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Short title: Overwintering strategy of Japanese raccoon dog

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Abstract

The raccoon dog, *Nyctereutes procyonoides*, is a canid with a passive overwintering strategy in northern Europe. However, the behavior and physiology of the Japanese subspecies, *N. p. albus*, which has fewer chromosomes than the other subspecies, remain unknown. We measured body temperature, body composition and blood biochemistry of wild free-ranging and fasted enclosure-housed *N. p. albus* during boreal winter in Hokkaido, Japan. Body temperature of *N. p. albus* decreased from 38 ºC in autumn to 35.9-36.7 ºC while maintaining a circadian rhythm in late February (n = 3). A transient 21-36 % decrease in resting heart rate occurred when body temperature was low (n = 2). Despite a 33-45 % decrease in body weight due to winter fasting, circulating glucose, total protein and triglyceride levels were maintained (n = 4). Serum urea nitrogen dropped by 43-45 % from autumn to spring, suggesting protein conservation during fasting. The overwintering survival strategy of *N. p. albus* in central Hokkaido is based upon large changes in seasonal activity patterns, winter denning and communal housing without a large decrease in body temperature that is characteristic of subarctic animals exhibiting hibernation or torpor.

Keywords

Autumnal fattening, body temperature, communal housing, *Nyctereutes procyonoides*, winter denning

Introduction

The raccoon dog (*Nyctereutes procyonoides*), a member of the family Canidae, is a small-sized nocturnal omnivore that inhabits temperate to sub-arctic environments. This species is native to northeastern Asia and there are six recognized subspecies; four live in the Chinese/Russian mainland and two inhabit the Japanese islands (Ward and Wurster-Hill 1990). In the 1930s, *N. p. ussuriensis*, originally native to northeastern China and southeastern Russia, was introduced into northwestern Russia to establish a new fur-bearing game animal. Feral animals are now widespread in central and northern Europe (Ward and Wurster-Hill 1990). It has long been thought that the raccoon dog was unique among canids in that the overwintering strategy of the species involves hibernation, partial hibernation, or winter lethargy (Asikainen et al. 2004; Kauhala and Saeki 2004; Ward and Wurster-Hill 1990; Ward et al. 1987). Such assumptions were based on observations that this species accumulated extensive fat stores in autumn (Korhonen et al. 1991), exhibited prolonged denning during winter (Ward and Wurster-Hill 1990) and was behaviorally torpid at this time (Kauhala and Saeki 2004), rather than on measurements of body temperature (T_b) or metabolic rate during winter denning. Recently, Mustonen et al. (2007b), utilizing data-logger temperature measurements and radio-tracking procedures, reported that feral raccoon dogs in Finland exhibited prolonged denning and decreased T_b in wintertime, but winter lethargy was intermittent and the decrease in T_b was less pronounced than in marmots and hedgehogs, suggesting that the raccoon dog in Finland does not hibernate.

Geographically isolated island populations often evolve different morphological and behavioral characteristics and are strongly influenced by founder effects. Cytogenic analyses of raccoon dog subspecies originally native to the Chinese/Russian mainland (Ward et al. 1987) and Finland (Mäkinen et al. 1986) demonstrate a diploid chromosome number of $2n = 54 + Bs$. In contrast, in raccoon dogs native to the Japanese islands, the diploid chromosome number is reduced to $2n = 38 + Bs$ (Ward et al. 1987) as a result of Robertsonian translocation. This suggests that the Japanese raccoon dog is a more recent form undergoing incipient speciation. On the basis of morphological (Haba et al. 2008) and genetic (Masuda 1994) distinctions and geographically non-overlapping distributions, the raccoon dogs of Japan are subdivided into two subspecies; *N. p. viverrinus* is native to the islands of Honshu, Shikoku and Kyushu, where the climate is relatively warm, while *N. p. albus* is native to the northern island of Hokkaido, which is separated from the main island of Japan by the “Blakiston’s line”, marking the transition to subarctic climate. In Hokkaido, *N. p. albus* has been traditionally considered to hibernate during winter, but no physiological recordings of T_b exist. The aim of this study was to clarify the overwintering strategy of *N. p. albus* by examining body composition, blood biochemistry and 24-h rhythms in T_b during winter denning in Hokkaido, northern Japan, and to make comparisons with the overwintering strategy of Finnish *N. p. ussuriensis*.

