

Core body temperature cycles in captive Svalbard rock ptarmigan (*Lagopus muta hyperborea*)

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Daniel Appenroth

BIO-3950 Master thesis in Biology, Arctic Animal Physiology
August 2016



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Cover photo by Stig Brøndbo

Photo of a male Svalbard ptarmigan (*Lagopus muta hyperborea*)

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Daniel Appenroth
Tromsø, August 2016

Abstract

The Svalbard rock ptarmigan (*Lagopus muta hyperborea*) is an arctic animal and lives as such in constant light and constant darkness for parts of the year. They are therefore an interesting subject for chronobiologists and have been thoroughly studied in terms of their biological rhythms. One rhythmic physiological factor, however, has received little attention in former studies: oscillation in core body temperatures (T_b).

In this experiment first-year birds were divided into two groups. While one group stayed permanently under a short photoperiod with 6 hours of light (SP), the other group went from SP to a long photoperiod with 16 hours of light (LP) and subsequently into constant light (LL). All birds under SP and LP showed clear cycles in T_b with high T_b in their active-phase during light phase and with decreased T_b during their rest-phase during dark. Furthermore, they showed rise in T_b in anticipation to light. Birds under LL, on the other hand, did not show convincing evidence for a sustained oscillation.

Besides T_b , locomotor activity was recorded in male birds. Activity and T_b correlated in all studied birds under SP, LP and with one exception under LL. It was also shown that the anticipatory rise in T_b was, in some recorded cases, phase advanced to activity, indicating that the T_b cycle is an actively controlled mechanism and not merely a side effect of activity cycles.

Furthermore, it was shown that birds constantly held under SP showed a reduction in rest-phase T_b over 52 days. Increased heat loss during the rest-phase due to dropping ambient temperature and decreasing body mass are possible explanations for this observation.

Keywords: *Lagopus muta hyperborea*, core body temperature, biological rhythms, locomotor activity, anticipation.

Table of Contents

Acknowledgements	i
Abstract	iii
1 Introduction	1
1.1 Biological rhythms.....	1
1.1.1 Circadian rhythms.....	2
1.1.2 Circannual rhythms.....	2
1.2 Arctic environments.....	3
1.2.1 Daily cycles in arctic environments.....	4
1.2.2 Annual cycles in arctic environments.....	5
1.3 Svalbard rock ptarmigan.....	6
1.3.1 Daily cycles in Svalbard ptarmigan.....	6
1.3.2 Annual cycles in Svalbard ptarmigan.....	7
1.4 Body temperature cycle.....	8
1.4.1 Daily cycles in body temperature.....	8
1.4.2 Annual cycles in body temperature.....	9
1.5 Aim of the study.....	10
2 Material and Methods	11
2.1 Housing.....	11
2.2 Light treatment.....	12
2.3 Body mass, plumage and blood sampling.....	13
2.4 Surgical implantation of iButton temperature loggers.....	13
2.5 iButton calibration.....	15
2.6 Illustration of recorded body temperature.....	15
2.7 Activity recording.....	16
2.8 Statistical analyses.....	17
2.8.1) Analyses of body mass, body temperature and activity.....	17
2.8.2) Analyses of periods for body temperature and activity oscillation.....	18
2.8.3) Analyses of changes in body temperature cycles over several weeks.....	18
3 Results	19
3.1 Body mass and plumage.....	19
3.2 Body temperature cycles under different light-dark regimes.....	20
3.2.1 Representative bird undergoing all three light-dark regimes.....	20
3.2.2 Mid-day body temperature vs. mid-night body temperature.....	22

3.2.3 <i>Individual hourly profiles of body temperature oscillation</i>	23
3.3 Correlation between activity and body temperature.....	25
3.4 Body temperature cycles during sickness.....	27
3.5 Body temperature cycles under constant short photoperiod.....	29
3.5.1 <i>Representative bird under constant short photoperiod</i>	29
3.5.2 <i>Change in rest-phase body temperature under constant short photoperiod</i>	31
4 Discussion	34
4.1 Body mass	34
4.2 Daily body temperature cycles	35
4.2.1 <i>Body temperature cycles under light-dark regimes</i>	35
4.2.2 <i>Body temperature cycles under constant light</i>	37
4.2.3 <i>Activity and body temperature</i>	38
4.3 Change in rest-phase body temperature under constant short photoperiod.....	39
4.3.1 <i>Ambient temperature and body mass in connection with rest-phase body temperature</i>	39
4.3.2 <i>Potential endocrine basis for change in rest-phase body temperature</i>	40
4.4 The case of the sick bird.....	41
5 Conclusion	41
References	42
Appendix	I

1 Introduction

1.1 Biological rhythms

The Earth is moving. Rotation around its own axis results in day and night whereas rotation around the Sun in combination with the Earth axis tilt of ca. 23.5° leads to seasons (**Figure 1**). This results in cycles of environmental conditions (temperature, humidity and light exposure) and variation in resources (food quality and quantity) on daily and annual basis. It is therefore beneficial for organisms living in this cyclic surrounding to be rhythmic. Biological rhythms are cyclic changes in behaviour and physiology. They are the means by which organisms keep in synchrony with the environment and help to anticipate rhythmic changes in nature. Biological rhythms can be categorized into different periods, circadian and circannual rhythms being the most studied.

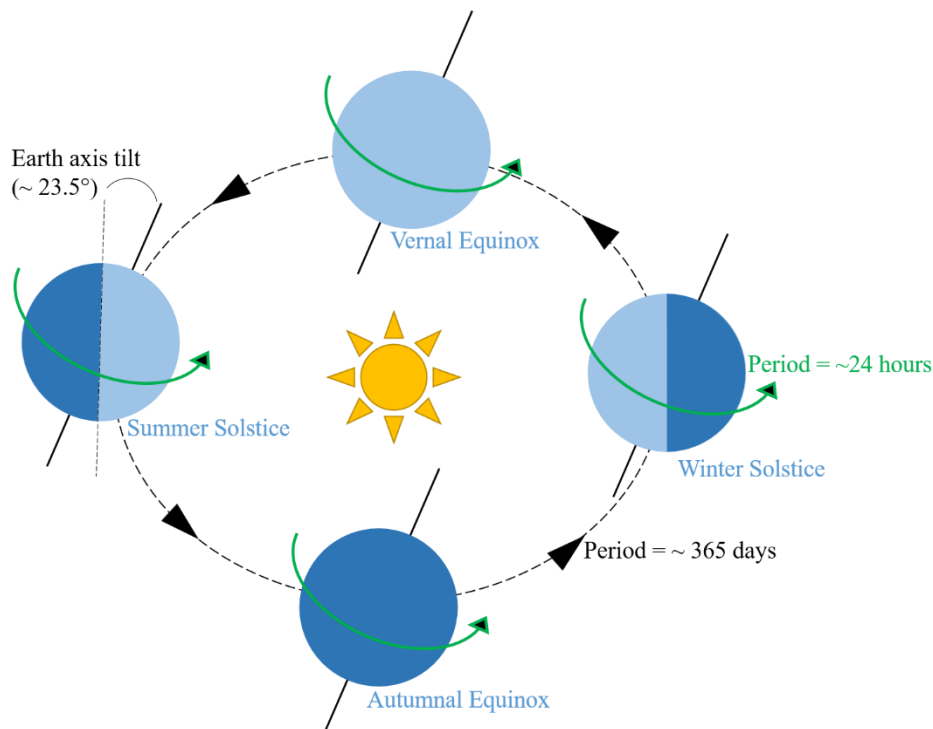


Figure 1. Earth rotation and revolution around the Sun. The Earth rotates around its own tilted axis with a period of ca. 24 hours whereas the cycle around the Sun takes ca. 365 days.

1.1.1 Circadian rhythms

Circadian rhythms describe cycles with a period of ca. 24 hours and are expressed by biochemical pacemakers, called circadian clocks. Circadian clocks can receive input from the rhythmic environment and are able to use this input to stay entrained to its environment. Environmental cues that entrain biological clocks are called *zeitgebers* (deriving from German word for time giver). *Zeitgebers*, in circadian sense, can be any external cues with a daily cycle, e.g. daily ambient temperature oscillation and food availability. However, the most potent and reliable *zeitgeber* is the recurring daily pattern of day and night. The entrained circadian clock is then expressing a rhythmic output in the form of cyclic physiology and behaviour.

Furthermore, circadian rhythms must be endogenous, meaning that they are able to persist in constant environment, i.e. in absence of entraining cues. The endogenous basis distinguishes the circadian rhythm from exogenously driven passive responses. Exogenously driven rhythms would not persist in absence of *zeitgeber*, e.g. in constant light or darkness, whereas an endogenous rhythm would be still oscillating with a period close to 24 hours (free-running period). Circadian rhythms must also be temperature compensated in order to keep the endogenously generated period constant regardless of ambient temperature (T_a) fluctuations.

Circadian rhythms can help organisms to anticipate daily events, e.g. presence of predators or food availability and are therefore thought to be beneficial for survival. Direct tests of this are limited, however. Mice (*Mus musculus domesticus*) with homozygous mutation in a gene that is involved in generating circadian rhythms (casein kinase 1 ϵ), showed reduced survival rates, compared to wild type mice in an outdoor enclosure (Spoelstra, Wikelski et al. 2016). Wild type mice showed free-running periods closer to 24 hours, while mutants displayed a shorter free-running period for activity. This led to hypothesis that circadian rhythms with endogenous period close to 24 hours are favoured by selection. In addition, lesion studies in free-living chipmunks (*Tamias striatus*) in the Appalachian mountain range showed that animals with lesion in the Suprachiasmatic Nucleus, location of the mammalian master clock (pacemaker which dictates its rhythm to other peripheral clocks) were more vulnerable to predation than control animals (DeCoursey, Walker et al. 2000).

1.1.2 Circannual rhythms

Circannual rhythms describe endogenous biological rhythms with a period of ca. 365 days. Their function lies in anticipation of seasonal changes in the environment and adjustment of an

animal's behaviour and physiology in preparation for upcoming seasons. Therefore, costly life events, like reproduction, moult and migration can be timed to match episodes with favourable environmental conditions. As circadian rhythms, true circannual rhythms have to be endogenous and have to have a mean to be entrained by the environment. In non-equatorial zones, the annual change in day-length (**Figure 2**), which results from the Earth's axial tilt and its revolution around the Sun, is the most potent *zeitgeber* to which circannual rhythms entrain.

As mentioned above, circannual rhythms must be endogenous. Tropical birds, like African stonechats (*Saxicola torquata axillaris*), provide examples for circannual rhythms with strong endogenous basis. Despite being held in constant equatorial photoperiod, African stonechats show rhythmicity in testicular width and moult with a circannual period of ca. 9 month, even for a duration of more than 12 years (Gwinner 1996).

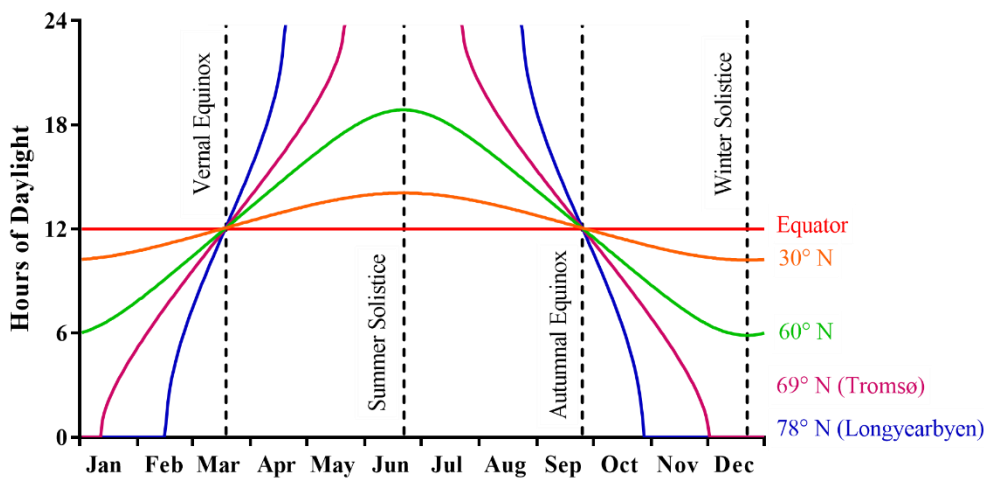


Figure 2. Annual variation in photoperiod over several latitudes north of the Equator.

1.2 Arctic environments

In the middle of the Arctic Ocean, where the axis of the Earth rotation meets the surface, one finds the North Pole surrounded by the Arctic region. Geographically, the Arctic is the area north of the Arctic Circle, which runs $66^{\circ} 33'$ north from the Equator. Yet, a biologically more

relevant characterisation defines the Arctic as region above the July 10°C isotherm. This describes also the climate in the Arctic. It means that summer temperatures do not exceed the 10°C mark. In the winter, temperatures can go as low as -40 and even to -60 °C, depending on the region. In general, the climate can be described as cold, dry and windy.

The Arctic (in the following defined as the area above the July 10° C isotherm) includes, besides the Arctic Ocean and its ice pack, northern parts of the Pacific and Atlantic Ocean. Greenland, Svalbard, most of Iceland and northern regions of Canada, Alaska, Russia and Norway make up the terrestrial portion of the Arctic Region. Vegetation of these terrestrial regions, if not ice covered or within the sparsely lichen and moss covered arctic desert, are dominated by erected shrublands, graminoid tundras and wetlands (Blix 2005, Walker, Raynolds et al. 2005). Trees on the other hand are rare; in fact the tree line is correlating well with the July 10° C isotherm.

Climate and vegetation might characterise the Arctic but are not exclusive to this area, since these descriptions are also met by high altitude ecosystems at lower latitudes. The unique feature of the frigid zones is the dramatic annual change in day-length. Latitudes above the Arctic Circle challenge organisms with periods of the year when the Sun does not set (polar day) and when it remains below the horizon (polar night). The resulting absence of daily photoperiodic cues at these phases of the year makes arctic species an interesting subject for studies of circadian and circannual rhythms.

As already mentioned true circadian and circannual rhythms require entrainment, must be temperature compensated and must be endogenous, meaning that they sustain their oscillation in constant conditions. In the following chapters, the terms circadian or circannual rhythm were, therefore, carefully applied to daily and annual cycles that met these criteria.

1.2.1 Daily cycles in arctic environments

Primary research has led to the speculation that arctic species may have circadian clocks with rather weak endogenous basis. Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and Svalbard ptarmigan (*Lagopus muta hyperborea*), both being permanent inhabitants of the Arctic, show diurnal activity in presence of a light-dark cycle but abolish their daily cycle of locomotion in constant conditions (Stokkan, Sharp et al. 1986, Reierth and Stokkan 1998, van Oort, Tyler et al. 2005). This has led to the speculation that in arctic animals, daily cycles during the portions of the year with daily light-dark cycles might just be passive responses to the environment.

However, other studies suggested that this may not always be the case. Bumblebees (*Bombus terrestris*) in northern Finland (69° N) showed robust diurnal foraging rhythms in the constant light of the arctic summer (Stelzer and Chittka 2010) and arctic ground squirrels (*Urocitellus parryii*) in northern Alaska (68° N) display robust circadian body temperature rhythms during 6 weeks of the polar day (Williams, Barnes et al. 2011). Furthermore migratory birds, which breed in Barrow, Alaska (71° N) under the midnight Sun, can show, depending on the species and breeding state, circadian locomotor rhythms of 24 hours (Steiger, Valcu et al. 2013).

This leads to the view that daily cycles have, depending on the species, an endogenous basis and may have some adaptive value, even in the constant environment of the Arctic during the polar day and polar night. But it is also possible that these animals are able to use other environmental cues as *zeitgeber* in absence of light-dark cycles, e.g. position and colour temperature of the Sun (Krüll 1976a, Krüll 1976b). Krüll (1976a) showed that snow buntings breeding in Svalbard (*Plectrophenax nivalis*) during the polar day were able to entrain their feeding activity to these *zeitgeber*.

1.2.2 Annual cycles in arctic environments

Circannual time keeping is believed to be a strong feature in arctic animals. Winter challenges arctic species with long nights, low temperatures and decreased food availability. This extreme change in environmental conditions and resources make it necessary for an organism to correctly anticipate upcoming seasons and prepare its physiology and behaviour accordingly.

The necessity of correct timing is highlighted by studies of seasonal mismatch. Due to global warming (resulting in earlier summers and later winters in northern latitudes) timing of food availability can shift. However, annual life events in non-equatorial animals are still timed by changing photoperiod, which can lead to mismatches between life events and the favourable environmental conditions.

Migrating pied flycatcher populations (*Ficedula hypoleuca*), for example, decline in some areas, because they fail to breed during the time of high food abundance (Both, Bouwhuis et al. 2006). Therefore, while the time of food abundance shifts, the birds are not able to account for that, leaving their new-born chicks struggling with low food quantity. Studied examples for the Arctic are muskoxen (*Ovibos moschatus*) and caribou (*Rangifer tarandus*) in Greenland. Their long gestation make it necessary to time conception correctly, so that birth occurs in a time of the year with favourable conditions and with high food abundance for the new-borns. Again mismatches of

birth and peaks in food abundance are leading to fitness decline in form of reduced calf production (Kerby and Post 2013).

These examples show on one hand that strict timing by photoperiod can have a negative impact when the environment rhythm changes, but it also shows the importance of correct timing.

