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FREDRIK ANDREASSON

DEPARTMENT OF BIOLOGY | FACULTY OF SCIENCE | LUND UNIVERSITY

List of papers

I. Andreasson F, Nord A, Nilsson J-Å (2016) Brood size constrains the development of endothermy in blue tits. Journal of Experimental Biology 219: 2212–2219.

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III. Andreasson F, Hegemann A, Nord A, Nilsson J-Å. Experimental facilitation of heat loss affects work rate and innate immune function in a breeding passerine bird. Manuscript.

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Avian thermoregulation across age and seasons

Fredrik Andreasson

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

Animals constantly need to relate to prevailing environmental conditions and regulate their body temperature accordingly. Body temperature regulation in birds and mammals is inherently linked to energy expenditure and, thus, thermoregulation is a physiological mechanism that could trade-off with other costly processes, with potential long-term fitness consequences.

In this thesis, I studied ecological and physiological sources of variation in body temperature across age and seasons, and how variation in thermoregulatory patterns trade-off with other processes within a life-history framework. More specifically, I studied how thermoregulatory development was affected by early life thermal environment and how body temperature was regulated and maintained outside of thermoneutrality. For this purpose, I used small, hole-nesting, passerine birds both during the nestling stage and as adult birds in winter and during breeding.

I found that nest environment affected body temperature regulation, short-term growth and development in nestlings but that there also might be long-term effects on apparent survival. More specifically, growing up in a small brood allowed for investment in both growth and thermoregulatory development, compared to nestlings that grew up in large broods. By experimentally increasing nest temperature I was also able to show that nestlings were well equipped to deal with increased nest temperatures, but that it came with a cost in lower body mass gain, and that increased nest temperatures could potentially have positive effects on long-term survival.

While working hard to feed their young, the risk of overheating can constrain parental work rate. By experimentally reducing this constraint, I found that females were able to increase innate immune function while reducing work rate, but without compromising nestling development.

In winter, small passerines routinely reduce body temperature during the night, thereby lowering energy expenditure. I found that young birds (in their first winter) had both higher metabolic rate and body temperature during the night compared to old birds (in their second winter or older). Such differences could be related to experience-based variation in assessment of predation risk or age-related differences in plumage quality. I also show that handling birds in winter caused a pronounced decrease in body temperature, highlighting the importance of plumage in maintaining body temperature in the cold for these small birds. Overall, these studies demonstrate the need for including age-effects into studies of thermoregulation and energy management in the cold.

I have, for the most part, studied avian thermoregulation outside of thermoneutrality, when additional energy is required to maintain body temperature. Given the current changes to our climate, with an expected increased frequency of extreme weather, studies like these can add important knowledge to our understanding of how animals respond physiologically to such changes and how this, potentially, could shape life-histories.

Key words
Ambient temperature, body temperature, *Cyanistes caeruleus*, energy expenditure, hypothermia, life-history theory, metabolic rate, *Parus major*, thermoregulation, trade-off, winter ecology

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Author contributions

I. FA, AN and J-ÅN jointly conceived and planned the experiment. FA, AN and J-ÅN performed the fieldwork. FA did the statistical analysis. FA led the writing of the manuscript with help from AN and J-ÅN.

II. FA and J-ÅN conceived the ideas and designed methodology with input from AN. FA and J-ÅN collected the data. FA analysed the data and led the writing of the manuscript with help from AN and J-ÅN.

III. FA, AH, AN and J-ÅN conceived the ideas. FA, AN and J-ÅN designed methodology. FA and J-ÅN collected the data. AH conducted all immunological assays. FA analysed the data and led the writing of the manuscript together with all authors.

IV. FA, J-ÅN and AN conceived the ideas. FA, J-ÅN and AN designed methodology. FA collected and analysed the data and led the writing of the manuscript with help from AN and J-ÅN.

V. FA, J-ÅN and AN conceived the ideas. FA and J-ÅN designed methodology with input from AN. FA and J-ÅN collected the data. FA analysed the data and led the writing of the manuscript with help from AN and J-ÅN.

VI. FA, J-ÅN and AN conceived the ideas. FA and J-ÅN designed methodology with input from AN. FA and J-ÅN collected the data. FA analysed the data and led the writing of the manuscript with help from AN and J-ÅN.

Authors

Fredrik Andreasson (FA), Andreas Nord (AN), Jan-Åke Nilsson (J-ÅN) and Arne Hegemann (AH).

All authors in the list of papers have given their consent for the use of their work in the thesis.

Abstract

Animals constantly need to relate to prevailing environmental conditions and regulate their body temperature accordingly. Body temperature regulation in birds and mammals is inherently linked to energy expenditure and, thus, thermoregulation is a physiological mechanism that could trade-off with other costly processes, with potential long-term fitness consequences.

In this thesis, I studied ecological and physiological sources of variation in body temperature across age and seasons, and how variation in thermoregulatory patterns trade-off with other processes within a life-history framework. More specifically, I studied how thermoregulatory development was affected by early life thermal environment and how body temperature was regulated and maintained outside of thermoneutrality. For this purpose, I used small, hole-nesting, passerine birds both during the nestling stage and as adult birds in winter and during breeding.

I found that nest environment affected body temperature regulation, short-term growth and development in nestlings but that there also might be long-term effects on apparent survival. More specifically, growing up in a small brood allowed for investment in both growth and thermoregulatory development, compared to nestlings that grew up in large broods. By experimentally increasing nest temperature I was also able to show that nestlings were well equipped to deal with increased nest temperatures, but that it came with a cost in lower body mass gain, and that increased nest temperatures could potentially have positive effects on long-term survival.

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extreme weather, studies like these can add important knowledge to our understanding of how animals respond physiologically to such changes and how this, potentially, could shape life-histories.

Populärvetenskaplig sammanfattning

Vilda djur påverkas ständigt av omgivningens temperatur som, tillsammans med ytterligare faktorer, styr många av de mönster och beteenden vi ser hos djur i naturen. Förändringar i temperatur kan till exempel påverka hur populationer och individer rör sig över tid och rum, när de väljer att fortplanta sig samt hur mycket föda som finns att tillgå.

För jämnvarma djur, fåglar och däggdjur, som producerar värme för att hålla en hög och någorlunda konstant kroppstemperatur är omgivningens temperatur också fysiologiskt viktig då energikostnaden för att bibehålla en given kroppstemperatur ökar då det är för kallt eller för varmt. En liten fågel som kurar ihop sig för att sova i ett svenskt vinterlandskap kan behöva bibehålla en kroppstemperatur som är mer än 50 °C över omgivningens temperatur. Därför behöver den producera mer värme, samtidigt som den måste minska sin värmeförlust via olika beteenden och fysiologiska anpassningar. Den kan till exempel söka upp en sovplats som är vindskyddad och där den dessutom är skyddad för rovdjur, samtidigt som den fluffar upp sina fjädrar så mycket som möjligt och minimerar exponerad kroppsyta. Allt för att öka sin isolering och minska sin värmeförlust. Ofta reducerar fågeln även sin kroppstemperatur under natten, vilket sparar energi. Dessa vinterförhållanden, med kalla temperaturer, långa nätter och liten födotillgång, är extra utmanande för små fåglar eftersom de har en hög kroppstemperatur (jämfört med däggdjur) och en stor kroppsyta i relation till sin volym, vilket gör att de lättare förlorar värme.

Samtidigt så riskerar dessa små fåglar att överhettas när de arbetar hårt för att mata sina ungar på våren. Som exempel kan nämnas blåmeshonor som under sin häckning gör över 600 holkbesök per dygn för att mata sina ungar, ibland med en kroppstemperatur på över 45 °C! Att arbeta med så höga kroppstemperaturer är förmodligen inte gynnsamt, åtminstone inte under långa perioder, då det ökar risken för cellskador.

Ruvningstemperatur och omgivningstemperatur under ungarnas tid i boet har effekter på ungarnas tillväxt, fysiologi och överlevnad. Både alltför kalla och alltför varma temperaturer under dessa perioder kan vara skadliga. Trots detta är det få studier som undersökt hur ungarnas tillväxt och utveckling under ungperioden påverkas av höga eller låga temperaturer i boet.

Mitt mål och syfte med denna avhandling var att studera vilka faktorer som orsakar variation i kroppstemperatur hos fåglar, över åldrar och årstider, och vad detta kan få för fysiologiska och ekologiska konsekvenser samt hur det påverkar fåglarnas livshistoria. Till detta syfte använde jag blåmesar och talgoxar som häckar (och övernattar) i våra holkar kring Krankesjön och Vomb, strax utanför Lund.

I första kapitlet (**Paper I**) studerade vi hur antalet ungar i boet (kullstorlek) påverkar ungarnas kroppstemperatursutveckling genom att undersöka hur enskilda ungar från olika kullstorlekar reglerar sin kroppstemperatur under nedkylning. Ungar från mindre kullar var bättre på att stå emot nedkylning tidigt i utvecklingen jämfört med ungar från större kullar. Detta var troligtvis inte relaterat till temperaturen i boet eftersom den inte skiljde sig åt nämnvärt under tidig utveckling. Mer sannolikt var detta en effekt av en bättre näringsmässig status hos ungar från mindre kullar vilket troligen tillät dem att lägga energi på både tillväxt och utveckling av termoreglering. I andra kapitlet (**Paper II**) byggde vi vidare på dessa idéer för att få en bättre förståelse för hur en hög temperatur i boet påverkar ungarnas tillväxt och termoreglering. Våra resultat visade att ungar har en god förmåga att bibehålla en relativt normal kroppstemperatur i experimentellt förhöjda temperaturer men att detta kostar i form av sämre tillväxt. Dessutom tyder våra resultat, mot vår förväntan, på att förhöjda temperaturer i boet till och med kan ha en positiv effekt på överlevnad fram till nästa vinter och häckningssäsong för dessa ungar.

I det tredje kapitlet (**Paper III**) underlättade vi värmeavgivning för hårt arbetande blåmesar under häckning för att studera hur detta påverkade deras kroppstemperatur, arbetskapacitet och immunförsvar. Bröstmusklerna genererar mycket värme vid hårt arbete och därför klippte vi bort fjädrar och dun över bröstmuskler och buk för att göra det lättare för fåglarna att bli av med överskottsvärme. Denna manipulering är ofarlig och övergående då fåglarna ruggar och byter ut gamla fjädrar mot nya efter häckning. Vi mätte sedan matningsfrekvens under den mest intensiva matningsperioden, efter vilken vi fångade föräldrarna och tog blodprov för att analysera deras immunförsvar. Klippta hannar matade lika mycket som oklippta och de hade också lika hög kroppstemperatur, medan klippta honor matade mindre än oklippta honor. Således gjorde den underlättade värmeavgivningen inte att honor ökade sin matningsfrekvens, men de investerade däremot mer i sitt immunförsvar, utan att kompromissa med ungarnas tillväxt.

