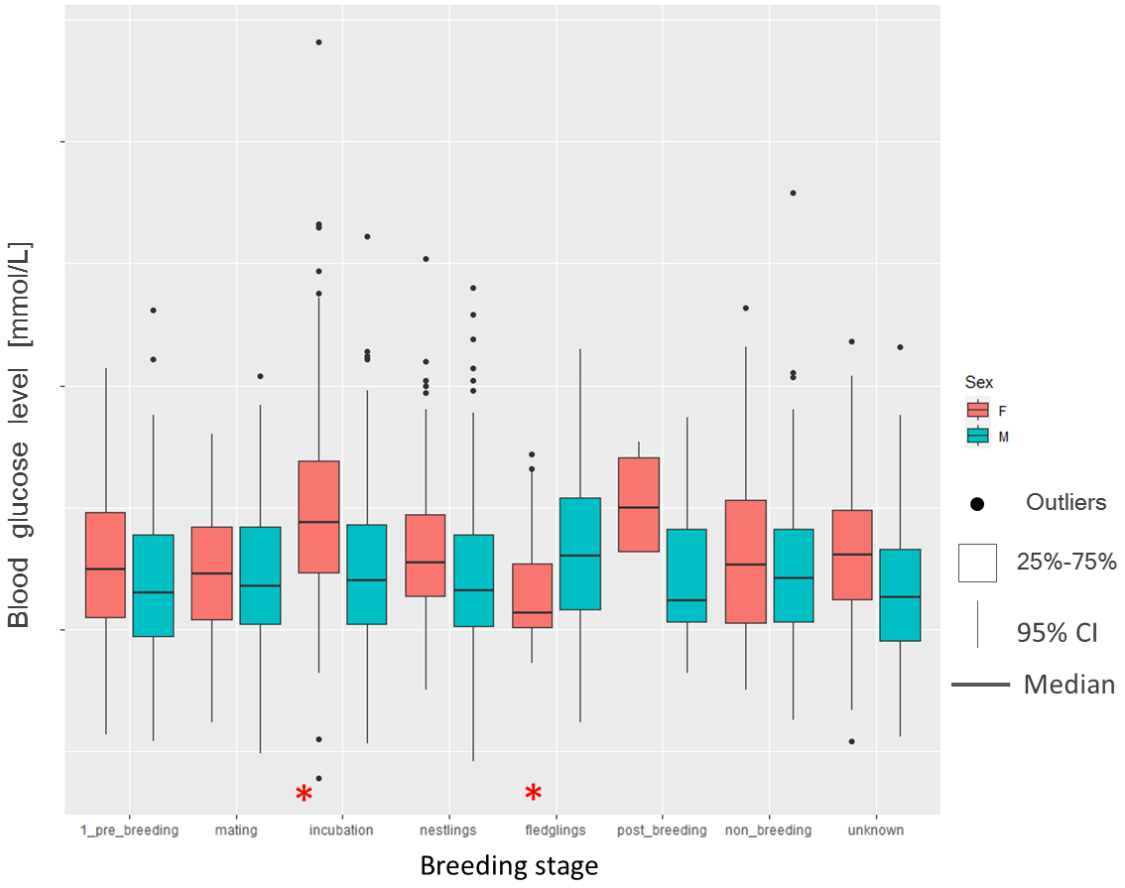

Figure 5: Blood glucose level during different breeding stages in female and male Barn Swallows. Red stars indicate breeding stages, in which females significantly differed in blood glucose level both from female pre-breeding values and from male values at the same stage.

The next individual aspect, which can influence physiology, is age. In the final model, quadratic effect of age was statistically significant, suggesting mild decline in blood glucose level towards old age (Table 5, Figure 6).

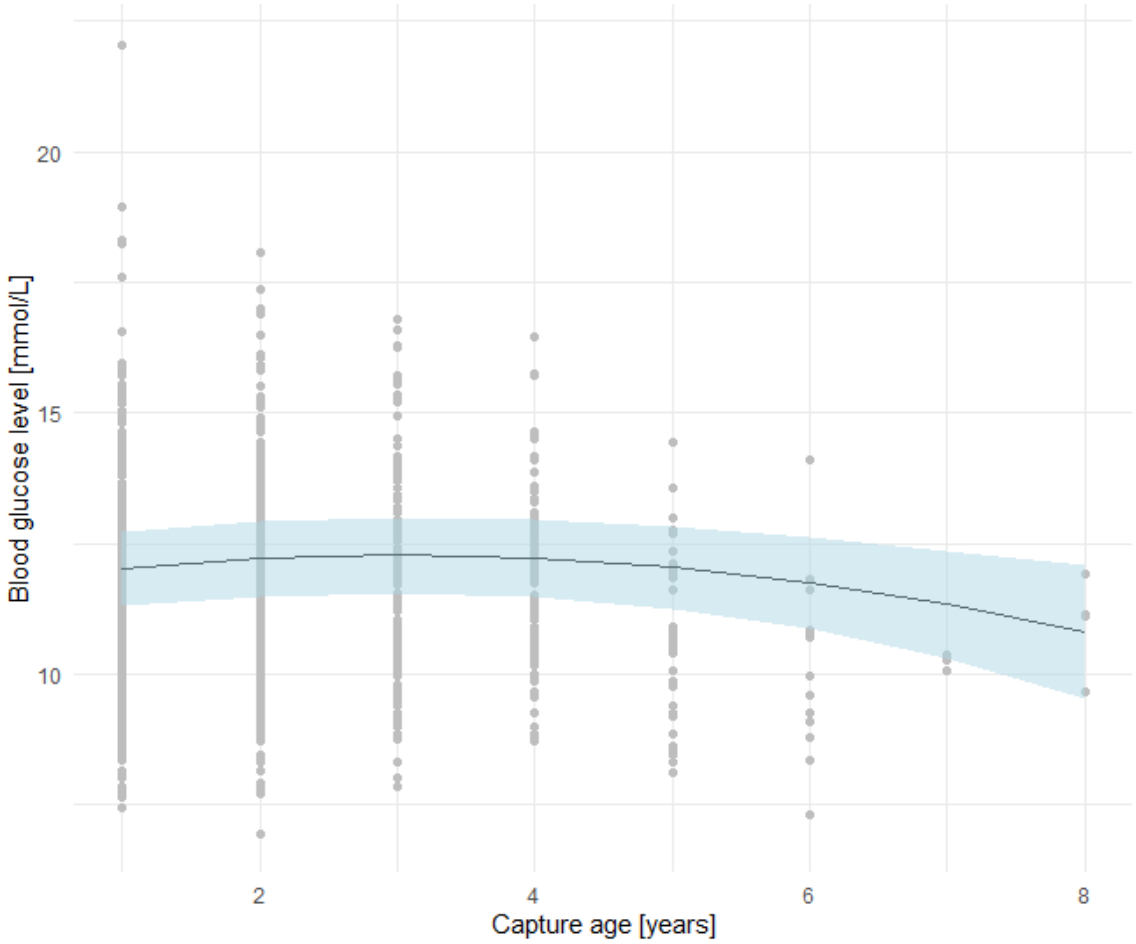

Figure 6: Quadratic effect of capture age on blood glucose level in the Barn Swallow. Line depicts predicted values from the final model, blue band depicts 95% confidence interval, and grey points are original data.

Apart from sex, breeding stage, and age, blood glucose level was also influenced by several environmental factors. First statistically significant factor was Julian day, indicating that blood glucose was increasing over the breeding season (Figure 7). Second statistically significant environmental predictor was temperature. Average temperature the day before day of capture negatively affects blood glucose level (Table 5, Figure 8). The last was time of day, whose linear and quadratic effects were statistically significant in the final model (Table 5, Figure 9).