1.3 Svalbard rock ptarmigan

One arctic species, which is a permanently resident of the Arctic region, is the Svalbard rock ptarmigan (*Lagopus mutus hyperborean*; **Figure 3**). They live, as their name suggests, on the Svalbard archipelago and stay there all year around. The islands range from 74° to 81° N and challenge its inhabitants, besides the climate, with intense day-length variation. From mid-November to February the Sun remains at least 6° below the horizon (border of civil twilight) resulting in the polar night, whereas from April until September the Sun does not set, resulting in the polar day. Svalbard is therefore a terrestrial environment with dramatic changes in day-length and where the primary *zeitgeber* for circadian rhythms, the light-dark cycle, is absent for parts of the year.



Figure 3. Captive male Svalbard ptarmigan.

1.3.1 Daily cycles in Svalbard ptarmigan

Svalbard Ptarmigan have diurnal locomotor activity in the presence of a light-dark cycle. Their onset and end of locomotor activity was shown to be correlated with the time of civil twilight (Reierth and Stokkan 1998). In constant light and in constant dark they display continuous intermittent feeding and locomotion with no apparent period (Stokkan, Mortensen et al. 1986, Reierth and Stokkan 1998), which suggests that in Svalbard ptarmigan daily locomotor activity and feeding rhythms are a passive response to the environment. This leads to the speculation that Svalbard ptarmigan have a circadian clock with a weak endogenous basis. However, circadian studies in Svalbard ptarmigan are only based on different forms of activity (i.e. just one output

factor for potential circadian clocks) and do not necessarily represent persistence or loss of circadian rhythm at a core level.

1.3.2 Annual cycles in Svalbard ptarmigan

Svalbard ptarmigan are strong seasonal animals. In anticipation of the arctic winter, Svalbard ptarmigan change to a highly insulative winter plumage, adjust their digestive tracts to digest shrubs and reduce their resting metabolic rate (Stokkan 1992). Furthermore there is an apparent deposit of a substantial amount of fat, functioning as an energy reservoir (Stokkan, Lindgård et al. 1995) and possibly increasing insulation (Mortensen and Blix 1986).

The timing of these physiological adjustments is of vital importance and achieved by changes in photoperiod. Change into winter plumage, for example, is triggered by exposure to decreasing day-length (Stokkan, Sharp et al. 1986).

Body mass is also under photoperiodic control. In natural conditions it increases rapidly from August until November and decreases from mid-November until April (Stokkan, Mortensen et al. 1986). Interestingly food intake changes run out of phase with the cycle in body mass. It was shown that food intake drops by ca. 33 % during the same time body mass increases and doubles during the time body mass declines (Stokkan, Mortensen et al. 1986). That suggests that the body mass cycle is not just a passive response to changing food availability. Furthermore it was shown that captive Svalbard ptarmigan under constant T_a and under constant short day-length maintained a constant high body mass, which decreased rapidly when confronted with long photoperiod (Stokkan, Lindgård et al. 1995). In the same study, captive birds under natural light-environment were starved mid-winter, thus lost body mass, but recovered, after re-feeding, to the same point shown by control birds. This suggests that the annual change in body mass is an actively regulated physiological feature.

This introduces the idea that Svalbard ptarmigan possess a strong circannual calendar, which uses changing photoperiod to accomplish physiological and behavioural changes in anticipation of upcoming seasons.

1.4 Body temperature cycle

The afore mentioned studies of daily and annual cycles in Svalbard ptarmigan have yielded a general understanding of biological rhythms in this species. However, one physiological factor, which is also known to be under circadian, or circannual control in other species, core body temperature (T_b), has so far not received much attention in the Svalbard ptarmigan.

1.4.1 Daily cycles in body temperature

It is known that T_b oscillates on a daily basis in numerous endothermic species, including all birds so far studied (Prinzinger, Pressmar et al. 1991). In general, birds have a T_b of ca. 41°C during their active-phase and ca. 38.5°C during their rest-phase, i.e. 1.9 to 2.5 °C higher than in mammals (Prinzinger, Pressmar et al. 1991).

Early studies under constant light conditions also revealed persistent T_b cycles in numerous species, including birds, indicative of circadian control of T_b (for review see, Refinetti and Menaker 1992). This persistent T_b cycle was also studied in Willow ptarmigan (*Lagopus lagopus*). It was shown that when a bird is transferred from a light-dark environment into constant darkness for two days, it keeps its T_b rhythm according to the prior days (Blix 2005); longer recordings are lacking, however. Direct evidence for the endogenous basis of T_b oscillation in mammals was provided by *tau* mutant golden hamsters (*Mesocricetus auratus*) which displayed an activity rhythm with a period of 20 hours (Ralph and Menaker 1988). The *tau* mutation also alters the period of the T_b oscillation to 20 hours (Refinetti and Menaker 1992).

It could be argued that the oscillation of T_b is a consequence of elevated locomotor activity, but studies showed that the rise in T_b is often phase advanced relative to the rise in activity, for example in humans (Aschoff, Gerecke et al. 1967). It was also possible to shift the relative phasing of T_b oscillation and locomotor activity, for example in rats by food and water deprivation (Bolles and Duncan 1969, Krieger 1974). Furthermore it was shown than human subjects instructed to stay at rest, show still a daily cycle in T_b (Marotte and Timbal 1981). Other studies showed that the T_b is actually a consequence of daily cycles in heat production and heat loss. The cycle in heat production was shown to be phase advanced while heat loss was phase delayed to the T_b cycle in laboratory rats (*Rattus norvegicus*) (Refinetti 2003). Similar results were found in pigeons (*Columba livia domestica*), where it was shown the T_b cycle is linked to active thermoregulation

in form of heat production and heat loss (Graf 1980). Daily T_b cycles can therefore be regarded as a distinct output of the circadian clock.

This leads to the question of the possible function daily T_b cycles have. Controlled heterothermic responses, like hibernation, daily torpor and rest-phase hypothermia are known to save the animal a considerable amount of energy by lowering T_b and metabolic expenditure. Rest-phase hypothermia and daily torpor are forms of hypothermia, which can be expressed on a daily basis and were found in a range of birds, including bird which overwinter in central Norway (Reinertsen and Haftorn 1986). Minimum T_b , reached during these forms of hypothermia, can go as far down as 4.3 °C in birds (McKechnie and Lovegrove 2002). Normal circadian oscillation in T_b works on a smaller scale with a reduction in T_b by just 2.5 to 1.3 °C (Prinzinger, Pressmar et al. 1991). Still, they might profit from the same principle: the reduction in T_b and metabolic heat production during rest to decrease energy expenditure.

Another function of daily T_b oscillation might be the synchronisation of peripheral tissue with the master clock, an idea which has been thoroughly studied in mammals. The master oscillator of mammals, the suprachiasmatic nucleus (SCN) is entrained by the environment and controls other biochemical oscillators in peripheral tissues. This includes, besides neural and hormonal signals, control through temperature change (Mohawk, Green et al. 2012). Oscillators in peripheral tissues including in the liver, lung and kidney are sensitive to temperature changes in the range of daily mammalian T_b oscillation while the SCN is not (Buhr, Yoo et al. 2010). Therefore, the master oscillator can employ the daily T_b cycle to entrain peripheral tissues, which are sensitive to temperature changes. It is reasonable to think that a similar mechanism may operate in birds, but I am unaware of any studies addressing this issue.

1.4.2 Annual cycles in body temperature

Besides circadian cycles in T_b there are some reports of circannual variations in birds. Male Japanese quail (*Coturnix japonica*) show differences in their active-phase T_b throughout the year: birds kept under constant T_a and natural photoperiod variation showed higher active-phase T_b in winter than in summer (Feuerbacher and Prinzinger 1981). Furthermore gray jays (*Perisoreus canadensis*), studied under field conditions showed higher differences in T_b between rest- and active-phase in winter than in summer (Veghte 1964).

These differences were argued to be connected with the sexual activity of the birds and the effect of the sex-hormone testosterone. Castrated male Japanese quail showed higher active-phase

T_b and lower rest-phase T_b than control birds. Both, intact and castrated birds had similar body masses. Testosterone substitution abolished this difference by lowering active-phase T_b and increasing rest-phase T_b in castrates. It was therefore postulated that winter bird (with low testosterone levels) decrease their rest-phase T_b to reduce the energy consumption and increase active-phase T_b to allow maximal activity during the short days. (Hänssler and Prinzinger 1979, Feuerbacher and Prinzinger 1981).

1.5 Aim of the study

In the following experiments I aimed to investigate T_b oscillation in Svalbard ptarmigan to get more insight in the rhythmicity of this arctic species on a daily and annual time scale. Cycles in T_b also provides an interesting and unique physiological feature, since it combines two important measures of how these animals survive in the Arctic: the study of thermoregulatory processes and the study of biological timing.

Based on the concept that as a high arctic species, Svalbard ptarmigan have a weak circadian system, I hypothesized that their daily T_b cycle has a weak endogenous basis and is a passive response to the environment. That means, that Svalbard ptarmigan would show a clear daily rhythmicity under a light-dark cycle but would lose this rhythm in constant light conditions, with no clear periodicity. In addition, I hypothesized that T_b cycle are not merely caused by daily activity cycles, but that they are distinct physiological feature.

Secondly, on the basis that arctic animals seem to have pronounced circannual time measurement and that seasonal changes in T_b were observed in other seasonal birds, I hypothesized that seasonal changes in T_b would also be observed in Svalbard ptarmigan.

To test these hypotheses, T_b was recorded in captive Svalbard ptarmigan under three different photoperiods: short photoperiod (SP), long photoperiod (LP) and constant light (LL). Furthermore locomotor activity, body mass and T_a were measured simultaneously, to evaluate potential influences on the T_b cycle.

2 Material and Methods

2.1 Housing

Svalbard ptarmigan eggs, laid by captive females at Tromsø Universtiy (69° 39' N, 18° 57' O), were hatched and raised in outdoor cages. Nine to ten weeks old birds were transferred into indoor cages with *ad libitum* access to standardized protein food (Norgesfor, Ref. No.: OK2400 070316) and water. The birds were divided into two groups in separate rooms, with nine individuals per room (2 older birds supplemented the stock, but were not used in experimentation). Each room contained three racks, each containing three cages in vertical orientation (**Figure 4**). The ambient temperature was kept constant between 3 and 7°C throughout the experiment.

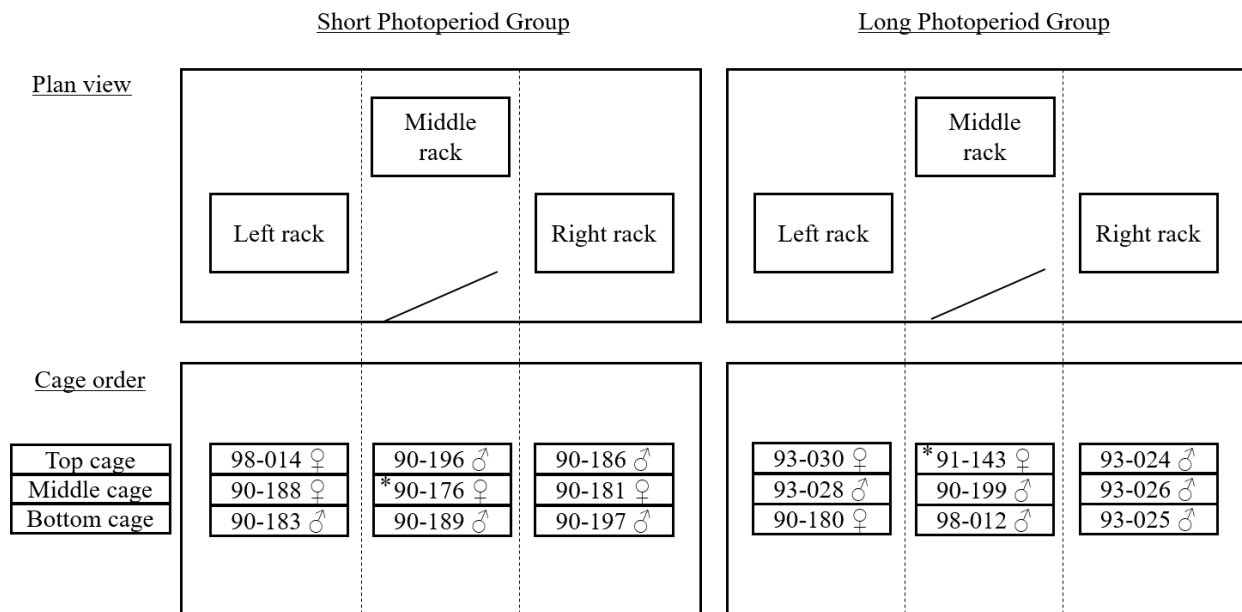


Figure 4. Room plan. Distribution of experimental birds with their identifications and gender. Older birds, indicated by * were housed with the experimental birds but were not used in the experiments.

2.2 Light treatment

The experimental birds were transferred from outside cages under natural light in Tromsø (sunrise/sunset 06:03/19:12) to inside cages with 12 hours of light and 12 hours of dark (LD 12:12) on the 17th September 2015. Inside light was provided by 85-watt fluorescent lamps emitting white light (OSRAM LUMILUX 58W/ 830). Top cages received in average 1238 lux, middle cages received 639 lux and bottom cages received 420 lux, these are mean values from measurements of three different rooms with similar cage arrangements as in this experiment. **Figure 5** shows the light schedules for both groups until the end of the experiment. Exact times and dates for the experimental photoperiods can be found in **Appendix A**. Light was turned on and off at corresponding times. During transitions between photoperiods, light duration was changed in half hour steps in the mornings and evenings over 8 to 12 days, thus resulting in a change in light duration of 1 hour per day.

Both groups were put under SP (LD 6:18) starting from the 29th September 2015. The long photoperiod group was subsequently put under LP (LD 16:8) and later to LL, whereas the short photoperiod group was kept at constant SP.

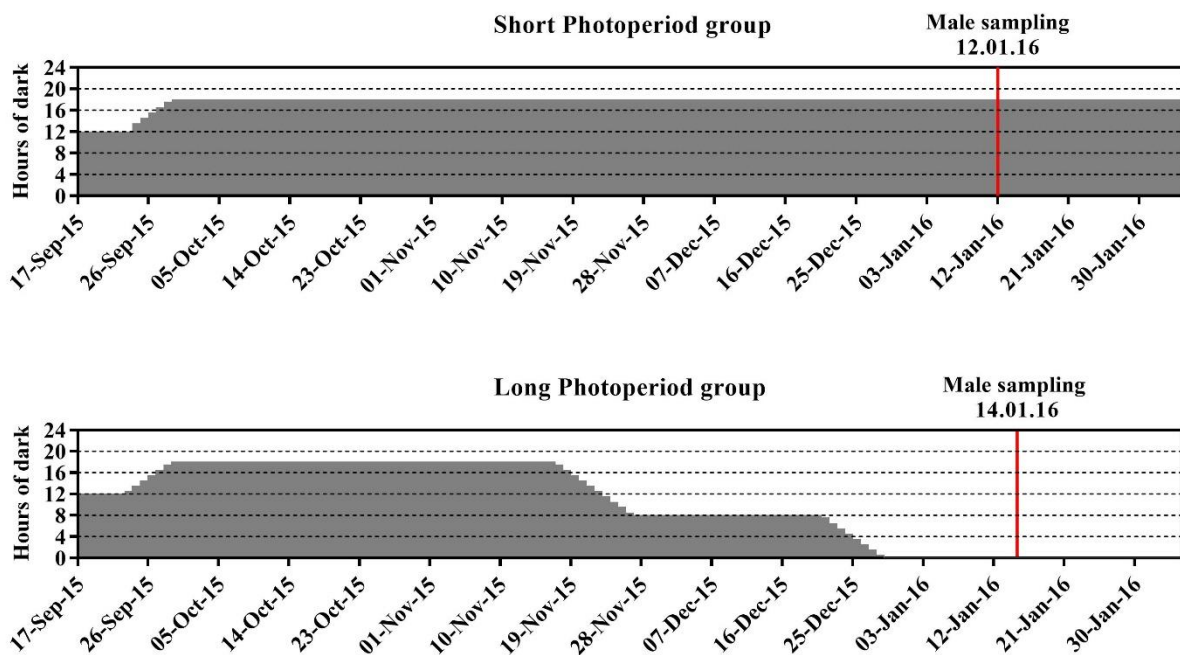


Figure 5. Light schedule throughout experiment. Grey bars symbolize darkness. Males were sampled on the 12th/ 14th January 2016. Females remained under same light schedules for further unrelated experimentation.

At the end of the experiment, males were euthanized by decapitation on the 12th/ 14th January 2016 between 10:30 and 12:00 to recover implanted temperature loggers and to sample brains for future *in situ* hybridisation analysis of gene expression. Female birds were used for further unrelated experimentation lasting until the 5th February 2016 and were euthanized afterwards for iButton recovery.

2.3 Body mass, plumage and blood sampling

Body mass and plumage pigmentation were measured weekly. Body mass was assessed with a laboratory scale (Bergman & Plesner, Ref. No.: 612421). Plumage was measured by taken pictures of the birds and measuring the amount of pigmentation by using the color threshold in ImageJ (Rasband, Version 1.50b)

Starting from 16th October 2015 blood was taken once a week for future hormone assays and DNA studies. Blood was sampled from the basilic vein on the underside of the wing a heparinized 2 ml syringe (BD, Ref. No.: 300185) and a sterile 0.45 x 10 mm needle (BD, Ref. No.: 300300). A maximum of 1 ml of venous blood was taken and transferred into a 1.5 ml microcentrifuge tube (BRAND, Cat. No.: 780500). Blood was subsequently centrifuged (VWR centrifuge, Ref. No.: CT15RE) for 20 minutes at temperature of 4 °C to separate plasma from the blood. Both components were subsequently frozen at -20 °C.