Det fjärde, femte och sjätte kapitlet handlar alla om hur dessa blåmesar och talgoxar reglerar sin kroppstemperatur vintertid. I det fjärde kapitlet (**Paper IV**) analyserade vi hur värmeproduktion och därmed energiförbrukning ökar när det blir succesivt kallare och hur denna ökning förhåller sig till reglering av kroppstemperatur. Vi fokuserade särskilt på olika ålderskategorier (fåglar under deras första vinter – unga, fåglar under deras andra vinter, eller äldre – gamla) och fann att både energiförbrukning, total värmeledningsförmåga samt kroppstemperatur var högre i unga fåglar. Detta skulle kunna vara en konsekvens av att unga fåglar är mer tidspressade vid sin första ruggning och därmed har en fjäderdräkt av sämre kvalitet.

Tidigare studier har visat att småfåglar, som till exempel blåmesar, gärna undviker att sänka sin kroppstemperatur under natten om de har god tillgång till föda eller om klimatet är milt. Det har länge spekulerats i att en ökad predationsrisk kan vara en orsak till att dessa fåglar gärna undviker att sänka sin kroppstemperatur under natten då en nedkyld fågel sannolikt skulle ha svårare att fly vid ett predationsförsök. I det femte kapitlet (**Paper V**) undersökte vi därför hur en ökad predationsrisk påverkar denna vanligt förekommande kroppstemperaturreducering under kalla vinternätter och fann att unga, men inte gamla, fåglar bibehåller en relativt hög kroppstemperatur när predationsrisken uppfattas vara hög. Denna effekt var dock temperaturberoende och bara synlig under de kallaste nätterna.

I det sjätte och sista kapitlet (**Paper VI**) studerade vi hur hantering av sovande fåglar under vintern, som till exempel vid en vanlig ringmärkning vintertid, påverkar talgoxars kroppstemperatur. Vi visade att hantering ger upphov till en dramatisk reducering av kroppstemperaturen som även fortsätter efter att fågeln har slutat hanteras och lagts tillbaka i holken. Denna reducering visar tydligt på vikten av fjäderdräktens isolerande egenskaper och hur hantering kan ge stora effekter på kroppstemperaturen hos dessa fåglar.

Gemensamt för våra studier är att de lägger fokus på åldersskillnader. Unga fåglar verkar vara mer ”reaktiva” i att de har högre kroppstemperatur, högre energiåtgång och reagerar mer på en ökad predationsrisk. Vi föreslår att detta kan bero på skillnader i fjäderdräkt eller på mer erfarenhetsbaserade effekter, vilket skulle behövas studeras mer i detalj i kommande studier.

Denna avhandling berör främst fåglars temperaturreglering i värme eller kyla, då de behöver lägga energi på att bibehålla sin kroppstemperatur. Med pågående klimatförändringar och kommande ökningar av extrema väderhändelser i åtanke kan studier som dessa vara värdefulla för att förutse hur djur påverkas fysiologiskt och vilka effekter det kan tänkas ha på deras ekologi och livshistoria.

Introduction

Wild animals constantly need to relate to and cope with prevailing, often changing, environmental temperatures. Therefore, it is not surprising that multiple thermoregulatory strategies have evolved as adaptations to cope or deal with thermal change.

Animals can be roughly divided into ectotherms and endotherms (in older literature; “cold-blooded” and “warm-blooded”) based on their thermoregulatory pattern and thermogenic capacity (Cowles 1962). Essentially, these two terms are rooted in the mode of heat generation; most ectotherms regulate body temperature mainly via behaviour while endotherms, in contrast, produce heat endogenously to maintain an elevated and sustained body temperature (IUPS Thermal Commission 2001). However, thermoregulation is a complex process involving several factors other than heat production. Thus, endothermy should not be used interchangeably with homeothermy (maintenance of body temperature within a narrow range), and ectothermy not with heterothermy (when variation in body temperature exceeds homeothermic levels) or poikilothermy (large variations in body temperature, coupled to variation in ambient temperature) (IUPS Thermal Commission 2001). More recent classifications have emphasized the continuous nature of thermal strategies and suggested that animals are better described as being somewhere on the continuum between thermoregulator (cf. endotherm) and thermoconformer (cf. ectotherm) (e.g. Angilletta 2009). Whatever terminology we decide to use, the thermal strategy of the animal trades off with other important traits (e.g. growth, parental care, foraging ability, energy expenditure, migratory capacity), making thermoregulatory strategies a crucial object of study in evolutionary ecology.

Birds are endothermic and rely on costly heat production to keep a sustained and elevated body temperature, which forces the organism to allocate energy into thermoregulation that otherwise could be invested in growth and maintenance. Efficient endothermy also requires a certain degree of insulation. Insulation in general is crucial for retaining endogenously produced heat (Scholander et al. 1950a) and feathers, which are important for determining thermal conductance (Novoa et al. 1994) are energetically costly to produce (Lindström et al. 1993).

Given the costs associated with endothermy, the benefits have to be substantial. The perhaps most important benefit (and arguably also the most likely target for selection in the evolution of endothermy; Nespolo et al. 2017) is the capacity to maintain a sustained work capacity and high activity levels irrespective of ambient temperatures, thereby facilitating improved foraging abilities and migratory capacity (Bennett & Ruben 1979, Ruben 1995, Clarke & Pörtner 2010). A high body temperature in itself also entails greater performance of high-stability enzymes, thereby enhancing general performance (Hochachka & Somero 2002).

Birds generally maintain a higher body temperature than mammals. Mean body temperature for placental mammals is 36.9 °C (Clarke 2017) while mean body temperature in birds is 38.5 °C during rest, 41.0 °C during normal activity, and 43.9 °C during high activity (such as when provisioning for nestlings) (Prinzinger et al. 1991). So despite being characterized as endothermic homeotherms, avian body temperature fluctuates diurnally and also varies substantially with activity levels (Prinzinger et al. 1991, McKechnie & Lovegrove 2002).

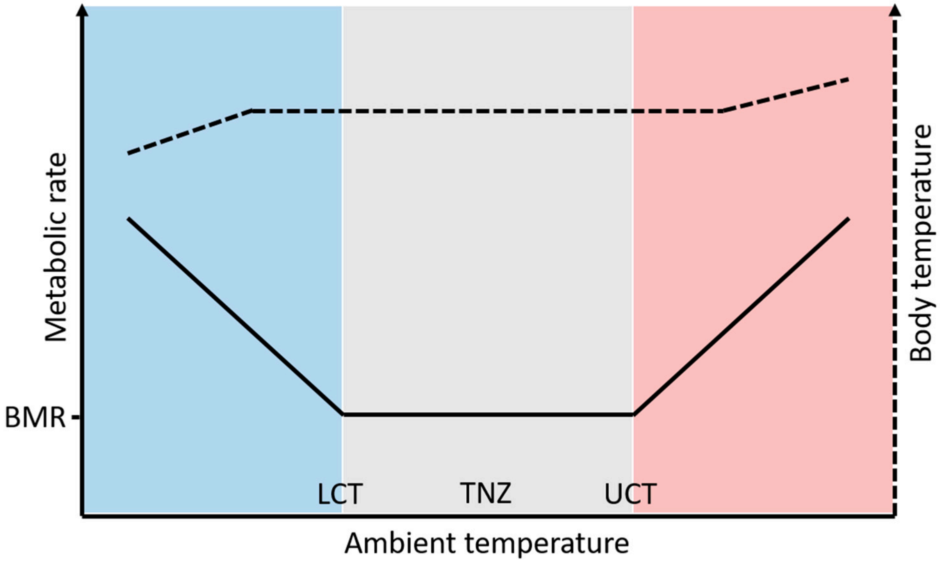


Fig. 1 A modified Scholander-Irving model showing how metabolic rate and body temperature change with ambient temperature. For explanations of abbreviations, see main text.

The theoretical relationship between metabolic rate, body temperature and ambient temperature in endotherms can be described by the classic Scholander-Irving model of thermoregulation (Scholander et al. 1950b) (Fig. 1). It is a simplified way of illustrating how a homeothermic endotherm respond energetically to changes in ambient temperature and can be useful as a framework for analysis, predictions and generation of new ideas (but see Boyles et al. 2019 for a discussion of its limitations). Basal metabolic rate (BMR) is defined as the minimum metabolism that is required for maintenance in a resting endotherm in thermoneutrality (in adult, postabsorptive, inactive and non-reproductive animals) (McNab 1997). Thus, in the thermoneutral zone (TNZ), no extra energy above BMR is required to maintain a preferred body temperature. However, as ambient temperature falls below the lower critical temperature (LCT) or increases above the upper critical temperature (UCT) the animal needs to increase heat production or heat dissipation (both of which cost energy), respectively, to defend body temperature. In birds, heat production below LCT has traditionally been mainly attributed to shivering thermogenesis (Marsh & Dawson 1989; Hohtola 2004). Shivering is mechanistically identical to normal muscle contractions, except that contractions are asynchronous, releasing chemical energy from ATP hydrolysis as heat instead of kinetic energy (Hohtola 2004). In birds, tremor amplitude during shivering is low (often not visible to the human eye) which minimizes convective heat loss (Hohtola 2004). Non-shivering thermogenesis in muscle have also been shown in birds (Duchamp & Barré 1993, Dumonteil et al. 1995) but the relative contribution to heat production compared to shivering remains largely unknown (cf. Teulier et al. 2010, see Nowack et al. 2017 for an overview of muscle non-shivering thermogenesis in mammals and birds). Above UCT heat needs to be dissipated if hyperthermia is to be avoided. The main physiological pathway, by which birds get rid of excess heat when ambient temperatures are high, is through evaporation (Dawson 1958, Dawson & Whittow 2000, Williams & Tieleman 2005, Talbot et al. 2017). Birds that are well-adapted to hot and arid environments lose heat through evaporation of water through cutaneous surfaces or gular fluttering (McKechnie & Wolf 2004, Smith et al. 2015, McKechnie et al. 2016, Talbot et al. 2017). Most passerines, however, mainly use panting (respiratory evaporative heat loss) which is energetically more expensive compared to cutaneous evaporation. Thus, it follows that facultative hypo- or hyperthermia can help reduce energetic costs associated with ambient temperatures outside of thermoneutrality as it would reduce the need for energetically expensive heat production or dissipation. However, these facultative responses are not without costs. Hyperthermia may increase the risk of physiological damage and hypothermia could increase predation risk in certain settings (**Paper V**).

In conclusion, the effect of ambient temperature on avian body temperature regulation is ever present; both across seasons and across life-stages. A small bird in winter will need to produce heat while simultaneously minimizing heat loss to defend its body temperature, while the same bird during breeding in spring and summer will need to dissipate heat generated from hard work while feeding its nestlings. Ambient temperature and nest thermal environment also affects the development of endothermy in growing nestlings.

Thus, body temperature regulation is an important physiological mediator of trade-offs in life-history theory.

Aims of the thesis

The main aim of this thesis was to explore how avian body temperature regulation can act as a physiological mediator of trade-offs across age and seasons. By using thermoregulation in a small bird as a model framework the aim was to study how body temperature regulation outside of thermoneutrality trades off with other ecophysiological parameters and how different thermal nest environments shape growth and development in nestlings. Small birds have a high metabolic rate and a large surface area to volume ratio (i.e. high heat loss rate) and are therefore subject to severe energetic challenges during winter. On the other hand, their high metabolic rates and high body temperatures makes them vulnerable to overheating when working hard during breeding, thus making them ideally suited for these types of questions.