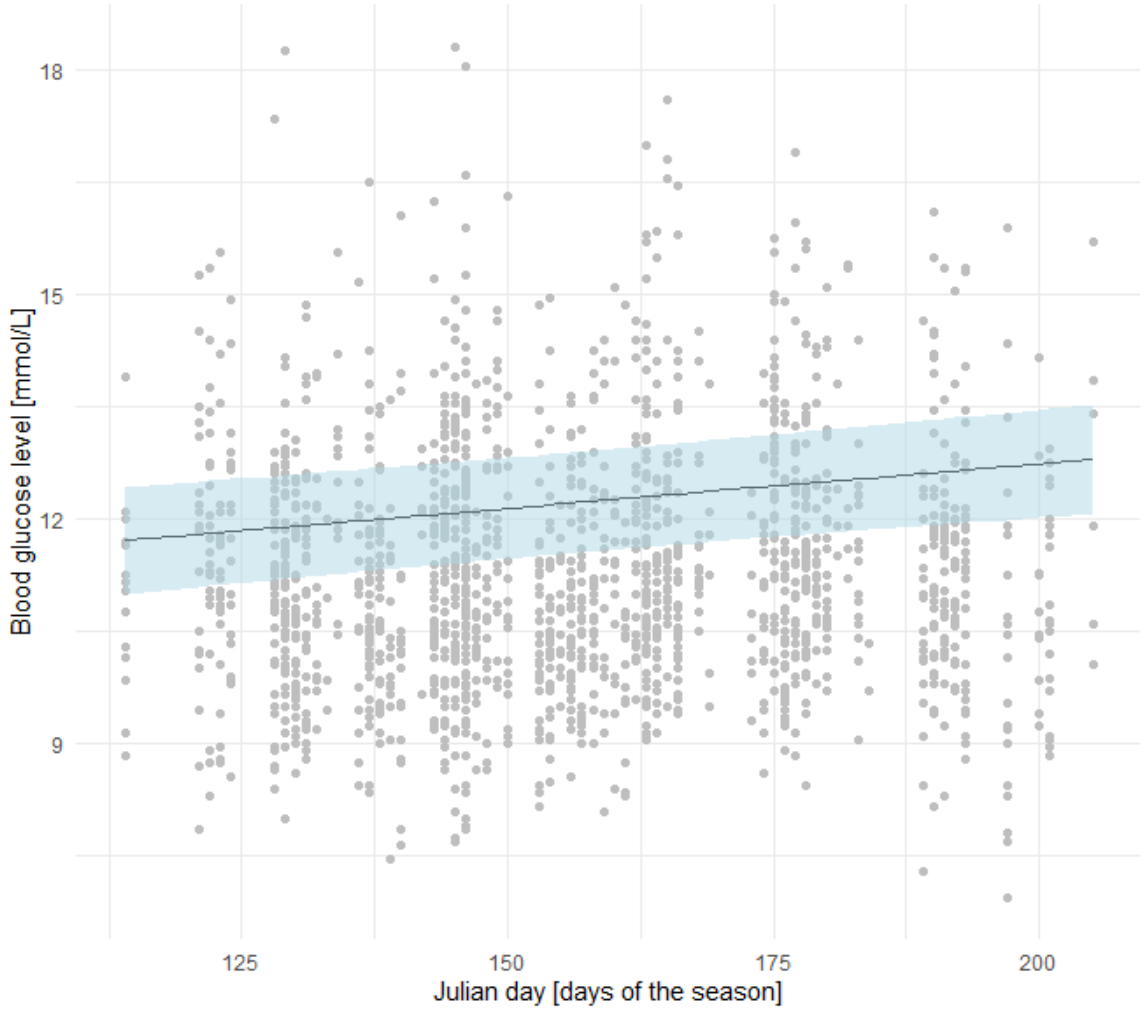

Figure 7: Effect of Julian day on blood glucose level in the Barn Swallow. Line depicts predicted values from the final model, blue band depicts 95% confidence interval, and grey points are original data.

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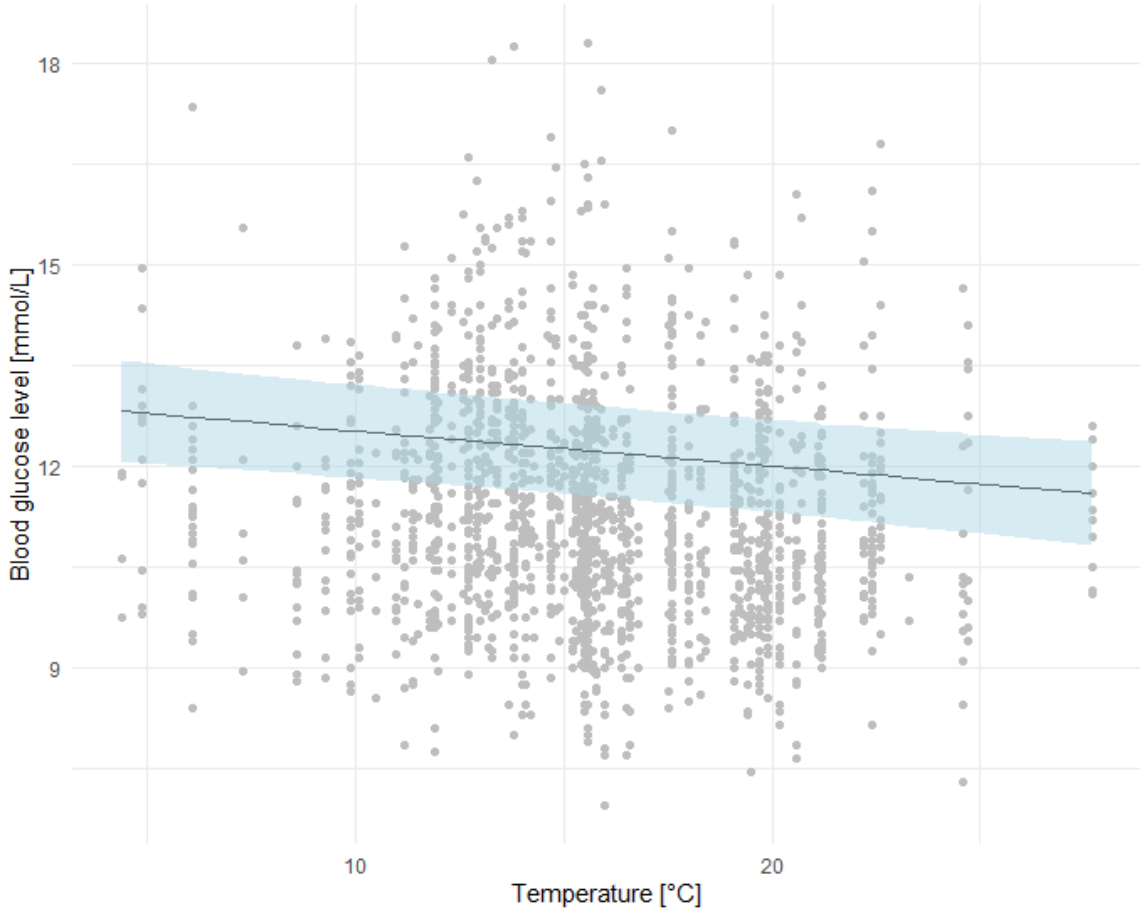

Figure 8: Effect of average temperature one day before capture on blood glucose level in the Barn Swallow. Line depicts predicted values from the final model, blue band depicts 95% confidence interval, and grey points are original data