Materials and Methods

Experiments were approved by the Japanese Ministry of the Environment and conformed to the ethical guidelines of Asahiyama Zoological Park, Asahikawa. Experiments were performed on one group of three free-ranging *N. p. albus* and a second group of four enclosure-housed *N. p. albus*.

Wild *N. p. albus* (two females WTF1, WTF2 and one male WTM3) were captured from farmland habitat on the outskirts of Asahikawa (43º46’N, 142º28’E) in autumn 2007. Under isoflurane anesthesia and butorphanol (0.025 mg/kg s.c.) and tepoxaline (10 mg/kg p.o.) analgesia, temperature data loggers (StowAway TidbiT, model TBI32-5+37, Onset Computer Corporation, Bourne, MA), which were covered with a sterilized silicon sheet, were surgically implanted onto the peritoneal wall inside the abdominal cavity. The loggers registered T_b below 38.2 ºC once every 8 min, yielding 20519-32520 data points per animal. Radio-transmitter collars were attached to each animal (Mammal collar, model M1830B, Advanced Telemetry Systems Inc., Isanti, MN) to monitor the size of home ranges and to enable recapture. After a postsurgical recovery period of 2 weeks, during which animals were housed and their postsurgical condition monitored daily at Asahiyama Zoological Park, the animals were released at the capture sites and located 1-2 times a week at 13:00-14:00 h with a portable radio-receiver (R410 Scanning receiver, Advanced Telemetry Systems, Inc.) and a hand-held antenna. In spring 2008, the animals were recaptured and the loggers recovered. Body weight and body fat percentage were determined before and after winter with standard scales and body fat scales (HealthLab, Kao Corporation, Tokyo, Japan) calibrated for dogs using bioelectrical impedance analysis. Upon the first capture and recapture after sedation with the mixture of medetomidine (50 µg/kg i.m.) and midazolam (0.15 mg/kg i.m.) and prior to feeding, blood was sampled from jugular vein and allowed to clot at room temperature. Afterward, it was centrifuged at 1000 g for 15 min and supernatant serum was obtained and stored at -80 ºC until analysis. Blood biochemistry was assayed in a commercial laboratory (Hoken-kagaku kenkyusho, Yokohama, Japan). Snow accumulation data were obtained from the Japan Meteorological Agency. Local ambient (T_a) and *N. p. albus* den temperatures (T_d) were recorded by thermosensitive loggers (StowAway TidbiT, model TBI32-20+50, Onset Computer Corporation).

In 2003 and 2007, four other *N. p. albus* (two females and two males) housed at Asahiyama Zoological Park were also tested under semi-natural conditions. Animals, involved in a traffic accident or captured around the zoo, were protected and housed in open-air cages (2 × 2 × 1.5 m) with wooden nestboxes (60 × 60 × 60 cm) either singly (2003) or in pairs (2007) under natural photoperiod and T_a. Prior to experiments foods (chicken, apples and potatoes) and water or ice were available *ad libitum*. In autumn, temperature and electrocardiogram (ECG) radio-transmitters (TA11CTA-F40, Data Sciences International, St. Paul, MN) were surgically implanted using surgical protocol described above. The body of the transmitter containing the temperature sensor was implanted into the peritoneal cavity and affixed to the peritoneal wall. Paired wire electrodes for ECG leads exited the peritoneal cavity and were sutured to the pectoral muscle under the skin of the ventral thorax. The animals were allowed a recovery period of 1 month. On December 25, they were deprived of food and their cages and nest boxes were covered with snow to simulate natural environmental conditions. T_b and ECG were recorded at a frequency of 0.5 Hz (T_b) and 400 Hz (ECG) using Power Lab Data Acquisition Systems (ADInstruments, Sydney, Australia) or Dataquest A.R.T.^{TM} system (Data Sciences International) until the following spring (March 4). Every 10 min, heart rates (HR) were calculated by the R-R intervals of the ECG recording. These parameters were recorded only when animals were in or near the nestboxes in which the radio-receivers were set up. As a result we were able to determine whether the animals were inside the nestboxes or moving outside. Two transmitters failed during use and recordings were obtained only in two animals (numbers of successful HR recording were 6440 for ZTF03 and 8683 for ZTM07). Body weight, body fat and blood biochemistry of four animals were recorded as previously described.