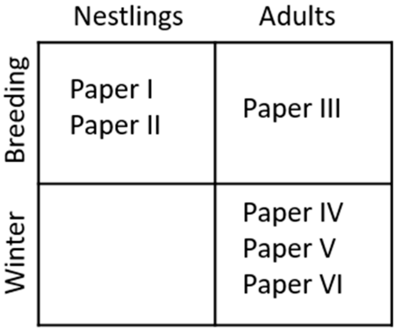
The main questions I wanted to answer were:

How does nest temperature affect development and growth in nestlings (**Paper I, II**)?

What ecological and physiological factors determine at what level body temperature is maintained when birds are forced to get rid of excess heat during hard work (**Paper III**) or when they have to produce heat in the cold (**Paper IV**, **V**, **VI**)?

How are other processes, e.g. immune function, affected by such trade-offs (**Paper III**)?

Table 1
A classification of the papers included in this thesis according to life-stage (nestling or adult) and season (winter or breeding).



	Nestlings	Adults
Breeding	Paper I Paper II	Paper III
Winter		Paper IV Paper V Paper VI

Materials and methods

Study species

We have used blue tits (*Cyanistes caeruleus* Linnaeus) and great tits (*Parus major* Linnaeus) in our studies. In addition to the physiological and ecological attributes that make them excellent study species, their use of nest-boxes, both during breeding in spring and while roosting in winter, makes them easily accessible and easy to work with while still being free-living, wild animals in a natural setting.

Blue tit

The blue tit (Fig. 2) is a small (11-13 g) hole-nesting passerine, common throughout Europe and western Asia. In southern Sweden, some blue tits remain resident, while some migrate south across the Baltic Sea in winter (Smith & Nilsson 1987). Blue tits nest in natural cavities, but readily use nest-boxes when provided. During winter, nest-boxes are also routinely used for non-communal night-roosts. In spring, nest building starts in late March and the first egg is usually laid mid-April. Average clutch size is 10-12 eggs, but clutches up to 18 have been recorded in our population. When the last egg is laid, the female incubates the eggs for 12-14 days (Nilsson 2000). The nestlings are intermittently brooded for about a week by the female (Perrins 1979). Nestlings are fed by both parents, sharing the provisioning duties. The major food-items delivered are foliage-living caterpillars (Perrins 1979), and thus, it is important for parents to match egg-laying and incubation with leafing phenology so that the nestling period, when food is most needed, is matched by peak caterpillar density (cf. van Noordwijk et al. 1995). The nestlings remain in the nest for almost three weeks before they fledge, but after fledging they are still tended for and fed by parents for almost two weeks before they are ready to leave the family group.

Fig. 2 A blue tit during breeding in May 2015. Photograph by Aron Hejdström ©, published with permission.

Fig. 3 A great tit photographed in conjunction with the experiment in **Paper VI** in February 2015. Photograph by Aron Hejdström ©, published with permission.

Great tit

The great tit (Fig. 3) shares many attributes with its smaller relative, the blue tit. They roost and breed in the same nest-boxes (but with a larger entrance hole) and have very similar food preferences and breeding ecology. However, they are almost twice the size of a blue tit (18-20 g) and lay fewer eggs (8-10).

Study area

All studies were done using wild birds from three nest-box populations (Fig. 4), east of Lund in southern Sweden; blue tits from the Revinge-area surrounding Lake Krankesjön (A; 55°70´N, 13°48´E) and great tits from Vomb (B; 55°65′N, 13°55′E). In **Paper III**, we also included nest-boxes from a population in Öved (C; 55°71´N, 13°61´E).

Fig. 4 A map of the study areas used. Map provided by Lantmäteriet.

The area around Lake Krankesjön (A) is mainly located on military training grounds where the Swedish Armed Forces, cattle farmers and biologists share the land for their respective purposes. The area consists mainly of deciduous forests mixed with cattle-grazed grasslands and wetter habitats close to Lake Krankesjön. About 500 wooden nest-boxes, inhabited by blue tits and marsh tits (*Poecile palustris*), are scattered across the area. The core part of this study area has been monitored since

1983, while the 150 additional nest-boxes in the managed beech forests (*Fagus sylvatica*) in Öved (C) have been monitored since 2005. With the odd exception of a coal tit (*Periparus ater*) or a few resourceful great tits, pied flycatchers (*Ficedula hypoleuca*) and nuthatches (*Sitta europaea*) that make use of slightly excavated entrance holes, the population usually contain ca. 200-250 breeding blue tit pairs and 80-100 breeding marsh tit pairs each year.

The ca. 300 nest-boxes, spread over 219 ha, used by great tits in Vomb (B) are located in a pine (*Pinus sylvestris*) plantation with a dense mixed-in deciduous understory, composed mainly of oak (*Quercus robur*), birch (*Betula pendula*), rowan (*Sorbus aucuparia*) and sallow (*Salix* spp.). The first 50 boxes were put up in the 1970s and additional boxes have been added through the years.

Methods

I have employed a wide array of techniques, ranging from field-based experimental manipulations to technical methods and laboratory analyses. Here I will present the main methods used (see Fig. 5 for examples).

Brood size manipulations

Ever since Lack (1947) suggested that optimal brood size is determined by the number of nestlings parents can successfully raise to independence, brood size manipulations have been used in many studies. Manipulating brood size (number of nestlings) or clutch size (number of eggs) is an easy and straightforward way to increase parental effort, either in terms of work rate during nestling feeding (brood size) or in incubation effort (clutch size). We know that tending to an enlarged brood increases parental effort and pushes parents toward maximal working rates (Nur 1984, García-Navas & Sanz 2010). In theory, reduced broods would allow for parents to invest more into each nestlings, but studies have shown that parents that suffer brood size reductions also decrease their work rate (Nilsson 2002, Griffioen et al. 2019) and that they might do so without a compensatory increase in prey size in the items delivered (Griffioen et al. 2019). Naturally, manipulation of brood size also affects nestlings. Nestlings often grow smaller in enlarged broods (e.g. Smith et al. 1989) and nestlings from reduced broods grow bigger and have higher survival (Smith et al. 1989, but see Nilsson & Nord 2017). Brood size manipulations are also an effective method for eliminating the effect of natural variation in quality and resource acquisition on variation in resource allocation (*sensu* van Noordwijk & de Jong 1986). For example, parents of high quality and/or with high quality habitats may have enough resources to invest in both current reproductive effort and self-maintenance simultaneously. Thus, brood size manipulations effectively separate effects of individual quality from resource allocation decisions, which are hard to disentangle using only natural variation in brood size.

In **Paper I** we used brood size manipulations as a method to manipulate thermal nest environment (Dunn 1976, Gilbert et al. 2010) and in **Paper III** we enlarged broods to increase parental work rate.

Nest temperature

Measurement of nest temperature was necessary to answer the research questions in **Paper I** and **II**. Thus, we wanted to have a measurement that was as close to the thermal environment of the brood as possible. This was accomplished by attaching a temperature logger (iButton DS1922-L), fixed to a small piece of chicken mesh, which was subsequently secured at the bottom of the nest cup (unattached temperature loggers are routinely thrown out or buried into the nest material by the parents) (Fig. 5E). This allowed us to obtain measurements of the thermal nest environment with high precision and temporal resolution.

Body temperature

The main aim through all studies was to study the role of body temperature in certain ecological settings. Thus, reliable and precise measurement of body temperature was essential. There are several ways to measure body temperature in free-living animals, each with its own set of advantages and possible disadvantages (McCafferty et al. 2015). When deciding which method to use several aspects needs to be taken into consideration as methods vary in costs, invasiveness, potential stress effects, precision and preferred site of measurement. Depending on the focus of the study, we have used one (or more) of the following methods.

Cloacal temperature

Measuring cloacal or rectal temperature via thermometry is the classical way of obtaining internal body temperature (Fig. 5D). It is fast, accurate, repeatable, cheap and non-invasive (McCafferty et al. 2015). However, it is not well suited for monitoring body temperature continuously over longer time periods in moving animals. Cloacal temperature was the main measurement method in **Paper II**, **V** and **VI**, but other types of measurements used in the other studies were always validated/calibrated against cloacal temperature in a subset of individuals.

Passive integrated transponder (PIT) tags

PIT-tags are temperature-sensitive transponders that will send a unique ID-number and attached temperature data to an RFID (radio frequency identification) reader unit when they are within the electromagnetic field created by the units’ antenna

(Bonter & Bridge 2011, McCafferty et al. 2015) (Fig. 5A). Since the transmitters are small (our PIT-tags are cylindrical, height: 13.0 mm; diameter: 2.1 mm), not powered by batteries and biologically inert (glass-casing), they can be implanted into the body cavity or subcutaneously in most birds (Fig. 5B). When implanted subcutaneously, it is minimally invasive (see Oswald et al. 2017 for a comparison of PIT-tagging subcutaneously vs. intraperitoneally), without bleeding and inflammation, and allows the researcher to monitor subcutaneous temperature as long as the animal remains in the vicinity of (such as when roosting or when in a metabolic chamber), or make regular visits close to (such as when feeding nestlings in a nest-box), the antenna. Perhaps the most obvious downside is that subcutaneous temperature measured at the periphery is not necessarily reflective of core body temperature, especially in large animals (Scholander et al. 1950c). However, we know that subcutaneous temperature in small birds such as blue- and great tits correlate well with core body temperature (Nord et al. 2013) when the birds are measured in a stable thermal environment. However, the gradient between core and periphery increases as ambient temperature decreases, but this can be accounted for as long as the change in gradient is known (**Paper IV**). PIT-tags have been used in **Paper III** and **IV**.

Thermal imaging

Thermal imaging is another option for measuring body surface temperature at a distance, without the need to handle the animal. A thermal imaging camera detects infrared radiation, emitted from all surfaces with a temperature above absolute zero, 0 K (McCafferty 2013). Thermography will only provide a measure of surface temperature and for animals with pelage or plumage it can rarely be used to estimate internal temperature. However, for small nestlings without feathers or down, surface temperature is a valid proxy for body temperature and we used this technique in **Paper I** (Fig. 5F), enabling us to measure body temperature in small nestlings in a non-invasive manner (cf. Bakken et al. 2005).

Measurement of metabolic rate

Metabolic rate was measured using flow-through respirometry. Birds were put in individual, sealed, chambers placed within a climate cabinet that allows for precise control of ambient temperature (Fig. 5C). Essentially, metabolic rate is estimated by measuring oxygen consumption (and/or carbon dioxide production), which is easily calculated by comparing oxygen concentration in incoming air with concentration in effluent air after it has passed the bird in the chamber. Oxygen consumption is then converted to metabolic rate assuming a certain oxyjoule equivalent (number of joules of heat energy produced per unit of oxygen consumption). There are many possible ways of setting up a respirometry system, depending on study species and the objectives of the study. We pushed pressurized air through the chambers, and after removing water vapour, the gas stream was analysed for both oxygen and carbon dioxide concentrations (for a detailed description of metabolic measurements, see **Paper IV**).