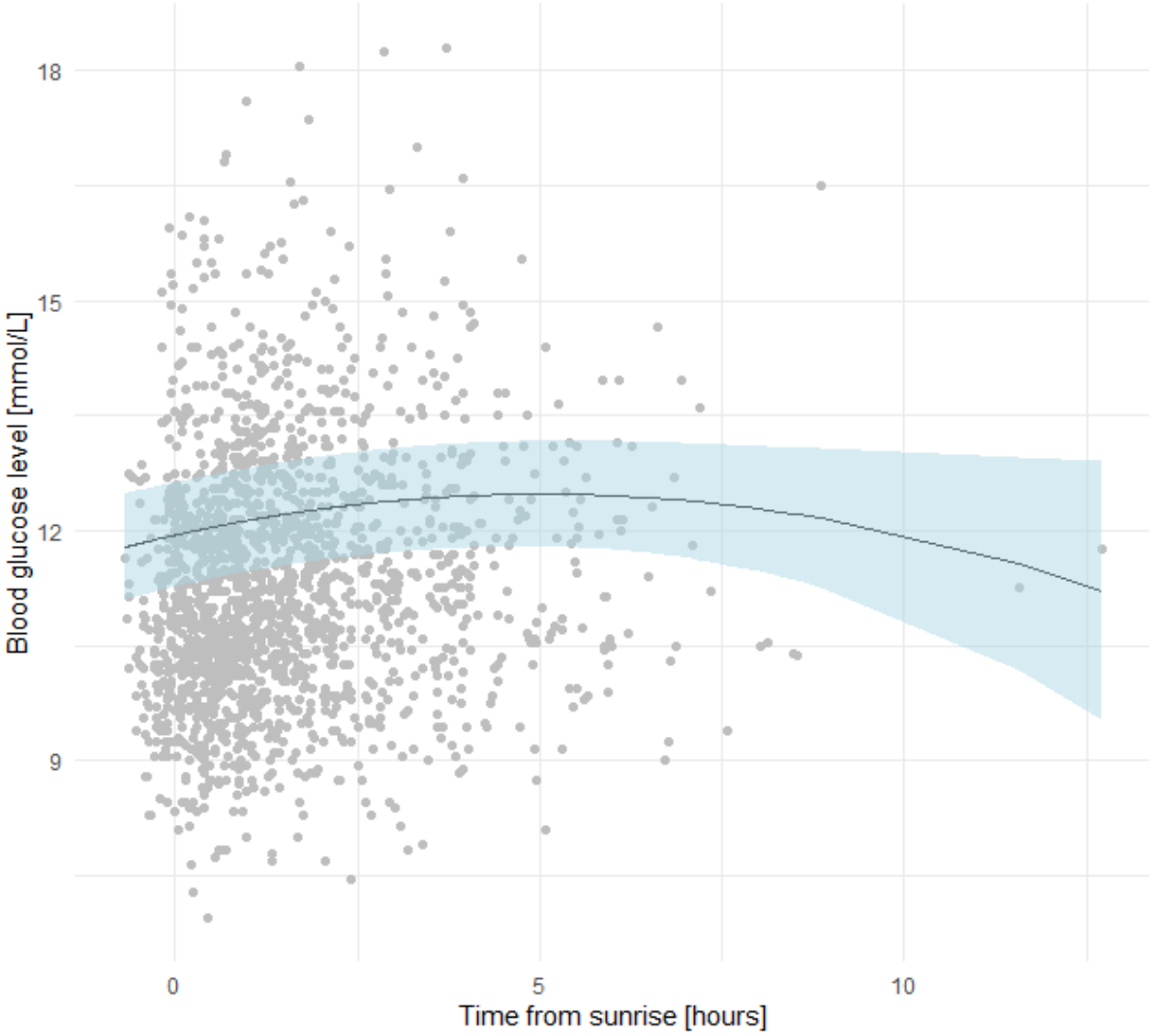

Figure 9: Quadratic effect of time of day on blood glucose level in the Barn Swallow. Line depicts predicted values from the final model, blue band depicts 95% confidence interval, and grey points are original data.

Next aspect that influences blood glucose level was stress. It can be caused by the capture and blood sampling. We tested the effect of blood sampling latency in the final model. Its polynomial effects (second and third order polynomial) were statistically significant (Table 5, Figure 10).

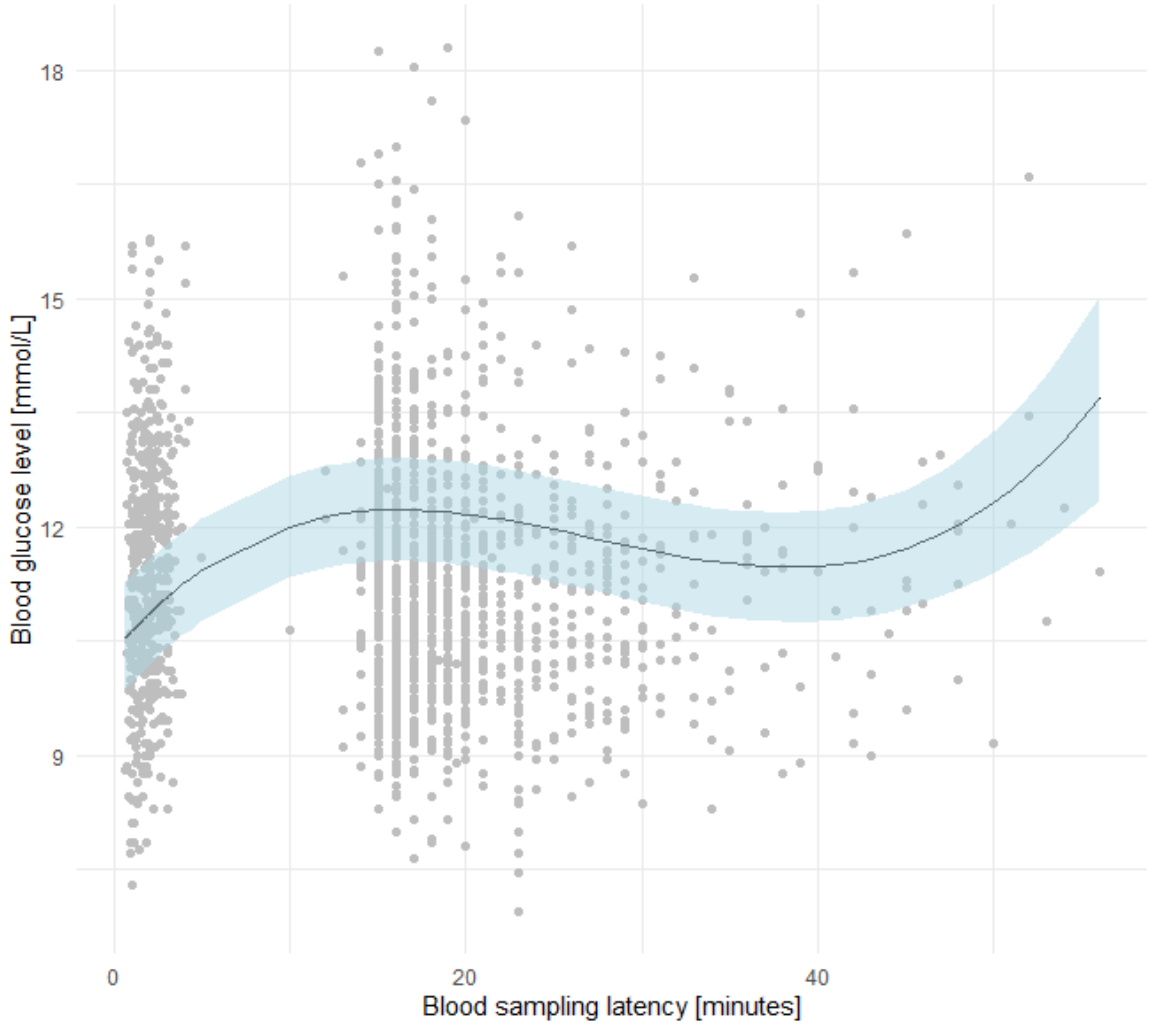

Figure 10: Effect of blood sampling latency on blood glucose level in the Barn Swallow. Line depicts predicted values from the final model, blue band depicts 95% confidence interval, and grey points are original data.

