Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Information Technology and Electrical Engineering
Department of Biology

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Torpor arousal rates in northern bats (*Eptesicus nilssonii*)

Master's thesis in Natural Science with Teacher Education
Supervisor: Clare Stawski
Co-supervisor: Mari Aas Fjelldal

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NTNU
Kunnskap for en bedre verden

Abstract

Endothermic animals often keep their body temperature high and stable in the face of many environmental conditions, which is energetically expensive. To avoid the energetic challenge many mammals use *torpor*, which is a process where their body temperature, metabolic rate and oxygen consumption is lowered. Animals in torpor must eventually arouse, a process which can also be energetically expensive, as it involves a rapid increase in body temperature, metabolic rate, and oxygen consumption. Induced and spontaneous arousals have previously been considered to be the same, however it has been shown that inducing arousal can change several aspects of torpor, which needs to be considered when using induced arousals in studies. There is little to no information of the arousal process of *Eptesicus nilssonii* (northern bat). This study aims to investigate the arousal process of *E. nilssonii* in the laboratory and compare this to what happens out in nature. The goal is to show that the arousal process is not just a process to rewarm as quickly as possible, and that the bats have a large thermogenic capacity that they do not always utilize when arousing.

Five male individuals of *E. nilssonii* were caught in Trondheim in June and July of 2021. The skin temperature of these five individuals were measured both in the laboratory at NTNU using a thermocouple, and in the field using temperature-sensitive transmitters. The arousals in the laboratory were induced while the field arousals were natural arousals. Arousal rate, peak arousal rate and durations were found from these measurements. Neither body mass nor duration influenced arousal rate or peak arousal rate in the laboratory. Start skin temperature and duration proved to have a negative effect on arousal rate in the field, while start skin temperature had a negative effect on duration in the field. When comparing arousal rate and peak arousal rate between laboratory and field, the laboratory arousals were significantly faster ($p < 0.01$), but no significant difference could be found for arousal durations ($p > 0.05$). These results point towards these bats having a larger thermogenic capacity than what they are utilizing when arousing naturally, and that the arousal process is not just a process to rewarm as fast as possible.

Sammendrag

Endoterme dyr holder ofte kroppstemperaturen sin høy og stabil i møte med mange miljøbetingelser, noe som krever mye energi. For å unngå energiutfordringene bruker mange pattedyr *torpor*, en prosess hvor kroppstemperatur, metabolsk rate og oksygenkonsum reduseres. Dyr som er i torpor må etter hvert varme seg opp igjen, en prosess som også kan kreve mye energi, fordi det involverer en rask økning i kroppstemperatur, metabolsk rate og oksygenkonsum. Induserte og spontane gjenoppvarminger har tidligere blitt sett på som like prosesser, men det har blitt vist at det å indusere gjenoppvarming kan endre flere aspekter ved torpor, noe som må tas i betraktning når man bruker induserte gjenoppvarminger i studier. Det finnes lite eller ingen informasjon om gjenoppvarmingsprosessen hos *Eptesicus nilssonii* (nordflaggermus). Denne studien ønsker å undersøke gjenoppvarmingsprosessen hos *E. nilssonii* i laboratoriet, og sammenligne dette med det som skjer ute i naturen. Målet er å vise at gjenoppvarmingen ikke alltid er en prosess hvor flaggermusene ønsker å varme seg opp så raskt som mulig, og at de har en stor termogen kapasitet som de ikke alltid benytter seg av.

Fem hannflaggermus av arten *E. nilssonii* ble fanget i Trondheim i juni og juli i 2021. Hudtemperaturen til disse fem individene ble målt både ved et laboratorium ved NTNU ved bruk av en thermocouple, og ute i felt ved bruk av temperatur-sensitive transmittere. Gjenoppvarminger i laboratoriet var induserte, mens gjenoppvarminger i felt var spontane. Gjenoppvarmingsrate, maksimal gjenoppvarmingsrate og varighet av gjenoppvarmingen ble funnet fra de samlede dataene. Verken kroppsvekt eller varighet påvirket gjenoppvarmingsrate eller maksimal gjenoppvarmingsrate i laboratoriet. Hudtemperatur ved start av gjenoppvarming og varighet hadde negativ effekt på gjenoppvarmingsrate i felt, og hudtemperatur ved start hadde også negativ effekt på varighet i felt. Ved sammenligning av gjenoppvarmingsrate og maksimal gjenoppvarmingsrate mellom laboratorium- og feltmålinger var målingene i laboratoriet signifikant raskere ($p < 0.01$), men ingen signifikant forskjell kunne finnes for varighet ($p > 0.05$). Disse resultatene peker mot at flaggermusene har en større termogen kapasitet enn hva de bruker når de spontant gjenoppvarmer, og at gjenoppvarmingsprosessen ikke bare er en prosess for å varme seg opp så raskt som mulig.

Acknowledgements

Writing my master thesis has been an extremely beneficial learning experience. It's been intriguing and entertaining to learn about the biology of bats and torpor. It has allowed me to delve further into biological topics of my interest and undertake independent work in the laboratory and field, which has given me a deeper understanding for the subject. Also writing the thesis has given me insight into academic writing. All of this has provided me with valuable experience and qualities that will help me become a better teacher in the future.

Firstly, I would like to thank my supervisors Clare Stawski and Mari Aas Fjelldal. Your advice, guidance and support has been invaluable for this project. Thank you for always staying positive in the face of challenges and answering my many questions. I would also like to thank Rune Sørås and Mari Aas Fjelldal for helping me while in the field and in the lab. Thank you for patiently teaching me about bats, your help was crucial for me being able to do this project.

I would also like to thank my fellow students and friends for support and motivation throughout these years. It has been five great years that would not have been the same without you. Thank you Marthe, my roommate, for all the laughs and always being there. Finally, I would like to thank Martinus. Your constant support through these five years of my teaching degree has been invaluable. Thank you!

Silje Huse Skauge

Trondheim, June 2022

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Abbreviations

T_a	=	ambient temperature
T_b	=	body temperature
TNZ	=	thermoneutral zone
T_{skin}	=	skin temperature

1 Introduction

All animals require energy to survive, as energy is used in all physiological processes. There must be balance in their energy turnover, as too much or too little energy can have consequences. When animals eat and drink they gain energy, and this energy can be stored in tissue and lost from the body as heat, external work or waste such as urine or faeces (Hill et al., 2017; Withers et al., 2016). An animal can overheat if it gains too much energy or cool down if it loses to much energy (Hill et al., 2017; Porter & Gates, 1969). When an animal exchanges energy with the environment, it can alter their body temperature (Porter & Gates, 1969).

Endothermy

All living birds and mammals are endothermic. Through thermoregulation these endotherms can keep a constant and high body temperature (T_b) over a range of ambient temperatures (T_a) (Geiser, 2021). Endothermic mammals usually keep a stable T_b, which for some mammals can be as low as 31ºC and for others as high as 38ºC. They also have a high metabolic rate and high ventilation rate, which is needed for them to keep their T_b high and stable in any thermal environment (Withers et al., 2016). Endotherms have a thermoneutral zone (TNZ), which is where their metabolic rate is low and they do not need to thermoregulate. When T_a is below or above their TNZ endotherms must increase their metabolic rate to keep their body temperature stable, because their heat loss increases. Small endotherms often have a high TNZ, starting around a T_a of about 30ºC (Geiser, 2021; Hill et al., 2017).

There are several advantages of being endothermic. It makes the animal capable of remaining active and allows them to forage over many different T_a, thus making them largely independent from the thermal conditions around them (Altringham, 2011; Hill et al., 2017). Endothermic animals also have a high stamina, and they can digest their food quite rapidly, allowing them to grow fast (Geiser, 2021). However, endothermy is energetically expensive, especially for smaller individuals because heat loss and heat production are both strongly affected by body mass. The heat exchange occurs over the body surface. In small animals, which have a high surface area to volume ratio, their body surface is relatively larger compared to bigger animals (Geiser, 2021; Withers et al., 2016). Being small also reduces the amount of fat that can be stored and thickness of fur for insulation (Dunbar & Tomasi, 2006; Withers et al., 2016), which gives smaller animals even more difficulty of keeping T_b high.

The T_a is a major influence on animals as it affects their metabolic rate, and because they are unable to avoid exchanging heat with the environment they inhabit (Hill et al., 2017; Withers et al., 2016). Endotherms constantly spend energy on heat generation to keep warm when T_a is below their TNZ. The energy they use for this is 'lost' to them as it does not provide them with any direct material benefit, like for example tissue growth (Speakman & Thomas, 2003). An animal's T_b is determined by the interaction of internal heat production within the animal and heat exchange with the environment (Withers et al., 2016). T_b affects the functions of tissues and thus the biological processes that occur in the tissues (Hill et al., 2017). To keep their T_b high and stable across thermal environments, animals need to thermoregulate. Insulation, morphology, internal heat production, behaviour and evaporative heat loss are all different parts

of thermoregulation. Animals regulate both heat gain and loss, as a constant T_b is attained when the heat production is adjusted to balance the loss (Withers et al., 2016).

Torpor

Maintaining a high and stable T_b is, as mentioned previously, energetically expensive and especially if the difference between T_a and T_b is large (Withers et al., 2016). In cold climates some animals solve this energetic challenge simply by avoiding it. Many birds and some mammals migrate to warmer areas to avoid the cold temperatures, but since the distances required to get away from the cold climate can be quite large, the option of migration is not possible for all species (Speakman & Rowland, 1999). These species that are unable to migrate can instead let their T_b decline to a lower set-point, to reduce the large difference between T_a and T_b. They go into a period of *torpor*, or adaptive hypothermia. The difference between hypothermia and torpor is that animals are unable to rewarm from hypothermia without getting help from an external heat source, but they are able to rewarm from torpor using heat that they produce themselves (Withers et al., 2016).

Torpor is used extensively by small mammals (Geiser, 2006). Its function is to reduce the cost of thermoregulation in the face of unfavourable conditions in their environment. Such conditions can be low T_a or periods of reduced food availability. It can also be used when conditions are favourable, and then its function could for example be to enhance fat storage for use later (Dunbar & Tomasi, 2006; Geiser, 2006). Nowack et al. (2017) presents a table with other functions of torpor, beyond energy conservation. These functions include for example increasing amount of fat stored before migration, enhancing storage of sperm and delaying birth, reduction of water loss and survival of droughts, to name a few.

When an animal is using torpor, they lower their metabolic rate to bring their T_b closer to the T_a. In addition some body processes are reduced, like for example the heart rate and respiratory rate (Hock, 1951). There is also peripheral vasoconstriction and the ability to spontaneously arouse using endogenous heat (Altringham, 2011). By lowering the T_b set point and halting metabolic heat production until the T_b has been reduced to this new set point, animals can save substantial amounts of energy (Willis, 2007). Using torpor allows mammals to escape the large energetic demands of endothermy. Water demands are also reduced because of the reduced breathing rate, and lower temperature which leads to a lower water vapor content of the air that is exhaled (Hill et al., 2017).

Torpor is usually split into two different types of torpor expressions: daily torpor and hibernation. Species are placed in either category largely based on the length of their torpor bouts (Geiser & Ruf, 1995). Hibernators are animals that are capable of bouts of multiday torpor lasting longer than 24 hours, while animals expressing daily torpor have torpor bouts lasting less than 24 hours. (Ruf & Geiser, 2015). Generally, hibernators also reach lower T_b during their torpor bouts than daily heterotherms, although minimum T_b during torpor is generally not suitable to alone distinguish between hibernation and daily torpor (Geiser & Ruf, 1995). Patterns of torpor for most mammals are often different between laboratory and field. There is evidence that suggests that torpor bouts in the field last longer and are deeper. In studies of captive animals the use of torpor might often be underestimated, along with the depth and length of torpor bouts (Geiser et al., 2000).