Immune function

In **Paper III**, we wanted to study whether reduced constraints on heat dissipation in adult blue tits were re-invested in nestlings or in measures of self-maintenance, e.g. immune function. We focused on innate immune function, which is the first line of defence against pathogens. It is also energetically costly (Råberg et al. 2002), which makes it a suitable measure for a trade-off with work rate and investment in current reproduction (nestling quality). However, variation in immune function is not easily captured by measuring single immune parameters (Adamo 2004, Martin et al. 2006, Matson et al. 2006). Thus, we measured four innate immune indices that are indicative of protection against a wide range of pathogens and summarized these in a principal component analysis (PCA). Specifically, we quantified titers of lytic enzymes of the complement system (lysis) and non-specific natural antibodies (agglutination) (Matson et al. 2005). Complement and natural antibodies both belong to the constitutive innate immune system (Schmid-Hempel & Ebert 2003, Matson et al. 2005). We also measured haptoglobin (or a functional equivalent, i.e. PIT54, see Wicher & Fries 2006), which is an acute phase protein and a general antioxidant that binds free hemoglobin that otherwise could harm cell membranes and produce reactive oxygen species (ROS) (Gutteridge 1987, Quaye 2008). Lastly, we also quantified total bacteria killing capacity (BKA) against *Escherichia coli*, which is an integrative measure of innate immune function that includes both natural antibodies and phagocytosis as well as complement activation (French & Neuman-Lee 2012).

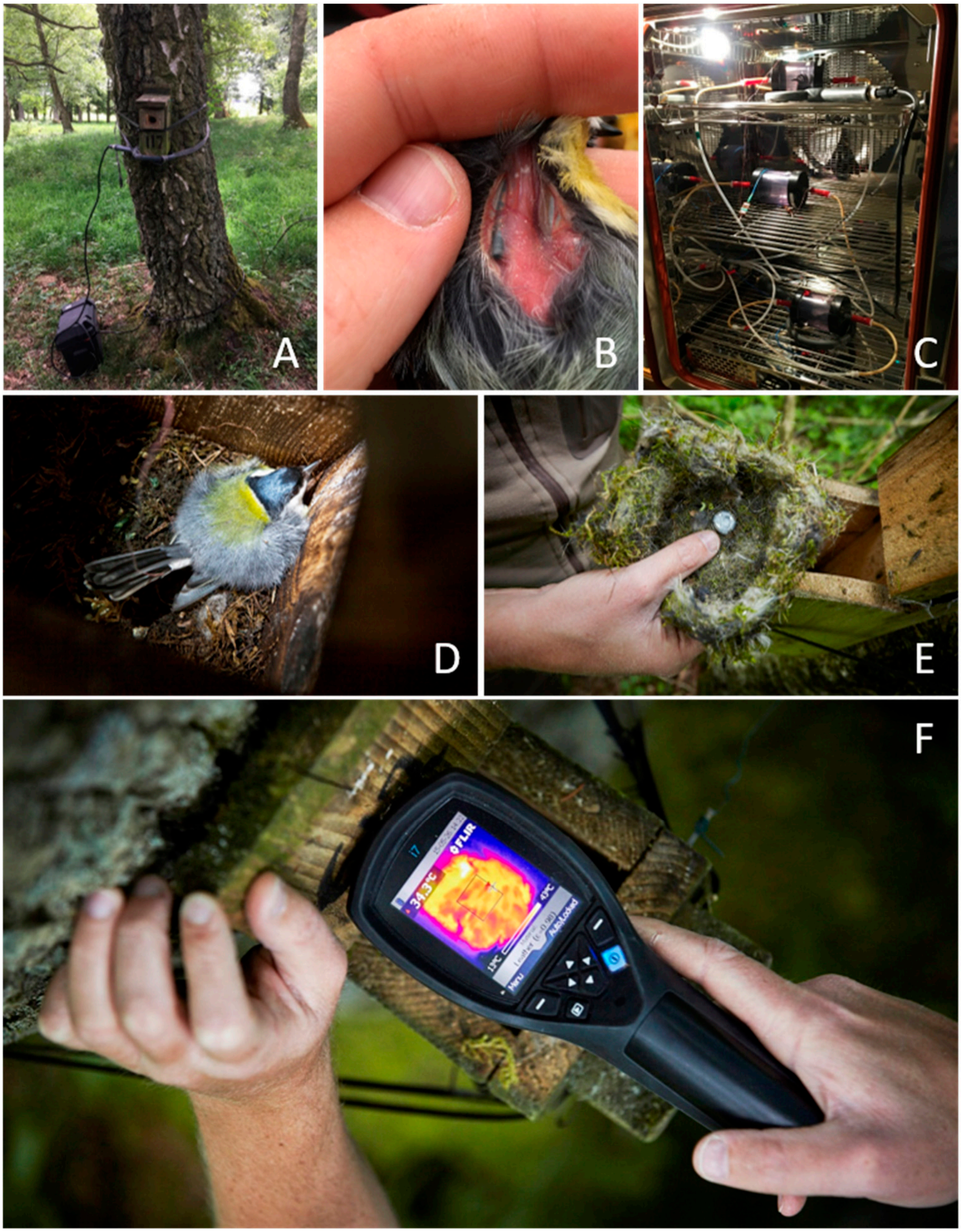


Fig. 5 A range of different methods and techniques used. A) A PIT-tag reader and antenna attached to a nest-box, recording feeding frequency and body temperature of blue tits feeding their nestlings. B) A subcutaneously implanted PIT-tag in the neck of a blue tit. C) The climate cabinet with metabolic chambers. D) A roosting great tit with a cloacally mounted thermocouple (attached to a data logger) taped to two tail feathers, recording body temperature throughout the night. E) A temperature logger attached to the nest cup floor measuring nest temperature. F) A handheld thermal imaging camera taking a thermal image of a whole brood of blue tit nestlings. Photographs D, E and F were taken by Aron Hejdström ©, published with permission.

Background, results and discussion

The effect of brood size and thermal environment on growth and development

The thermal environment of the nest both during incubation (Nilsson et al. 2008, Pérez et al. 2008) and post-embryonic development shape both growth and development (Lloyd & Martin 2004, Ardia 2013), with potential carry-over effects to later life (Hepp & Kennamer 2012, Berntsen & Bech 2016). Both temperatures above (Nord & Nilsson 2016, Clauser & McRae 2017) and below (Berntsen & Bech 2016) optimal incubation temperature affects survival negatively and also post-embryonic growth requires a certain range of nest temperatures. A number of studies have documented positive effects of increasing ambient or nest temperatures on nestling growth rate and/or survival (McCarty & Winkler 1999, Eeva et al. 2002, Ardia 2013) while others have found opposite results (Murphy 1985, Lloyd & Martin 2004, Catry et al. 2011, 2015, Salaberria et al. 2014, Larson et al. 2015, Rodríguez and Barba 2016). A large part of the variation in the effect of temperature on nestling growth and development is likely attributed to the global environment, i.e. high ambient temperatures are beneficial for development in cold climes (Eeva et al. 2002) whereas high ambient temperatures can be detrimental in an already warm environment (Salaberria et al. 2014). Other factors are, of course, also important in shaping the patterns of growth and development in altricial nestlings. Aside from the rather obvious effect of food availability (reviewed in Martin 1987), predation risk exerts a strong selection pressure on decreased time spent in the nest, i.e. species with a high predation risk have faster nestling growth rates and nestlings leave the nest at earlier developmental stages (Remeš & Martin 2002). However, high growth rates are not without costs as it decreases immunocompetence (Brommer 2004, Pihlaja et al. 2006) and increases oxidative stress (Alonso-Alvarez et al. 2007).

Determining the effect of temperature on growth and development is problematic when only relying on natural variation in temperature. Direct temperature effects are hard to disentangle from indirect effects via food availability and parental effort (McCarty & Winkler 1999). Thus, experimental manipulation of the nest thermal environment is crucial in pinpointing the effect of temperature per se on growth and development. **Paper I** and **II** were designed to further our understanding of how the nest environment affects the transition from poikilothermic hatchling to endothermic nestling and how it affects growth and survival.

Paper I: Brood size and the development of endothermy

Altricial birds, such as blue tits, hatch without plumage and are unable to maintain body temperature when exposed to ambient temperatures below thermoneutrality (Visser 1998, Baarendse et al. 2007, **Paper I**) and are therefore completely dependent on heat provided by brooding parents (Ricklefs 1984, Węgrzyn 2013). However, thermogenic capacity starts to develop when heat-producing tissues (in altricial nestlings mainly the pectoral muscle; Hohtola & Visser 1998) begin to mature (Morton & Carey 1971, Visser & Ricklefs 1993). Together with the decreased thermal conductance created by developing plumage and the decreased surface area to volume ratio as nestlings grow larger (Morton & Carey 1971, Visser & Ricklefs 1993) this constitutes the beginning of the transition from a poikilothermic hatchling to endothermic nestling, ready to leave the nest.

The development of endothermy is costly (Visser & Ricklefs 1993) and requires maturation of skeletal muscles (Hohtola & Visser 1998) and could therefore theoretically trade-off with growth rate (Olson 1992, Visser & Ricklefs 1993, Brown & Downs 2002, Węgrzyn 2013).

Brood size can influence the thermoregulatory development in such a way that nestlings in large broods will benefit more from the higher thermal inertia and the effect of huddling (Dunn 1976, Gilbert et al. 2010) than those in small broods. Consequently, whole broods reach effective homeothermy sooner than individual nestlings (Dunn 1976, 1979). However, it is not known whether brood size has an effect on thermoregulatory development in individual nestlings. Thus, we wanted to know whether a more favourable thermal environment in large broods would allow nestlings to invest more in growth and development (without the risk of prolonged hypothermia due to the higher thermal inertia of the brood) and consequently if nestlings in small broods would be forced to develop effective homeothermy sooner than those in large broods.

To test this, we subjected nestlings from reduced, control and enlarged broods to cooling trials when they were 4, 6, 8 and 10 days old, i.e. when the development of endothermy should be most pronounced. We cooled nestlings individually at ca. 14 °C, i.e. moderate cooling but well below thermoneutrality for nestlings of that age, for 5 min and measured body surface temperature, using a thermal imaging camera, immediately before and after each cooling trial.

Our results show that nestlings from large broods had higher cooling rates compared to nestlings from reduced broods during early development (day 4 and day 6) and that nestlings from reduced broods reached effective homeothermy (homeothermy index calculated according to Ricklefs 1987, where a value of 1 would represent a completely homeothermic bird) sooner, just as predicted (Fig. 6).