2.4 Surgical implantation of iButton temperature loggers

Temperature loggers (iButtons, Maxim Integrated, Ref. No.: DS1922L) were implanted in the middle of November. Surgeries were performed under a fume hood, to avoid gas exposure to personnel. The surgical equipment (**Figure 6 A**) and the bench was cleaned with 70 % Ethanol (Sigma Aldrich, Ref. No.: 24106-5L-R) and covered with a sterilized surgical cloth. Birds were put on the bench and a purpose-built plastic mask was placed over the beak. Initially an air mix with 4 % Isoflurane (KDG Baxter, Ref. No.: 9623) was applied with the help of an Ohmeda vaporizer (BOC Health Care, Ref. No.: 058294) and an Isoflurane vaporizer (Vapor 2000, Ref. No.: ARXH-1225). The isoflurane proportions was set according to the state of the bird, during the surgery.

Isoflurane percentage was usually lowered to 3.5 % as soon as the bird showed muscle relaxation and no response to external stimuli.

The anaesthetized bird was put on its back and feathers were plucked from the surgical area ventrocaudal from the sternum (**Figure 6 B**). Besides clearing the surgical area, plucking feathers is a good indicator for muscle relaxation and consequently a sign of a sufficient anaesthesia. After plucking, the skin was cut along the *linea alba* with a surgical scissors and the exposed pectoralis muscle was opened. The muscle was minimally cut and the opening was expanded by gently teasing apart to avoid unnecessary bleeding. By this point the isoflurane percentage was usually lowered to 3 %. The iButton was then inserted sub-muscularly by pushing it into the opening. Muscle tissue was sutured with an absorbable 2-0 Polysorb string (Syneture, Ref. No.: CL-811), setting stitches ca. 5mm away from the cut (**Figure 6 C**). Muscle tissue was disinfected with 2 % iodine (Apotekenes, Ref. No.: 332452) and Isoflurane was usually set to 2 %. The skin was sutured with an absorbable 0 Dexon string (DG, Ref. No.: 7232-61) and again disinfected with iodine. Feathers in proximity to the surgical area were laid over the plucked skin to avoid heat loss. The surgeries took 18 to 23 minutes per bird. After surgery, the facial mask was removed and the bird was monitored until active and moving freely again.

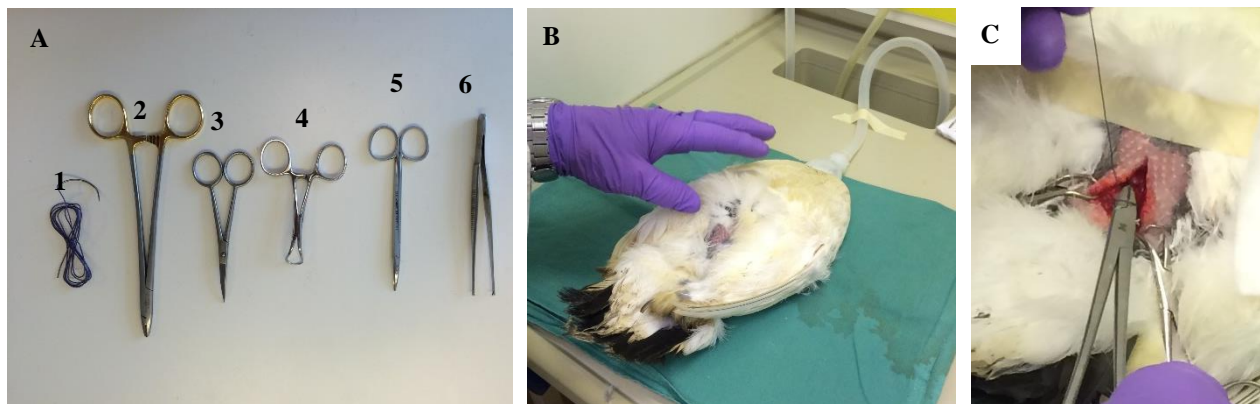


Figure 6. **A:** Surgical instruments used for the iButton implantation. The following instruments were used: absorbable string with needle (1) and needle holder (2) to suture tissue, towel clip to hold tissue (4), surgical scissors (3,5) to open tissue and thumb forceps to grasp tissue (6) **B:** Anaesthetized bird with plastic mask over beak and plugged feathers. **C:** Closure of muscle tissue after successful implantation of iButton.

The iButtons were programmed to record hourly and recording lasted from 38 to 90 days. They were recovered at the end of the experiment and were read by a USB iButton adapter (Maxim Integrated, Ref. No.: DS9490R) and extracted with OneWireViewer (Maxim Integrated, Version 1.5).

Additionally iButtons were installed in experimental rooms to record T_a . However, the iButton in the room of the LP-group failed to record. T_a is therefore only available for the SP-group.

2.5 iButton calibration

Before the surgical implantation, iButtons were calibrated. The iButtons were put in a water bath with different water temperatures (35, 40 and 45°C). True temperature was monitored with a factory calibrated Testo 925 digital thermometer (Testo AG, Germany) equipped with a 1.0 mm type K thermocouple and corrected for the thermocouple-specific measurement error. Simultaneously the iButtons in the water bath recorded temperature. The equation for the slope between true and iButton temperature was used to correct the data recorded by iButtons, after they were recovered from euthanized birds. An example for a calibration curve is shown in **Appendix B**.

2.6 Illustration of recorded body temperature

Body temperature was illustrated by actogram-like diagrams created using the ImageJ plugin ActogramJ (Schmid, Helfrich-Förster et al. 2011): T_b was plotted with stacked blocks between 40 °C and 42°C (**Figure 7 A**). T_b under 40.24°C do not show up as bar and all T_b over 41.75 °C are comprised by the highest bar. The chosen upper and lower limits are defined by the recorded T_b displayed by Svalbard ptarmigan (see **3.2 Body temperature cycles under different light-dark regimes**).

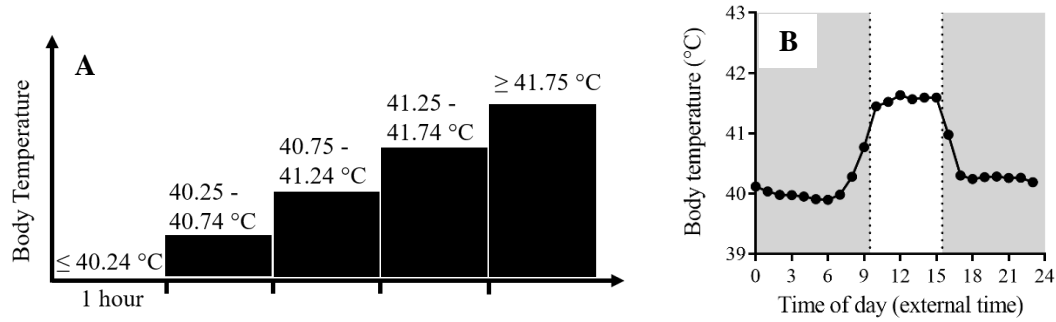


Figure 7. T_b illustrations. **A:** Actogram-like T_b diagrams with increasing bars. One bar represents the hourly recorded T_b . **B:** Time-profiles with T_b expressed either as mean or as actual measured value.

Besides the actogram-like diagrams, T_b was illustrated by average time-profiles with a period of 24 hours. **Figure 7 B** illustrates an average time-profile, every dot is the T_b mean of a given hour for a certain time segment. Grey shades indicate hours of dark.

T_b was also illustrated by normal time-profiles. In these diagrams every dot represents the measured value during the specified day and hour. These normal time-profiles were used to illustrate the shape of T_b and to illustrate T_b together with simultaneously recorded locomotor activity and T_a .

All times in illustration are given in external time, meaning the time of day for Norway (summer time).

2.7 Activity recording

Locomotor activity was recorded simultaneously with T_b from the middle of December until the end of the experiment. Four male birds from the SP-group and three male birds from the LP-group were randomly chosen for these recordings. Standard passive infrared detectors with a homemade circuit coupled to a 9 V DC power supply were used to record locomotor activity. The sensors were installed on the cage door over the food bowl (**Figure 8**). Change in infrared were detected and signalled by a 0/9 V binary signal. Signals were collected using an Actimetrics CL200 USB interface coupled to a ClockLab acquisition package (Version 2.61) running on a PC laptop. The software recorded number of movements detected per minute.

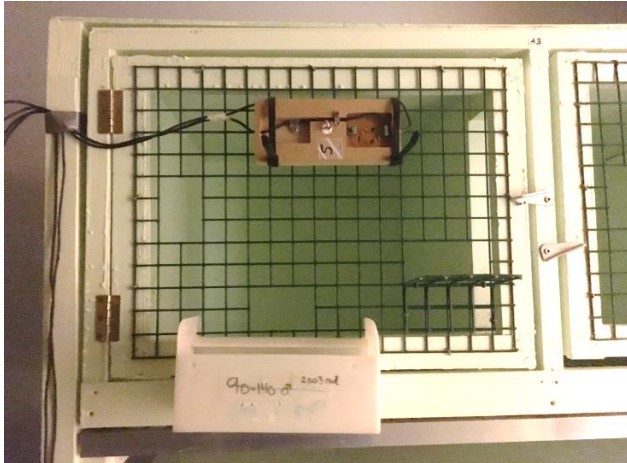


Figure 8. Passive infrared detectors with homemade circuits installed on the cage indoor above the food bowl and facing inwards.

2.8 Statistical analyses

All statistical tests were performed with GraphPad Prism 7. Not all experimental birds could be used for analyses, since some birds lost their iButtons or died before the end of the experiment.

2.8.1) Analyses of body mass, body temperature and activity

Temporal changes in body mass were analysed with repeated measures ANOVA and means of the measuring dates were compared by Tukey's multiple comparisons test. Data of body mass was expressed as mean \pm Standard Error of the mean (SEM).

The T_b data did not pass the D'Agostino-Pearson omnibus normality test. Means of T_b were therefore compared by the Wilcoxon signed rank test (a nonparametric alternative to the paired Student's t-test) and the Mann-Whitney U test (a nonparametric alternative to the unpaired Student's t-test). T_b was expressed as mean \pm Standard Deviation (SD). T_b was also correlated with either T_a or locomotor activity with the non-parametric Spearman r correlation.

2.8.2) Analyses of periods for body temperature and activity oscillation

Periods of the T_b and locomotor activity cycles were calculated with the ImageJ plugin ActogramJ (Schmid, Helfrich-Förster et al. 2011) and the chi-square periodogram (Sokolove and Bushell 1978):

$$Q_P = \frac{K \sum_{h=1}^P (\bar{X}_h - \bar{X})^2}{N^{-1} \sum_{i=1}^N (X_i - \bar{X})^2}$$

P = set period

K = number of section with
period P

h = hour in periods

\bar{X}_h = Mean of hour h

i = time point of undivided
recording

X_i = value at time point i

\bar{X} = total mean

The algorithm calculates the index Q_p for a range of period lengths set by the user, here from 14 to 34 hours. Data is thereby compared between sections, which are defined by the chosen periods. That means a high Q_p indicates a period in which sections are similar to each other. Furthermore, the Q_p indices follow a chi-squared distribution (Sokolove and Bushell 1978). The true period was therefore considered to be the value with the highest Q_p value with a significant P -value under 0.05.

2.8.3) Analyses of changes in body temperature cycles over several weeks

T_b for six birds (two females and four males) under SP were recorded for 52 days. Simultaneously T_a and body mass were documented. Changes in the active- and minimum rest-phase T_b , rest-phase T_a and body mass over the period of 52 days were analysed with linear regression analysis. Subsequently daily means of rest-phase T_b was correlated with daily means of rest-phase T_a and body mass for each bird by non-parametric Spearman r correlation.

3 Results

3.1 Body mass and plumage

Body mass was measured weekly (**Figure 9**). Repeated measures ANOVA indicated temporal changes in body mass in the SP-group ($P = 0.005$) and LP-group ($P < 0.001$). Subsequent Tukey's multiple comparisons test for the SP-group showed statistical significant differences between middle of September and the peak at the beginning of November (502.8 ± 21.6 g vs. 637.7 ± 11.2 g, $P = 0.006$). Body masses after November until January did not vary significantly from each other ($P > 0.05$). However, it is visible from the graph that body mass slightly decreases after November in the SP-group.

The LP-groups also gained weight from the middle of September until the beginning of November (September: 564.6 ± 15.35 g vs. November: 683.3 ± 16.0 g, $P < 0.001$) and lost body mass from the beginning of November until the end of the experiment (November: 683.3 ± 16.0 g vs. January 526.5 ± 14.03 , $P < 0.001$).

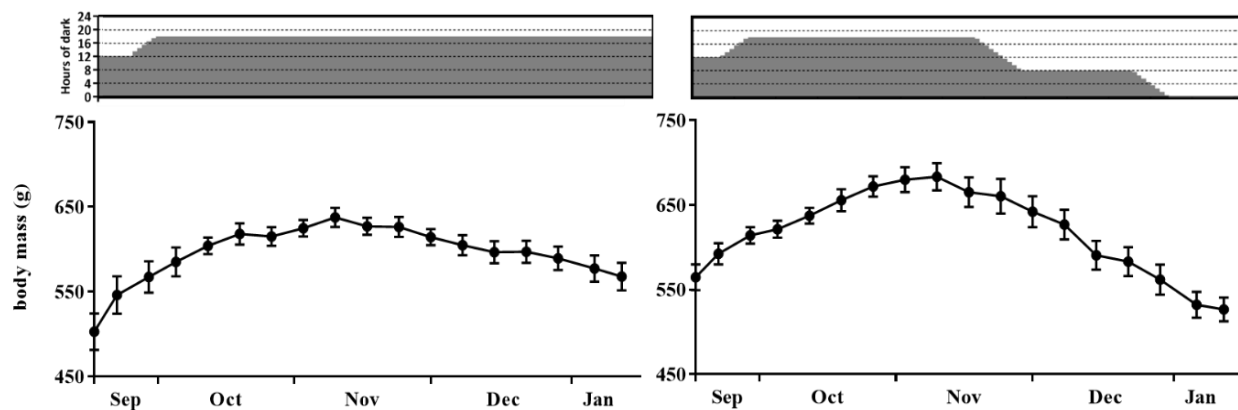


Figure 9. Body masses measured throughout the experiment with corresponding light treatments. Grey bars in the photoperiod graphs indicate hours of darkness. Data is shown as mean \pm SEM.

Plumage pigmentation was also recorded weekly. The birds have completed the change into white winter plumage by the beginning October and the white plumage was maintained by the SP-group until the end of the experiment. Birds that underwent transfer to LP and LL started to show

few pigmented feathers by the end of December and beginning of January, shortly before the end of the experiment (**Appendix C**).

3.2 Body temperature cycles under different light-dark regimes

3.2.1 Representative bird undergoing all three light-dark regimes

Temperature loggers were implanted into the abdominal cavity of Svalbard ptarmigan that underwent different light regimes. Recordings lasted from 38 to 90 days. Birds were separated into a SP-group and a LP-group. While the SP-group was kept under SP (LD 8:16) throughout the whole recording, the LP-group went from SP to LP (LD 16:8) and ultimately to LL (constant light).

Figure 10 shows a representative female bird of the LP group with an uninterrupted T_b recording for 79 days. The representative female bird went through all three experimental light regimes (SP, LP and LL) during the recording. The bird showed a clear cycle in T_b in a rhythmic light-dark environment. T_b rose in anticipation of lights on and fell with the onset of darkness. The sequence was divided into SP, LP and LL segments and chi-squared periodograms were calculated for each segment. This revealed a main period of 24 hours under SP and LP conditions ($P < 0.05$). All other experimental birds under SP and LP, tested with chi-squared periodograms, also showed significant periods of 24 hours (**Appendix D**; $P < 0.05$). Under LL, the exemplary bird showed no evidence of a periodic daily T_b cycle ($P > 0.05$). However, some experimental birds under LL seem to have maintained a weak rhythm with a significant period of 24 hours (**Appendix D**).

T_b of the exemplary female bird during the three light regimes was also used to create average time-profiles with a 24-hour period (**Figure 10**). Time-profiles were defined as plots with the average body temperature of a given hour (external time) over the days within each segment of SP, LP and LL. Again, T_b is shown to be rhythmic under light dark condition, but becomes arrhythmic under LL.