Arousal

One torpor cycle is made up of three phases: the cooling period as they go into torpor, maintenance of torpor and arousal from torpor (Chia-Huang Wang & Hudson, 1970; Menzies et al., 2016). In this study, the arousal phase is investigated. The arousal part of torpor is interesting because it requires the animals to reverse the changes they went through to go into torpor, e.g., low T_b, low metabolic rate and reduced heart rate. During an active (endogenous) arousal there are a series of physiological events, where the animal rewarms to normothermic temperatures using heat produced by their own body (Fishman & Lyman, 1961). Depending on the T_b the animal is defending while in torpor, it can increase this with more than 20-30ºC during the arousal process, within a relatively short period of time (Utz et al., 2007).

Although using torpor is a way for the animal to reduce their energy consumption, arousals from torpor using endogenous rewarming are energetically expensive (Thomas et al., 1990). Most of the fat that is stored for hibernation is used for arousals (Geiser, 2007; Thomas et al., 1990). Active arousal is generally achieved by shivering and/or non-shivering thermogenesis (Geiser, 2021). The arousal process begins with increasing heart- and breathing rate. Breathing rate and oxygen consumption increases before the T_b increases noticeably, and not all the arousal processes are restarted at the same time or rate (Chia-Huang Wang & Hudson, 1970; Fishman & Lyman, 1961; Utz & van Breukelen, 2013). Some blood is sent to the brown adipose tissue, which is fat that is rich in mitochondria and is specialized for producing heat. The blood passes through the brown adipose tissue and is warmed, and then carries this warmth to the rest of the body when it circulates (Altringham, 2011). The energy that the brown adipose tissue uses is directly converted to heat, and thus it provides a very efficient method for rewarming (Nespolo et al., 2001). The thermogenic capacity that brown adipose tissue has stems from proton leakage in the mitochondria through uncoupling protein 1 in placental mammals, which is contrary to how the mitochondria produces energy with coupled oxidative phosphorylation (Cannon & Nedergaard, 2004; Staples, 2014; Utz & van Breukelen, 2013; Withers et al., 2016). In addition to this the animals can also use shivering of skeletal muscles, in which warmth that is generated by uncoordinated activity of the muscles (Altringham, 2011).

Animals generally must periodically arouse from torpor even while in deep hibernation, although the trigger for arousing is yet unknown (Dunbar & Tomasi, 2006; Speakman & Thomas, 2003). There is evidence of some animals hibernating at high T_a not having interbout arousals (Dausmann et al., 2004, 2005), so there might be some relationship between T_b and T_a that allows animals to avoid arousal. The arousal process and returning to normothermic T_b is costly and arousing from hibernation too frequently can result in rapid loss of body mass if the animal is not taking in energy in between the torpor bouts (Dunbar & Tomasi, 2006). As such, the duration of the arousal, and the frequency of arousals are important factors in the total energy budget. This is especially important in small mammals, like bats, which do not have capability to store as much fat as larger animals (Altringham, 2003; Dunbar & Tomasi, 2006).

The large cost of arousal is a disadvantage of torpor and can potentially cancel out the energy savings of short torpor bouts (Menzies et al., 2016). The arousal process is also extremely demanding on the cardiovascular system, as the heart rate rapidly increases from as low as 10 beats per minute to over 700 beats per minute (in small mammals) in a relatively short amount

of time (Currie, Noy, et al., 2015). The large cost of arousals can be reduced by passively rewarming, which can be done for example by sharing heat between individuals in the same roost (huddling), increases in T_a, or basking in the sun (Geiser et al., 2004). A study by Lovegrove et al. (1999) of the marsupial *Sminthopsis macroura* found that energy expenditure of the whole torpor bout was about halved when the animals were heated passively.

Increase in T_b over time during arousals in mammals that use torpor generally takes a sigmoid form (Stone & Purvis, 1992). A curve of the arousal for an individual that is rewarming from torpor will likely take this sigmoid shape as shown in Figure 1.1. The shape might stem from the rate of reactions increasing exponentially as T_b increases, in combination with decrease in thermoregulatory drive when the T_b gets closer to normothermic values (Nicol & Andersen, 2008). Because of the sigmoid form of the rewarming curve, determining where warming starts and stops might be challenging, which can have a major impact when estimating arousal duration and average arousal rates (Nicol & Andersen, 2008; Nicol et al., 2009).

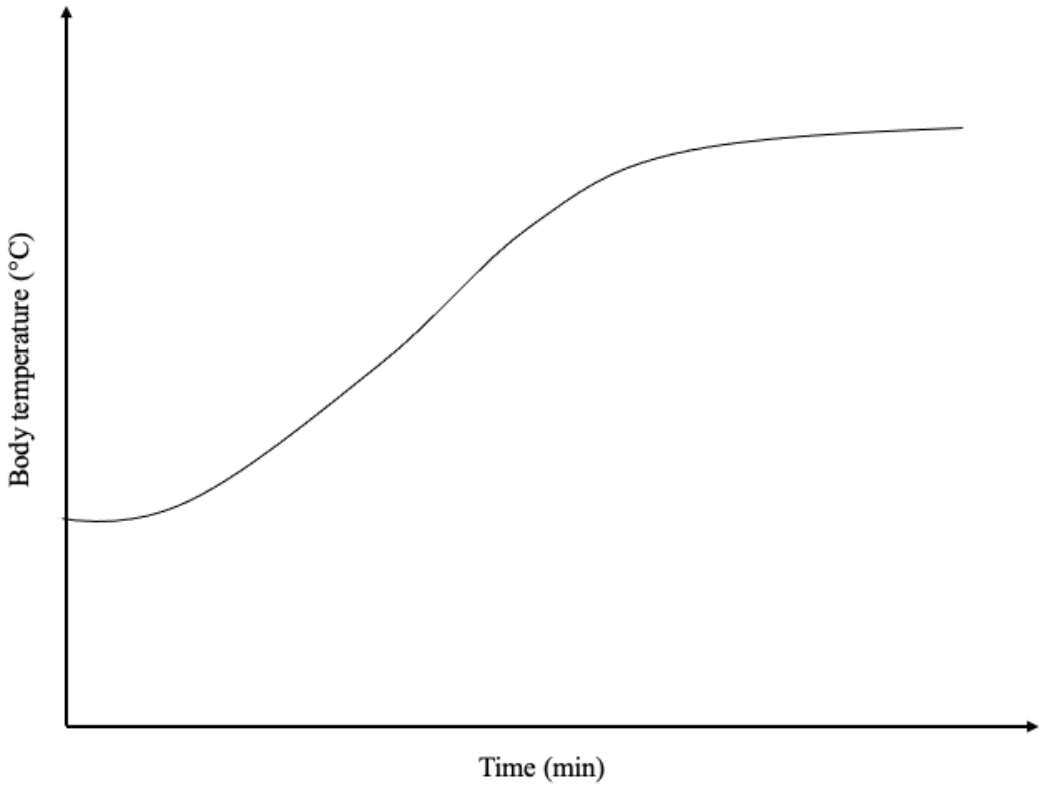

Figure 1.1: An example curve showing what an arousal from torpor could look like, with time (min) on the x-axis and body temperature (ºC) on the y-axis.

When studying torpor previously, natural and induced arousals have often been considered the same (Utz & van Breukelen, 2012), but inducing arousal in animals can actually result in a faster maximum arousal rate than if the arousal is natural (Utz & van Breukelen, 2013). This suggests that animals might not be using their full potential when they are arousing spontaneously in nature. Some other aspects of the arousal are changed when it is induced, for example the arousal duration is longer and the interbout arousal period (time between torpor

bouts) is shorter (Utz & van Breukelen, 2013). Tähti and Soivio (1977) found that when the arousal was induced the thermogenesis was 'stronger', and that the increase in breathing rate, heart rate and blood pressure happened faster and approximately at the same time, shortly after the induction of arousal. As mentioned previously, that does not normally happen in a spontaneous arousal (Chia-Huang Wang & Hudson, 1970; Fishman & Lyman, 1961; Utz & van Breukelen, 2013). There are few publications that directly compare induced vs. natural arousals (Utz & van Breukelen, 2013). Utz and van Breukelen (2012) presented a sample of 50 random publications that considered arousals. They noted that even though 35 of these studies used induced arousals, only 8 of them used them intentionally. Considering that several aspects of torpor are altered when the arousal is induced, one should interpret the data carefully when studying arousal by using induction of the arousal.

Generally, arousals are fast events compared to the entrance into torpor. The rate at which the animal is rewarming increases along with the increase in T_b, until normothermic values are reached (Utz & van Breukelen, 2012). The arousal rate is the result of the rate of heat that the animal produces and the heat that it loses (Stone & Purvis, 1992). Species at higher latitudes tend to have higher rewarming rates than those at lower latitudes, meaning there is a positive relationship between latitude and rewarming rates (Menzies et al., 2016). This might be an adaption, due to the colder and more variable climate at higher latitudes, to reduce the cost of arousal, as rewarming at slower rates increases energy expenditure (Stone & Purvis, 1992). There has also been found a negative relationship between rate of rewarming and body mass (Geiser & Baudinette, 1990; McKechine & Wolf, 2004; Stone & Purvis, 1992), and Geiser and Baudinette (1990) found that most small mammals that weighed about or less than 10 grams had a rewarming rate at about 1.0ºC/min.

Bats

Generally speaking, most bats are small in size which means that they have a large surface area to volume ratio. That results in high heat loss when T_a is below TNZ (Geiser, 2021). Because of the large costs of thermoregulation due to their size and their ability to fly, bats are probably the order of mammals with the largest amount of heterothermic species and are very likely to exhibit torpor (Geiser, 2021; Willis et al., 2005). In temperate areas, the ability to use torpor and thus lower energy expenditure seems to be crucial for insectivorous bats (Willis, 2006). Temperate bats also often use torpor year-round (Davis & Reite, 1967; Wojciechowski et al., 2007). Bats show a variety of diets (fruit, nectar, blood etc.), but using torpor appears more characteristic for insectivorous bats, because of their high heat loss due to their small size and their fleeting supply of food (Altringham, 2011).

As mentioned previously, all animals using torpor must eventually arouse. This applies to bats as well, who even during their long winter hibernation are not constantly in one torpor bout. They rarely remain torpid for longer than a period of two weeks at a time, even when conditions are ideal (Altringham, 2003, 2011). The cost of active arousals might be higher in bats than they are in other mammals that use torpor, which is due to the large surface area of the wing membranes that contributes to a high heat loss, in addition to the fact that some species tend to roost alone (Currie, Noy, et al., 2015), and thus cannot share heat between themselves.

Eptesicus nilssonii (the northern bat) belongs to the family of Vespertillinoidae, and is the northernmost bat species in the world (Altringham, 2003; Rydell, 1993). It is the only species of bat found breeding well above the Arctic Circle (66ºC) (Rydell et al., 1994; Schober & Grimmberger, 1997). It is one of the most common bat species found in Scandinavia and in Norway and it is also found throughout Europe and Russia. The adults usually have a body mass of approximately 9 to 12 grams. Their fur is dark, with golden tips on their back and head (juveniles do not have these golden tips). *E. nilssonii* is insectivorous, as the rest of the Vespertillinoidae also are. They usually start hibernating in early winter when temperatures drop, and insects become scarce, and the hibernation period usually lasts until around April (Rydell, 1993; Schober & Grimmberger, 1997). *E. nilssonii* is quite understudied, and there does not exist any arousal data for this species to my knowledge.

Aim of this study

The aim of this study is to investigate the arousal part of torpor in *E. nilssonii*. More precisely, it will focus on the active arousal where the bats do not use an external heat source to rewarm. This will be studied in individuals both in the laboratory and the field. I aim to show the thermogenic potential that these bats can have (i.e., in the laboratory when forced to arouse) and compare this to arousals when they are in their natural roosts and are arousing on their own terms.