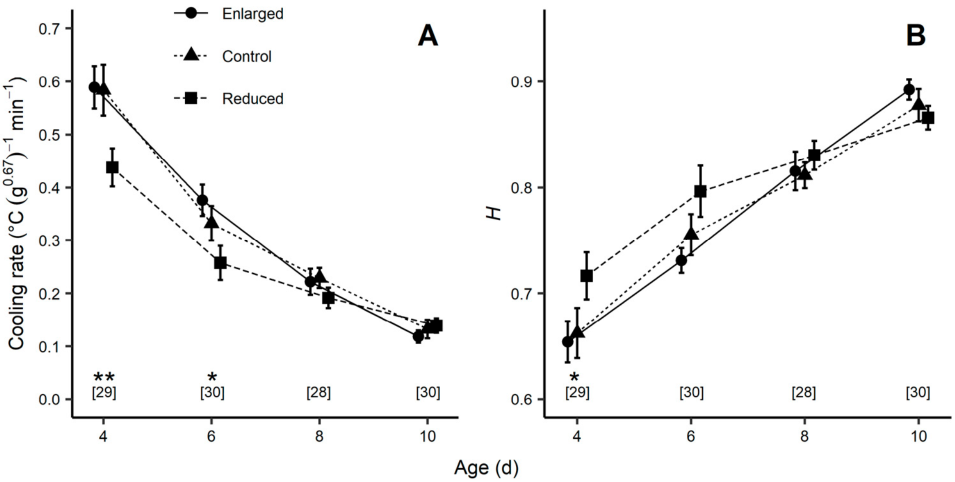


Fig. 6 A) Cooling rate (± SE) and B) homeothermy index (± SE) in blue tit nestlings from differently sized broods during 5 min of cooling as a function of age.

The nestlings from reduced broods were better able to withstand cooling and defend body temperature during early development. In a natural setting, where females may need to take long recesses from brooding, this could prove to be very important as low body temperatures could render nestlings unresponsive and attenuate begging intensity (Choi & Bakken 1990). Brood size manipulations were done on day 3 and the largest difference in cooling rate was observed already on day 4, indicating that heat production capacity can improve rapidly early in development despite the thermal detriment of being both small and without insulating plumage. It seems that working thermoreceptors are present in altricial nestlings already in early post-hatch development (Østnes & Bech 1997), but the hypothalamic-pituitary-thyroid (HPT) axis (which mediates thermoregulatory development) usually does not become fully functional until after a week (or more) after hatching (Debonne et al. 2008). However, our results suggest that the early increase in resistance to cooling in nestlings from reduced broods could be a consequence of earlier maturation of the HPT-axis.

It is unlikely that the observed earlier onset of endothermy in nestlings from reduced broods was a consequence of differences in nest temperature, as these were most evident in late development when cooling rates between nestlings from differently sized broods were similar. Instead, we suggest that the likely higher food intake and possible superior nutritional status of nestlings from reduced broods, indicated by their higher body mass, could have allowed for these nestlings to invest energy into increased thermoregulatory development as well as into growth.

However, to unambiguously determine if differences in cooling rates reflect an ontogenic shift in heat production capacity (presumably via shivering thermogenesis), measurements of nestlings’ metabolic rate are needed.

Paper II: The effect of increased nest temperature on nestling growth and physiology

To evaluate the direct effect of nest temperature on growth and development of nestlings, experimental manipulations of temperature are needed. Given the expected increase in extreme weather events, such as heat waves (IPCC 2012), studies of animals' physiological tolerance and responses to heat stress will prove valuable when trying to estimate how biodiversity will adjust under climate change (Urban et al. 2016). In light of this it is perhaps surprising that we know little about how increased nest temperatures affects nestlings while in the nest and little about potential long-term consequences of heat stress in wild birds.

For nestlings, heat stress could potentially be detrimental to growth as energy being allocated to avoid hyperthermia could otherwise be allocated into growth (Cunningham et al. 2013). In ambient temperatures above body temperature, nestlings must lose heat via evaporation (Dawson 1958, Dawson & Whittow 2000, Williams & Tieleman 2005) which is costly both in terms of energy expenditure (Wolf & Walsberg 1996, Whitfield et al. 2015, Smith et al. 2017) and in terms of water loss (Albright et al. 2017). In addition, letting body temperature rise and using facultative hyperthermia is not without costs either, as production of reactive oxygen species increase (Boyles et al. 2011) and as enzymes and proteins may even lose activity and degrade (Daniel et al. 2010, Del Vesco et al. 2015).

Only two studies have experimentally heated nests to assess effects on nestling growth and fledging success and they found contrasting results. Dawson and colleagues (2005a) found that heating tree swallow (*Tachycineta bicolor*) nests had positive effects on growth and fledging success, while Rodríguez and Barba (2016) found reduced body mass in great tit nestlings as a response to heating. The difference in results is most likely explained by ambient conditions as Dawson and colleagues heated nests in British Columbia, Canada (temperate climate) while Rodríguez and Barba did so in Valencia, Spain (Mediterranean climate). However, neither of these studies measured body temperature of nestlings which makes it difficult to, for example, know whether heated nestlings in Rodríguez and Barba (2016) suffered reduced growth due to sustained hyperthermia, increased cost of thermoregulation, or both.

We wanted to test whether experimentally increased nest temperature forced nestlings to invest more in thermoregulation at the expense of growth and also to test if this had any long-term effect on apparent survival. To do this, we heated nest-boxes between nestling day 8 and 13 by adding heat-packs to the bottom of the nest-box and measured growth and body temperature over the experimental period, ending on day 14. The added heat-packs elevated nest temperature for ca. 6 h.

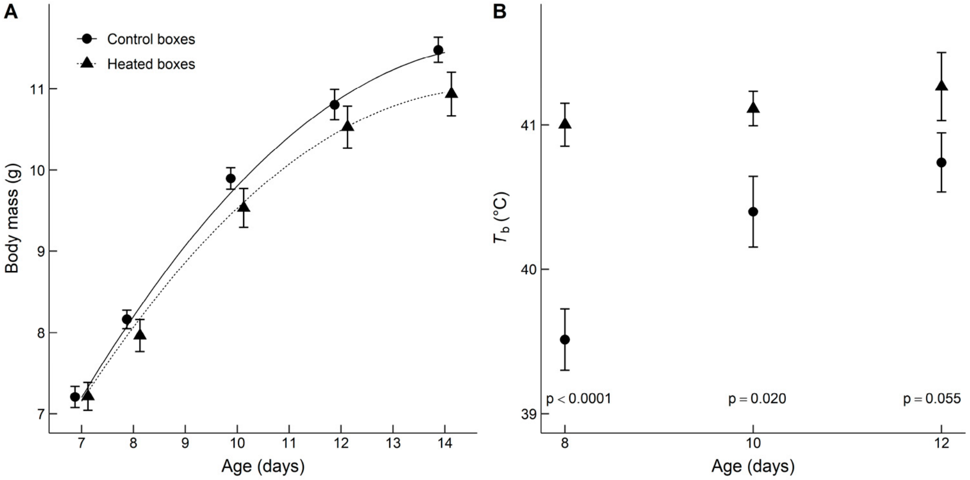


Fig. 7 A) Body mass (± SE) in nestlings from control- and heated boxes over the experimental period and B) body temperature (T_b) (± SE) in nestlings from control- and heated boxes at nestling day 8, 10 and 12.

Body temperature was higher in nestlings from heated nests on day 8 and 10, and tended to be so also at day 12 (Fig. 7B), but despite facing very high nest temperatures (mean nest temperature across days was 44.1 °C and could reach temperatures > 50 °C occasionally) no nestlings exceeded 43.8 °C in body temperature. Thus, nestlings effectively dissipated heat and maintained only a slightly elevated body temperature, most likely avoiding costs associated with hyperthermia.

However, keeping body temperature below detrimental levels came with a cost as nestling from heated boxes gained body mass slower (Fig. 7A) compared to control nestlings. Tarsus- and wing lengths did not differ between nestlings, so nestlings from heated boxes prioritized structural growth over body mass under adverse thermal conditions (cf. Rodríguez & Barba 2016).

Body mass at fledging is usually positively correlated with increased survival (Perrins 1965, Smith et al. 1989, Both et al. 1999, Råberg et al. 2005, Rodríguez et al. 2016), which is why we were surprised to find that nestlings from heated boxes (that had lower body mass gain) had a higher apparent survival to adulthood compared to control nestlings. Thus, any potential benefit of developing in a heated nest would have to be substantial to compensate for the lower body mass gain. We suggest that the higher survival could potentially be related to lower parasite pressure, as an elevated nest temperature could reduce the number of nest parasites (e.g. blowflies *Protocalliphora* spp. Dawson et al. 2005b, Castaño-Vázquez et al. 2018). Alternatively, the experimental heating could have formed a mild stressor which increased resilience to similar stressors in later life (i.e. hormetic priming *sensu* Costantini et al. 2012, 2014).

Work rate, risk of overheating and immune function

One of the central tenets of life-history theory is the trade-off between current and future reproduction. It states that there is a "cost of reproduction" (Williams 1966) and that increased investment in current reproduction therefore reduces survival and reproductive success later in life (Lessells 1991, Stearns 1992). This theoretical prediction has been confirmed experimentally, often using birds as model species (e.g. Askenmo 1979, Reid 1987, Gustafsson & Sutherland 1988, Daan et al. 1996; but see Santos & Nakagawa 2012).

Successfully raising a brood of nestlings to fledging can be energetically expensive and parents work hard to provide nestlings with food. What limits the parents' capacity to work? Traditionally, environmental limitations such as food availability have been considered the principal limiting factor (Stearns 1992, McNab 2002) but physiological limitations to energy absorption (Drent & Daan 1980), delivery capacity to tissues (West et al. 1999, Brown et al. 2004) and working capacity of peripheral tissues (Hammond et al. 1994, Hammond et al. 1996) have also been proposed as limits to how hard an animal can work. However, recently, it has been suggested that also the capacity to get rid of excess heat can limit maximum energy expenditure (heat dissipation limit (HDL) theory; Speakman & Król 2010). In its essence, it states that animals are constrained in their capacity to work by the risk of overheating, which has been confirmed in several laboratory experiments (Król et al. 2007, Simons et al. 2011, Sadowska et al. 2016) and recently also in free-living blue tits (Nord & Nilsson 2019). Nord and Nilsson (2019) showed that by removing the constraint of overheating (via removing ventral feathers covering pectoral muscles and abdomen), parents were able to invest more in both current reproduction (larger nestlings) and self-maintenance (lower body temperature and less body mass loss).

Immune function is a costly and crucial part of self-maintenance and therefore it is also an important factor in the trade-off between current and future reproduction. The immune system consists of two parts; the innate and the adaptive immune system. The adaptive immune system gives rise to a pathogen-specific response, both through production of memory antibodies and helper- and cytotoxic T-cells when a pathogen is encountered more than once. The innate immune system is the, non-specific, first line of defence against any pathogen. The importance of immune function in the trade-off between current and future reproduction has been shown in several studies where experimentally increased parental effort (often through brood size manipulations) decrease both adaptive (Nordling et al. 1998, Hasselquist et al. 2001, Ardia et al. 2003, Hanssen et al. 2005) and innate (Hegemann et al. 2013, Neggazi et al. 2016) immune function.

Given the potential for immune function to shape the trade-off between current and future reproduction we wanted to quantify if the potential benefits to self-maintenance following reduced constraints on heat dissipation rate were reflected in increased investment in immune function.