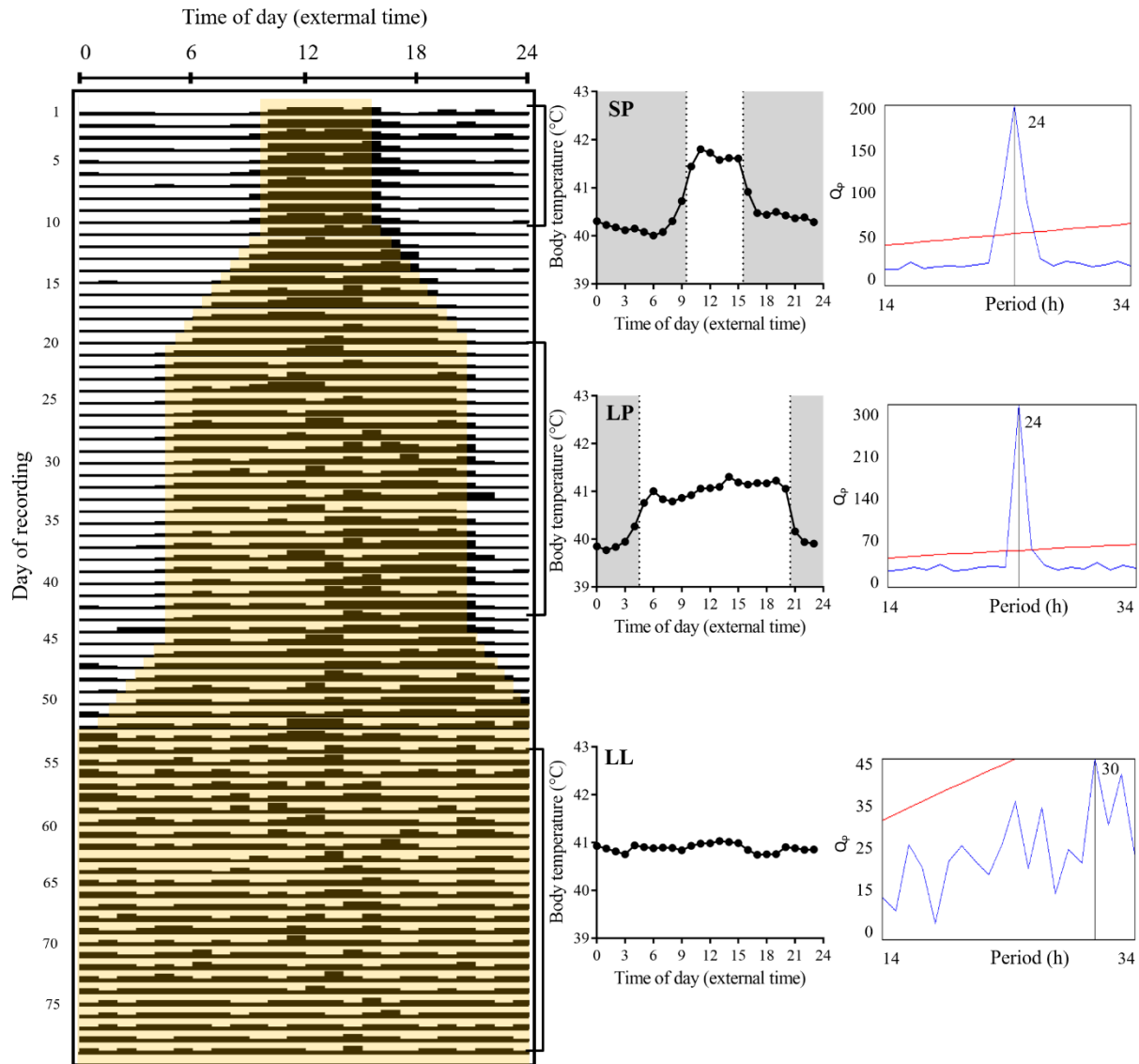


Figure 10. Actogram-like T_b cycle plot of representative female Svalbard Ptarmigan (ID: 90-188) with corresponding periodograms and time-profiles. Increasing black bars indicate increasing T_b . Recording started on 07th November 2015 and ended on the 24th January 2016 (79 days). The bird underwent all experimental light regimes and transitions. Chi-squared periodograms and time-profiles were plotted for SP, LP and LL. Significant periods of 24 hours ($P < 0.05$) are evident in SP and LP. The period in LL with the highest Q_P value was 30 hours but was not significant ($P > 0.05$). Yellow shading indicates light. The red line in the periodograms marks the threshold for significance ($P = 0.05$).

3.2.2 Mid-day body temperature vs. mid-night body temperature

Time points at mid-day (11:00, 12:00 and 13:00, Norway summer time) and mid-night (23:00, 00:00 and 01:00) were used for all experimental birds to assess the peaks and amplitude of the T_b cycle (**Table 1**). The average of mid-day and mid-night is the overall mean of T_b during mid-day and mid-night hours over all recorded days and all recorded birds under the respective light regime.

The means were compared with the Wilcoxon matched-pairs signed rank test (**Figure 11**). While there is a highly significant difference in T_b between mid-day and mid-night hours under SP and LP ($P < 0.001$), these values do not differ significantly under LL ($P = 0.136$). The amplitude under SP and LP were compared with the Mann-Whitney U test and showed to be significant higher under SP than under LP ($P < 0.001$).

Differences between genders were tested with the Wilcoxon matched-pairs signed rank test for the mid-night and mid-day phase. There was a difference in gender for birds under SP during mid-night ($P < 0.001$) and mid-day ($P = 0.016$) with higher T_b in female birds. Furthermore, there was a difference in gender for birds under LP during mid-night ($P < 0.001$) and mid-day ($P < 0.001$) and for birds under LL during mid-day ($P < 0.001$) with higher T_b in male birds. However, there was no significant sex differences for birds under LL during mid-night ($P = 0.843$).

Table 1. Average T_b of Svalbard ptarmigans during mid-day and mid-night. Data points collected during handling were excluded. The amplitude was calculated as the difference between mid-night mean and mid-day means. Asterisks mark significant differences between genders during mid-night and mid-day.

Photo period	Days recorded	Number of birds	Mean mid-night phase ($^{\circ}\text{C} \pm \text{SD}$)	Mean mid-day phase ($^{\circ}\text{C} \pm \text{SD}$)	Amplitude ($^{\circ}\text{C} \pm \text{SD}$)
SP	52	6 (4 ♂, 2 ♀)	39.89 \pm 0.32 *	41.56 \pm 0.23 *	1.67 \pm 0.39
LP	23	7 (4 ♂, 3 ♀)	39.94 \pm 0.42 *	41.07 \pm 0.31 *	1.13 \pm 0.38
LL	14	7 (4 ♂, 3 ♀)	40.90 \pm 0.24	40.97 \pm 0.28 *	0.07 \pm 0.37

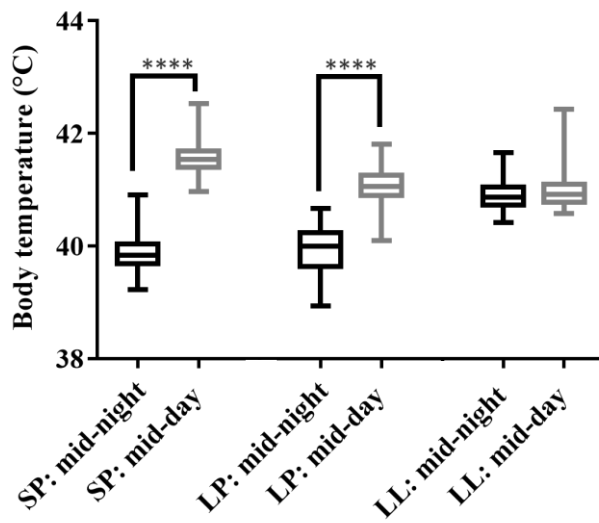


Figure 11. Average T_b of Svalbard ptarmigans during mid-night and mid-day under the three different photoperiods. Wilcoxon matched-pairs signed rank test was applied to compare mid-day and mid-night phase under reach light-dark regime. Data is shown as median with upper and lower quartile as box, whiskers mark minimum and maximum T_b s. **** $P < 0.0001$.

3.2.3 Individual hourly profiles of body temperature oscillation

Time-profiles of three continuous days for all available experimental birds were plotted to illustrate the shape of T_b cycles in Svalbard ptarmigan (**Figure 12**). While birds under SP showed a peak in T_b during the six hours of light, birds under LP showed different and more diverse patterns. Some of the birds, regardless of gender, under LP showed two peaks in T_b during the 16 hours of light, indicated by arrowheads in **Figure 12**. The first peak was typically expressed shortly after light onset whereas the second daily peak seemed to be expressed before light off switch. However, this is not a general pattern for all experimental birds. Bird under LL show relatively constant T_b with no apparent rhythm. However, short peaks in T_b , with durations between 1 and 3 hours, are evident.

Nocturnal rise in T_b in anticipation to light was again visible under SP and LP.

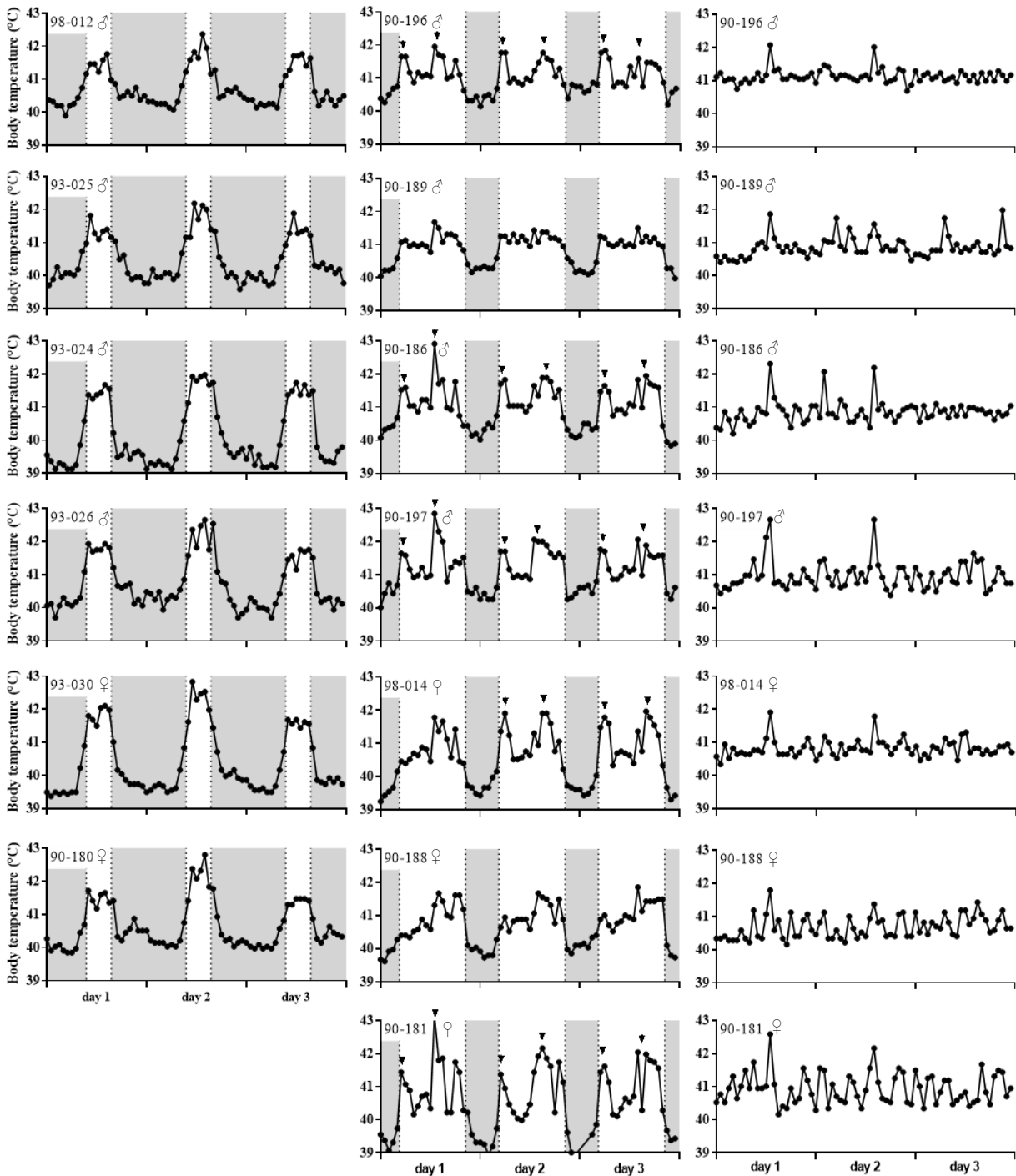


Figure 12. Time-profiles for three continuous days for each bird under their corresponding light-regime. Three days when there was no handling (weighing and blood sampling) were chosen. ▼ indicate two peaks in T_b during one day in LP birds.

3.3 Correlation between activity and body temperature

From mid-December onwards, locomotor activity was recorded simultaneously to T_b in seven male birds. Three continuous days without handling, i.e. blood sampling or weighing, were chosen for representative birds under each light-dark regime (**Figure 13**). Activity and T_b correlated significantly in all three experimental light regimes (nonparametric Spearman r correlation; **SP**: $R = 0.645$, two tailed $P < 0.001$; **LP**: $R = 0.490$, two tailed $P < 0.001$; **LL**: $R = 0.703$, two tailed $P < 0.001$). All other recorded male birds showed also significant correlations between T_b and activity (**Appendix E**), except one bird under LL (nonparametric Spearman r correlation; $R = 0.056$, two tailed $P = 0.639$). Activity was mostly detected during light hours but sometimes occurred before lights on in SP- and LP-birds. However, the rise in T_b before lights on is always evident, even in the absence of activity.

T_b was also correlated to T_a in the same SP-bird and the same three days as for the correlation with activity (**Figure 13**). However, even though T_a and T_b are correlating for this bird (nonparametric Spearman r correlation; $R = 0.677$, two tailed $P < 0.001$), it is clearly visible that T_a rises after the rise in T_b . T_a is not available for the LP-group, since the recorder in this room malfunctioned.

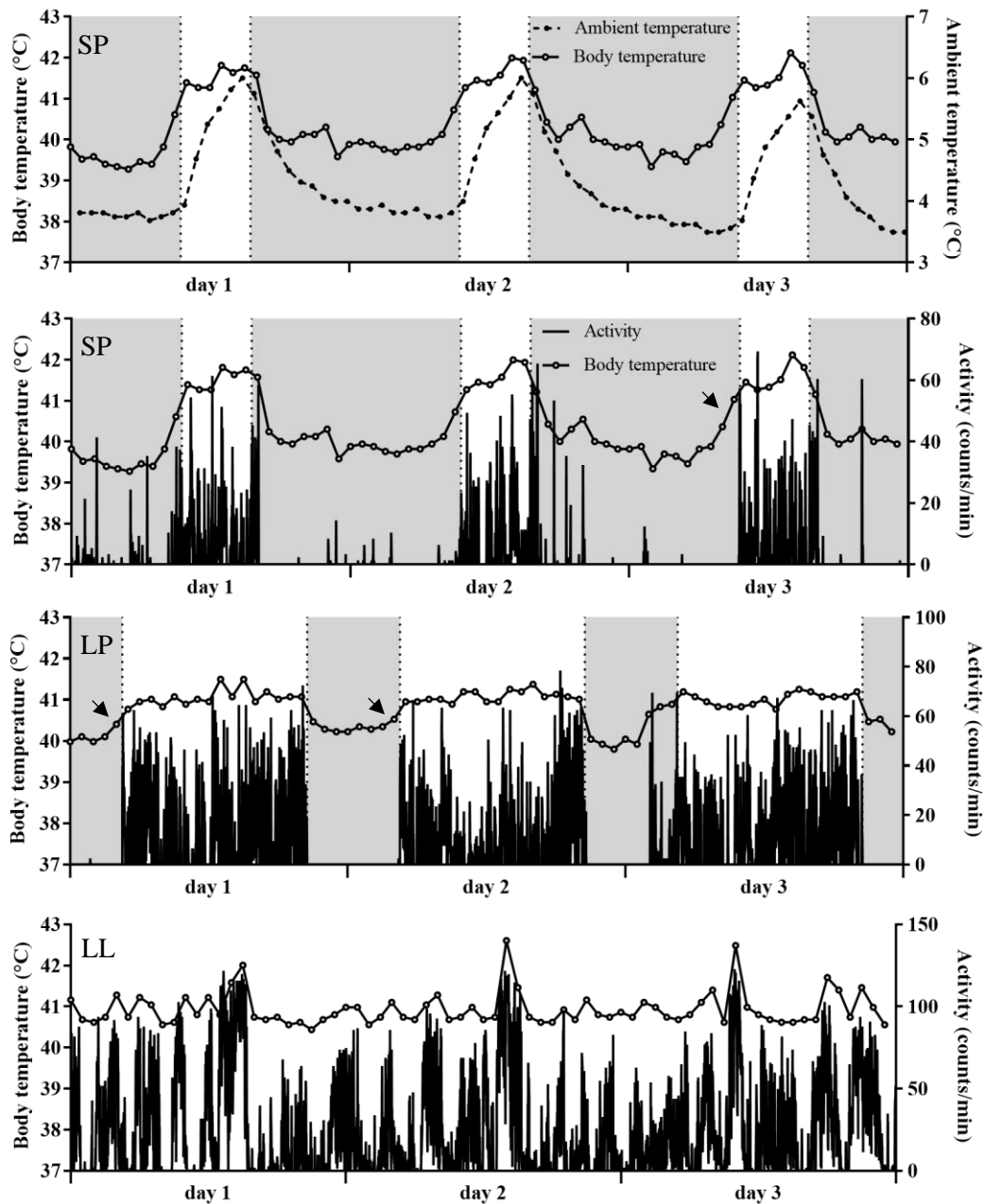


Figure 13. Simultaneous recordings of T_b with T_a and locomotor activity in three different male birds under different light regimes. Correlation was tested with non-parametric r Spearman correlation and was evident for T_b vs. T_a ($R = 0.677$, two tailed $P < 0.001$) under **SP** and for T_b vs. activity under **SP** ($R = 0.645$, two tailed $P < 0.001$), under **LP** ($R = 0.490$, two tailed $P < 0.001$) and under **LL** ($R = 0.703$, two tailed $P < 0.001$). Three days when there was no handling (weighing and blood sampling) were chosen for each shown bird. Arrows indicate anticipatory rise in T_b in absence of activity. Bird IDs: 93-026 for SP, 90-189 for LP and 90-197 for LL.