I hypothesize that body mass and duration will affect *E. nilssoni's* arousal rate in the laboratory, with the prediction that the arousal rate will be slower with increased body mass and duration. I also hypothesize that both skin T_{skin} and T_a at the start of the arousal will affect arousal rate and duration in the field. Here I predict that with higher T_{skin} and T_a there will be a slower arousal rate and shorter duration. I also hypothesize that duration in the field will affect the arousal rate, with the prediction that the arousal rate will be slower with longer durations. In the comparison between laboratory and field data my predictions are that there will be faster arousal rates and shorter durations in the laboratory.

2 Materials and Methods

2.1 Skin temperature measurements of *E. nilssonii* in the laboratory

2.1.1 Animal capture and handling

Five male *E. nilssonii* were caught in Trondheim during summer 2021 (June, n=2, July, n=3) using mist nets. All bats were weighed, and their forearm lengths were measured, and then they were brought to a laboratory at Norwegian University of Science and Technology (NTNU) the same night they were caught. In the flight cage they were kept in, each bat was placed in an individual plastic box with a towel they could hang and/or hide in. The room with the flight cage was kept at a constant 10°C, to encourage the bats to enter torpor. A temperature data logger (0.5 °C, DS 1921G Thermochron iButtons, Maxim Integrated Products, Inc., Sunnyvale, CA, USA) was placed in the room during measurements to monitor that the temperature in the room was stable at 10ºC. As the data logger was kept in another warmer room between measuring days and was placed in the flight cage not long before the measurements started each morning, the temperature recorded by the data logger had not stabilized when some of the measurements had started. A mean of the T_a for the whole arousal was calculated to see if it was stable throughout measurements.

The skin temperature during arousal was first measured the morning after each individual bat was caught and then measured again on the next morning for a repetitive measure. All bats were measured within the same timeframe on the two days (between 09:00h and 13:00h), but no bat was measured at the exact same time on consecutive days. The measurements were conducted without feeding the bats first, to avoid a premature arousal. After the measurements the bats were weighed to the nearest 0.1g. Then they were fed with mealworms and water, weighed again, and placed back in their plastic box. This feeding procedure was repeated in the evening. Each bat was kept for two days in captivity before releasing them at the capture location after sunset on the second day.

2.1.2 Temperature measurements

The bats' arousal was induced when they were removed from their roosts. The handling was kept to a minimum to try to avoid heat transfer between handler and bat, and the bats were held loosely in a gloved hand while taping a thermocouple (1200 series Remote Squirrel Meter/Logger, Grant Instruments Ltd., Cambridge, UK) on their abdomen. As there is little fur on the stomach area, this approximately measures T_{skin}. T_{skin} has been found to be a good proxy for T_b across environmental temperatures for small mammals (Audet & Thomas, 1996; Barclay et al., 1996; Dausmann, 2005). Then they were placed with their ventral side down into a shallow plastic box, with a cardboard plate to sit on (Figure 2.1). This was to ensure minimal heat transfer from the bat to the table the box was placed on, because of cardboards' poor thermal conductivity. The time and temperature were recorded as soon as the bats were placed on the cardboard (minute 0 of arousal). While the measurements were running the bats were not touched until their skin temperature had stabilized, upon which the wire was taken off or they flew away from the wire themselves.

Figure 2.1: The laboratory setup used in the study. *E. nilssonii* was placed with ventral side down on a cardboard plate inside a plastic box, with the wire of the thermocouple (1200 series Remote Squirrel Meter/Logger, Grant Instruments Ltd., Cambridge, UK) taped to its abdomen.

2.2 Skin temperature measurement of *E. nilssonii* in the field

2.2.1 Capture and transmitters

The five same male *E. nilssonii* were equipped with temperature-sensitive transmitters (~0.5 g, PIP31, Lotek Wireless Inc., Dorset, U.K), that were calibrated in a water bath against a precision thermometer before use and released where they were captured. The transmitters were attached to the dorsal region of the bats by trimming a small patch of fur and using a skin-adhesive (B-530 Adhere Adhesive and Sauer-Hautkleber 50.01, Manfred Sauer GmbH, Lobbach, Germany). The bats were tracked with radiotelemetry to locate their day-roost. When a roost was found an external data logger was installed outside, recording pulse-intervals from the transmitters every 10 minutes when the bats were within signal-range. These pulse-intervals could afterwards be converted to T_{skin} measurements using data from the transmitter-calibration. To measure T_a in the field a temperature data logger (0.5 °C, DS 1921G Thermochron iButtons, Maxim Integrated Products, Inc., Sunnyvale, CA, USA) was placed in a nearby tree inside a paper cup (to avoid direct sunlight). This data logger measured the ambient temperature near the roost at 10-minute intervals.

2.2.2 Determining torpor and arousals from field-data

From the data from the transmitters, torpor-bouts were determined. Willis (2007) presented this equation used to calculate a torpor onset temperature, that was used to determine torpor bouts:

$$T_{onset}\text{-}1\ SE = (0.041) \times \text{Body mass} + (0.040)T_a + 31.083 \qquad (1)$$

An active arousal from torpor is here defined as a period when a bat rewarms from torpor to normothermic temperatures, and the increasing T_{skin} values are not caused by simultaneously increasing T_a. Here, the start of an arousal is defined as when the T_{skin} has an increase of more than 0.5ºC between two consecutive datapoints (10-minute period) at the end of a torpor bout. Similarly, an arousal has ended when T_{skin} decreases with less than 0.5ºC between two consecutive datapoints.

2.3 Defining arousal rates

2.3.1 Laboratory

Arousal rate is here defined as the mean of the change in T_{skin} over the duration of the arousal, i.e., calculating the change of each minute (subtracting previous T_{skin} value to current T_{skin} value) and finding the overall mean. In the calculation of arousal rate, negative values below −1.0ºC were excluded. This was because of the likeliness of this being due to movement that exposed the wire of the thermocouple to a colder temperature (e.g., pushing themselves up) instead of a true measure of T_{skin}. Laboratory arousal duration was defined as starting the moment the bat was placed in the box and the first temperature was measured and lasting until the temperature stabilized or the bat flew away from the thermocouple.

Peak arousal rate, i.e., the fastest change in temperature during the arousal, was determined by subtracting the previous T_{skin} value from the current T_{skin} value, to get the change in T_{skin} between two points, and evaluating the arousal to find the fastest point. To make the peak arousal rates between laboratory data and field data comparable, peak arousal rate over 10 minutes was also calculated. This was done by subtracting T_{skin} measurements 10 minutes apart and locating the fastest change.

One of the arousals was excluded from the laboratory data because the bat stopped rewarming at about 17ºC and flew away, and thus not being comparable to the others as a true endogenous arousal.

2.3.2 Field

Field arousal rate was calculated slightly differently, due to the manner of measurements. As the data was taken in 10-minute increments, the T_{skin} change was calculated for each of these increments and these values were summed across the full arousal. This was then divided by the total arousal-duration. In this calculation, negative values below –1.0ºC were excluded to make the data comparable with laboratory data. Peak arousal rate was defined as the fastest change over 10 minutes.

2.4 Statistical analyses

All statistical analyses were carried out using R 4.0.2 (R Development Core Team, 2020). A p-value lower than 0.05 was considered significant. In the thesis 'n' equals number of individuals, while 'N' equals number of measurements.

To test arousal rate and peak arousal rate for the laboratory data, linear mixed models were fitted using the function lmer from the package *lmerTest* (Kuznetsova et al., 2017), with individual ID as a random effect. The fixed effects included were individual body mass (g) and duration (minutes) of the arousal. However, the random effects were estimated to be approximately zero in the mixed models, and thus these models were changed into linear models using the lm function instead.

To analyse the field data, mixed effect models were again first tested, but later exchanged for linear models as the random effects of ID did not explain any of the variation in the data. Models were constructed to test arousal rate and duration. The model for arousal rate included start T_a (ºC), start T_{skin} (ºC) and duration (minutes), while the model for duration included start T_a (ºC) and start T_{skin} (ºC). Ranking of the models was done based on AIC values. ΔAIC had to be reduced with >2 to be considered a better fit. If two models had ΔAIC<2, the concept of parsimony was used to select the model, meaning the one with less explanatory variables (Burnham & Anderson, 2002). The ranking of all tested models can be found in Table A3 and A4 in the Appendix.

One-sided t-tests were carried out to compare the laboratory and field data, using a mean value for each individual. As the same five individuals were measured in the laboratory and field, paired t-tests were used for comparing arousal rates, arousal duration and peak arousal rate.

3 Results

3.1 Skin temperature measurements of *E. nilssonii* in the laboratory

The results in the laboratory are based on five individuals and nine measurements in total (Table A1). When the bats started arousing (i.e., the first temperature measurement when they were placed in the box) their average T_{skin} was 9.9±0.8ºC. Generally, their T_{skin} was quite close to the T_a, most of them from 9.5ºC to 10.5ºC, but one of the bats had a skin temperature as low at 7ºC. The curve of the active arousals during my study sometimes had a sigmoid shape (Figure 3.1). As such, an active arousal can be separated into three periods:

1. A period of slow rewarming as the arousal is at its beginning (marked in grey).
2. A period of quite rapid rewarming, where the increase in T_{skin} is accelerating (marked in blue).
3. A period where it slowly stops increasing and stabilizes when the bat reaches desired (normothermic) temperature (marked in black).

In some of the arousals, where the bat flew away from the wire before T_{skin} stabilized completely, the last period where the curve flattens out is absent giving it more of an exponential shape (Figure A1).

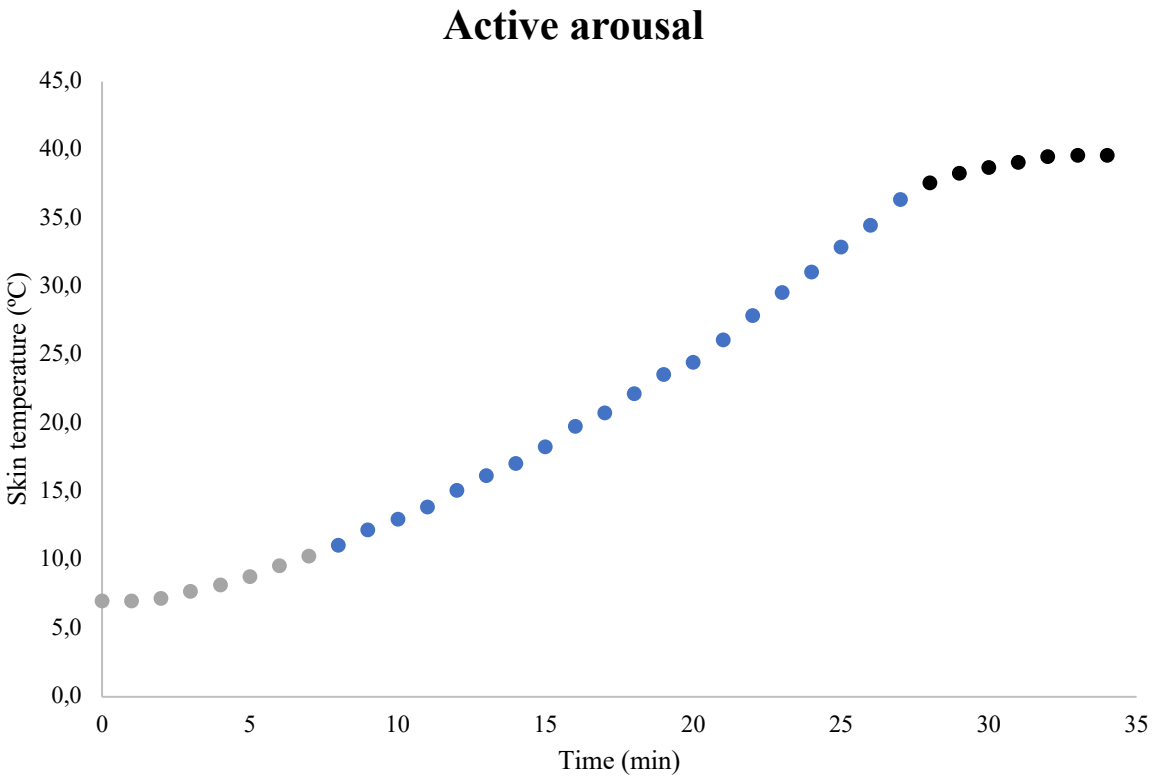

Figure 3.1: Example of an active arousal of one individual bat (*E. nilssonii*) at T_a=10ºC from my dataset. The different periods of the arousal are marked in grey (slow rewarming), blue (rapid rewarming) and black (stabilization).