We further calculated conditional and marginal coefficient of determination of the final model (Table 5). The marginal R^2 = 0.17, and conditional R^2 = 0.59. We also partitioned variation explained by each random effect, while controlling for all the fixed-effect predictors. This was done using calculation of their intra-class correlation coefficient (repeatability) from the final model. The variance explained by capture day was r = 0.05, and the variance explained by capture year was r = 0.24.

We also calculated individual repeatability during season and within season, while controlling for all the fixed-effect predictors. In this model, we included capture year among the fixed-effect predictors to control for its effect. Individual repeatability during season was r = 0.34 and within season was r = 0.28.

To test the effect of body mass we used the final model structure, adding body mass as another predictor and using the subset of 1239 individuals (722 males, and 517 females) with available body mass data. The effect of body mass proved to be statistically significant (β = 0.16, SE = 0.42, t_{1195} = 4.35, $p < 0.001$) (Table S2, Figure 11). In general, females have higher body mass than males (Figure 12), when we compared all data of body weight (female: mean = 19.1 g, males: mean = 18.2 g).

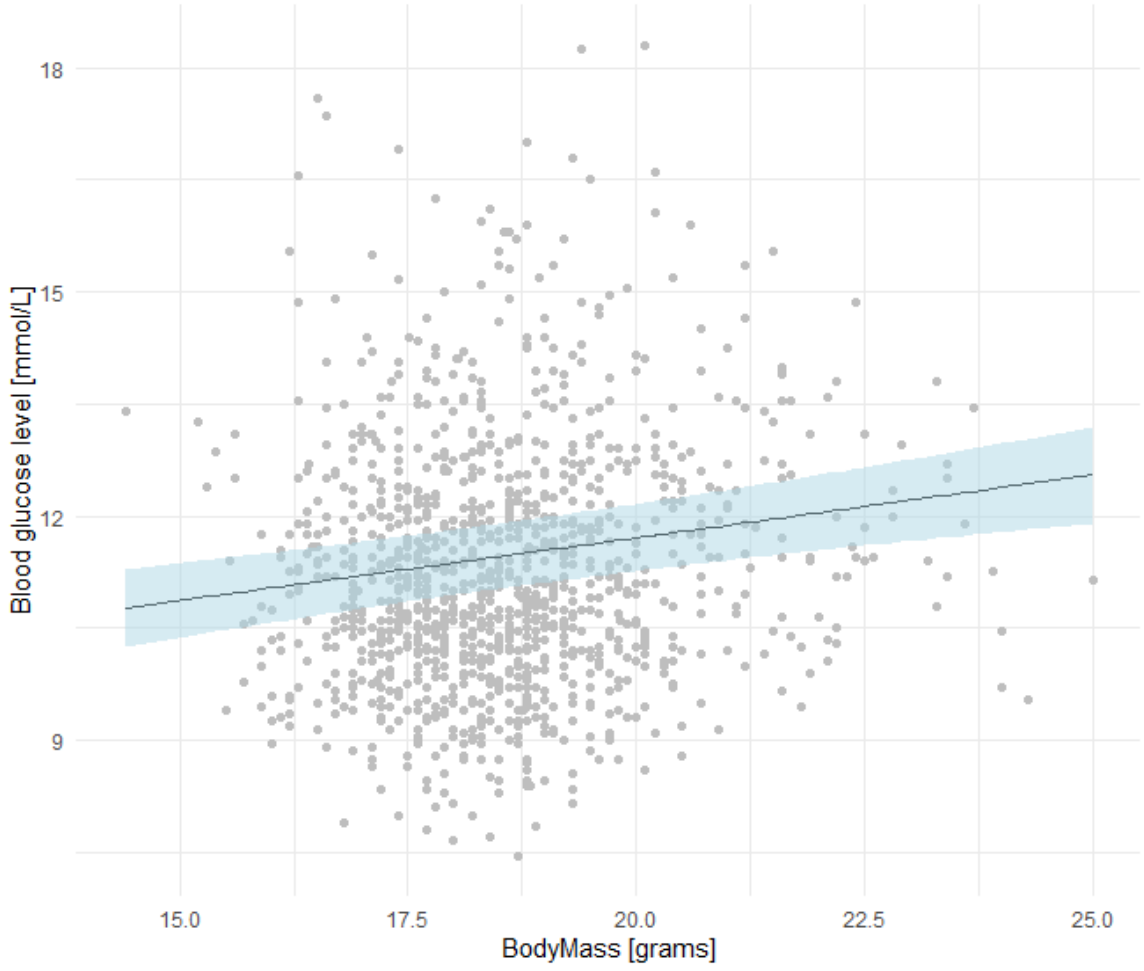

Figure 11: Effect of body mass on blood glucose level in the Barn Swallow. Line depicts predicted values from the final model, blue band depicts 95% confidence interval, and grey points are original data.

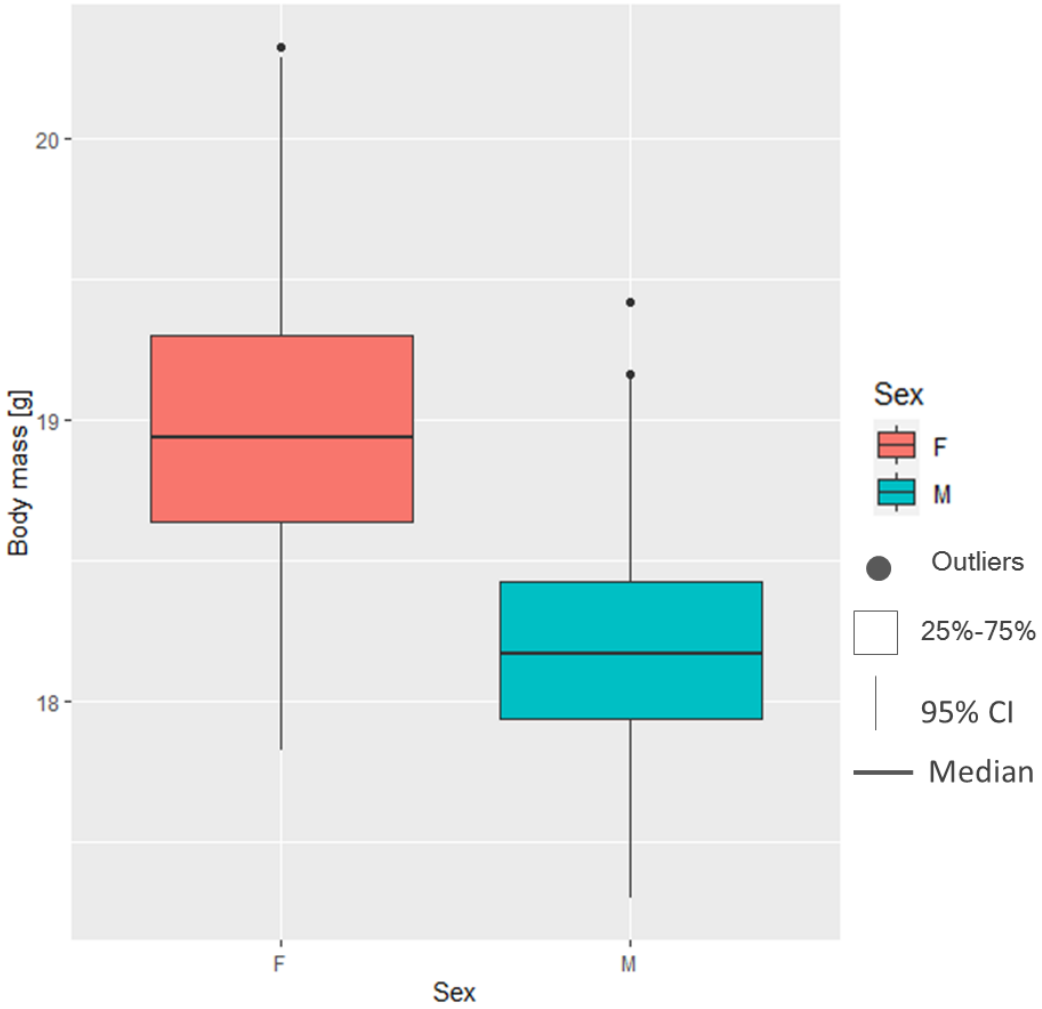

Figure 12: Difference in body mass in females and males in the Barn Swallow.