3.4 Body temperature cycles during sickness

During the T_b recording, one bird got sick, showing signs of diarrhoea. Consequently, the bird was taken out of the experiment, not handled anymore and was offered additional high protein food. However, the animal's condition deteriorated fast and the bird died 7 days after the first signs of sickness. The iButton was recovered *post mortem* and recordings were plotted with activity recordings. Chi-squared periodograms were created for T_b and activity oscillation for 7 days before diarrhoea onset and for 7 days from the onset of diarrhoea until the death of the bird.

Before the onset of diarrhoea (**Figure 14**) the bird showed a T_b and activity cycle with a period of 24 hours ($P < 0.05$).

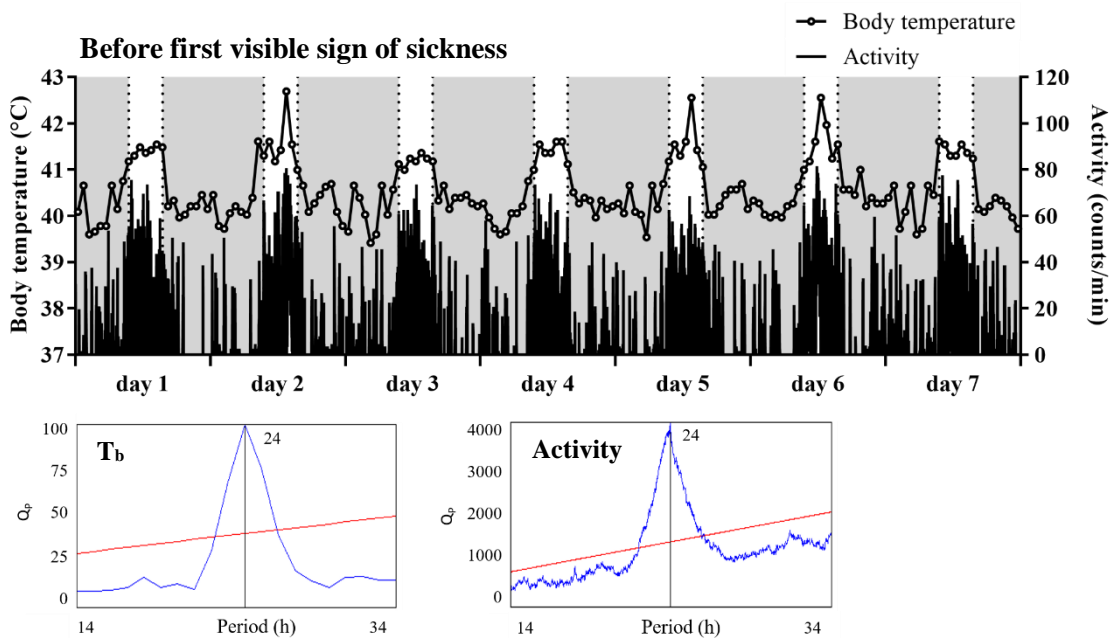


Figure 14. Time-series for sick bird (ID: 90-199) seven days before visible signs of sickness. Chi-squared periodograms showed significant periods for the T_b and activity cycle ($P < 0.05$). Grey bars indicate dark hours and the red line in the periodograms marks the threshold for significance ($P = 0.05$).

During the period with observable diarrhoea (**Figure 15**), the bird continued to show oscillation in locomotor activity with a significant period of 24 hours ($P < 0.05$), but there was no significant period for the T_b oscillation ($P > 0.05$). Furthermore, the amount of activity counts

decreased from $10\,338 \pm 1\,422$ per day (mean \pm SD) before diarrhoea onset to $6\,172 \pm 1\,766$ per day.

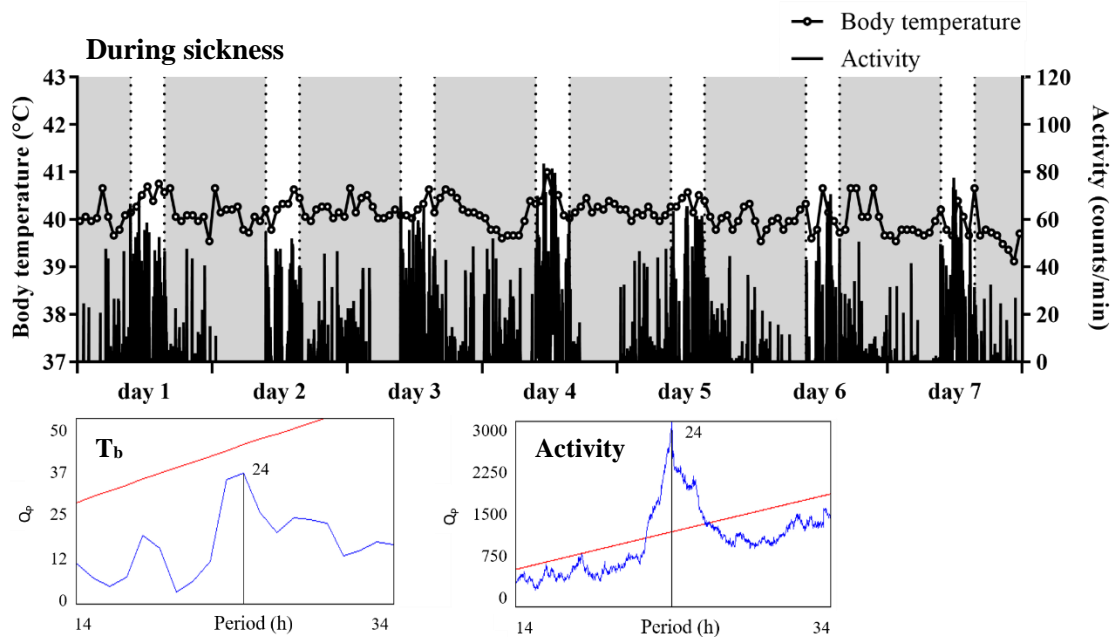


Figure 15. Time-series for sick bird (ID: 90-199) seven days within the period of visible symptoms. Chi-squared periodograms showed significant periods for the activity cycle ($P < 0.05$) but not for T_b cycle ($P > 0.05$). Grey bars indicate dark hours and the red line in the periodograms marks the threshold for significance ($P = 0.05$).

The sick bird showed also regularly activity during the dark hours before the onset of diarrhoea (**Figure 14**) and between the onset of diarrhoea and its death (**Figure 15**). This continuative pattern of nocturnal activity was not observed in birds which did not show any signs of sickness throughout the experiment (**Figure 16**, see also **Appendix E**).

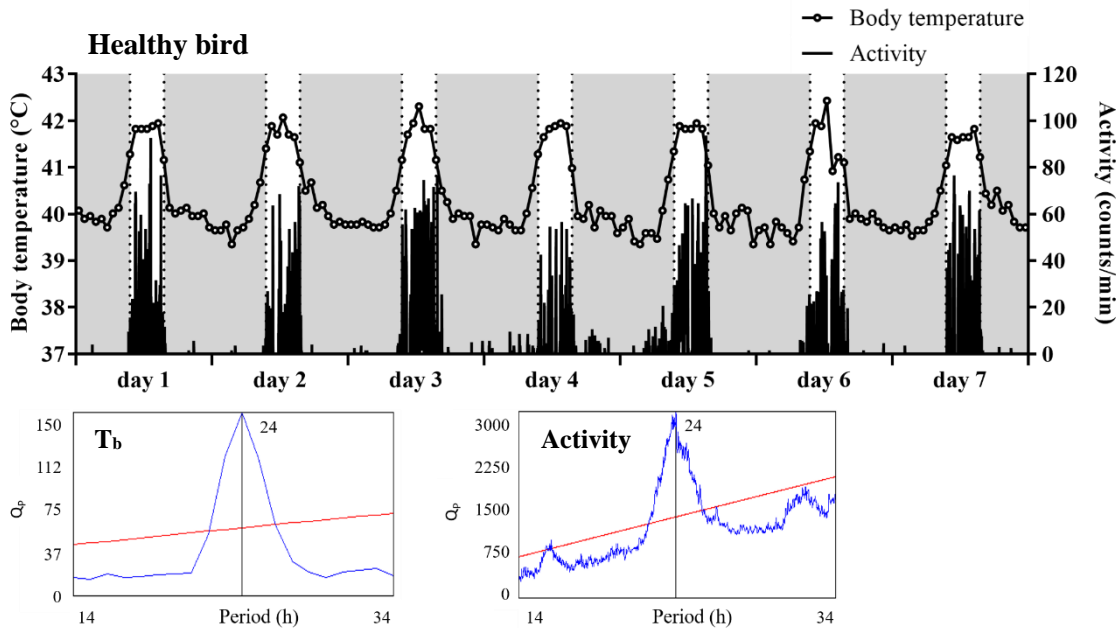


Figure 16. Time-series for bird without any sickness (ID: 98-012) Chi-squared periodograms showed significant periods for the T_b and activity cycle ($P < 0.05$). Grey bars indicate dark hours and the red line in the periodograms marks the threshold for significance ($P = 0.05$).

3.5 Body temperature cycles under constant short photoperiod

3.5.1 Representative bird under constant short photoperiod

After the initial transition from natural light into SP (LD 6:18), the photoperiod of the SP-group was kept constant throughout the whole experiment. **Figure 17** shows a representative female bird with an uninterrupted recording for 90 days. As before the rise and fall of T_b is closely connected to the light on- and offset, with anticipatory rise in T_b before lights are switched on and with a significant period of 24 hours (chi-squared periodogram; $P < 0.05$).

The exemplary T_b recording was visualised by actogram-like graphs. A change in T_b during the rest-phase can be observed over the duration of the recording, as the rest-phase T_b seems to decrease throughout the recording. This change in T_b under constant experimental photoperiodic conditions was further investigated under the next subheading.

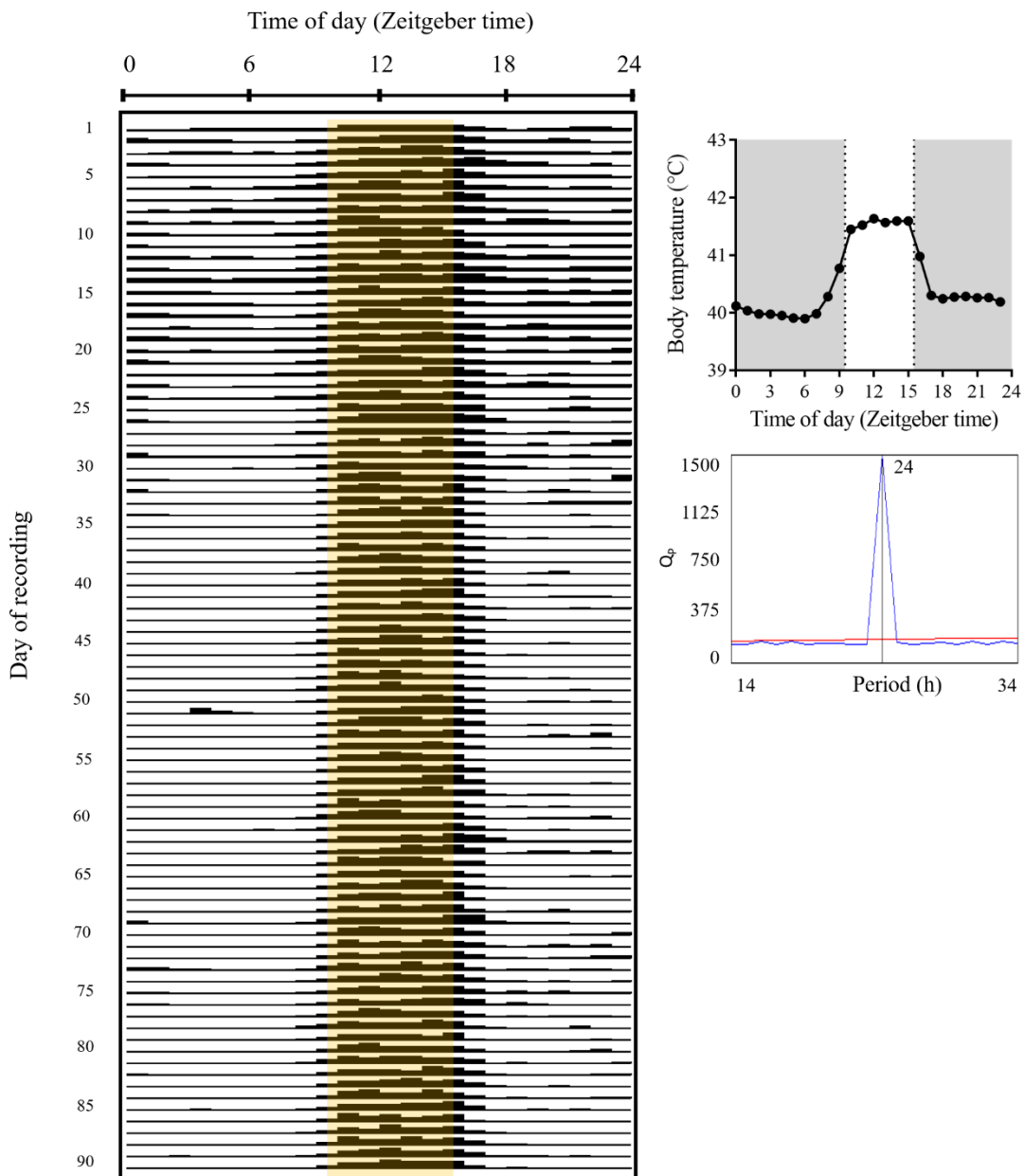


Figure 17. Actogram-like plot for T_b cycle of a representative female Svalbard ptarmigan (ID: 90-180). Increasing black bars indicate increasing T_b . Recording started on 07th November 2015 and ended on the 4th February 2016 (90 days). The bird was kept under short photoperiod (LD 6:18) throughout the experiment. A chi-squared periodogram and a time-profile were plotted for the whole T_b recording. The chi-squared periodogram showed a significant period of 24 hours ($P < 0.05$). Yellow shading indicates light. The red line in the periodogram marks the threshold for significance ($P = 0.05$).

3.5.2 Change in rest-phase body temperature under constant short photoperiod

Six birds from the SP-group with a continuous recording for 52 days (from the 21st November 2015 to the 11th January 2016) were analysed together. Time-profiles were visualized in one plot with hourly means for every week (**Figure 18**). Hour 1 to 6 (external time, Norway summer time) were chosen to analyse the decrease in rest-phase T_b , because during this time of the day the birds had the lowest T_b . Throughout the 7 weeks of recording, the birds seem to decrease in rest-phase T_b .

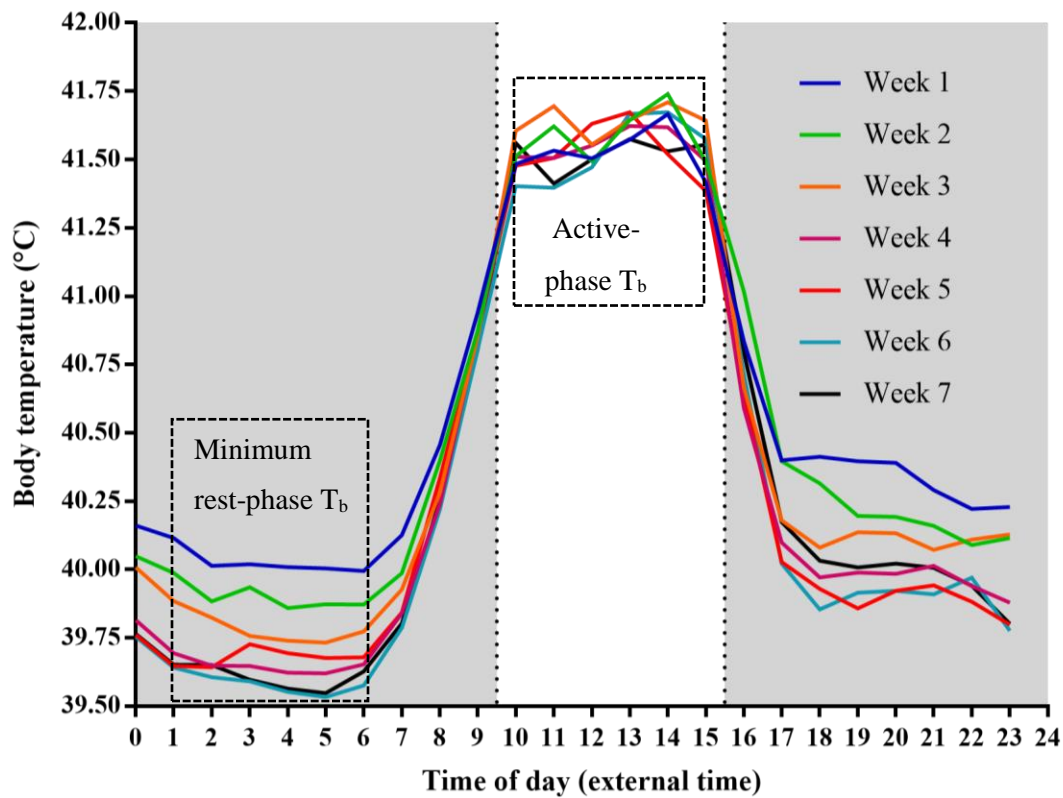


Figure 18. Weekly time-profiles of six birds of the SP-group. Each line in the plot represents the hourly mean of six birds over 7 days. Active-phase T_b (hour 10 to 15) and minimum rest-phase T_b (hour 1 to 6) are indicated by rectangles.