One of the arousals from the laboratory data was removed from analysis due to it not being a 'proper' endogenous arousal. It started out at 8.0ºC and increased slowly, with an average arousal rate of 0.3ºC. Its peak arousal rate was 0.8ºC/min which was reached after 24 minutes. The bat flew away from the wire at 16.8ºC, after 36 minutes (Figure A2).

The overall change during the first five minutes was quite slow, as can be seen in Figure 3.2. All the individuals increased around 1ºC (on average 1.2±0.5ºC) during the first five-minute period. Peak arousal rate was reached after 29.8±4.58 minutes on average for the five individuals, as indicated by triangles in Figure 3.2, with a peak arousal rate of 2.0±0.49ºC/min on average. The triangles for bat1 and bat5 are almost directly on top of each other in the figure. The T_{skin} at the end of the arousal was on average 34.3±2.1ºC. The mean arousal rate for the individuals in the laboratory was 0.72±0.16ºC/min and varied from 0.54 to 0.98ºC/min (Table 3.1, Table A1). Mean arousal duration of the arousals was 35.2±4.7 minutes and varied from 28 to 44 minutes (Table 3.1, Table A1). In the models tested, none of the predictor variables had a significant effect on either arousal rate or peak arousal rate, meaning that neither body mass or duration of the arousal had a significant effect on arousal rate, or peak arousal rate in these five individuals while in the laboratory.

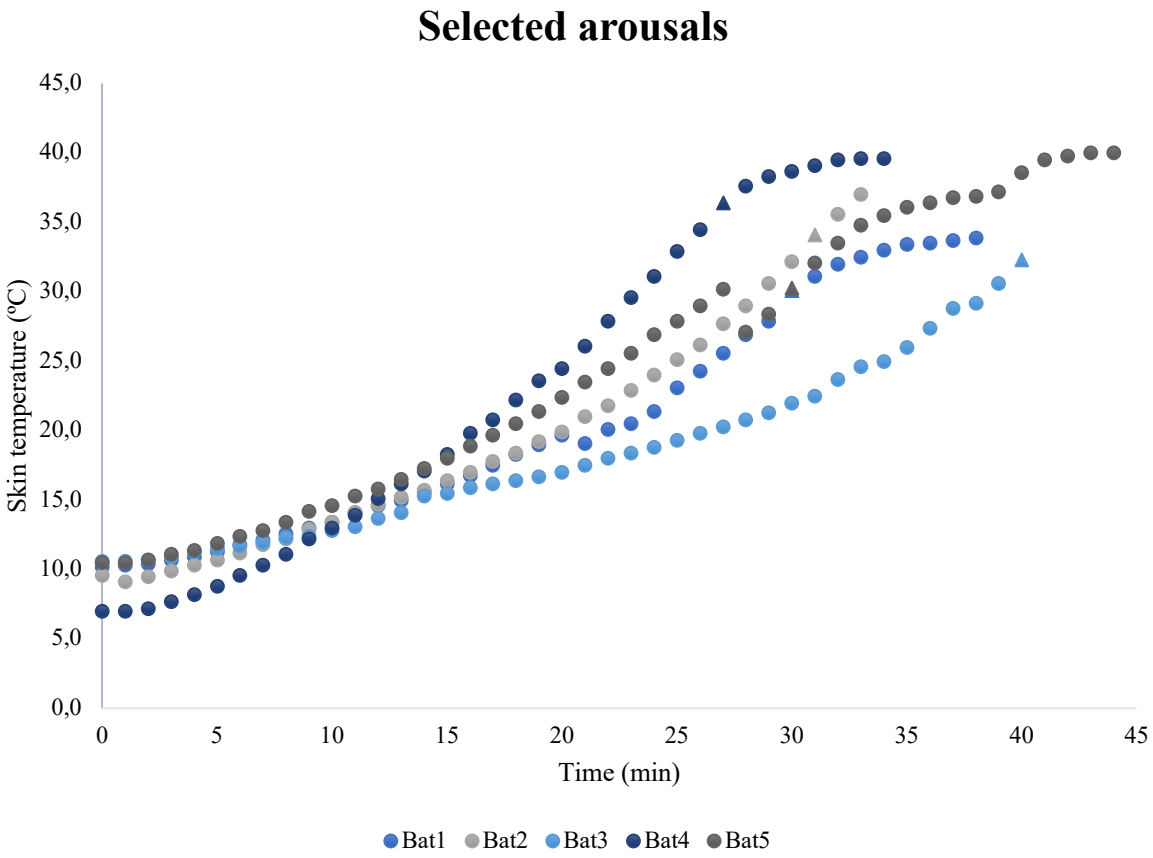

Figure 3.2*:* One selected arousal for each bat (*E. nilssonii*) at T_a=10ºC. Fastest point of arousal (peak arousal rate) indicated by triangles on each curve (triangle for bat1 is almost directly under the one for bat5).

Table 3.1: The mean body mass (g), mean arousal rate (ºC/min) and mean duration of the arousal (min), mean T_{skin} (ºC) at start and stop of arousal, and mean T_a (ºC) during measurement for each individual of *E. nilssonii* measured in the laboratory. The number of measurements used for each mean is presented in brackets.

Individual	Body mass (g)	Arousal rate (ºC/min)	Duration (min)	T_{skin} start (ºC)	T_{skin} stop (ºC)	T_a (ºC)
Bat1 (N=2)	9.1±0.4	0.73±0.15	33.0±7.1	9.9±0.5	33.5±0.6	10.2±0.6
Bat2 (N=2)	7.6±0.2	0.72±0.16	32.5±0.7	10.1±0.6	33.5±5.0	10.0±0.2
Bat3 (N=1)	8.2±0.0	0.54±0.0	40.0±0.0	10.6±0.0	32.3±0.0	10.0±0.0
Bat4 (N=2)	7.8±0.1	0.97±0.01	30.0±5.7	8.6±2.2	37.7±2.8	10.3±0.6
Bat5 (N=2)	8.0±0.1	0.63±0.18	40.5±5.0	10.5±0.1	34.5±7.8	10.0±0.0
Mean	**8.1±0.6**	**0.72±0.16**	**35.2±4.7**	**9.9±0.8**	**34.3±2.1**	**10.1±0.1**

3.2 Skin temperature measurements of *E. nilssonii* in the field

The results are based on measurements of the same five individuals that were used in the laboratory, with a total of 15 measurements from the field (Table A2, Figure A3). When the bats started arousing their average T_{skin} was 22.5±2.2ºC, while the average T_a was 17.8±3.5ºC. Mean arousal rate in the field was 0.3±0.05ºC/min and varied from 0.19 to 0.44ºC/min, while mean duration of arousal was 42.6±12.5 minutes and varied from 20 to 90 minutes (Table 3.2, Table A2). In the models considering arousal rate both duration and T_{skin} did have a significant effect ($p<0.05$). The highest ranked model (Table A3) showed that when correcting for duration or T_{skin}, the other variable had a negative effect on arousal rate (Table 3.3). This means that with higher T_{skin} and duration, the slower the arousal rate was. For the models considering duration, start T_{skin} had a significant effect on duration ($p<0.05$). The highest ranked model (Table A4) showed that the effect of start T_{skin} was negative, meaning that the higher skin temperature the bats start out at, the shorter their duration was (Table 3.4).

Table 3.2: Mean arousal rate (ºC/min)**,** mean duration of arousal (min), mean T_{skin} at start and stop of measurements (ºC) and mean T_a at start of measurements (ºC) for each individual of *E. nilssonii* measured in field. The number of measurements used for each mean is presented in brackets.

Individual	Arousal rate (ºC/min)	Arousal duration (min)	T_{skin} start (ºC)	T_{skin} stop (ºC)	T_a start (ºC)
Bat1 (N=4)	0.28±0.04	55.0±5.8	20.8±2.6	36.2±2.2	15.5±2.1
Bat2 (N=2)	0.26±0.03	35.0±7.1	24.0±4.5	32.9±5.2	14.5±2.1
Bat3 (N=2)	0.38±0.09	36.0±7.1	22.1±1.5	35.7±4.2	17.5±0.7
Bat4 (N=4)	0.33±0.07	30.0±8.2	25.5±1.5	34.4±1.5	23.5±4.5
Bat5 (N=3)	0.27±0.09	57±31.2	20.1±3.3	33.6±4.3	18.0±2.0
Mean	**0.30±0.05**	**42.6±12.5**	**22.5±2.2**	**34.6±1.4**	**17.8±3.5**

Table 3.3: Estimates, Std. Error and P-value of explanatory variables included in model for arousal rate.

Variable	Estimate	Std. Error	P value
Intercept	0.739	0.15	<0.001
Start T_{skin}	-0.013	0.005	<0.05
Duration	-0.003	0.0009	<0.05

Table 3.4: Estimates, Std. Error and P-value of explanatory variable included in model for duration.

Variable	Estimate	Std. Error	P value
Intercept	122.291	30.081	<0.01
Start T_{skin}	-3.510	1.325	<0.05

3.3 Comparing laboratory and field arousals

The five measured individuals had on average 0.42ºC/min faster arousal rates in the laboratory than in the field (Table 3.5, Figure 3.3). A paired, one-tailed t-test between laboratory arousal rate (mean=0.72ºC/min, SD=0.16) and field arousal rate (mean=0.30ºC/min, SD=0.07) showed that individuals in the laboratory had a significantly faster arousal rate ($p<0.01$). All the bats had a faster rate in the laboratory than in the field. A paired, one-tailed t-test comparing peak arousal rate over 10 minutes (Table 3.6, Figure 3.4) in the laboratory (mean=11.98ºC/10 min, SD=2.58) and in the field (mean=5.84ºC/10 min, SD=1.2) showed a significant difference, with individuals in the laboratory having a faster peak arousal rate ($p<0.01$). The arousals lasted on average 6.9 minutes longer in the field than in the laboratory (Table 3.7, Figure 3.5). A paired, one-tailed t-test comparing arousal duration in laboratory (mean=35.7 min, SD=5.3) and the field (mean=42.6 min, SD=18.78) yielded no significant results, indicating that arousals in the field were not significantly longer than the arousals in the laboratory for the five individuals tested.

Table 3.5: Mean arousal rate (ºC/min) in field and laboratory for the five individuals of *E. nilssonii*. Arousal rate for each arousal was found and a mean for each bat is presented, the number of measurements used for each mean is presented in brackets.