$t_{409.64}$= –8.55, p <0.001

Finally, we tested the effect of the sample order (i.e. first or subsequent sampling on the day), adding this categorical variable into the model (Table S4), the structure of which was otherwise the same as the final model in Table 5. The dataset comprised 2286 samples. Blood glucose level was significantly higher in both the second and the third samples compared to the first sample (second: $\beta = 0.92$, $SE = 0.21$, $t_{578.6} = 4.47$, $p < 0.001$, third: $\beta = 2.01$, $SE = 0.42$, $t_{1666} = 4.79$, $p < 0.001$) (Figure 13).

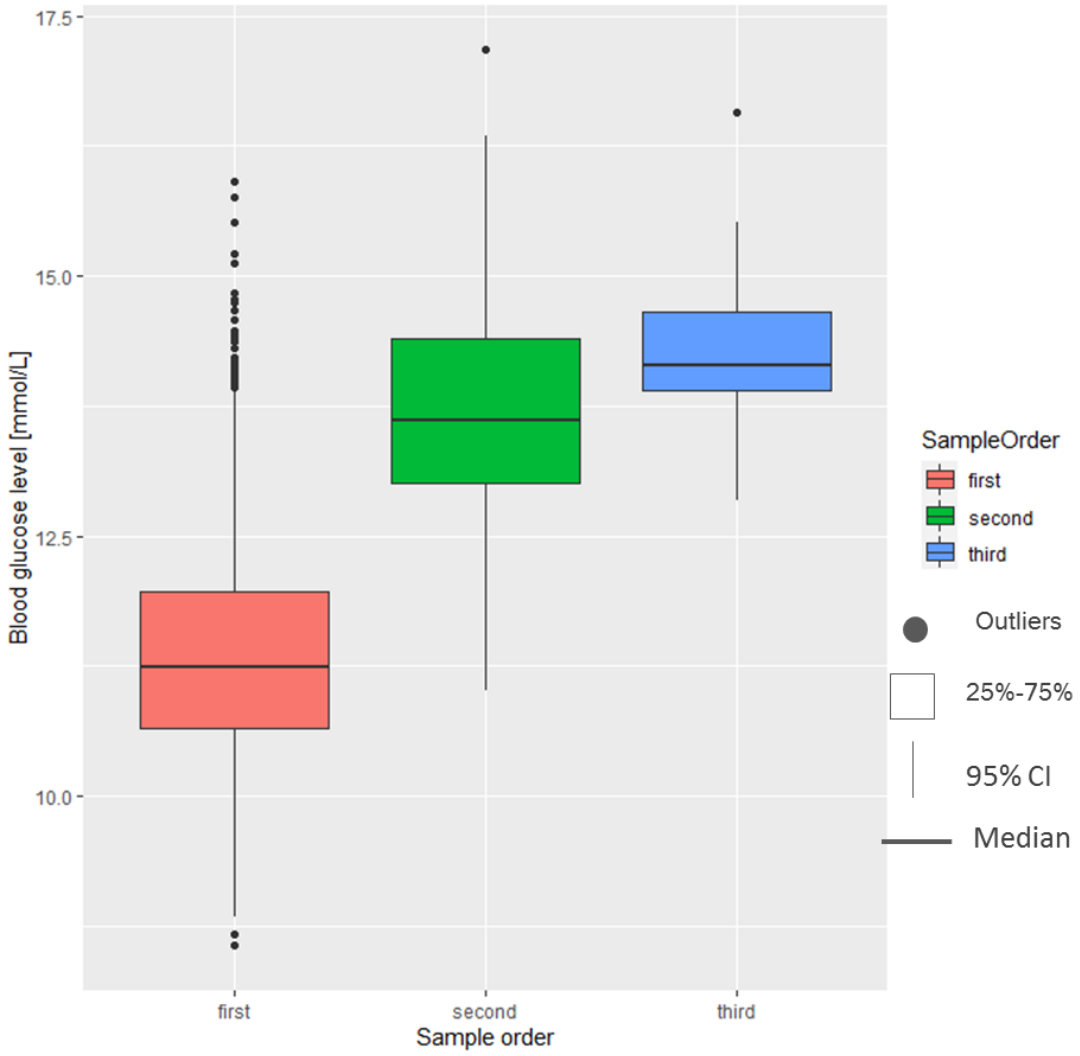

Figure 13: Effect of first, second, and third blood sample on blood glucose level in the Barn Swallow. Box and whiskers plot of predicted blood glucose level and sample order. Blood glucose level data are predicted data from model (Table S4).

5 Discussion

Glucose is a crucial molecule in energy metabolism. It is main source of energy for intense activities of short duration and stressful situations. However, it can cause severe tissue damages in high concentration at least in mammals. Glucose is an indicator of ability to mobilize energy in stress situations and seems to be one of the important aspects for individual fitness. Despite the indisputable importance of glucose, there are only a few short-term studies observing only small number of individuals. In our study, we analysed wild populations of the Barn Swallow comprising hundreds individuals over their lifetimes.

Blood glucose concentration is considerably higher in birds compared to mammals of the same size (Polakof, Mommsen and Soengas, 2011). Barn Swallows in our population showed the mean blood glucose level 11.2 mmol/L, with the range from 6.0 to 23 mmol/L. This mean is lower than the mean 15.6 for 511 avian species (Polakof, Mommsen and Soengas, 2011). This blood glucose level is comparable with blood glucose concentrations measured in comparative study of 30 passerine birds which ranged from 10.1 mmol/L in Scarlet Rosefinch (*Carpodacus erythrinus*) to 15.2 mmol/L in Northern Wren (Tomasek *et al.*, 2019).

In this thesis, we investigated the effect of several possible variables on blood glucose level in adult Barn Swallows. Firstly, we considered these environmental factors: Julian day, time from sunrise, current and long-term temperature, relative humidity, and precipitation. Next paragraphs describe selected predictors in more details.

Blood glucose level is a labile physiological feature which quickly responds to intrinsic and environmental stimuli in minutes. Stressful stimuli cause production of stress hormones, such as catecholamine (e.g. adrenalin) and later glucocorticoid (corticosterone) (Wingfield and Romero, 2011). Both of them are known to elevate blood glucose level, besides other physiological effects. The level of adrenalin increases in seconds. On the other hand, corticosterone is detectable in approximately three minutes following an exposure to a stressor and its effect last for hours (Sapolsky, Romero and Munck, 2000).

We took blood samples in different time after capture which could cause changes in blood glucose level due to stress hormones production. However, we took into account this time in the final model and we controlled for its effect. In resulting graph depicting effect of blood sampling latency (Figure 10) on blood glucose level, we can see three stress phases of

glucose mobilization. In first wave of stress reaction blood glucose level was increasing from the basal level (approximately 1–3 minutes; Romero and Reed, 2005)) to the peak at 15 minutes after capture. Secondly, after 15 minutes the blood glucose level slightly decreased. Thirdly, after 40 minutes blood glucose level was increasing to the higher level than at 15 minutes. We consider blood glucose level after 15 minutes to be already on stress level. The mild decrease after 15 minutes is not strong and blood glucose values seem to be comparable. The final increase could possibly be caused by prolonged capture stress. However, the limited sample size precludes any strong conclusion about this later increase.