Subsequently, means of rest-phase and active-phase T_b for each day and each bird were calculated over a recording of 52 days and analysed by linear regression. Rest-phase T_b was significantly decreasing with a slope of -0.01 °C/ day ($R^2 = 0.195$, $P < 0.001$; **Figure 19 A**), while active-phase T_b remains constant ($R^2 = 0.032$, $P = 0.208$; **Figure 19 B**). Linear regression for T_a during rest-phase ($R^2 = 0.201$, $P < 0.001$; **Figure 19 C**) and body mass ($R^2 = 0.254$, $P < 0.001$; **Figure 19 D**) showed also significant decreases over the recording. Change in T_a in the experimental room seemed to be a result of changing air temperatures in Tromsø (**Appendix F**).

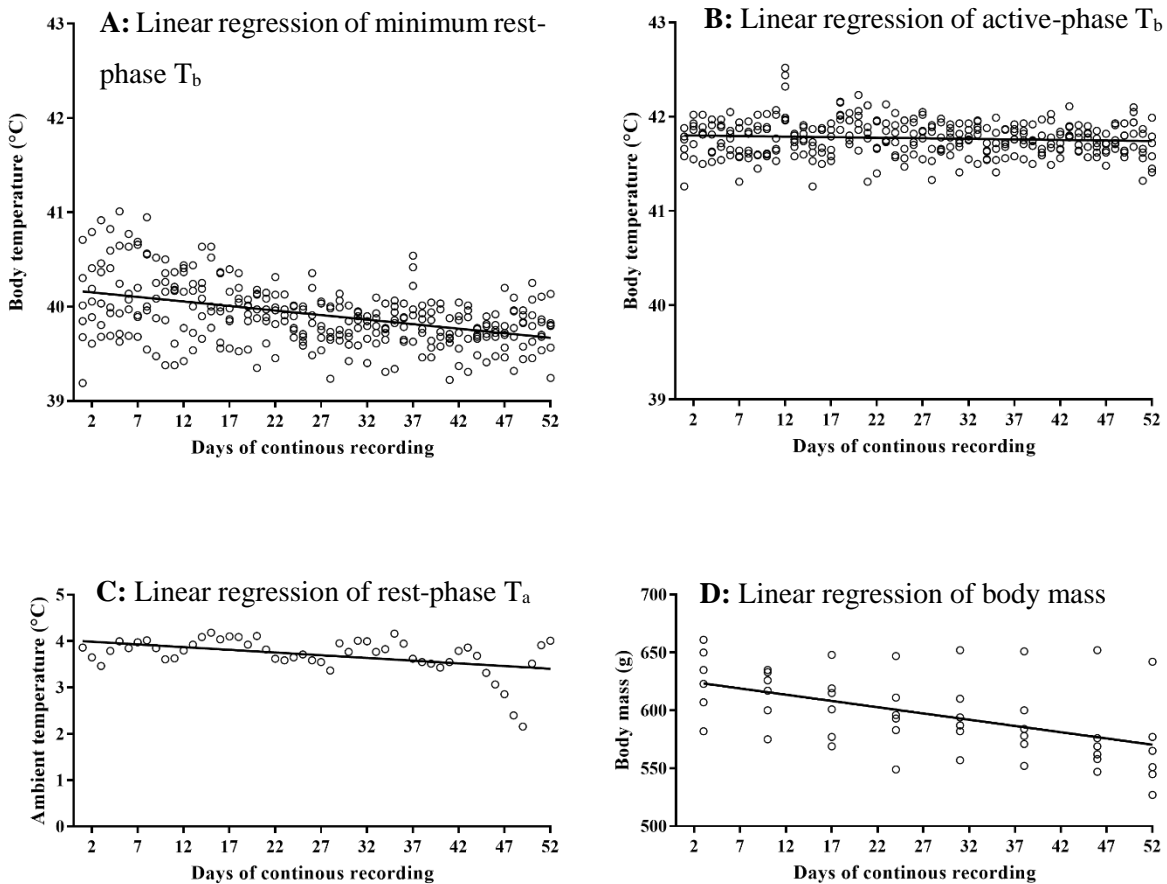


Figure 19. **A:** Linear regression of minimum rest-phase T_b over 52 days. The slope is significant different from zero (slope = -0.01 , $P < 0.001$; $R^2 = 0.195$). **B:** Linear regression of active-phase T_b over 52 days. The slope is not significant different from zero ($P = 0.208$; $R^2 = 0.032$). **C:** Linear regression of T_a during rest-phase over 52 days. The slope is significant different from zero (slope = -0.01 , $P < 0.001$; $R^2 = 0.201$). **D:** Linear regression of body mass over 52 days. The slope is significant different from zero (slope = -1.08 , $P < 0.001$; $R^2 = 0.254$).

The birds of the SP-group were also analysed separately by linear regression and non-parametric Spearman r correlation. Five out of six birds showed a significant decrease in rest-phase T_b and five out six birds showed a significant decrease in body mass (**Table 2**). Out of the five birds, showing a rest-phase T_b decrease, two birds showed a significant correlation with body mass and three birds showed a significant correlation with T_a (**Table 3**).

Table 2. Changes in rest-phase T_b and body mass for each bird of the SP-group with 52 days of T_b recording. Changes were analysed with linear regression.

Bird ID	Sex	Decrease in rest-phase T_b (P -value)	Coefficient of determination (R^2)	Decrease in body mass (P -value)	Coefficient of determination (R^2)
93-030	female	No (0.844)	0.001	Yes (< 0.001)	0.969
90-180	female	Yes (< 0.001)	0.457	Yes (< 0.001)	0.874
98-012	male	Yes (< 0.001)	0.469	Yes (< 0.001)	0.933
93-024	male	Yes (< 0.001)	0.248	Yes (< 0.001)	0.978
93-026	male	Yes (< 0.001)	0.336	Yes (< 0.001)	0.871
93-025	male	Yes (< 0.001)	0.561	No (0.606)	0.047

Table 3. Correlations between T_b and body mass and between T_b and T_a for each bird of the SP-group with 52 days of T_b recording. Correlation was tested with nonparametric Spearman r correlation.

Bird ID	Sex	Correlation between T_b and body Mass (P -value)	Correlation coefficient (R)	Correlation between T_b and T_a (P -value)	Correlation coefficient (R)
93-030	female	No (0.399)	0.347	No (0.549)	0.085
90-180	female	No (0.096)	0.643	Yes (0.022)	0.315
98-012	male	Yes (0.005)	0.905	Yes (0.002)	0.426
93-024	male	No (0.171)	0.548	No (0.113)	0.222
93-026	male	Yes (0.005)	0.905	No (0.187)	0.186
93-025	male	No (0.332)	0.395	Yes (0.029)	0.029

4 Discussion

4.1 Body mass

Body mass in both groups increased from mid-September (start of measurement) until the beginning of November and decreased subsequently. However, birds held in LP and LL decreased much faster than birds kept under SP, whose body masses decreased slightly and non-significantly. Similar results were obtained by previous studies about annual changes in body mass: Svalbard ptarmigans, transferred into short photoperiod after they experienced continuous daylight during the Arctic summer, gained body mass after the transfer and kept a constant high body mass until stimulation through long photoperiod (Stokkan, Lindgård et al. 1995).

I did not conduct food intake measurements in this experiment, but it was already established in former studies that food intake runs out of phase with the body mass cycle in Svalbard ptarmigans (Stokkan, Mortensen et al. 1986, Lindgård, Stokkan et al. 1995). It is reasonable to assume that the relation between food intake and body mass was the same in this experiment as in former ones. SP-birds probably had a low food intake, despite a high body mass, while LP-birds probably increased their food intake, despite decreasing body mass. This out of phase relationship in addition to the observation that the here studied birds were held in relative constant T_a suggests that body mass is actively regulated and not a mere passive consequence for increased food intake or changes in energy expenditure due to changing T_a .

Therefore, I assume that the here observed temporal changes in body mass are due to the different photoperiods: birds kept under SP remained in their winter phenotypes with relative high body masses, while the birds under LP and LL lowered their body masses, to reach the summer phenotype with relative low body mass.

The fact that the experimental groups start from different body masses is because birds from the SP-group were one week younger than birds from the LP-group.

4.2 Daily body temperature cycles

4.2.1 Body temperature cycles under light-dark regimes

Body temperature oscillates in Svalbard ptarmigan under light-dark cycles, with elevated T_b during the active-phase and low T_b during the rest-phase. Measurements during mid-night (SP-group: 39.89 ± 0.32 °C and LP-group: 39.94 ± 0.42 °C) and mid-day (SP-group: 41.56 ± 0.23 °C and LP-group: 41.07 ± 0.31 °C) correspond well with the means for studied *Galliformes* (rest-phase: 38.9 ± 1.16 °C; active-phase: 41.4 ± 0.92 °C) (Prinzinger, Pressmar et al. 1991). The T_b cycle under light-dark cycle has also a stable period of 24 hours. Female birds display normally a slightly higher T_b than males (Prinzinger, Pressmar et al. 1991). In this experiment, differences between genders were evident but were not consistent between the groups. Females had a higher T_b in the SP-group, while it was the opposite in the LP-group.

The recordings showed also anticipatory rise in T_b before the lights were switched on. This anticipatory rise in T_b possibly ensures optimal temperature for biochemical body functions during the active-phase. It also shows that Svalbard ptarmigan must have means of endogenous time measurement to anticipate light, since no other cue could have helped to correctly foretell the lights on switch. Changes in T_a could have acted as cue, but T_a rises after the lights were switched on. The rise in T_a was probably due to a combination of rising air temperatures, higher activity in birds and heat output from the lamps.

The amplitude between mid-day and mid-night was significantly higher under SP than under LP. This difference can be mostly attributed to changes in the mid-day mean, which changes from SP to LP. Birds kept under LP often showed two T_b peaks during their active-phase: a peak after light onset and one later in the day. The means for the active-phase were taken from the hours 11:00, 12:00 and 13:00, and these hours usually fell in the time between the two peaks. The birds under SP, on the other hand, showed often just a single T_b peak and had therefore a more elevated T_b during the hours used in the analysis. Two peaks have been reported previously in activity profiles of Svalbard ptarmigan (Reierth and Stokkan 1998). Reierth and Stokkan (1998) demonstrated a morning and evening bout in locomotion. Two peaks in activity were also found in a variety of other birds, such as greenfinches (*Chloris chloris*), bramblings (*Fringilla montifringilla*) and Chaffinch (*Fringilla coelebs*) and are considered characteristic for daily activity

cycles (Aschoff and Meyer-Lohmann 1955, Dancker 1964, Aschoff 1966). Since increased locomotor activity elevates T_b in birds (Torre-Bueno 1976, Butler 1991), I suggest that the two peaks in T_b are the result of activity bouts and that the higher active-phase T_b in the SP-group is a result of photoperiod driven merging of the two T_b peaks seen under LP.

Other heterothermic responses with higher magnitude than the normal daily T_b cycle, like daily torpor and rest-phase hypothermia are known for a range of birds, including species with comparable size to Svalbard ptarmigan, such as the tawny frogmouth (*Podargus strigoides*) (Körtner, Brigham et al. 2000). However, I found no evidence of these forms of controlled heterothermic responses in this experiment. The amplitude of T_b oscillation under all light regimes was under 2 °C, which is considered to be within the range of normal daily cycle observed in birds (Prinzinger, Pressmar et al. 1991, Reinertsen 1996). This is not surprising, since the birds were housed indoors at a constant T_a well within their thermoneutral zone and had unlimited access to food, conditions in which these more severe heterothermic responses are not displayed (Reinertsen and Haftorn 1986, Nord, Nilsson et al. 2009). I am unaware of any studies demonstrating rest-phase hypothermia or daily torpor in Svalbard ptarmigan or other rock ptarmigan subspecies, so the question if these birds display daily torpor or rest-hypothermia is still unanswered.

After showing clear oscillation in T_b under light-dark regimes, the question arises why Svalbard ptarmigan have a daily T_b cycle. Heterothermic responses like daily torpor and rest-phase hypothermia are known to lower an animal's energy need by decreasing metabolic expenditures. The daily T_b cycle could have the same function, but on a smaller scale.

In an attempt to quantify the energetic savings deriving from the T_b cycle, I calculated the metabolic heat production. I thereby assumed that heat production equals the rate of heat loss. For the calculation, I used the mass-specific conductance for Svalbard ptarmigan with winter phenotype from Mortensen and Blix (1986). I assumed a theoretical bird with a body mass of 600 g (approximate body mass for a bird under SP in this experiment) and a constant T_a of 4 °C (typical T_a experienced by experimental birds). The calculations can be found in **Appendix G**. The daily metabolic saving, by lowering the T_b by 1.67 °C during rest-phase (mean for birds under SP) was calculated to be 1.44 kcal/ day under SP, compared to a bird which would have a constant high T_b . This equals 2.81 % of the daily resting metabolic rate of a Svalbard ptarmigan during winter, which is 51.18 kcal/ day for a 600 g bird (Mortensen and Blix 1986). Naturally, this saving would be less

under LP. If these relative small energy savings are the real reason for the existence of T_b cycle is debatable.

Another function for daily T_b cycle is the synchronization of peripheral clocks with the master clock, an mechanism thoroughly studied in mammals (Buhr, Yoo et al. 2010, Mohawk, Green et al. 2012). Unlike mammals, birds do not appear to have a single master circadian pacemaker, but rather a system of three interacting components: pineal gland, retina and hypothalamic oscillator. However, the idea for the synchronization of peripheral clocks remains a plausible function for the T_b cycle, seen in Svalbard ptarmigan.

4.2.2 Body temperature cycles under constant light

In constant light T_b between mid-day and mid-night did not differ significantly from each other and chi-squared periodograms analysis showed no clear periodicity in the T_b cycle in four out of seven birds under LL (**Appendix C**).

Some arctic species show stable daily cycles in locomotor activity and in T_b under constant light during the polar day, like bumblebees in northern Finland (Stelzer and Chittka 2010) and arctic ground squirrels in Alaska (Williams, Barnes et al. 2011). It was already known that Svalbard ptarmigan abolish their locomotor activity rhythm in constant light conditions (Stokkan, Mortensen et al. 1986, Reierth and Stokkan 1998), here I have shown that captive Svalbard ptarmigans abolish also their T_b cycle under constant artificial light and *ad lib* food. Unfortunately, it was not possible to determine the exact time point when the T_b cycle broke down, because of the step-wise transition from LP to LL. Studies from willow ptarmigan transferred from a light-dark treatment into constant dark sustained their T_b cycle for two recorded days (Blix 2005). I therefore think that Svalbard ptarmigan would also not abolish their T_b cycle instantly after a transfer into LL, but this assertion still needs to be investigated.

Svalbard Ptarmigan live from April to September under constant light. The observation that they keep a relative constant T_b during continuous illumination supports the thesis that Svalbard ptarmigan are adapted to be arrhythmic under constant conditions. However, it has to be considered that the birds were held inside under artificial light. Light colours and position of the Sun can act as *zeitgeber* for high arctic birds. Snow buntings (*Plectrophenax nivalis*) which breed in Svalbard were shown to keep an constant cycle in feeding activity by entraining to theses *zeitgeber* (Krüll 1976a, Krüll 1976b). I can therefore not disregard that Svalbard ptarmigan exposed to constant natural light have a daily cycle in T_b . On the other hand, Svalbard ptarmigan held under constant

natural light abolished their activity cycle (Reierth and Stokkan 1998), which would suggest that they do not entrain to these *zeitgeber*.

Yet single diurnal peaks in T_b were observed and in some birds these peaks occurred roughly at the same time of the day. This added up to a statistically significant period of 24 hours. However, these peaks are most likely related to stress reactions due to daily food and water refill by animal care staff. It is known that stress does increase T_b in birds (Cabanac and Guillemette 2001, Carere and van Oers 2004) and daily maintenance procedures were approximately performed at the same times of the day. This most likely led to stress, resulting in T_b peaks during the same times of day, resulting to significant periodogram peaks in three out of seven LL-birds.

4.2.3 Activity and body temperature

It is known that activity raises T_b in birds (Torre-Bueno 1976, Butler 1991) and Svalbard ptarmigan show a clear diurnal activity rhythm in light-dark environments (Stokkan, Mortensen et al. 1986, Reierth and Stokkan 1998). In this experiment, I also showed that locomotor activity and T_b peaks correlate with each other in birds under LL. Activity could therefore be a reasonable explanation for the diurnal rise in T_b in Svalbard ptarmigan.

However, recordings of activity and T_b shows that the rise in T_b starts before light onset. Activity also occurred occasionally before light onset but, importantly, the anticipatory rise in T_b is also evident when activity is absent. Additional evidence that T_b is separately regulated from activity is provided by the case of the sick bird. T_b recordings from the last seven days showed that the sick bird abolished its circadian T_b oscillation, the activity rhythm, however, still showed a clear periodicity of 24 hours.