Mean arousal rate

	Laboratory (ºC/min)	Field (ºC/min)
Bat1	0.73 (N=2)	0.28 (N=4)
Bat2	0.72 (N=2)	0.26 (N=2)
Bat3	0.54 (N=1)	0.38 (N=2)
Bat4	0.97 (N=2)	0.33 (N=4)
Bat5	0.63 (N=2)	0.27 (N=3)
Mean	**0.72**	**0.30**
St.dev	**0.16**	**0.07**

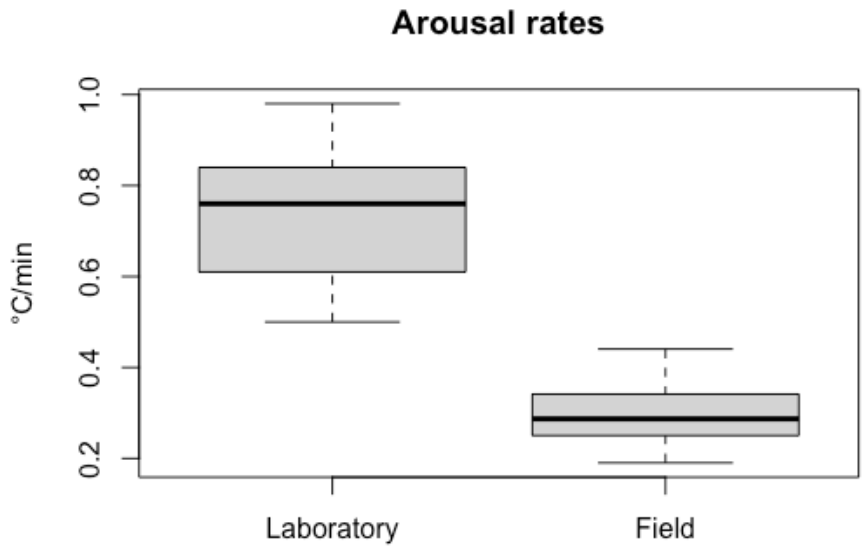

Figure 3.3: Boxplot of arousal rates (ºC/min) for individuals in the laboratory (n=5, N=9) and field (n=5, N=15). The line in the box is the median of the data, while the lower and upper limits of the box is the 25th and 75th percentile, respectively. The whiskers represent the minimum and maximum.

Table 3.6: Mean peak arousal rate over 10 minutes (ºC/10 min) for laboratory and field for the five individuals of *E. nilssonii*. Peak arousal rate over 10 minutes was found for each arousal and a mean for each bat is presented. The number of measurements used for each mean is presented in brackets.

	Peak arousal rate	
	Laboratory (ºC/10 min)	Field (ºC/10 min)
Bat1	12.30 (N=2)	6.76 (N=4)
Bat2	11.90 (N=2)	4.19 (N=2)
Bat3	10.30 (N=1)	6.46 (N=2)
Bat4	15.30 (N=2)	6.84 (N=4)
Bat5	9.25 (N=2)	4.95 (N=3)
Mean	**11.98**	**5.84**
St.dev	**2.58**	**1.2**

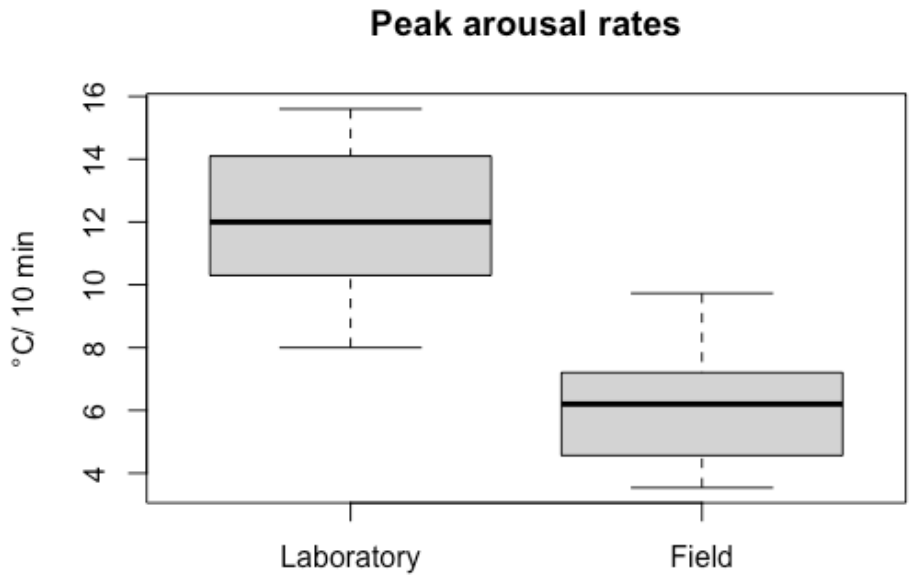

Figure 3.4: Boxplot of peak arousal rates (ºC/10 min) for individuals in the laboratory (n=5, N=9) and field (n=5, N=15). The line in the box is the median of the data, while the lower and upper limits of the box is the 25th and 75th percentile, respectively. The whiskers represent the minimum and maximum.

Table 3.7: Mean duration of arousal (min) in field and laboratory for the five individuals of *E. nilssonii*. Duration of each arousal was found and a mean for each bat is presented. The number of measurements used for each mean is presented in brackets.

Mean duration of arousal

	Laboratory (min)	Field (min)
Bat1	33.0 (N=2)	55.0 (N=4)
Bat2	32.5 (N=2)	35.0 (N=2)
Bat3	40.0 (N=1)	36.0 (N=2)
Bat4	30.0 (N=2)	30.0 (N=4)
Bat5	40.5 (N=2)	57.0 (N=3)
Mean	**35.3**	**42.6**
St.dev	**4.7**	**18.78**

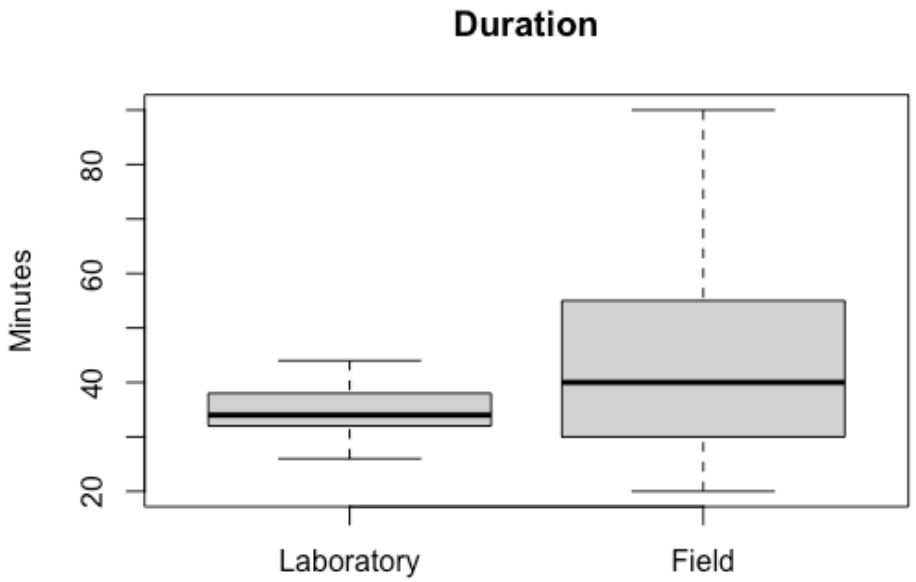

Figure 3.5: Boxplot of duration (min) of arousals for individuals in the laboratory (n=5, N=9) and field (n=5, N=15). The line in the box is the median of the data, while the lower and upper limits of the box is the 25th and 75th percentile, respectively. The whiskers represent the minimum and maximum.

4 Discussion

In this study, skin temperatures of *E. nilssonii* were measured during active arousals from torpor both in the laboratory and field to quantify the arousal process of this high-latitude living species. The aim of the study was to investigate the active arousal in this species to find their capacity for rewarming, and then compare the arousals measured in the laboratory and in the field to see if the process differ when they are in their natural environment. Neither body mass nor duration was found to have any effect on arousal rate in the laboratory, while start T_{skin} and duration affected arousal rate in the field. Arousal rate and peak arousal rate in the laboratory was found to be faster than in the field, which could point to animals not using their full capacity when rewarming spontaneously. This is interesting, as it raises questions about why there are such large differences between the two situations investigated in this study. Duration of the arousals however was not found to be significantly different in the two situations, but start T_{skin} was found to affect the duration in the field arousals.

4.1 Skin temperature measurements of *E. nilssonii* in the laboratory

When the bats started their arousal in the laboratory their T_{skin} was close to the ambient temperature (although there was some variation between the individuals). Some of the arousals showed a clear sigmoid shape when graphed, as Stone and Purvis (1992) noted that they generally do, while others did not (Figure A1). The arousals that did not have this shape lacked the last period of stabilization on the curve, and rather resembled exponential curves. Seeing as the manner of measurement, which was minimally invasive, was to tape the thermocouple to the animal and then leaving them be, it allowed the animals to fly away when they wanted to. Bats finishing the last part of their torpor arousals by generating heat through activation of the flight muscles has already been documented (Willis & Brigham, 2003), and is probably the cause of the non-sigmoid shapes in these arousals, as bats do not need to reach euthermic T_b to fly (O'Farrell & Bradley, 1977; Reeder & Cowles, 1951). It is likely that had the bats not been able to get away from the wire, the last tail of the curve would show this stabilization in all the arousals.

When the animals either stabilized their temperature or flew away from the wire, their average T_{skin} was 34.3±2.1ºC. That is approaching the normothermic T_b of placental mammals, which generally is around 37-38ºC (Hill et al., 2017; Withers et al., 2016). The observed difference could be due to the measurements being skin temperature and not body temperature. T_{skin} has been found to be lower than T_b during normothermia (Willis & Brigham, 2003), and the difference is often around 2ºC (Audet & Thomas, 1996). However, it is perhaps more likely that the average T_{skin} at the end of the measured arousals is underestimated, given that some of the arousals were cut short before reaching the stabilizing period. There were large variations between bats, from 29.0ºC to 40ºC, but generally around 34.3ºC seems to be a temperature at which they are warm enough to fly away.

The mean arousal rate of the bats when measured in the laboratory was 0.72±0.16ºC/min (Table 3.1). Comparing results from this study to the findings of Geiser and Baudinette (1990) it seems that the bats measured here are close to their observation that small mammals (about 10 grams)

rewarmed at about 1ºC/min, but the bats measured here have somewhat slower rates. There is a lot of variation in the arousal rates measured in the laboratory, as there were values as low as 0.5ºC/min and up to 0.98ºC/min (Table A1). It is worth noting that Geiser and Baudinette (1990) used measurements mostly taken at 'room temperature', approximately 20ºC, while the measurements in this study are taken at 10ºC. But considering the results from both Geiser and Baudinette (1990) and Utz et al. (2007) that show that there is negative effect of T_a on arousal rate, it would be expected that the arousal rates obtained in this study should be higher than those obtained in their study. There is also lot of variation in the data from Geiser and Baudinette (1990), for example in the measurements of different bat species there are arousal rates ranging from 0.15ºC/min to 1.58ºC/min. Thus, it looks like it might be difficult to determine what would be a 'normal' rate for a bat as it could depend on species and the environment they are measured in.

The peak arousal rate obtained in the laboratory study was on average 2.0±0.5ºC/min. Comparing this to a study by Menzies et al. (2016) on peak arousal rate, the peak arousal rate obtained in this study is faster than the overall average for all bat species that they presented, which was 1.02ºC/min. Due to some uncertainty in which types of rates Menzies et al. (2016) used in their study, this comparison may be debatable. It was not clear what definition their paper used regarding maximum rewarming rate and whether it is the same used in this study, therefore, the peak arousal rates may have been obtained using different methods and not be directly comparable. In their study Menzies et al. (2016) found that there was an effect of latitude on the arousal rate, where species at higher latitudes had higher arousal rates. Their study included 41 different bat species over a large range of latitudes, but none were found as high as Trondheim (63º). The highest latitude included in their study was 51.09º, which was for *Plecotus auritus*. This species had a peak rewarming rate of 0.70ºC/min and a body mass of 12.0 grams. As *E. nilssonii* is the only bat found far above the Arctic circle (Rydell, 1993; Rydell et al., 1994; Schober & Grimmberger, 1997), it would be expected that it will have a faster rate of rewarming because of the positive effect of latitude Menzies et al. (2016) found in their study. Although it is worth noting that comparison with *P.auritus* should be done with caution as its body mass is slightly higher than the bats in this study, which is proven to have negative effect on arousal rate (Geiser & Baudinette, 1990; McKechine & Wolf, 2004; Stone & Purvis, 1992). The effect of body mass on arousal rate in this study will be discussed in more detail below.