Statistical analyses were performed with InStat software (GraphPad Software Inc., San Diego, CA). The minimum daily T_b (mT_b) was calculated for each date and individual, and the mean mT_b, representing the mean values of mT_b during each time period described below, were compared by repeated measures analysis of variance (ANOVA). The mean mT_b values of the wild free-ranging animals were calculated over four representative time periods which corresponded to the snow-free period (November 8-14), early winter period (January 1-7), late winter period (February 12-18) and early spring period (March 25-31). The mean mT_b values of the enclosure-housed animals, fasted from December 25 to March 4, were calculated over three representative time periods which corresponded to the early fasting period (December 25-31), mid-fasting period (January 25-31) and late fasting period (February 23-29). Differences in body weight, body fat and blood biochemistry were analyzed by paired Student's t-test. $P < 0.05$ was considered statistically significant. The results were expressed as means or means ± SD.

Results

Home ranges and dens of the three wild free-ranging raccoon dogs

WTF1 was captured in an orchard and spent much of her time there and in the adjacent cemetery in autumn. In November, WTF1 travelled up to 2 km from the orchard and the animal appeared to spend most of her time foraging. A den could not be located at this time. Once snow accumulated (mid-November) the animal spent almost all of her time in concrete trenches and drains of about 30 cm deep, under the cemetery. Up to eight other *N. p. albus* also utilized this system of subterranean trenches. Snow cover was continuous from December to March with a depth of about 50 cm. The temperature inside the trenches (T_d) where the animal usually stayed was nearly constant (-2.4-+1.8 ºC from mid-January to early March) despite T_a decreasing to -18 ºC (Figure 1). By mid-March WTF1 had begun foraging in the orchard and by a stream nearby.

WTF2 and WTM3, which were captured on separate days in another orchard, were a cohabiting pair. In November they foraged and lived separately in adjacent forested valleys. Dens could not be precisely located at this time. In December both animals cohabited in a den previously occupied by the male (WTM3). This den had two exits at the same end. It was a single tunnel at least 5 m long that ended in a chamber about 1 m below the ground. The T_d of this den was 1.5-3.9 ºC from mid-January to early March and was warmer than that of WTF1. In early February, in response to observer interference, both animals moved to a den in the next valley that had been previously occupied by the female (WTF2). This den was also more than 5 m in length with two or three entrances at the same end. WTF2 resided in this den until recapture in early April, by which time she had begun to forage up to 1.5 km from this den. In late March WTM3 began to forage and reside in another den about 500 m away. This den was similar in structure and length to the others examined.