Paper III: Heat loss, work rate and immune function

We facilitated heat loss in male and female blue tits, during the most work-intensive period in nestling feeding, by removing feathers covering the abdomen and pectoral muscles (see Fig. 8) and measured resultant investment in self-maintenance (body mass loss over the experimental period, immune function, body temperature) and reproductive effort and output (feeding rate and nestling size).

When nestlings were 6 days old, all broods were enlarged to 15-17 nestlings, which is 5 nestlings above normal brood size. On day 7, nests were either assigned to the experimental category where both parents were captured and clipped and the males were implanted subcutaneously with a temperature sensitive PIT-tag. To minimize the risk of nest abandonment, we did not implant females with PIT-tags, but instead taped PIT-tags to two colour rings on the tarsus. Control parents were treated identically but for the clipping. Starting on day 10, we recorded feeding rate (via PIT-tags) and body temperature (in males) for approx. 24 h. Then, on day 14, parents were captured again and blood samples were taken for immune assays. All nestlings were ringed, measured and weighed at day 14.

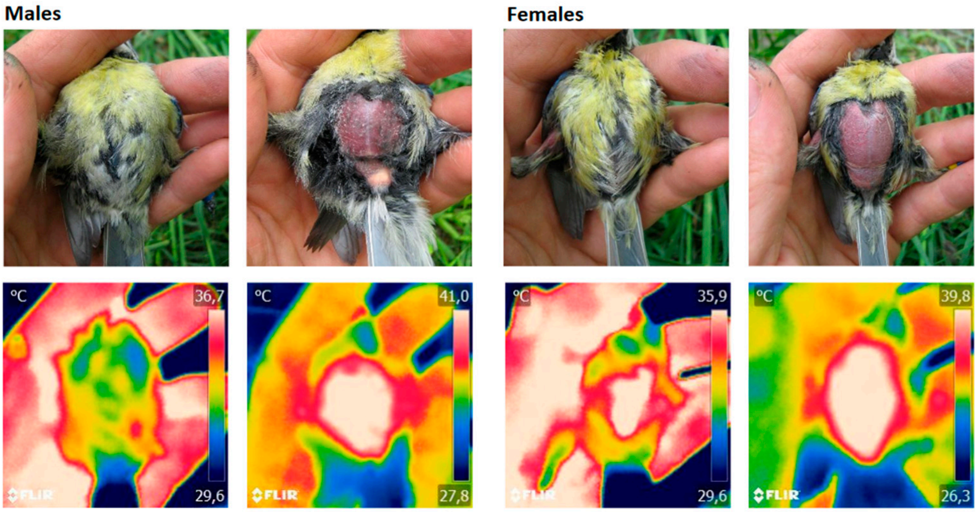


Fig. 8 Photos and thermal images of clipped and unclipped male (left) and female (right) blue tits. The effect of feather clipping on surface temperature can be seen in the thermal images where lighter areas indicate warmer surfaces (see scale at right hand side). Fig. S1 from Supporting Information in Nord and Nilsson (2019), republished with permisson.

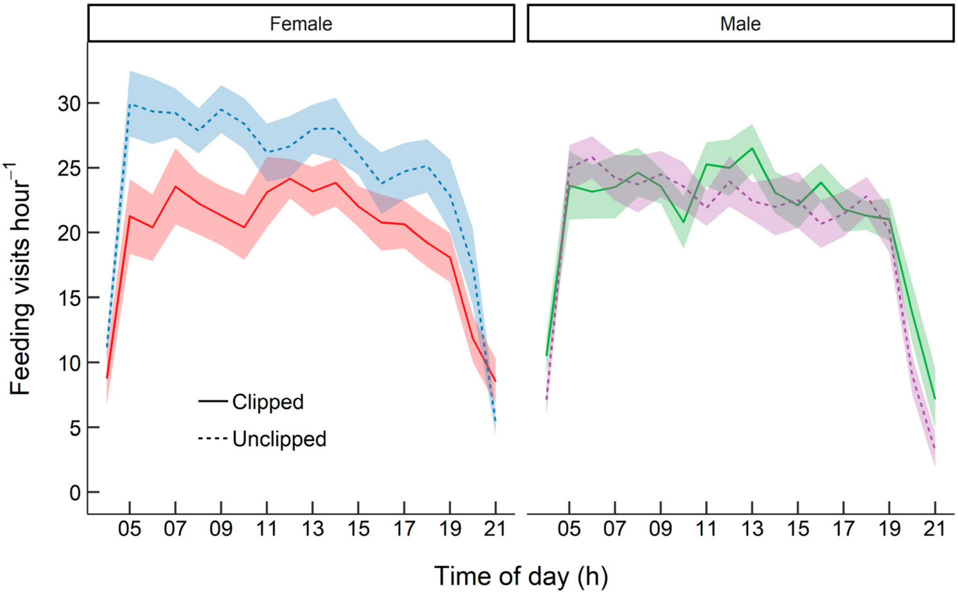


Fig. 9 Mean (± SE) number of feeding visits hour^{-1} in clipped and unclipped blue tits as a function of the time of day.

Clipped females reduced their number of feeding visits compared to unclipped females while clipping had no effect on feeding rate in males (Fig. 9). Despite the reduced feeding rates in clipped females, nestling size did not differ between the experimental groups. However, clipped females increased their investment in innate immune function (Fig. 10). Thus, although clipped females invested more in self-maintenance (increased immune function, reduced work rate), they did so without compromising nestling development.

Our results differ somewhat from the preceding experiment (Nord & Nilsson 2019) in that clipped males did not operate at lower body temperatures and overall, clipped parents did not lose less mass. However, females invested more in self-maintenance in both studies while still not compromising current reproductive output (Nord and Nilsson also found increased investment in nestling size).

Our results show that constraints of overheating can also influence innate immune function. Most likely, detailed analyses of additional physiological variables can further help us add pieces to the puzzle of current vs. future reproduction trade-offs.

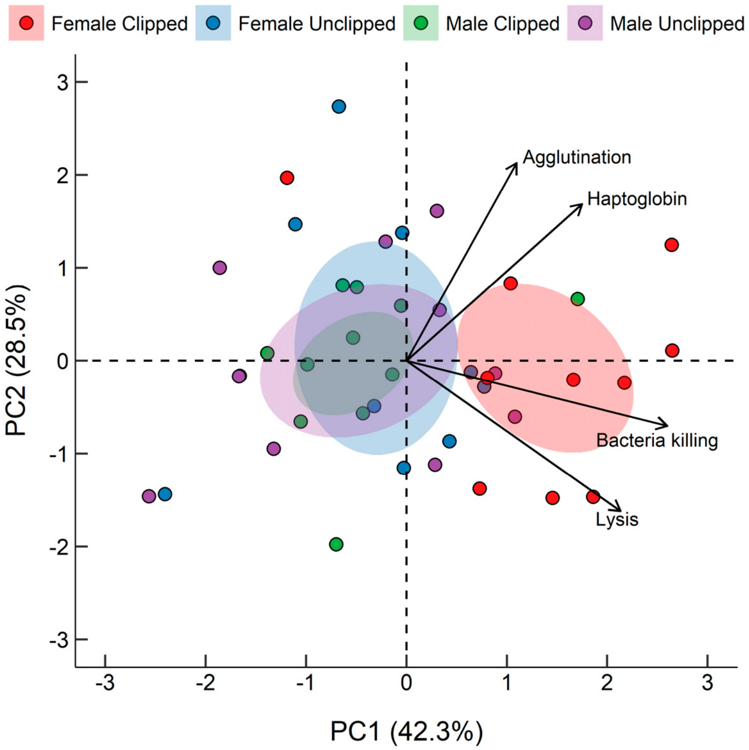


Fig. 10 Biplot from principal component analysis (PCA) showing scores of individuals (dots) and loadings of variables (vectors) in relation to the first two principal components.

In addition, our results also show that annual variation can have large effects, relevant in light of current climate change.

Ecological and physiological sources of body temperature variation in winter

There are several factors that pose energetic challenges for animals that overwinter at cold, northern latitudes. This is especially true for a small bird. It is often cold and the small size, high body temperature, high metabolic rate and high heat loss rate of a small bird makes it essential to minimize heat loss while simultaneously optimizing energy intake. The days are also short, meaning that time for foraging is limited and that food might also be in short supply, especially if there is thick snow cover. The challenge to maintain a positive energy balance in such a setting is further complicated by the trade-off between predation and starvation: i.e. a bird that forages extensively will be more exposed to predators (e.g. Lima 1985) and carrying extra fat will also reduce the chance of escaping diurnal and crepuscular predators as it can impair flight performance (Metcalfe & Ure 1995) and take-off ability (Kullberg et al. 1996).

Birds use several different behaviours to cope with cold ambient conditions and energy shortage. Some species huddle (e.g. Ancel et al. 1997) and/or roost communally (Walsberg 1990, Du Plessis & Williams 1994, Hatchwell et al. 2009), while others choose thermally favourable microclimates for roosting, such as snow burrows (Irving et al. 1967, Marjakangas et al. 1984). Postural adjustments, covering poorly insulated areas such as bill, head and legs with feathers, are also common (Pavlovic et al. 2019, Ferretti et al. 2019). Small passerines are also able to create an insulating layer of warmer air by fluffing up and erecting their feathers (i.e. ptiloerection; Steen 1958, Hill et al. 1980, Hohtola et al. 1980).

There are also physiological adaptations, some of which are components of seasonal adjustments. For example, deposition of fat, to be used for fuel, increase in winter (Lehikoinen 1987) and plumage insulation (Dawson & Carey 1976, Swanson 1991, Cooper 2002), cold tolerance and heat production capacity (reviewed in Swanson 2010) often increase from summer to winter.

Additionally, birds can use short-term physiological adjustments, such as peripheral vasoconstriction or facultative hypothermic responses, to reduce heat loss and save energy. Shallow reductions in body temperature that exceeds normal diurnal variation are usually termed rest-phase or night-time hypothermia (for an overview of facultative hypothermic responses in birds, see McKechnie & Lovegrove 2002). It acts as an energy saving mechanism as it reduces the gradient between the body and the colder surrounding air, thereby reducing heat loss, as well as reducing the need for costly heat production. In addition, the physical effect of temperature also contributes to reduced energy expenditure as biochemical reaction rates decrease with temperature (temperature coefficient, Q_{10}). The energy savings associated with the use of hypothermia can be significant (Reinertsen & Haftorn 1986, Clark & Dukas 2000, Pravosudov & Lucas 2000, Cooper & Gessaman 2005) and using

Fig. 11 Winter in Revinge.

hypothermia throughout the winter is predicted to increase winter survival substantially even if energy savings each night are moderate (Brodin et al. 2017). Facultative hypothermic responses are widespread across the avian phylogeny (McKechnie & Lovegrove 2002), but passerines generally exhibit moderate reductions (< 10 °C) compared to different species of nightjars, hummingbirds and swifts that can enter truly torpid states (reviewed in McKechnie & Lovegrove 2002, Ruf & Geiser 2015).