It was shown that thermoregulatory mechanisms are responsible for the daily T_b cycle in pigeons (Graf 1980) and rats (Refinetti 2003), i.e. increasing heat production and decreasing heat loss at the entry of the active-phase and *vice versa* for the rest-phase. I therefore conclude that the observed T_b cycle in Svalbard ptarmigan is a result of active thermoregulation, rather than a mere consequence of activity.

However, I do not want to suggest that activity does not affect T_b at all. Activity is known to raise T_b (Torre-Bueno 1976, Butler 1991) and activity peaks occur often simultaneously to T_b peaks in birds under LL.

4.3 Change in rest-phase body temperature under constant short photoperiod

Svalbard ptarmigan kept under constant short photoperiod showed a decrease in rest-phase T_b over a period of 52 days. Body mass and T_a were simultaneously recorded throughout the experiment, to evaluate potential influences on the changing rest-phase T_b .

4.3.1 Ambient temperature and body mass in connection with rest-phase body temperature

The drop in T_a could be a plausible cause for the visible decrease in rest-phase T_b . The decrease in T_a led to a bigger gradient between T_a and T_b . This could have increased heat dissipation during rest-phase and consequently to lower rest-phase T_b . In fact, studies about the influence of T_a on daily T_b cycles in tree shrews (*Tupaia belangeri*), gray mouse lemurs (*Microcebus murinus*) and Malachite sunbirds (*Nectarinia famosa*) showed that T_a does influence the amplitude of the daily T_b oscillation (Refinetti 1997, Aujard and Vasseur 2001, Downs, Brown et al. 2002). In all cases, decreasing T_a lowered rest-phase T_b , but did not change active-phase T_b .

It is also plausible that the difference in body mass had an influence on the rest-phase T_b . Decreasing body mass in birds and mammals increases mass-specific conductance and leads therefore to increased heat loss (Herreid and Kessel 1967). The balance between heat loss and heat production regulates the daily cycle in T_b (Graf 1980, Brown and Refinetti 1996, Refinetti 2003). It is therefore plausible that reduction in body mass could have increased heat loss during the rest-phase and led therefore to lower rest-phase T_b s. The plumage of the SP-birds did not change during the recording duration of 52 days, concluding that there was no effect on conductivity due to changed plumage insulation.

The correlation tests of T_b with either T_a or body mass gave diverse results across the studied birds. However, four out of five birds, which showed rest-phase T_b decline, correlated with at least one of the two factors. I therefore propose that the observed decrease in rest-phase T_b might be the result of a combination of T_a and body mass decrease, rather than the result of one factor. I do not exclude the possibility that body mass or T_a by themselves can have an influence on the rest-phase T_b in Svalbard ptarmigan, however. The observed changes in T_b , T_a and body mass were relatively small. Therefore, tests with more controlled variation in T_a and body mass are necessary to determine the exact effect sizes of T_a and body mass on rest-phase T_b in Svalbard ptarmigan.

4.3.2 Potential endocrine basis for change in rest-phase body temperature

Another explanation for the decrease in rest-phase T_b would be that it is an actively controlled feature as response to the corresponding season. There are some studies suggesting seasonal variation in T_b in birds (Veghte 1964, Hänssler and Prinzinger 1979, Feuerbacher and Prinzinger 1981, Wu, Zhou et al. 2015). Reduction in rest-phase T_b and metabolic rate could thereby contribute to lower energy demands in the winter.

Feuerbacher and Prinzinger (1981) showed decreased rest-phase T_b in castrated Japanese quail compared to control birds with same body mass. Testosterone substitution abolished this difference. They therefore postulated that male birds in winter (with low testosterone levels) have lower rest-phase T_b to save energy during the cold long nights.

Stokkan *et al* (1988) studied endocrine changes in captive Svalbard ptarmigan under artificial light simulating natural seasonal photoperiod changes. Male birds under LD 6:18 showed low plasma testosterone concentration, which rose with increasing day length (Stokkan, Sharp et al. 1988). In the present study, birds were already exposed to SP for nearly two months before the actual recording period of 52 days started, in which they were also held under SP. It is therefore reasonable to think that plasma testosterone levels are constantly low throughout the recording in the studied male birds, indicating that testosterone might be not involved in the observed lowering in rest-phase T_b . However, it has to be considered that the annual endocrine changes in Svalbard ptarmigan were measured on different photoperiodic circumstances and can therefore not lead to any definite conclusions. Blood was taken weekly during the whole period of the recording, so future hormone assays will reveal more information about potential endocrine mechanisms involved in the here observed decrease of rest-phase T_b .

In conclusion, it is possible that the decrease in rest-phase T_b is an actively regulated feature in the winter phenotype of Svalbard ptarmigan. It could be an adaption timed by an endogenous calendar, to reduce metabolic cost during the severe winters. However, this assertion and possible mechanisms behind it needs further thorough investigation.

4.4 The case of the sick bird

During the experiment, one bird got sick and died seven days after the first signs of diarrhoea. Besides the breakdown of the T_b cycle and the reduction in locomotor activity after the onset of diarrhoea, the bird showed also unusual high activity during the rest-phase. This locomotor activity during the rest-phase was not seen in other birds. Admittedly, occasional activity during the rest-phase was not unusual during the experiment, but the sick bird showed rest-phase activity consistently throughout its whole recording (eighteen days from start until death). Unfortunately, the recording for the bird did not reach back further, so it is not clear whether this nocturnal activity was characteristic for this particular bird or if it was a sign of sickness.

If it was a sign for sickness, then it would have indicated the sickness even before diarrhoea onset, which means locomotor activity recordings could be used for monitoring the health status of captive Svalbard ptarmigans.

5 Conclusion

In this experiment I have shown that captive Svalbard Ptarmigan under artificial light and *ad lib* food display a clear daily cycle in T_b under light-dark conditions, but abolish this cycle under constant light. This supports the hypothesis that daily cycles in Svalbard ptarmigan are under little endogenous control and are rather environmentally driven responses. However, it was also shown that T_b rises in anticipation to light before any change in locomotor activity, which argues for some sort of time measurement for the T_b cycle in this arctic species. That also means that the T_b cycle is not a mere side effect of elevated activity. The function of the T_b cycle in this species is still debatable and needs further investigation.

On annual scale, I have shown that the amplitude and shape of the T_b cycle changes between birds under different photoperiods. Furthermore, a decrease in rest-phase T_b was noted in birds, which were kept under constant SP. The simultaneous decrease in T_a and body mass provide a reasonable explanation for this observation. However, an endogenous circannual control on rest-phase T_b cannot be excluded at this point.

As a side note, it was shown that sickness can disrupt the T_b cycle in Svalbard ptarmigan and it was speculated that consistent activity during dark hours might be connected to the sickness.

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Appendix

Appendix A: Dates and times for experimental photoperiods

Table A. Summary of light-dark schedules throughout the experiment for both groups. Birds were transferred into indoor cages on the 17th September 2016. The table corresponds to **Figure 5**.

Date	Short photoperiod group (light hours:dark hours)	Long photoperiod group (light hours:dark hours)
17th September 2015	Sunrise/sunset: 06:03/19:12 Civil twilight: 05:01/20:14 Tromsø (69° 39' N, 18° 57' O)	Sunrise/sunset: 06:03/19:12 Civil twilight: 05:01/20:14 Tromsø (69° 39' N, 18° 57' O)
17th September 2015- 22nd September 2015	12:12	12:12
22nd September 2015- 29th September 2015 (Transition)	12:12 → 6:18	12:12 → 6:18
29th September 2015- 16th November 2015	6:18	6:18
16th November 2015- 27^h November 2015 (Transition)	6:18	6:18 → 18:6
27^h November 2015- 20th December 2015	6:18	18:6
20th December 2015- 30th December 2015 (Transition)	6:18	18:6 → 24:0
30th December 2015 - 12th/14th January 2016	6:18	24:0
After 12th/14th January 2016	6:18	24:0

Appendix B: Example of a calibration curve

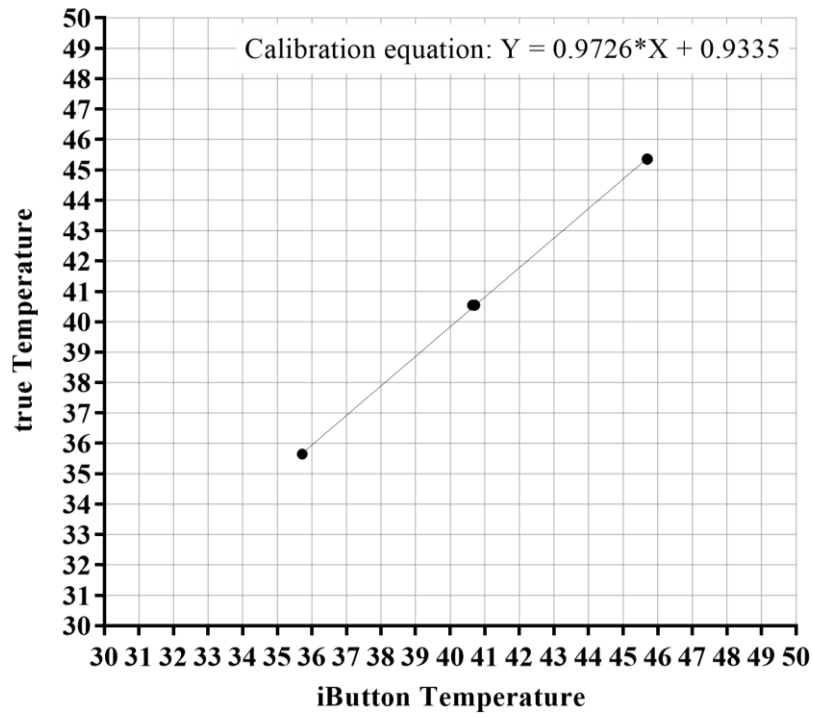


Figure B. Calibration curve for bird 93-030. Calibration equations were used to correct iButton recordings for each bird.

Appendix C: Plumage change during the experiment

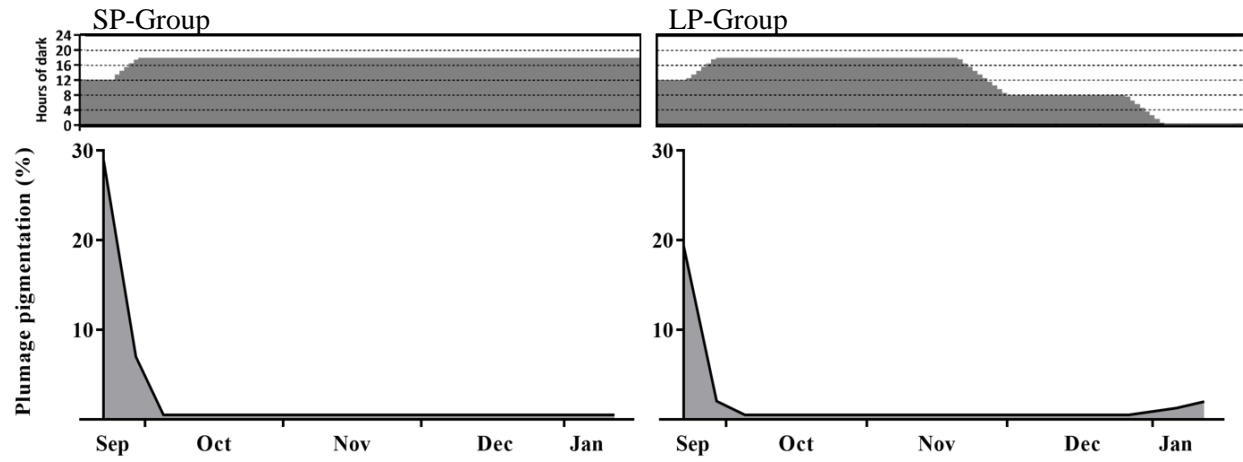
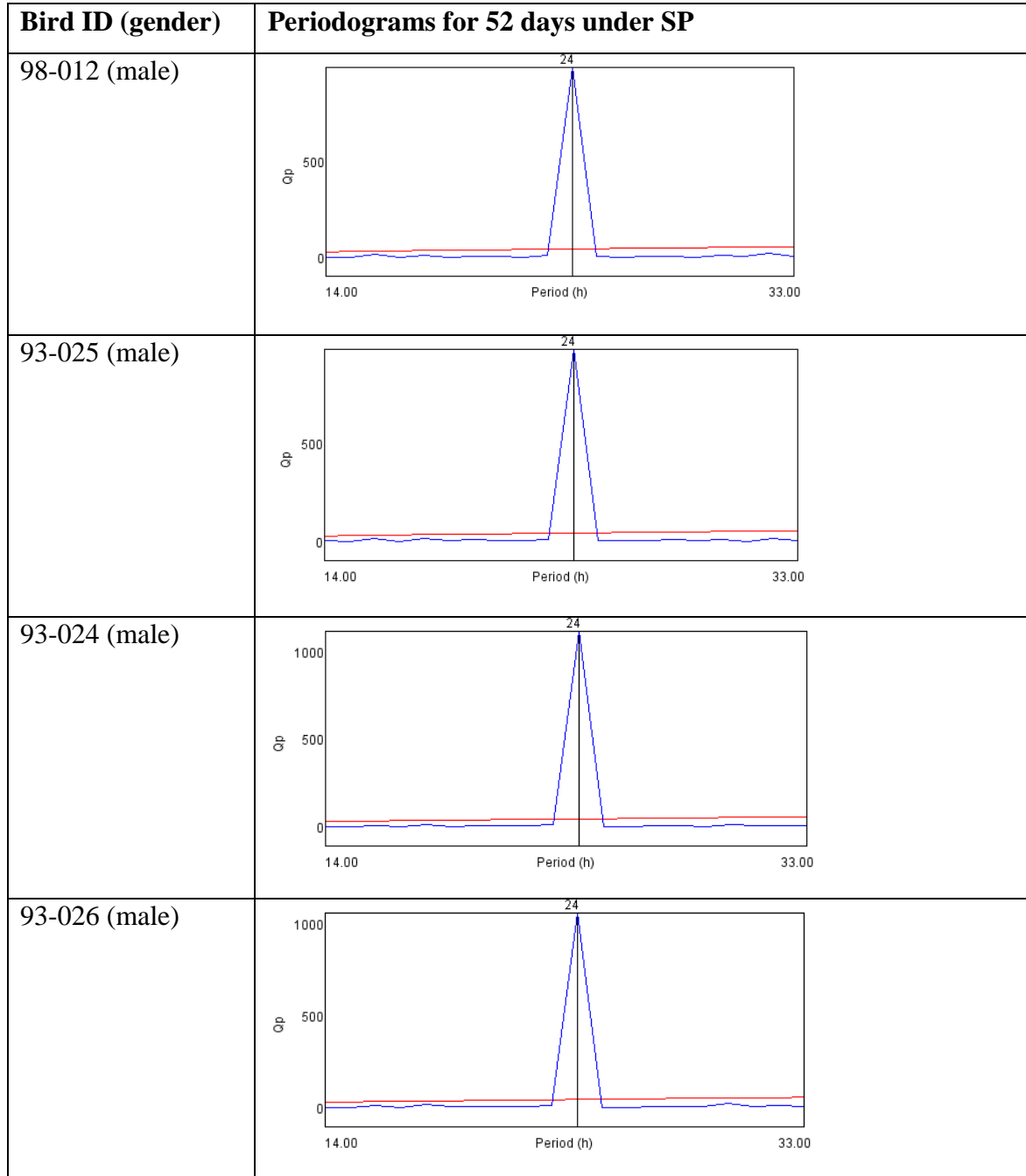
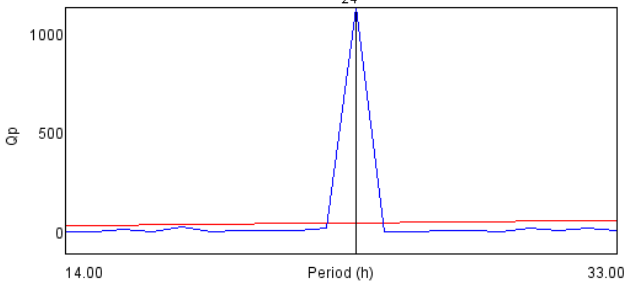
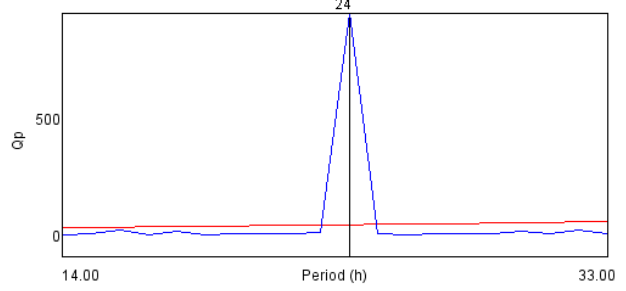
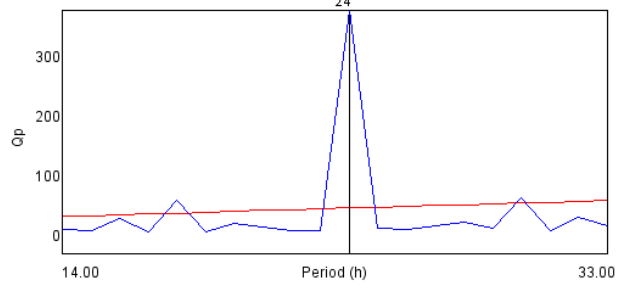
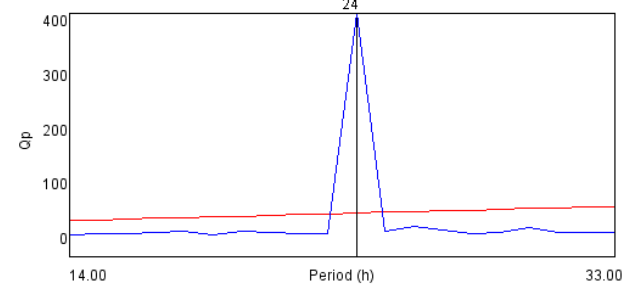
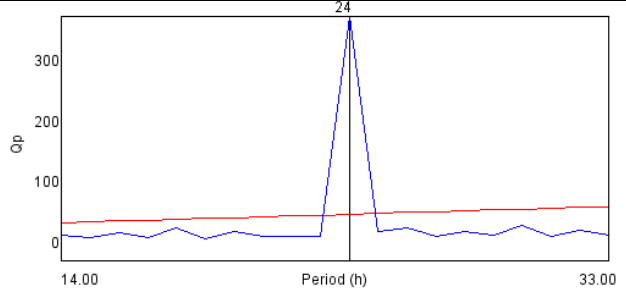