The next statistically significant environmental predictor that influenced blood glucose level in the Barn Swallow was quadratic effect of time of day. The minor increase in blood glucose level over the first five hours from sunrise could be associated with bird circadian rhythm. Study of captive Starlings (*Sturnus* vulgarius) showed similar tendency, when blood glucose level peaked in midday (Remage-Healey and Romero, 2000). However, Frelin (1974) found significantly lower blood glucose level between 11 and 13 o'clock in Redpolls (*Acanthis flammea*). Effect of circadian rhythm was also suggested by the study of chickens (Twiest and Smith, 1970). Twiest and Smith found out that blood glucose level in chickens was lower during the dark period, when the lights were off and higher during the light period. Blood glucose level was stable within both the light and the dark period, respectively. During the dark period, chickens were asleep and woke up one hour before light, when the light was still off. Nevertheless, the blood glucose level in this moment was already comparable with the level of light period. This finding indicates that increase of blood glucose level could be associated with higher activity after night sleep and fasting. The next study supported this suggestion, showing that the nocturnal fasting is linked with lower energy expenditure and decreased metabolic rate in the chicken (Buyse *et al.*, 1993). We observed similar tendency in the Barn Swallows, when blood glucose level was rising after the sunrise. These observations support increasing blood glucose level with increasing activity. In contrast, the study of Montoya *et al.* (2018) did not reveal any changes in blood glucose level associated with time of day.

Not only circadian rhythm, but also the effect of the season could be seen in blood glucose level in the Barn Swallow. The significant effect of Julian day showed increasing blood glucose concentration throughout the season. In the study of captive Garden Warbler (*Sylvia borin*), Bairlein (1983) found that blood glucose concentration was significantly higher during the autumn and spring migratory periods compared to the phase of the winter low

body weight. However this study was conducted on captive birds which were not allowed to migrate. He also supposed that increase in blood glucose level could be caused by body weight gain. However, body mass did not increase significantly during the breeding season in our data. Other possible explanations could be increasing breeding activity, higher stress, or repeated capture which could increase stress reaction.

Another point of view on Julian day is the changing day length during season. In study of captive Starlings, Remage-Healey and Romero (2000) found that the season had significant effect on the blood glucose level. Birds held on short days had lower level of blood glucose compare to the birds held on long days with high blood glucose level. These findings are quite consistent with our data, where we observed similar tendency. In contrast, Montoya *et al.* (2018) reported that blood glucose level in captive Zebra Finch was lower on longer days. This difference could be caused by the fact that Zebra Finch originate from south hemisphere and Starling as well as the Barn Swallow from north hemisphere.

Interestingly, only one of the potential weather aspects, namely average temperature of the day preceding the day of capture, was significantly associated with blood glucose concentration. The two other weather characteristics, relative humidity and precipitation, were not supported as statistically significant during the predictor selection. Hence, only temperature negatively affected blood glucose level in the Barn Swallow. Low temperature could cause smaller amount of flying insect, which is a main food source in Barn Swallows (Barrentine, 1980). Moreover, maintaining body temperature could be more energy demanding when environmental temperature is low. Birds are able to warm up by non-shivering thermogenesis which is considered to be the mediated by the hormone glucagon (Barre and Rouanet, 1983). Glucagon is known to increase blood glucose level in birds (Hazelwood, 1973). Exogenous glucagon injected in experiments also induced increases in metabolic rates (Barré, Cohen-Adad and Rouanet, 1987). Vaillancourt et al. (2005) present that metabolic rate in birds is elevated due to low temperature during night or at high altitude. Similarly, Montoya *et al.* (2018) found that blood glucose level in adult Zebra Finches was low when the surrounding temperature was high and the foraging costs were low. They inferred that blood glucose is maintained at lower level during low energy turnover. Surrounding weather condition influences not only the adult birds, but also nestlings. Facey et al. (2020) found that body mass of the Barn Swallow nestlings and fledglings was negatively correlated with temperature. Nevertheless, rainfall had different effect according to the stage in that study. Nestlings were heavier on dry days, fledglings on

days with low rain. Their results showed that environmental effects varies during early lifetime and have impact on individual body condition (Facey *et al.*, 2020). The effect of environmental conditions of body condition can mediate the negative covariation of blood glucose level with temperature observed in our study.

Apart from environmental condition, blood glucose is influenced by intrinsic factors. Our results showed that sex is an important predictor of blood glucose concentration. On average, female Barn Swallows have significantly higher blood glucose level than males (mean difference is 0.7 mmol/L). Our finding is in agreement with the results of the study comparing 30 passerine bird species (Tomasek *et al.*, 2019). However, in adult captive Zebra Finch, sex did not affect blood glucose level (Montoya et al., 2018). The higher blood glucose level in females Barn Swallow could be due to higher energy demands associated with breeding, especially with egg production and incubation because eggs are only incubated by females in the Barn Swallow. This assumption is supported by the fact that females Barn Swallows showed elevated blood glucose level during egg laying and incubation compare to the pre-breeding stage, as well as compared to males during egg laying and incubation. The reason why we observe higher blood glucose level in females compared to males can be traced to the fact that incubation and egg production, the most demanding tasks, are done solely by female Barn Swallows. Another reason could be the hormonal changes or enlargement and elevated activity of reproductive organs (Pick *et al.*, 2016). We could not find exactly the same pattern in other published studies that considered breeding stage and blood glucose levels. For example, significant increase of blood glucose level during courtship, mating, and feeding offspring was reported in both sex of pigeons (Gayathri, Shenoy and Hegde, 2004). Interestingly, they mentioned that females both body mass and blood glucose level did not differ during egg laying compare to the other stages of reproductive cycle. Pigeons feed their offspring by pigeon ‘milk’, what could be the explanation of higher blood glucose level in stage of feeding. On the other hand, in turkey hens blood glucose level decrease during incubation (Wentworth *et al.*, 1983). These discrepancies could be caused by the diverse breeding behaviour of different species.

Next significant predictor of blood glucose level in the Barn Swallow was body mass which was positively correlated with the blood glucose level. Although blood glucose level is negatively correlated with body mass at the interspecific level according to some studies (Braun and Sweazea, 2008; Tomasek *et al.*, 2019), at the intraspecific level the results of previous studies have been inconsistent. For example, in the study of Lill (2011) body mass

was only associated with blood glucose level in two of six studied bird species and, on top of that, these two species showed inverse directions of glucose-body mass covariation. Specifically, in the Grey-brown White-eye (*Zosterops ponapensis*), body mass was negatively correlated with blood glucose level, but in the Welcome Swallow (*Hirundo neoxena*), the correlation was positive (Lill, 2011). This is the interesting concordance which could potentially insinuate that blood glucose level of Swallows, in general, would be condition-dependent traits. However, this suggestion would be necessary to examine in more details.The association between blood glucose level and body mass may be species specific. In our study, blood glucose level was positively correlated with body mass, suggesting that glucose level is probably a condition dependent trait in the Barn Swallow.

Another important individual aspect, affecting physiology, morphology, or behaviour, is age. The effect of age on a trait is often non-linear, with initial increase in early adulthood due to maturation, followed by a plateau during mid-life, and decline in old age as a result of senescence. However, senescence is rarely studied in the wild as long-term studies following individuals throughout their lifetimes are needed. Moreover, only a small fraction of individuals survive until old age and it is thus necessary to follow large numbers of individuals to obtain a reasonable sample sizes in older age classes. In this study, the cubic effect of chronological age was not supported and we only observed a significant quadratic effect. Blood glucose concentration increased until the age of approximately three years, followed by decline in late age. Similar dynamics of age-related changes is present in several reproductive traits in the same Barn Swallow population, including clutch size, number of successful broods per season, and number of offspring per season (Pazdera, 2020). Balbontín *et al.* (2007), who also studied population of the Barn Swallow, reached the results with the same pattern in two reproductive traits, namely laying date and annual fecundity. Decrease in several aspects with age could indicate that the physical condition probably deteriorates during aging.

During the predictor selection, chronological age was better supported compared to years before death. Such a result suggests lack of differences in the effect of age on blood glucose concentration. The association of blood glucose concentration with lifespan was also not supported. The non-significant effect of lifespan indicates that survival is not associated with blood glucose concentration in the Barn Swallow. However, this effect of lifespan is not flexible enough to detect if specials pattern, such as certain concentrations cause better survival or vice versa.