The peak arousal rate of 2.0ºC/min is not the fastest rate found for bats, as Menzies et al. (2016) presents a rate of 2.7ºC/min for *Lasionycteris noctivagans*, a species found at 43.80º with a weight of 10.5 grams. This shows that there are other species that have a quite large thermogenic capacity. Also the findings in this study that peak arousal rate is on average 2.0ºC/min is found when inducing the bats to arouse, which is proven to affect the maximum rewarming rate of the animal (Utz & van Breukelen, 2013). Thus, there must be taken care here as well when comparing the results to Menzies et al. (2016) findings.

Stone and Purvis (1992) point out that mean arousal rate and peak arousal rate are dissimilar ways of describing the arousal. The mean arousal rate describes the whole arousal process, while peak arousal rate is more of a description of maximum thermogenic ability of the individual. The mean arousal rate here was as mentioned 0.72ºC/min while the peak arousal

rate was 2.0ºC/min, which is a difference of approximately 1.28ºC/min. This shows that although the bats overall rate might not be very fast given the different phases of an arousal, they do have quite a large thermogenic capacity to produce heat during just one minute.

The mean duration of the arousals in the laboratory was 35.2±4.7 minutes (Table 3.1). No effect of duration on the arousal rate was found from the five individuals used in this study. This could be because there were not large variations in the durations of the laboratory arousals (the shortest lasted 26 minutes, while the longest lasted 44 minutes). This could be because the bats might perceive the measuring situation as a threatening situation, as they are physically removed from their roosts and handled for a short amount of time. Due to this they might be trying to arouse as fast as possible to get away, and thus their durations are kept somewhat short. They might also not rewarm until complete normothermia, as they on average reached 34.3ºC in the end of their arousal as they in several cases flew away before the arousal was completed.

Many characteristics of mammals are associated with body mass, like for example metabolic rate that scales allometrically with body mass (Hill et al., 2017; Withers et al., 2016). There is also a tendency for heterothermy to be associated with or be influenced by body mass (Cooper & Geiser, 2007; Withers et al., 2016). Having a smaller body enhances heat loss and species that are smaller cool down faster, and the cost of rewarming is also smaller with smaller body masses (Speakman & Thomas, 2003). Earlier literature has further shown that there is a negative correlation between the speed at which an animal rewarms and body mass (Geiser & Baudinette, 1990; McKechine & Wolf, 2004; Stone & Purvis, 1992), meaning that animals with more body mass should rewarm slower.

In this study, there was no relationship between body mass and arousal rate. In Geiser and Baudinette (1990) there was a significant relationship between body mass and rewarming rate for all mammals, although when bats were singled out as a group they did not have a significant relationship. Willis (2008) also did not find a significant relationship for bats. Geiser and Baudinette (1990) hypothesized that this lack of relationship could be due to other factors, e.g., climate being a more important factor for arousal rate of bats than body mass. There is also the fact that having a faster rate of rewarming is less energetically costly, compared to a slow rate (McKechine & Wolf, 2004), and thus smaller mammals may have evolved mechanisms to arouse faster (Altringham, 2011). Having a fast arousal rate reduces the amount of heat that is lost to the environment, which is important for saving energy stores (Utz & van Breukelen, 2012). This should be important for smaller individuals because of their limited capacity to store fat (Dunbar & Tomasi, 2006; Withers et al., 2016). Individuals with more body mass would have more energy reserves, and thus more saved energy for the costly arousals. These might be reasons as to why no relationship was found between body mass and arousal rate in this study.

That these five individuals do not show any relationship between body mass and arousal rate could also possibly be due to the variation in body mass not being that large, ranging from 7.4 to 9.3 grams. It would possibly be more likely to see this relationship when comparing individuals with more variation in body mass. This part of the study also considers just five individuals and a total of nine measurements, which is not a large sample size. It does not mean

that there is not a relationship between body mass and arousal rate, but the relationship is not present in the measurements of these individuals, and finding a relationship likely requires a larger sample size.

The arousals in the laboratory are induced, seeing as the animals are physically removed from their 'roost' to do the measurements. This is an unavoidable consequence of how the measurements were done. Seeing as inducing arousals changes some of the different characteristics of torpor (Utz & van Breukelen, 2013), this needs to be taken into consideration when interpreting the data and also when comparing the laboratory arousals and the field arousals. On another side, Currie, Körtner, et al. (2015) mentioned that as bats generally are small and thus rewarm very quickly naturally, the effects of inducing the arousal might not be as prominent.

4.2 Skin temperature measurements of *E. nilssonii* in the field

The average T_{skin} of the bats when measured in the field was ~4.7ºC above the average T_a recorded at the beginning of their arousal. This could be because the iButtons measuring the ambient temperatures were not inside the bats' roosts and thus are not reflecting the actual environmental temperature they are experiencing. This is a limitation to this study as it is impossible to know the actual temperature of the roosts, as the roost temperature could be higher than the T_a recorded. Utz et al. (2007) points out that T_a has a negative effect on arousal rate, and thus the roost temperature potentially being higher could have effect on the arousal rates found for the field arousals. At the end of the arousal, the individuals had an average T_{skin} of 34.6±1.4ºC, which was almost identical to the average T_{skin} recorded at the lab (34.3±2.1ºC). This is, as mentioned previously, not quite what is 'expected' normothermic values for placentals (Hill et al., 2017; Withers et al., 2016), but the bats could also in this case be using flight to finish rewarming (Willis & Brigham, 2003).

The mean duration of arousals in the field was 42.6±12.5 minutes (Table 3.2), with quite a lot of variation from as short as 20 minutes to as long as 90 minutes (Table A2). The model testing if duration and T_{skin} influenced arousal rate showed a significant negative effect (Table 3.3), meaning that the longer the bats used to arouse and the higher their T_{skin} the slower their arousal rate was. This relationship was not found in the laboratory data, which could be due to the lack of large variation in the durations in the laboratory. The individuals showed a larger variation in duration in the field than in the laboratory, which could be because the arousals in the laboratory are induced while the ones in the field are spontaneous. Body mass could possibly explain some of the variation in arousal duration in the field, as previously mentioned that body mass has been seen to have negative effect on arousal rate (Geiser & Baudinette, 1990; McKechine & Wolf, 2004; Stone & Purvis, 1992). However, body mass was not considered when analysing the field data. This was because the only body mass measurements were from capture and after each measurement in the laboratory, which would not accurately represent their body mass for the field arousals.

T_{skin} when the arousal started was found to have a negative effect on the arousal duration, meaning that when the T_{skin} at the start of the arousal was higher, the arousal duration decreased.

In other words, the animals that started arousing at a higher skin temperature had shorter arousal duration. This matches what Phillips and Heath (2004) found in their study, where the animals that took the longest time to arouse were the ones that started with the lowest T_b. This might be a plausible explanation for why there is so much variation in the field arousal durations. All the individuals in the laboratory started with approximately the same T_{skin}, while in the field the T_{skin} varied a lot due to variations in T_a.

It must be pointed out that T_{skin} in the field was measured every ten minutes, instead of every minute as with the laboratory data. This could to some extent affect the accuracy of the duration of the arousals, as it is only possible to evaluate the temperature every ten minutes and not in between each measurement. It is possible that some of the arousals started or ended 'in between' two of the measurements and thus should be longer or shorter than what was recorded. It also hides possible fluctuations in the T_{skin} happening between two points of measurement, which could affect for example the peak arousal rate calculated over ten minutes.

It also must be mentioned that the assumption in this study is that these field arousals are natural, i.e., they are not induced as the ones in the laboratory is. However, there is no way to verify this assumption, other than that only active arousals (no increase in T_a along with T_{skin}) were chosen for the study. The arousals in the field could be induced by sounds or light near their roosts, as such things have been proven to induce arousals (Thomas, 1995). If induced, some of the aspects of the torpor bout may be altered, such as duration of arousal or the maximum rewarming rate (Utz & van Breukelen, 2013). It is also not known whether the bats are roosting alone or together with other bats. If that was the case, conspecifics could be helping them arouse by reducing heat loss and enabling some passive rewarming (Boratyński et al., 2015; Boyles et al., 2008), although Menzies et al. (2016) found that the size of a colony was not an important predictor of arousal rate.

4.3 Comparing laboratory and field arousals

When the bats were in the laboratory, they started arousing at a much lower average T_{skin} (9.9±0.8ºC) than they did in the field (22.5±2.2ºC). This difference is due to the different environmental conditions between the measurements in the laboratory and field. The temperature in the laboratory was controlled at 10ºC, while in the field the T_a outside of the roosts fluctuated between 13ºC to 26ºC in the different measurements (Table A1 and A2). Analysis showed that T_{skin} had a significant effect on the field arousal duration (Table 3.3). This might be a reason why the field arousals are not found to be significantly longer than the laboratory arousals. It could be that if the bats were starting out at similar temperatures in the laboratory and the field, the durations in the laboratory could have been shorter and there might have been found a difference. Utz and van Breukelen (2013) also found that the duration of the arousals increased when the arousal was induced, meaning that the arousals in the laboratory possibly could be longer due to this. This might also factor into that the durations in the laboratory were not found to be significantly shorter than the field.

Despite dissimilar ambient conditions and captive vs. free ranging, the bats ended up at very similar skin temperatures at the end of their arousals (34.3±2.1ºC vs. 34.6±1.4ºC). This shows

that the animals in the laboratory were not 'satisfied' with a lower stop temperature even though they were forced to arouse and were in a situation which might be perceived as threatening. It is not required to reach such high temperatures to be able to fly either, as it has been shown that several species of bats are capable of flight at T_b as low as 24ºC and some even lower (O'Farrell & Bradley, 1977; Reeder & Cowles, 1951). This is also illustrated by the removed arousal (Figure A3) where the bat flew away well before reaching normothermic values. A possible explanation is, as Willis and Brigham (2003) found, that bats can use their flight musculature to rewarm the rest of the way, ensuring that this energy is not wasted on the arousal process but used on flight instead. There could also be some underestimation, as T_{skin} has been shown to be a bit below T_b during normothermia (Audet & Thomas, 1996; Willis & Brigham, 2003).

When comparing the arousal rates between laboratory arousals and field arousals, a significant difference was found between both arousal rates (ºC/min) and peak arousal rates (ºC/10 min). The average laboratory arousal rate was about 0.42ºC/min faster than field (Table 3.5) and the average laboratory peak arousal rate was about 6.14ºC/10 min faster than field (Table 3.6). The difference that is found to exist between induced and natural arousals could possibly be an explanation for this result (Utz & van Breukelen, 2013). Still, it is quite interesting that there is so much difference between arousals in the same individuals. Considering the data obtained in the laboratory, one can see that the animals have the capacity to arouse rather quickly when the situation requires it. Having rapid arousal rates means that less energy is used, because the arousal happens quickly (McKechine & Wolf, 2004; Stone & Willmer, 1989) and could also be beneficial to deal with external disturbance, like predators or disturbance by human activity (Muise et al., 2018). Bats have also been shown to be able to arouse in response to smoke, suggesting that they are able to arouse in response to environmental cues, like fires, as well (Doty et al., 2018).

Due to the difference in ambient temperature between the measurements in the laboratory and field, it cannot be concluded with certainty that this faster arousal rate in the laboratory just comes from the effect of inducing the arousal. Utz et al. (2007) described a negative relationship between T_a and maximum rewarming rate, meaning that as T_a rises the maximum rewarming rate will decrease. So, animals at higher T_a probably do not need to have such a rapid rate to rewarm as they are helped along by the environmental temperature, as their torpid T_{skin} is higher due to the higher T_a. This negative relationship could also be the cause for the result we are seeing here, where the fastest rates are obtained in the laboratory where the T_a is lowest. It is not possible to point at just one thing that is causing these results, as it likely could be a combination of multiple factors that could not be controlled due to the limitations of this study.