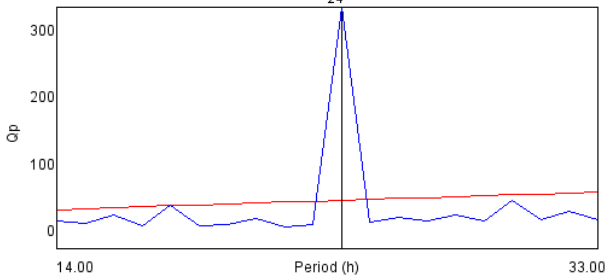
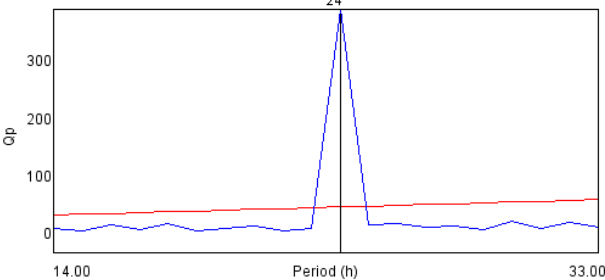
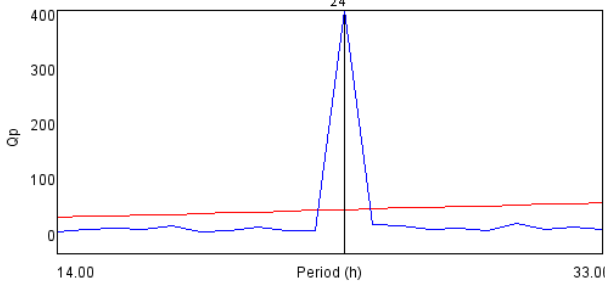
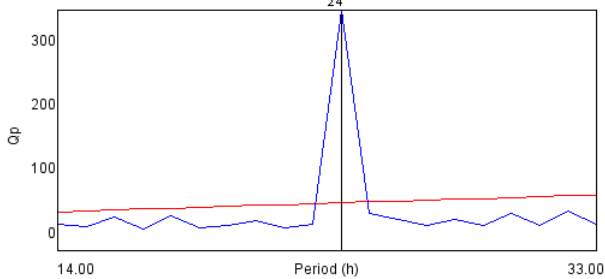
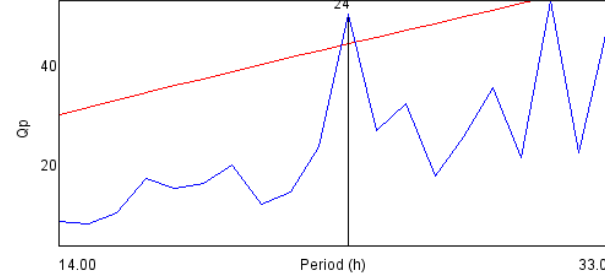
Figure C. Temporal change in plumage pigmentation for both experimental groups. Data is shown as mean.

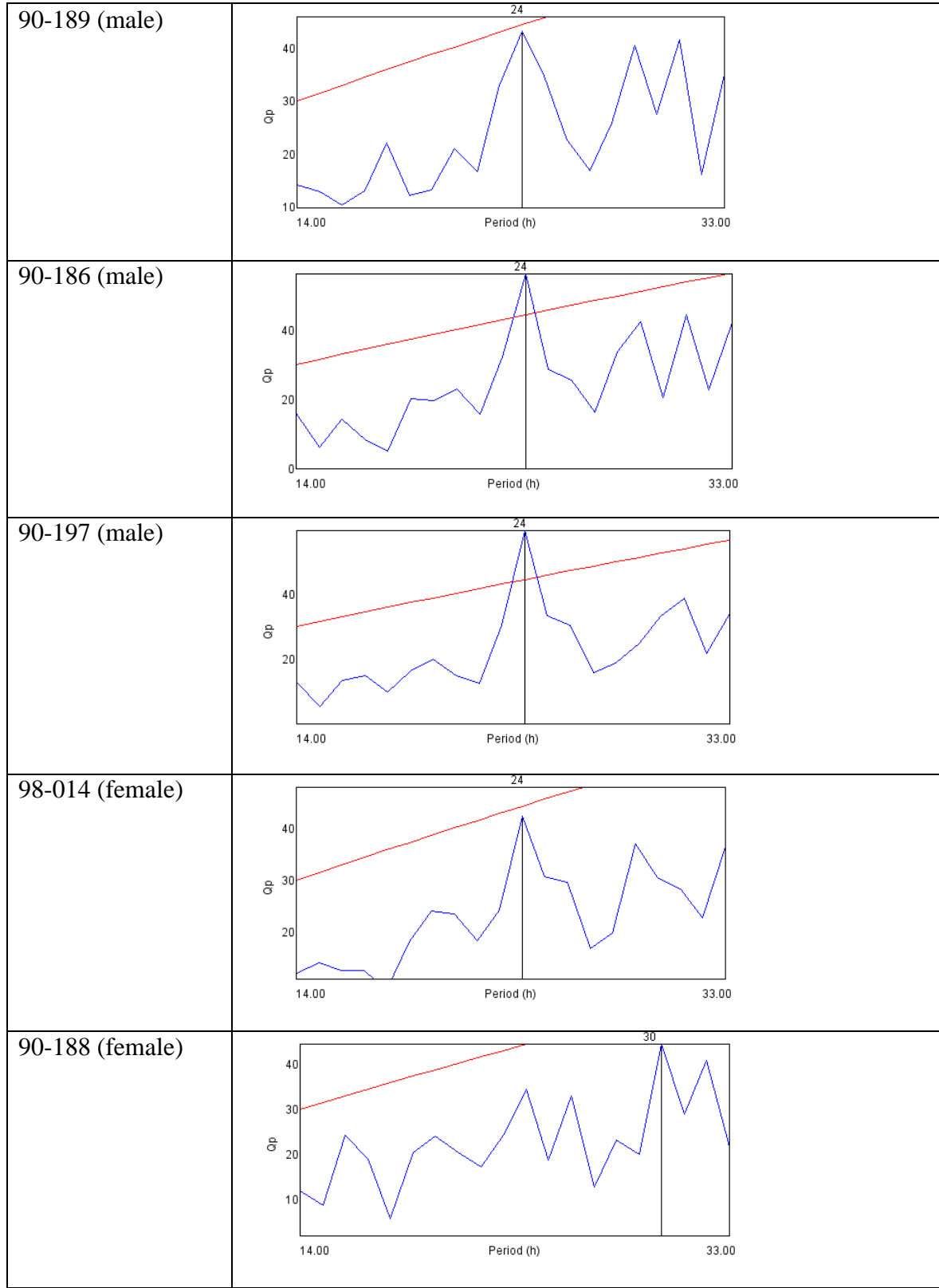
Appendix D: Chi-squared periodograms for all experimental birds

Figure D. Chi-squared periodograms for all experimental birds under each corresponding light regime. The red line in the periodograms marks the threshold for significance ($P = 0.05$).

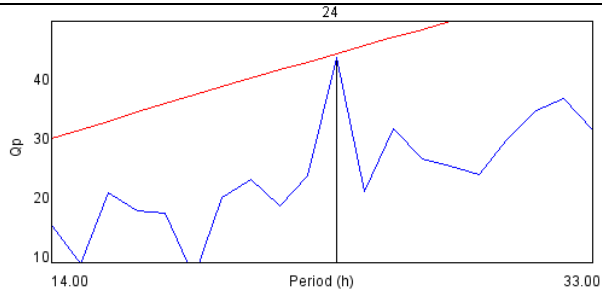


93-030 (female)	
90-180 (female)	
Bird ID (gender)	Periodograms for 23 days under LP
90-196 (male)	
90-189 (male)	
90-186 (male)	

90-197 (male)	
98-014 (female)	
90-188 (female)	
90-181 (female)	
Bird ID (gender)	Periodograms for 14 days under LL
90-196 (male)	



90-181 (female)



Appendix E: Correlation between activity and body temperature

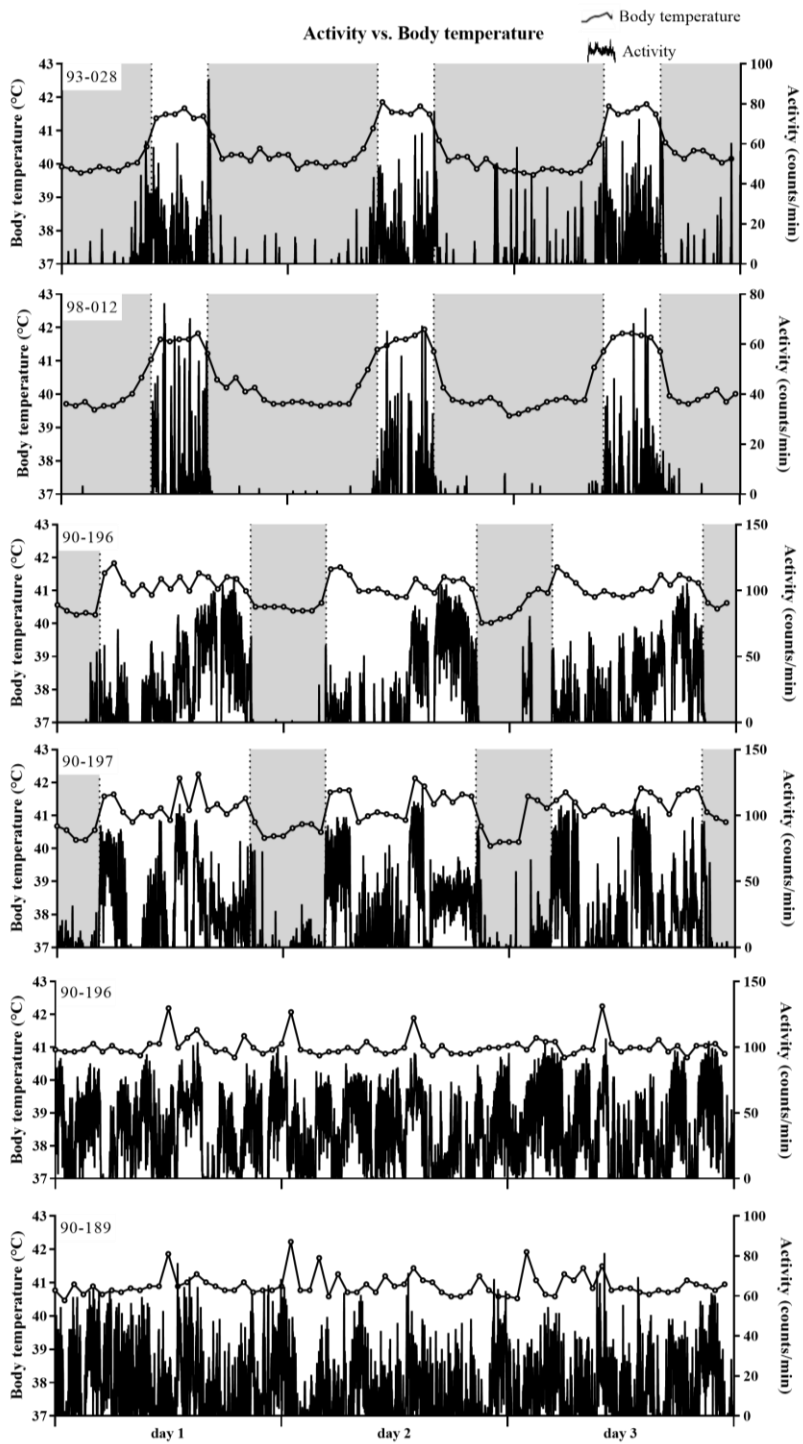


Figure E. Simultaneous recordings of T_b with locomotor activity in male birds. Correlation was tested with non-parametric Spearman correlation and was evident in all birds for the shown days (two tailed $P < 0.05$), except in bird 90-189 under LL (two tailed $P = 0.64$). Three days when there was no handling (weighing and blood sampling) were chosen for each shown bird.

Appendix F: Average air temperature throughout recording for birds under short photoperiod

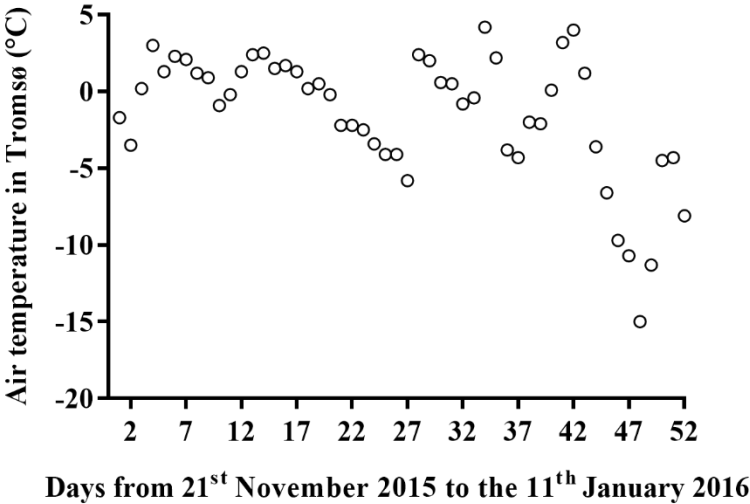


Figure F. Air temperature for Tromsø, Norway from the 21st November 2015 to the 11th January 2016. Data is shown as daily means. Source: <https://www.yr.no>

Appendix G: Calculation of energetic saving deriving from the body temperature cycle

The metabolic heat production was assumed to equal the heat loss and rate of heat loss was calculated by following equation:

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

Q = rate of heat loss
 C = conductance
 T_b = body temperature
 T_a = ambient

Mass-specific conductance for Svalbard ptarmigan in winter was used from Mortensen and Blix (1986) and equals $0.078 \text{ cal g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$. This equals $46.8 \text{ cal h}^{-1} \text{ }^\circ\text{C}^{-1}$ for a bird with a theoretical body mass of 600 g. In order to calculate the metabolic saving deriving from the daily T_b cycle I calculated the heat loss during rest-phase with low T_b and during active-phase with elevated T_b . I thereby used the T_b means of the SP-group from the chapter 3.2.2. T_a was assumed to be $4 \text{ }^\circ\text{C}$ in both phases. Following calculation derived from these assumptions:

$$Q_{rest-phase} = 46.8 \text{ cal h}^{-1} \text{ }^\circ\text{C}^{-1} (39.89 \text{ }^\circ\text{C} - 4 \text{ }^\circ\text{C}) = 1.68 \text{ kcal h}^{-1}$$
$$Q_{active-phase} = 46.8 \text{ cal h}^{-1} \text{ }^\circ\text{C}^{-1} (41.56 \text{ }^\circ\text{C} - 4 \text{ }^\circ\text{C}) = 1.76 \text{ kcal h}^{-1}$$

From the hourly heat loss I calculated the daily heat loss for a bird with a normal T_b cycle under SP assuming 6 hours of active-phase T_b and 18 hours of rest-phase T_b :

$$Q_{cycle} = 6 \text{ h} \times 1.76 \text{ kcal h}^{-1} + 18 \text{ h} \times 1.68 \text{ kcal h}^{-1} = \underline{40.80 \text{ kcal d}^{-1}}$$

To quantify the potential saving I calculated the daily heat loss for a theoretical bird with a constant high T_b :

$$Q_{no\ cycle} = 24 \text{ h} \times 1.76 \text{ kcal h}^{-1} = \underline{42.24 \text{ kcal d}^{-1}}$$

Heat loss can be assumed to equal metabolic heat production. Therefore, the metabolic saving by lowering T_b during rest-phase equals 1.44 kcal d^{-1} (difference between $Q_{no \text{ cycle}}$ and Q_{cycle}) for a bird with a body mass of 600 g, a constant T_a of 4°C and under SP.

In order to put this saving in relation I compared it with the resting metabolic rate of a Svalbard ptarmigan. Mortensen and Blix (1986) calculated a resting metabolic rate of $85.3 \text{ kcal kg}^{-1} \text{ d}^{-1}$ for winter adapted bird, which equals $51.18 \text{ kcal d}^{-1}$ for a 600 g bird. The metabolic saving of 1.44 kcal d^{-1} equals therefore 2.81 % of the daily resting metabolic expenditure.