Night-time hypothermia has been the subject of study in many northern passerines (e.g. Palmgren 1944, Udvardy 1955, Steen 1958, Haftorn 1972, Hill et al. 1980, Reinertsen & Haftorn 1983, Reinertsen & Haftorn 1986) but we still know relatively little about how increased heat production below thermoneutrality actually affects body temperature maintenance (i.e. what is the shape of the dashed body temperature line below LCT in Fig. 1?). Metabolic rate and body temperature has been continuously measured below LCT in black-capped chickadees (*Poecile atricapillus*: Chaplin 1976, Grossman & West 1977), willow tits (*Poecile montanus*: Reinertsen & Haftorn 1983, Reinertsen & Haftorn 1984) and greenfinches (*Chloris chloris*: Saarela et al. 1995), providing valuable information on annual and circadian variation in body temperature and metabolic rate and their relationship with food availability, ambient temperature and shivering. However, the effect of intrinsic state variables, such as age, on body temperature and metabolic rate in “the little bird in winter” (*sensu* Brodin 2007) is yet to be tested. This was the aim of **Paper IV**.

What determines the depth and use of night-time hypothermia in a small passerine in winter? Previous studies have showed that night-time hypothermia is attenuated when body condition is high and ambient conditions are relatively mild (Reinertsen & Haftorn 1986, Dolby et al. 2004, Nord et al. 2009, 2011) and birds that are fed *ad libitum* maintain a body temperature that does not decrease below the normal circadian variation (Laurila & Hohtola 2005, Nord et al. 2009) of 1-2.5 °C (Reinertsen & Haftorn 1986, Prinzinger et al. 1991). This suggests that there are costs associated with the use of hypothermia as an energy saving strategy. The main ecological cost is widely believed to be an increased predation risk as a deeply hypothermic bird would be slower to respond (Haftorn 1972) and to escape a potential predator (Carr & Lima 2013). There is partial evidence for this theory, based on domestic pigeons (*Columba livia*). Fasted pigeons attenuate night-time hypothermia when exposed to increased predation risk (goshawk *Accipiter gentilis*) during the day (Laurila & Hohtola 2005). However, as far as we know, there are no studies on the effects of increased predation risk on night-time hypothermia in birds in their natural setting. This was the aim of **Paper V**.

Handling, or other types of stressors, typically induce an increase in core body temperature as preparation for a fight-or-flight response in animals. This stress-induced hyperthermia has been shown in both mammals (e.g. Cabanac & Briese 1991, Moe & Bakken 1997) and birds (e.g. Cabanac & Aizawa 2000, Carere & van

Oers 2004) but seems to be contradicted by small passerines in winter where handling instead reduces body temperature (Udvardy 1955, Lewden et al. 2017). However, data on the duration and magnitude of the hypothermic response to handling is lacking, which we sought to add in **Paper VI**.

Paper IV: Age differences in metabolic rate and body temperature in the cold

We measured metabolic rate and subcutaneous body temperature in blue tits in ambient temperatures ranging from 25 °C to -15 °C during winter nights to study how body temperature is maintained below LCT when heat production and metabolic rate increases. We were specifically interested in whether there were differences between age-groups, as previous studies have indicated that time-constraints during moult can produce winter plumage of inferior quality (Broggi et al. 2011, Nord & Folkow 2018).

Resting metabolic rate in thermoneutrality (i.e. BMR) was 0.27 W, which is similar to previous studies in blue tits (Broggi et al. 2019) and black-capped chickadees (Cooper & Swanson 1994, Petit & Vézina 2014) in winter. LCT was estimated at ca. 14 °C and below this ambient temperature metabolic rate increased in a linear fashion (Fig. 12A).

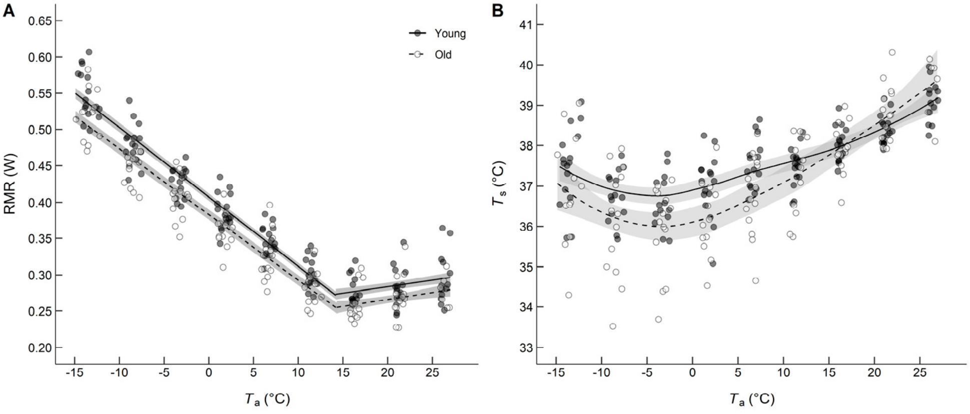


Fig. 12 A) Resting metabolic rate (RMR) and B) subcutaneous body temperature (T_s) as a function of ambient temperature (T_a) in young (in their first winter) and old birds (in their second winter or older). In A), age-specific regressions are model estimates (± SE) from separate linear mixed models above and below LCT and in B) age-specific fitted curves are LOESS (locally estimated scatterplot smoothing, ± 95% CI).

At -15 °C, resting metabolic rate was twice that of BMR, indicating that blue tits were far from using their full metabolic capacity for heat production as summit metabolic rate (M_{sum} – maximum metabolic rate under cold exposure) usually range from 5–8 × BMR in passerines (Dutenhoffer & Swanson 1996).

Overall, the body temperature reduction across the 40 °C ambient gradient was moderate. Body temperature decreased below LCT, but most birds did not reduce body temperature more than 4–5 °C from day-time levels and at the coldest ambient temperatures body temperature increased again (Fig. 12B). We validated the measure of subcutaneous body temperature (via implanted PIT-tags) with simultaneous measurements of cloacal temperature in three birds that were not part of the main experiment. The two measures correlated well, but the gradient between core and periphery increased as it got colder, from ca. 1 °C in thermoneutrality to 2 °C in an ambient temperature of -15 °C (Fig. 13). Thus, for most birds, it seems that the increase in heat production was enough to maintain a shallow, but relatively stable, hypothermic body temperature across the ambient range.

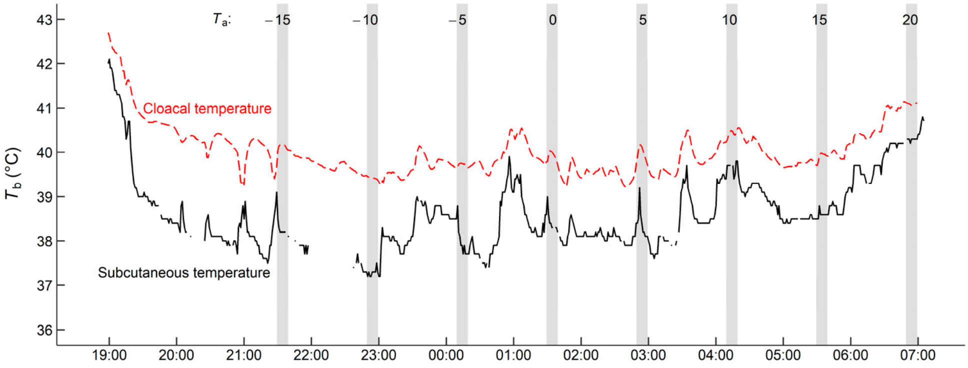


Fig. 13 Example of body temperature (T_b) during a whole night of measurement where body temperature was continuously measured both subcutaneously (black) and in the cloaca (red). Grey bars indicate incremental increases in ambient temperature (T_a).

We found that young birds (in their first winter) had higher metabolic rate and higher thermal conductance below LCT compared to old birds (in their second winter or older). One possible explanation is that young birds, with possibly inferior plumage, had to offset the higher heat loss rate with increased heat production. However, young birds also had higher body temperature in relation to increased perceived predation risk, a pattern that was observed also in **Paper V**. If the higher body temperature in this study was related to predation risk (as handling and subsequent confinement in a metabolic chamber likely also constitutes a comparable stressor) or other forms of variation in life-history strategies needs further study.

Paper V: The effect of increased perceived predation risk on night-time hypothermia

We increased perceived predation risk for free-living, roosting blue- and great tits in winter and measured resultant nightly body temperature to study if and how predation risk could act as an ecological cost of night-time hypothermia.

For blue tits, we prepared nest-boxes during the day with olfactory cues, which have previously been used to successfully assess responses to increased perceived predation risk in passerines (Amo et al. 2008, 2011, 2015, 2017, Mönkkönen et al. 2009, but see Godard et al. 2007, Johnson et al. 2011). We either added a predatory olfactory cue (mink *Mustela vison* urine), a control olfactory cue (acetic acid) or water (control) to nest-boxes and then returned late in the evening/night and measured cloacal body temperature. Great tits were handled (ringed, weighed and measured) when night-roosts had started after which we attached a thermocouple in the cloaca by taping the thermocouple wire to two tail feathers. The thermocouple was attached to a data-logger that recorded body temperature throughout the night. Minimum body temperature during the night was used as a measure of night-time hypothermia. Handling is most likely perceived as a predation attempt (cf. Nord et al. 2014, Nilsson & Nord 2017) and thus, for great tits, we were only able to evaluate how an increase in perceived predation risk affected night-time body temperature differently in young and old birds (not having a true control group as all birds were handled, **Paper VI**).

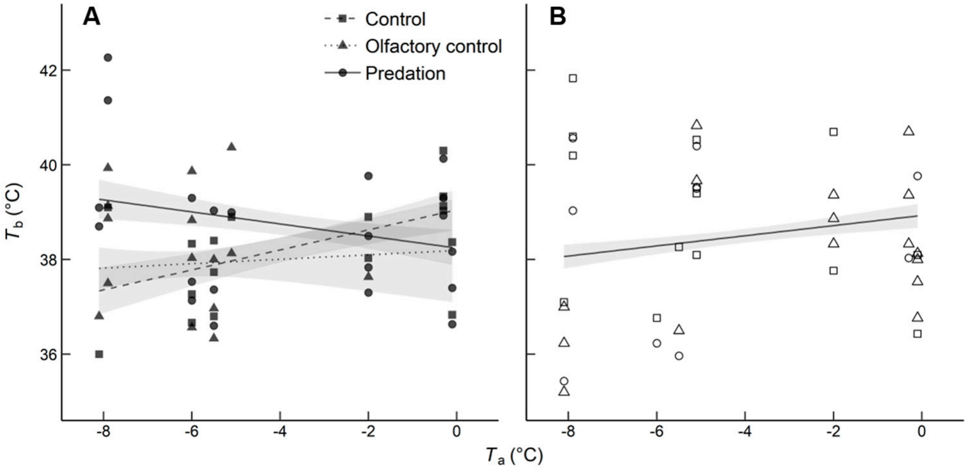


Fig. 14 Nightly body temperature (T_b) (± SE) in A) young (in their first winter) and B) old (in their second winter or older) blue tits exposed to different olfactory cues as a function of ambient temperature (T_a). In B), the regression line is based on all old birds.