It is quite interesting that when testing models to see which variables influenced the arousal rate, none of the tested variables in the laboratory had any significant effect on arousal rate. When testing the field data, it showed that both start $T_{skin,}$ and duration had an effect on the arousal rate. This could show that the bats are potentially maxing out their capacity for rewarming when they are forced to arouse in the laboratory, and thus they are not significantly affected by these variables. Had arousals in the laboratory been tested over multiple T_a there might have been found a relationship between T_{skin} and the arousal rate here as well.

Even though the true causes of these results cannot be concluded with certainty, the differences between arousal characteristics in the laboratory vs. the field are still of interest. As the arousals in the laboratory both had a faster arousal rate and faster peak arousal rate than in the field, the individuals showed a capacity to rewarm quite rapidly. The difference suggests that the animals possibly are able to regulate their arousal rate to match their 'situation' or environmental conditions, rather than the arousal simply being a process of arousing as fast as possible (Utz & van Breukelen, 2013). It emphasizes that arousing from torpor is not a process in which they rewarm as quickly as possible each time, but that there is some regulation happening that can modify the arousal rate when it is needed.

This raises the question, why is there such a large difference between induced and spontaneous arousals? There must be some downsides of having such a rapid arousal rate, seeing as they are not using this rapid rate every time they arouse. The advantages, as mentioned previously in this study, could be that a rapid arousal could protect them from stressors they are vulnerable to in their torpid state, like predators or fires (Doty et al., 2018; Muise et al., 2018), and in addition that a quick arousal uses less energy (McKechine & Wolf, 2004; Stone & Willmer, 1989). So why would animals have slower rates when arousing spontaneously? There are likely some harmful effects of having rapid rates. There might be an increase in production of reactive oxygen species when the animals arouse, as metabolic rate and T_b increases rapidly (Brown & Staples, 2011; Orr et al., 2009; Toien et al., 2001). There is also some evidence suggesting that during interbout euthermic episodes (the time between two torpor bouts) the reactive oxygen species are being removed to protect animals from oxidative damage (Wei et al., 2018). It is possible that using passive rewarming is helpful for not only reducing the cost of endogenous arousal but also limiting oxidative stress (Currie, Noy, et al., 2015). Spontaneous arousals from torpor often include a passive component (Geiser, 2021), and limiting energy use and oxidative stress could be a reason as to why this is.

4.3.1 Limitations of the comparison between laboratory and field

To improve comparisons between arousal rates measured in the laboratory vs. field, future studies should aim to make the methods as similar as possible. For example, in this study the measuring devices are placed differently on the body, as in the laboratory the thermocouple is placed ventrally while in the field the transmitter is placed dorsally. There have been studies showing that the anterior portion of the body can rewarm faster than the posterior (Currie, Körtner, et al., 2015; Lyman & O'Brien, 1963). This means that there could possibly be a difference between the two measures, but it is also possible that since bats generally are small the difference might not be as pronounced as it could be in larger animals.

Another thing to note is that the peak arousal rate is compared over ten-minute periods, due to the manner of measurement for the field data. It would have been preferable to compare peak arousal rate per minute, as the long measurement intervals could possibly mask fluctuations in T_{skin}. Long measurements intervals could likely underestimate rewarming capacity (Currie, Körtner, et al., 2015). Thus, the peak arousal rate from the field could be underestimated making the difference between laboratory and field even more pronounced, and it would likely be better to aim for similarity in the measurement intervals. In addition, this study also only considers

five individuals, with a total of nine measurements in the laboratory and fifteen measurements in the field. When using small sample sizes the interpretation of the data should be done with care, for example using t-tests with small sample sizes can be difficult (de Winter, 2013). And of course, a small group of individuals could not possibly represent the entire species. Although general conclusions are impossible to draw with certainty, conclusions made with these five individuals are possible. Smaller studies can be used to see if there are interesting findings, and then larger studies can be carried out in the future to draw more general conclusions (Hackshaw, 2008). As such, for future studies an increase in sample size could be a good choice to be able to conclude with more certainty.

5 Conclusion and future directions

5.1 Conclusion

Body mass or duration of arousal did not affect arousal rate or peak arousal rate for the five individuals in the laboratory. When measuring the same five individuals in the field, start T_a did not affect arousal rate nor duration in the field, but duration and start T_{skin} did have negative effect on arousal rate. In addition, T_{skin} had negative effect on duration of arousals in the field. There was no significant difference between durations in the laboratory and field. This study shows that when comparing induced arousals in the laboratory and natural arousals in the field, bats do seem to have a much larger capacity for rewarming than they utilize in the field. This is shown through the significant difference between laboratory and field arousal rate and peak arousal rate, and that body mass and duration did not affect arousal rate in the laboratory as they are seemingly arousing as fast as they can. This must mean that there is a reason as to why the rates are quite different, which is interesting. There are probably some costs or harmful effects of having a rapid rate, and thus the bats cannot do this every time they arouse even though there certainly are pros to having a fast rate as well. There are of course differences between methods of field and laboratory, such as inducing arousal in the laboratory, variation in T_a in the field, and different placement of measuring device, all of which could play some part in the differences observed in this study.

5.2 Future directions

As the results of this study could be influenced by the induction of arousal in the lab, avoiding the induction of arousal could be an avenue for further research. However, it could potentially be difficult to create an environment where the bats would want to act as in nature while in captivity. If a good study design, allowing for the bats to arouse spontaneously in the laboratory, could be devised it would be interesting to see if the differences seen in this study are equally pronounced when the animal is not forced to arouse. The additional benefit of doing this in the laboratory is that one would still be able to control the T_a and could thus get spontaneous arousals at lower temperatures than those obtained in this study and more variation in temperature in the laboratory as well. Another possibility could be to try to induce the arousals in the field as well, and then compare to see if induction in nature would have the same types of effects as the ones observed in the laboratory or if the field arousals still are not as rapid.

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Appendix

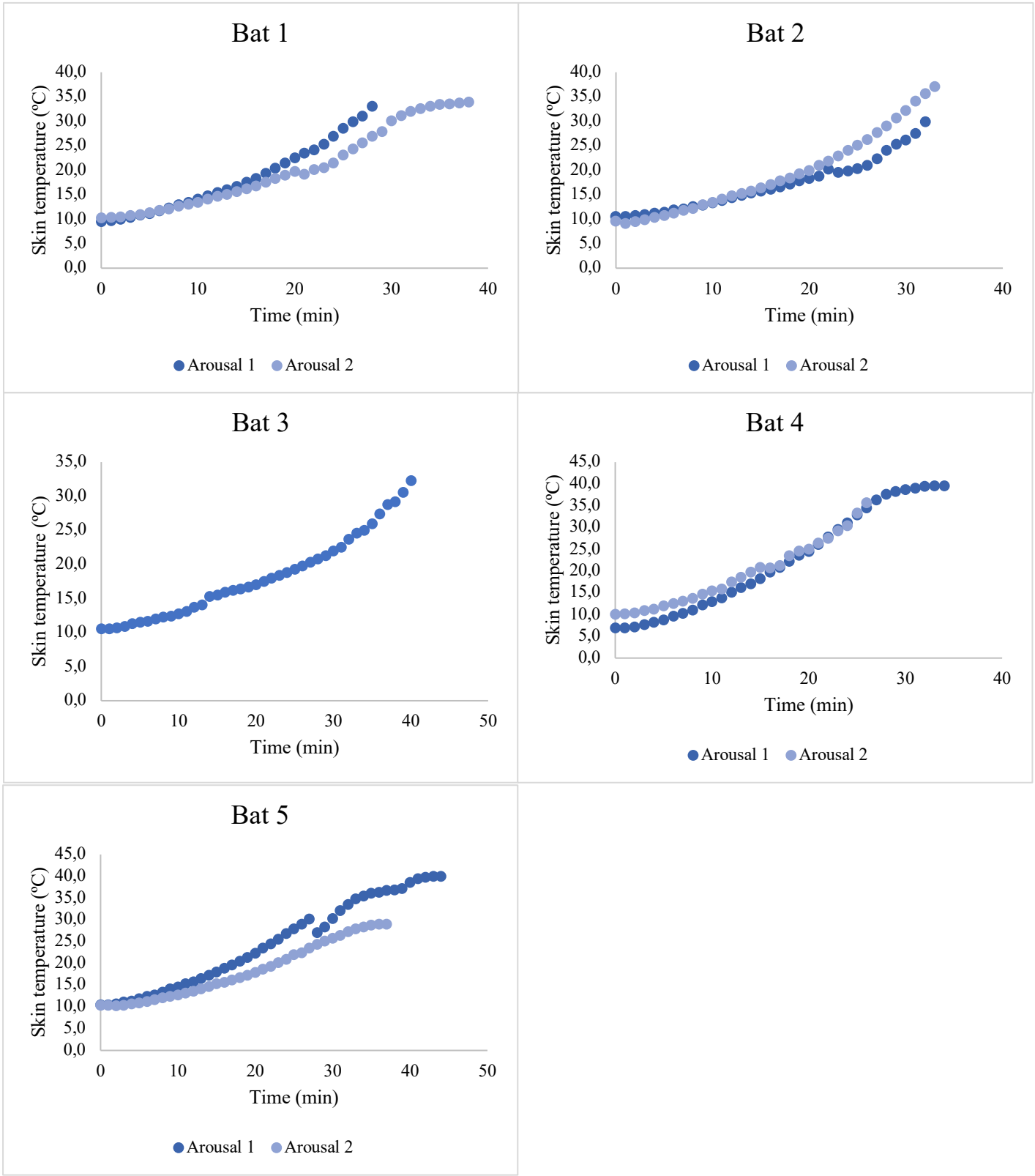


Figure A1: Both arousals for all five individuals in the laboratory (with exception of Bat 3 where one of the arousals were removed from the dataset).

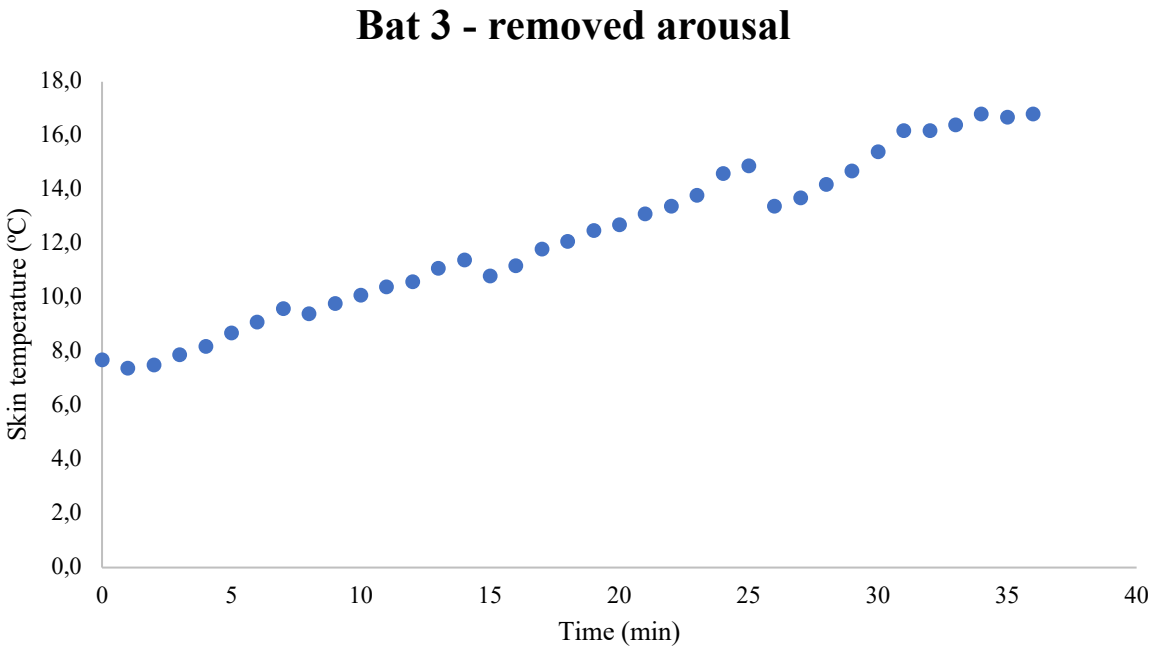


Figure A2: Removed arousal from Bat3 (laboratory).