Body mass, which is considered to be indicator of condition, was positively correlated with blood glucose level in Barn Swallow. This could potentially indicate better survival of individual with higher blood glucose level. This finding is interesting in the context of the fact that high blood glucose level causes the increase of the advanced glycation end products, which are connected with ageing in humans (Semba, Nicklett and Ferrucci, 2010). However, in the study of Montoya et al. (2018), blood glucose level was negatively associated with survival probability in captive Zebra Finches. On the other hand, the study comparing 30 passerine bird species did not find any connection between blood glucose concentration and longevity at the interspecific level (Tomasek et al., 2019). The discrepancies at the intraspecific level may suggest the differences between species. At the interspecific level, the lack of association between blood glucose level and lifespan suggests that, at the macroevolutionary scale, evolution of adaptations preventing adverse effects of high blood glucose is possible (Tomasek et al., 2019).

Although the tissue damage associated with hyperglycaemia is the most often found in humans due to inappropriate lifestyle, there are evidence that even wild birds could be affected by protein glycation. The study of Collared Flycatchers (*Ficedula albicollis*) supported the detrimental effect of high glucose concentration (Sibeaux *et al.*, 2016). Sibeaux reported the selective disappearance of individuals with the highest level of glycated haemoglobin. This finding suggests high glucose concentration could cause lower survival even in birds.

The each venepuncture is strong stressor for bird. In our study we revealed significant increase of blood glucose level after the first and the second venepuncture while controlling for the effect of blood sampling latency. The rise between the first and the second sample was considerably higher than the rise between the second and the third sample. This difference could be caused by smaller number of individuals in which we took three samples (twenty). The second possible explanation could be the strong stress reaction after the first sample that later did not allow as strong reaction during subsequent sampling. First venepuncture caused rapid increase in adrenaline and corticosterone, which caused increase in blood glucose level. After the second venepuncture new dose of adrenalin augmented blood glucose level while the corticosterone concentration was still high and potentially supressing blood glucose level. This could explain that the rise between the second and the third blood sample was lower than between the first and the second. Nevertheless, we cannot support this conclusion due to the lack of actual stress hormones measurements. Given that a

loss of blood volume is known stressor, another explanation of different increment could be much smaller volume taken in the second sample (a drop of blood) compared to the first sample. In other words, smaller blood loss could in the second sample might have induced milder stress reaction.

We tested individual repeatability of blood glucose level to assess whether and to what extent it is an individual-specific trait. This is important as the less the trait is individual-specific, the less scope is there for natural selection on that trait. We analysed data from 715 individuals, revealing that individual blood glucose level reaches 37% repeatability within the breeding season and 28% across years during individual's lifetime. Blood glucose concentration is dynamic trait and thus this rough repeatability could be decreased by effect of many intrinsic and environmental aspects (mentioned above). On the other hand, sex differences can increase estimates of individual repeatability; however, sex-related trait repeatability does not express a potential for selection between individuals. For these reasons, we calculated repeatability in the context of all the selected fixed-effect predictors, including capture year. This analysis revealed individual repeatability to be 34% within season and 28% across season. The figures seem to be similar despite the fact we controlled on the other predictors. Nevertheless, we eliminated both variables introducing noise (such as sampling latency, temperature, age etc.) and variables increasing individual repeatability artificially (sex). This can explain why controlled repeatability was similar as the rough repeatability. Nevertheless, the repeatability controlled for confounding factors should better reflect individual variability in genetic background, which is a substrate for selection.

However, based on this level of repeatability, we can consider that blood glucose level is an individual-specific trait, and thus it can be subject to selection provided that this variability is heritable. Similar repeatability was observed in the study on captive zebra finches (*Taeniopygia guttata*), where within-year and between-years repeatability was 29.7% and 27.4%, respectively (Montoya *et al.*, 2018). In a study focused on 14-day-old nestling Pied Flycatchers, blood glucose concentration exhibited 32% within-brood repeatability (Glądalski *et al.*, 2015). This finding also corresponds with the inter-species study that show ca. 30% repeatability at the species level (Tomasek *et al.*, 2019)

In contrast, repeatability of blood glucose level associated with capture day and season was quite low when we did not control for the other predictors (10% and 5.5% respectively). However, when we calculated this repeatability after factoring out the variability associated

with other fixed-effect predictors, the repeatability of the capture day decreased to only 5%, and the repeatability of the year increased to 24%. The decrease in capture day related repeatability probably reflects the effects of temperature and Julian day, which were controlled for in the latter model. The increase in within-year repeatability indicates that, on average, blood glucose concentration differs between years but this difference is only detectable when controlling for the other sources of variation. It also suggests that the factors responsible for the between-year variability are not related to variables fitted as fixed-effect predictors. The observation of between-year differences in blood glucose level in adult birds, adds to the findings of a previous study, showing a significant effect of the year on blood glucose level in Blue Tit nestlings (Skwarska *et al.*, 2014). Based on this study, we also supposed a significant effect of the year, which finally turned to be true.

In this thesis we attempted to reveal most of aspects which can potentially influence blood glucose level in the adult Barn Swallow. Although we examined individual and environmental factors, we were probably not able to take into account all of them. However we think, that our model contained majority of the most important predictors, which is supported by high proportion of explained variation of blood glucose level ($R^2 = 0.59$).

6 Conclusion

This thesis investigated blood glucose concentration in the wild populations of the Barn Swallow over seven years. The great benefit of the study is longitudinal data comparison of the same individuals with known age over their lifetimes observed in natural condition. Although blood glucose level is a labile trait, we found that it is an individual-specific trait and, hence, can be subject to selection. Glycaemia is affected by both intrinsic and environmental factors, namely sex, breeding stage, body mass, chronological age, day time, sampling time, day of the season, temperature, and stress. The connection between blood glucose level and the survival or the live expectancy has not been observed. However, the body mass was positively correlated with blood glucose level, which indicated that glucose level is probably a condition-dependent trait. Significantly higher blood glucose concentration was observed in females during egg laying and incubation which indicates its importance for reproduction. We assume that stress induced increase of blood glucose is associated with stress hormones, namely adrenalin and corticosterone. We plan to verify this presumption in a follow-up research. Similarly, we intend to investigate the second main source of energy, free fatty acids which complement each other with glucose in avian energy metabolism.

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8 Supplement

Table S1: Summary of final model reverse position of male and females

	Estimate	SE	df	t value	p
(Intercept)	0.263	0.367	7.71	0.717	0.494
Sex Females	0.500	0.202	1695.00	2.478	0.013
BreedingStage - Mating	-0.048	0.214	1617.00	-0.225	0.822
BreedingStage - Incubation	0.029	0.163	1641.00	0.177	0.860
BreedingStage - Nestlings	-0.151	0.173	1657.00	-0.875	0.382
BreedingStage - Fledglings	0.216	0.255	1550.00	0.849	0.396
BreedingStage - Post_breeding	-0.584	0.481	1589.00	-1.215	0.225
BreedingStage - Non_breeding	0.065	0.153	1739.00	0.424	0.672
BreedingStage - Unknown	-0.029	0.210	1679.00	-0.137	0.891
Capture Age	**0.171**	**0.069**	**1781.00**	**2.476**	**0.013**
Capture Age ^{2}	**-0.063**	**0.019**	**1701.00**	**-3.361**	**0.001**
Day time (hours)	**0.144**	**0.031**	**1700.00**	**4.624**	**< 0.001**
Day time (hours) ^{2}	**-0.017**	**0.008**	**1492.00**	**-2.113**	**0.035**
Blood Sampling Latency	-0.012	0.009	1529.00	-1.452	0.147
Blood Sampling Latency ^{2}	**-0.004**	**0.001**	**1189.00**	**-8.161**	**< 0.001**
Blood Sampling Latency ^{3}	**0.000**	**0.000**	**1630.00**	**6.695**	**< 0.001**
Lifespan	0.007	0.048	806.90	0.136	0.892
Julian Day	**0.012**	**0.003**	**95.45**	**4.083**	**< 0.001**
Temperature 1day before capture	**-0.057**	**0.015**	**96.26**	**-3.667**	**< 0.001**
SexF:BreedingStage - Mating	-0.228	0.332	1655.00	-0.687	0.492
SexF:BreedingStage - Incubation	**0.777**	**0.238**	**1671.00**	**3.269**	**0.001**
SexF:BreedingStage - Nestlings	0.054	0.248	1641.00	0.217	0.828
SexF:BreedingStage - Fledglings	**-1.029**	**0.394**	**1524.00**	**-2.614**	**0.009**
SexF:BreedingStage - Post_breeding	1.214	0.859	1659.00	1.413	0.158
SexF:BreedingStage - Non_breeding	-0.130	0.267	1659.00	-0.488	0.626
SexF:BreedingStage - Unknown	0.220	0.270	1724.00	0.816	0.414

Table S2: Summary of model with body mass – variables with fixed effects.