The effect of an increased perceived predation risk on night-time hypothermia was age-specific. Old blue tits did not differ in their response to the olfactory cues, while young birds were less prone to use hypothermia as an energy-saving strategy at low ambient temperatures when exposed to the predatory scent (Fig. 14). Great tits showed a similar age-related effect of ambient temperature on body temperature.

So, why do young birds maintain a higher body temperature in the cold when exposed to an increased predation risk compared to old birds? Other studies have showed that age can influence decision-making, where older animals perhaps are more experienced and better at risk assessment (or alternatively that they are willing to accept a higher predation risk given their lower residual reproductive value) while young individuals are more likely to adopt a "better safe than sorry"-strategy (Ramakrishnan & Coss 1999, Rajala et al. 2003, Rodriguez-Prieto et al. 2008). Older birds are also dominant (Krams et al. 2013) and it is likely that they outcompete subordinate, young birds, for territories and roosting-sites. If older birds are able to select roosting-sites where nocturnal predation risk is low, they could benefit from deeper night-time hypothermia to be able to exit the nest-box in the morning with larger reserves, thereby reducing the risk of predation from crepuscular predators.

In conclusion, our results confirm the idea that predation risk is a likely ecological cost of night-time hypothermia, but that this cost is age-specific and varies with ambient temperature.

Paper VI: Body temperature responses to handling in the cold

We searched nest-boxes for roosting great tits during winter nights. When a bird was found we immediately measured cloacal body temperature (T_1, see Fig. 16) after which we proceeded to ring the bird and take biometric measurements. After handling, during which we did not record body temperature, we reinserted the thermocouple (T_2) and put the bird back into its nest-box. The bird was then left overnight with the mounted cloacal thermocouple attached to two tail feathers connected to a data-logger, recording body temperature continuously, until we arrived before sunset to remove the thermocouple and data-logger. We extracted the lowest body temperature after handling (T_3) and the maximum body temperature attained after T_3 (T_4) from the temperature data (see Fig. 16).

Body temperature decreased rapidly during handling (0.55 °C/min handling time) and body temperature continued to decrease after birds were put back in their nest-boxes. Minimum body temperature at T_3 was 34.6 °C before birds started to rewarm to T_4 at 41.3 °C, which is close to day-time levels. The whole response from T_1 to T_4 took, on average, 20 minutes.

Fig. 15 A great tit in Vomb. Photograph taken by Aron Hejdström ©, published with permission.

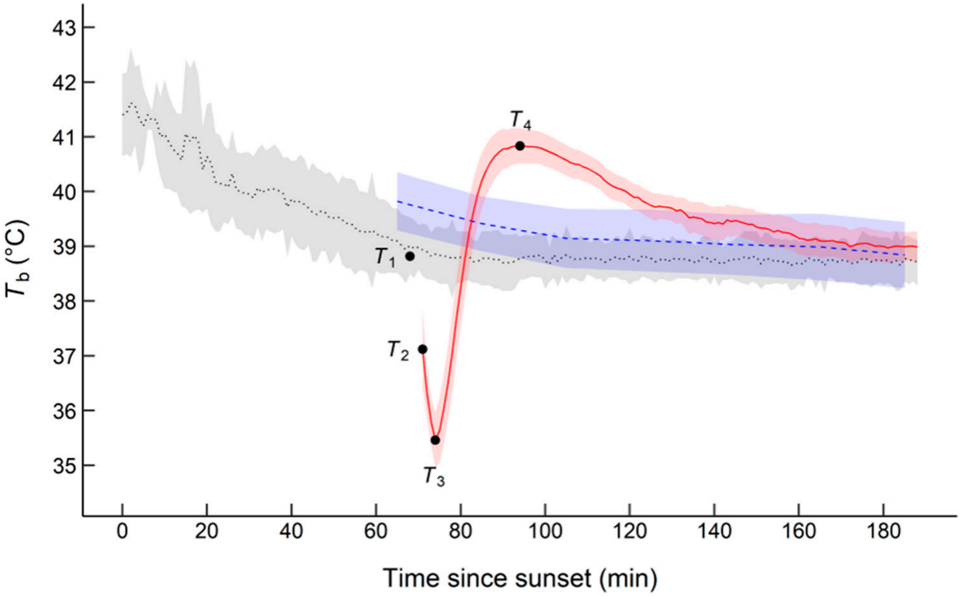


Fig. 16 Mean body temperature (T_b) of great tits as a function of time since sunset. The red (solid) line shows body temperature in handled great tits, and the blue (dashed) and black (dotted) lines show body temperature in undisturbed great tits from the same population kept in outdoor aviaries under similar ambient conditions to this study (data from Supporting Information and Nord et al. 2013). Shaded bands represent 95% confidence intervals.

Thus, any potential stress-induced hyperthermia was either overshadowed or delayed by our handling, which increased heat loss rate. If any stress-induced hyperthermia occurred during the handling, which lasted 4 min on average, we would have been unable to detect it.

It is likely that the observed, initial body temperature reduction was caused by disruption of the insulating layer of plumage created by erected feathers, and further exacerbated by conductive cooling from cold hands of the investigator (suggested by Lewden et al. 2017). Small birds, challenged by their unfavourable surface-area to volume ratio and lack of fat insulation, are especially vulnerable to this disruption of feathers and it seems that handling can reduce body temperature substantially, both during day (Lewden et al. 2017) and night (**Paper VI**) as long as ambient temperature is below thermoneutrality (Lewden et al. 2017, **Paper VI**). This is contrasted by the classical stress-induced hyperthermia observed in larger birds, despite being handled in the cold (Nord & Folkow 2019). It is intriguing that body temperature continued to decrease after the birds were put back into their nest-boxes, to levels that are well below expected nightly body temperature for unhandled great tits in this population (see Fig. 16). Birds either lost heat at a faster rate and/or reduced heat production during this time. Handling can trigger tonic immobility, where heart rate is attenuated (Gabrielsen & Smith 1985) and where muscular shivering is discontinued for a short time period (Hohtola 1981). In addition, it would not be energetically prudent to start extensive heat production until postural changes and feather erection were regained, which also takes some time (cf. Hohtola 1981). However, since we did not score activity or record behaviour after putting the birds back into the nest-boxes, this would need further study. We also found that rewarming was strongly correlated with T_3, suggesting that it was mainly driven by a need to restore body temperature rather than a delayed stress-response. In addition, T_4 was higher in young birds and females, which could be explained if females and young birds were constrained in their foraging due to lower social rank, which could produce a plumage of inferior quality.

Conclusions and future perspectives

The aim of this thesis was to study ecological and physiological sources of variation in avian body temperature across age and seasons. I did so by studying free-living, wild, blue tits and great tits, using mainly experimental approaches. Thus, I believe that the patterns, mechanisms and trade-offs observed are good representations of patterns that could arise in nature, and that they are likely to be of a general character, in most cases not only applicable to the current study species.

Based on the work presented in this thesis, it is clear that:

- Brood size and thermal environment of the nest have effects on short-term growth patterns and body temperature regulation, with possible fitness consequences related to long-term survival.
- Immune function is an important factor to incorporate in the heat dissipation limit (HDL) framework, especially when analysing trade-offs between current and future reproduction.
- Body temperature regulation and heat production as well as responses to increased predation risk and handling in winter is age-specific, where birds in their first winter generally maintain a higher body temperature in cold ambient temperatures compared to birds that are in their second winter (or older).

More specifically, I have shown that one of the key factors in avian life-history theory, brood size, shapes the development of endothermy in nestlings of an altricial species (**Paper I**). Thus, onset of endothermy is not ontogenetically fixed but varies with brood size, most likely due to differences in nutritional status. Growing up in a small brood, compared to being raised in a large brood, seemingly allows for energy allocation into both growth and thermoregulatory development, which could have potential fitness effects through decreased time spent in the nest and increased long-term survival. In addition, I have also shown that nestlings that are more than a week old effectively avoid severe hyperthermia when growing up in a warmer environment than normal, but that it comes with a cost – reduced body mass gain (**Paper II**). This could potentially also have carry-over effects to later life-stages as nestlings from heated nests had a higher apparent survival compared to nestlings from control nests. However, we still know little about long-term consequences of the thermal environment during development. New studies should seek to determine whether changes in brood size or nest temperature have long term effects on metabolic phenotypes and if these effects carry-over to adult life in winter and coming breeding seasons. In addition, growth and thermoregulatory development of nestlings will also affect the energy budget of parents as the need for provisioning and brooding will vary depending on nestling status.

It is known from before that another central trade-off in life-history, current vs. future reproduction, can be constrained by the risk of overheating. In **Paper III** I developed this idea further and showed that such trade-offs also include investment in innate immune function. When constraints are reduced, females invest more into immune function, but without jeopardizing nestling size. Thus, incorporating immune function into the HDL-framework allowed us to refine our understanding of how this limit to maximum energy expenditure could shape the trade-off between current and future reproduction. In future studies it would be valuable to add further physiological parameters of interest (e.g. measures of oxidative stress) and, if possible, expand such experiments over multiple seasons to include and assess the effect of annual variation.

When ambient temperature gets increasingly colder, heat produced just by sustaining vital body functions is not enough if body temperature is to be maintained, so metabolic heat production starts to increase. I have shown that this rate of increase (which was more or less constant below the lower critical temperature) is sufficient to maintain a stable, albeit slightly hypothermic body temperature (**Paper IV**) across a large ambient temperature gradient in blue tits. Young birds, however, have both higher metabolic rate and body temperature, suggesting that they produce more heat to offset increased heat loss due to inferior insulation (indicated by the higher thermal conductance). In addition, higher body temperature could also be attributed to experience-based variation in risk assessment, similar to what I found in **Paper V** where young birds maintain a higher body temperature in the cold compared to old birds when exposed to an increased perceived predation risk.

In **Paper VI** I showed that handling birds in winter causes a pronounced body temperature response with an initial decrease during handling with subsequent rewarming after being put back into the nest-box. The striking body temperature reduction during and directly after handling highlights the importance of plumage in maintaining body temperature in the cold. Even though the total energetic cost of such a handling event is modest, handling time should be minimized to prevent dramatic decreases in body temperature.

My results from winter all illustrate the need for including age-effects into the framework of energy management and body temperature regulation in the cold. I found age-specific effects of predation risk, ambient temperature and handling on body temperature regulation which could be attributed to differences in plumage quality or other age-related factors such as risk assessment or habitat quality. Future studies could therefore benefit from adding more detailed analyses of plumage quality in the different age-groups to help pinpoint their role in body temperature regulation and contribution to overall thermal conductance. Moreover, additional measurements, such as thermal imaging of plumage and modelling of heat loss rates and measurement of muscular shivering could help us further understand the relationship between body temperature and metabolic rate in the birds' ecological setting.

Finally, much of the work in this thesis has dealt with avian thermoregulation at the ambient "extremes", when additional energy is required to maintain body temperature. Given the expected increase in extreme weather events in the coming years, in light of current climate change, these types of studies can prove useful in the difficult task of predicting how animals and populations will respond to such changes.

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