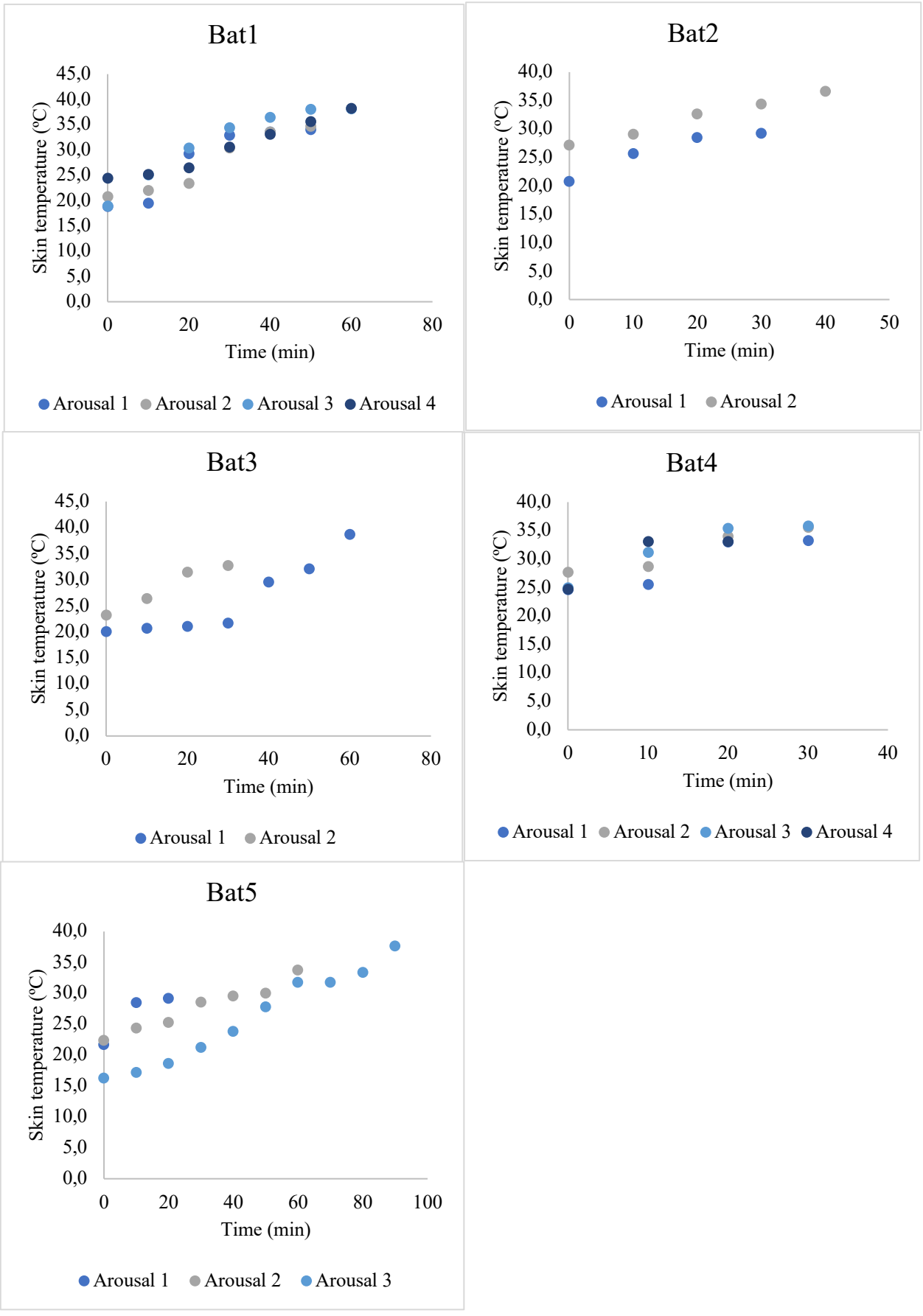


Figure A3: All arousals for each bat in the field.

Table A1: All values for each arousal for each individual in the laboratory.

	Bat1	**Bat2**	**Bat3**	**Bat4**	**Bat5**
Body mass (g)	8.8	7.4	8.2	7.8	8.1
	9.3	7.7	n/a	7.7	7.9
Arousal rate (ºC/min)	0.84	0.61	0.54	0.96	0.76
	0.62	0.83	n/a	0.98	0.50
Duration (min)	28	32	40	34	44
	38	33	n/a	26	37
Peak arousal rate (ºC/min)	2.0	2.4	1.7	1.9	1.9
	2.2	1.9	n/a	2.9	1.1
Peak arousal rate (ºC/10 min)	12.6	9.7	10.3	15.6	10.5
	12.0	14.1	n/a	15.0	8.0
T_{skin} start (ºC)	9.5	10.5	10.6	7.0	10.5
	10.2	9.6	n/a	10.1	10.4
T_{skin} stop (ºC)	33.0	29.9	32.3	39.6	40.0
	33.9	37.0	n/a	35.7	29.0
T_a (ºC)	10.6	9.9	10.0	10.5	10.0
	10.0	10.0	n/a	10.0	10.0

Table A2: All values for each arousal for each individual in the field.

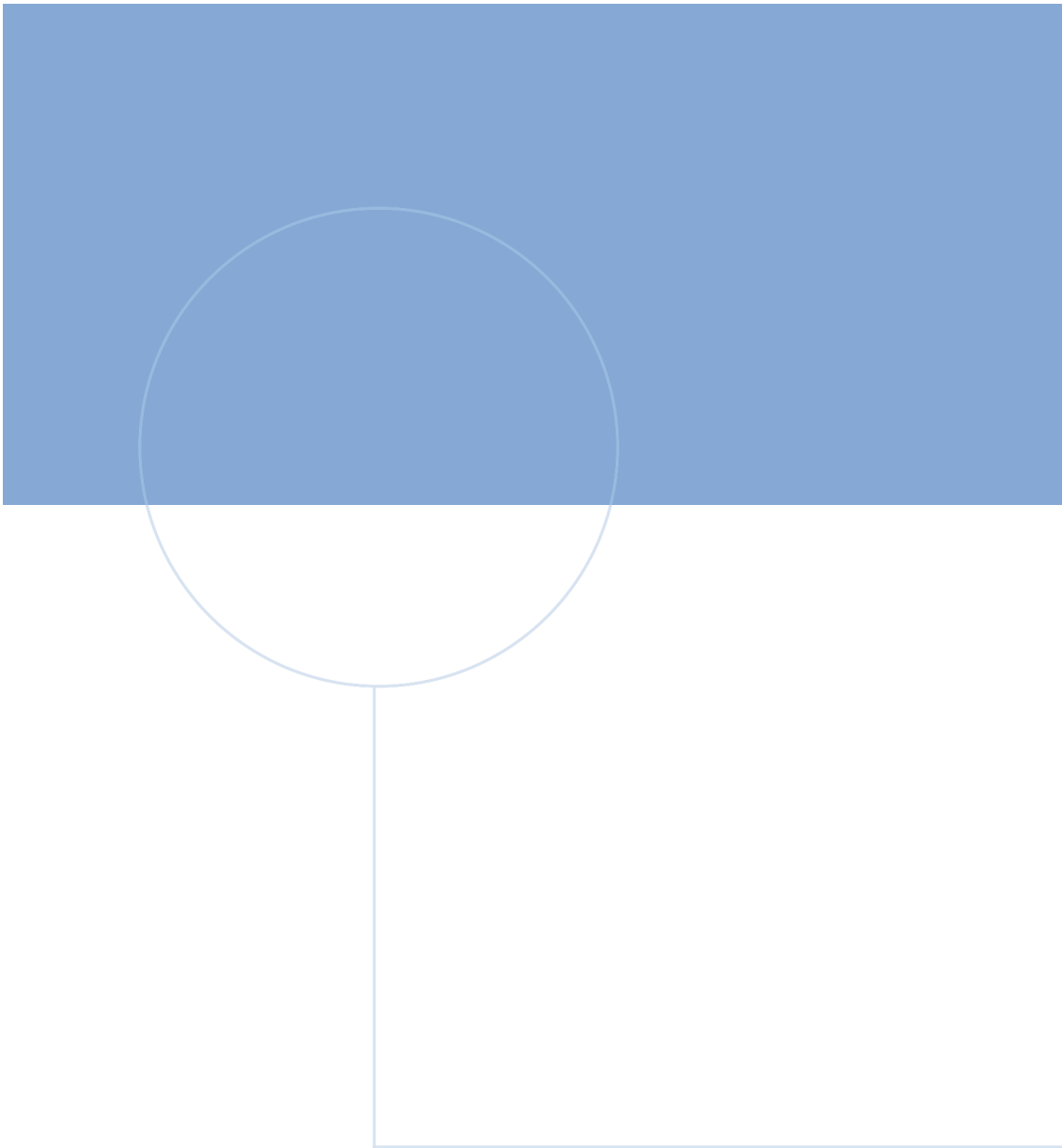
	Bat1	Bat2	Bat3	Bat4	Bat5
Arousal rate (ºC/min)	0.30	0.28	0.44	0.29	0.37
	0.28	0.24	0.32	0.26	0.19
	0.32	n/a	n/a	0.36	0.24
	0.23	n/a	n/a	0.42	n/a
Duration (min)	50	30	40	40	20
	50	40	30	30	60
	60	n/a	n/a	30	90
	60	n/a	n/a	20	n/a
Peak arousal rate (ºC/ 10 min)	9.73	4.84	7.88	6.26	6.80
	6.93	3.54	5.05	7.48	4.29
	6.20	n/a	n/a	5.28	3.76
	4.18	n/a	n/a	8.33	n/a
T_{skin} start (ºC)	18.90	20.8	21.0	24.6	21.7
	20.8	27.1	23.2	27.7	22.4
	18.9	n/a	n/a	24.9	16.3
	24.4	n/a	n/a	24.7	n/a
T_{skin} stop (ºC)	34.0	29.2	38.7	33.2	29.2
	34.6	36.6	32.7	35.5	33.8
	38.1	n/a	n/a	35.8	37.7
	38.2	n/a	n/a	33.1	n/a
T_a (ºC)	13	13	18	27	16
	18	16	17	24	20
	16	n/a	n/a	26	18
	15	n/a	n/a	17	n/a

Table A3: Ranking of the models tested for field arousal rate.

Rank	Model	df	AIC	ΔAIC
1	Arousal rate ~ Start T_{skin} + Duration	4	-41.34	0
2	Arousal rate ~ Start T_a + Start T_{skin} + Duration	5	-40.32	1.02
3	Arousal rate ~ Duration	3	-37.72	3.62
4	Arousal rate ~ Start T_a + Duration	4	-35.83	5.51
5	Arousal rate ~ Start T_{skin}	3	-31.80	9.54
6	Arousal rate ~ Start T_a	3	-31.78	9.56
7	Arousal rate ~ Start T_a + Start T_{skin}	4	-29.80	11.54

Table A4: Ranking of the models tested for duration of field arousals.

Rank	Model	df	AIC	ΔAIC
1	Duration ~ Start T_{skin}	3	129.03	0
2	Duration ~ Start T_a + Start T_{skin}	4	130.42	1.39
3	Duration ~ Start T_a	3	135.26	6.23



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