	Estimate	SE	df	t value	p
(Intercept)	0.28	0.33	11.89	0.83	0.421
SexM	-0.38	0.26	1173	-1.43	0.152
BodyMassctr	**0.16**	**0.04**	**1195**	**4.35**	**< 0.001**
BreedingStage - Fledglings	**-0.86**	**0.37**	**1039**	**-2.30**	**0.022**
BreedingStage - Incubation	**0.54**	**0.26**	**1169**	**2.10**	**0.036**
BreedingStage - Mating	-0.41	0.34	1150	-1.20	0.230
BreedingStage - Nestlings	-0.06	0.26	1137	-0.25	0.804
BreedingStage - Non_breeding	-0.04	0.30	1085	-0.15	0.881
BreedingStage - Post_breeding	0.40	0.78	1177	0.51	0.610
BreedingStage - Unknown	-0.10	0.30	1000	-0.32	0.751
Capture Age	0.07	0.09	877.7	0.73	0.463
I(Capture Age ^ 2)	**-0.05**	**0.02**	**1194**	**-2.02**	**0.043**
Time From Sunrise	**0.18**	**0.04**	**1128**	**4.98**	**< 0.001**
I(TimeFromSunrise ^ 2)	-0.01	0.01	940.5	-1.67	0.096
BloodSamplingLatencyctr	**-0.04**	**0.01**	**34.27**	**-3.85**	**< 0.001**
I(BloodSamplingLatencyctr^2)	-3.05E-04	1.12E-03	11.98	-0.27	0.789
I(BloodSamplingLatencyctr^3)	4.86E-05	3.20E-05	24.97	1.52	0.141
Lifespanctr	0.04	0.06	533.7	0.65	0.514
JulianDayctr	**0.02**	**0.00**	**50.93**	**4.31**	**< 0.001**
Temperature 1day before capture	**-0.06**	**0.02**	**54.69**	**-3.27**	**0.002**
SexM:BreedingStage - Fledglings	0.67	0.48	1011	1.41	0.160
SexM:BreedingStage - Incubation	**-0.61**	**0.31**	**1114**	**-1.98**	**0.048**
SexM:BreedingStage - Mating	0.32	0.42	1110	0.75	0.456
SexM:BreedingStage - Nestlings	-0.20	0.31	1073	-0.64	0.521
SexM:BreedingStage - Non_breeding	0.11	0.34	1104	0.32	0.753
SexM:BreedingStage - Post_breeding	-1.83	0.96	1191	-1.90	0.057
SexM:BreedingStage - Unknown	-0.12	0.31	1127	-0.37	0.714

Table S3: Summary of model with body mass for only female dataset – variables with fixed effects.

	Estimate	SE	df	t value	p
(Intercept)	0.42	0.42	9.09	1.01	0.340
BodyMassctr	0.16	0.05	487.30	3.18	**0.002**
BreedingStage - Fledglings	-0.92	0.43	444.30	-2.13	**0.034**
BreedingStage - Incubation	0.45	0.30	463.90	1.48	0.141
BreedingStage - Mating	-0.48	0.38	481.30	-1.26	0.207
BreedingStage - Nestlings	-0.07	0.31	407.80	-0.22	0.824
BreedingStage - Non_breeding	-0.11	0.32	461.90	-0.33	0.742
BreedingStage - Post_breeding	0.04	0.88	456.50	0.05	0.960
BreedingStage - Unknown	-0.32	0.36	440.40	-0.89	0.375
Capture Age	0.34	0.15	355.50	2.28	**0.023**
I(Capture Age ^ 2)	-0.15	0.08	445.40	-1.94	0.053
Time From Sunrise	0.25	0.07	475.10	3.76	< **0.001**
I(TimeFromSunrise ^ 2)	-0.03	0.01	414.30	-1.79	0.074
Blood Sampling Latency	-0.05	0.02	51.62	-2.63	**0.011**
I(Blood Sampling Latency ^ 2)	-2.76E-04	1.81E-03	14.48	-0.15	0.881
I(Blood Sampling Latency ^3)	5.62E-05	6.05E-05	34.37	0.93	0.360
Julian Day	0.02	4.85E-03	43.53	3.39	**0.002**
Temperature 1day before capture	-0.03	0.03	44.58	-1.24	0.221

Table S4: Summary of model with blood sample order – variables with fixed effects.

	Estimate	SE	df	t value	p
(Intercept)	9.13	0.61	57.83	15.04	**< 0.001**
SexM	-0.50	0.21	1859.00	-2.43	**0.015**
SampleOrder second	0.92	0.21	578.60	4.47	**< 0.001**
SampleOrder third	2.01	0.42	1666.00	4.79	**< 0.001**
BreedingStage - Fledglings	-0.93	0.35	2211.00	-2.67	**0.008**
BreedingStage - Incubation	0.75	0.20	2221.00	3.81	**< 0.001**
BreedingStage - Mating	-0.44	0.27	2229.00	-1.63	0.103
BreedingStage - Nestlings	-0.11	0.21	2252.00	-0.49	0.623
BreedingStage - Non_breeding	-0.18	0.24	1756.00	-0.77	0.443
BreedingStage - Post_breeding	0.66	0.83	2191.00	0.79	0.428
BreedingStage - Unknown	0.19	0.26	2236.00	0.73	0.467
CaptureAge	0.32	0.12	2244.00	2.60	**0.009**
I(CaptureAge^2)	-0.05	0.02	2240.00	-2.33	**0.020**
TimeFromSunrise_hodiny	0.14	0.05	2073.00	2.64	**0.008**
I(TimeFromSunrise_hodiny^2)	-0.01	0.01	1848.00	-1.31	0.190
BloodSamplingLatency	0.26	0.04	964.70	7.38	**< 0.001**
I(BloodSamplingLatency^2)	-0.01	1.67E-03	1642.00	-6.93	**< 0.001**
I(BloodSamplingLatency^3)	1.42E-04	2.30E-05	1964.00	6.17	**< 0.001**
JulianDay	0.01	0.00	100.20	3.59	**0.001**
Temperature 1day before capture	-0.06	0.02	100.10	-3.37	**0.001**
SexM:BreedingStage - Fledglings	1.06	0.41	2165.00	2.60	**0.010**
SexM:BreedingStage - Incubation	-0.83	0.24	2225.00	-3.38	**0.001**
SexM:BreedingStage - Mating	0.34	0.34	2217.00	1.00	0.318
SexM:BreedingStage - Nestlings	-0.16	0.26	2227.00	-0.61	0.540
SexM:BreedingStage - Non_breeding	0.20	0.28	1821.00	0.71	0.480
SexM:BreedingStage - Post_breeding	-0.80	0.95	2200.00	-0.85	0.396
SexM:BreedingStage - Unknown	-0.27	0.29	2229.00	-0.93	0.352