Body temperature of the three wild free-ranging raccoon dogs

In the snow-free period before winter, the 1-week mean mT_b (which was usually recorded between 04:00 and 10:00 h) of the three wild free-ranging animals was 37.9-38.1 ºC. Figure 1 shows that the mT_b of WTF1 began to decrease once snow had accumulated (mean mT_b was 36.1 ºC in early winter period) and it remained low during the period of snow accumulation (mean mT_b was 35.5 ºC in late winter period). The lowest T_b (34.8 ºC) was recorded on February 22, 1 week after the maximum snow accumulation. The mT_b began to increase as the snow began to melt (mean mT_b was 37.4 ºC in early spring period), and became constant in early April. The mT_b profiles of WTF2 and WTM3 were very similar (mean mT_b was 36.1-36.3 ºC in early winter period, 35.9-36.5 ºC in late winter period and 37.1-37.6 ºC in early spring period) and the mean mT_b of all three animals was significantly lower in early and late winter periods than in the snow-free period and in early spring period ($P < 0.05$). The lowest T_b of WTF2 and WTM3 in winter (35.5 ºC) was recorded on January 28, 2 days after the lowest T_a, and again on February 24 (WTF2) and February 8 (WTM3), respectively. The mean monthly values for T_a during winter for this research period were -4.4 ºC (December), -8.6 ºC (January) and -7.4 ºC (February). During the period of snow accumulation, when all animals were denning, T_b exhibited a circadian rhythm and mT_b was recorded between 06:00 and 11:00 h, except for early January and early February in WTF2 and WTM3.

Body temperature and heart rate of the two enclosure-housed raccoon dogs

The mean mT_b of the two enclosure-housed animals in the early fasting period was 36.4 ± 0.1 ºC (ZTF03) and 37.6 ± 0.2 ºC (ZTM07), and was usually recorded between 17:00 and 00:00 h. There was little change in mT_b until the end of January (36.4 ± 0.1 ºC in ZTF03 and 37.2 ± 0.1 ºC in ZTM07), but in the late fasting period, the mean mT_b decreased significantly to 35.9 ± 0.3 ºC (ZTF03) and 36.7 ± 0.3 ºC (ZTM07) compared to the previous fasting period ($P < 0.05$). The lowest T_b [35.4 ºC (ZTF03) and 36.3 ºC (ZTM07)] was recorded in late February between 08:00 and 10:00 h. A circadian rhythm of T_b was clearly evident and indistinguishable from the free-ranging animals. HR was recorded simultaneously along with T_b, and at the time of the lowest T_b the minimum daily HR (mHR) decreased by 18-36 %, relative to the mHR during the early fasting period (see Table 1). Figure 2 summarizes the 1-week T_b and HR fluctuations of ZTM07 in the early and late fasting periods.

Body composition and blood biochemistry

As shown in Table 2, the body weight and body fat percentage of both wild free-ranging (n = 3) and enclosure-housed (n = 4) animals decreased from autumn to spring. While the serum glucose, total protein and triglyceride concentrations were unchanged, the serum urea nitrogen levels decreased markedly by 43-45 %. Although considerable individual differences in the cholesterol levels were observed in autumn, the high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol concentrations decreased in both groups of animals by spring. The data from one enclosure-housed female in 2003 were atypical and excluded from analysis.

Discussion

This study on wild free-ranging and enclosure-housed *N. p. albus* in central Hokkaido demonstrated that the overwintering survival strategy of this subspecies consists of large changes in seasonal activity pattern, winter denning and communal housing without the large decrease in mT_b characteristic of subarctic animals that exhibit hibernation or torpor.

Autumn was characterized by an intensive foraging effort that resulted in the accumulation of considerable subcutaneous and intraperitoneal body fat reserves. The onset of snow accumulation induced a distinct decrease in the activity of the free-ranging and enclosure-housed *N. p. albus,* as both groups of animals spent large periods of time in their dens or nestboxes. From December to February, observations of the free-ranging *N. p. albus* were restricted to daylight hours; however the animals were never observed outside their dens. Tracks indicated that the animals sometimes spent 2-3 consecutive days inside their dens. Our recordings indicated that the enclosure-housed *N. p. albus* spent 18-24 h per day in their nestboxes and rarely moved in daylight hours during winter.

No previous studies have recorded T_b of Japanese raccoon dogs in nature or on farms during winter. One limitation of this study was that T_b data above 38.2 ºC could not be recorded for wild animals; however the mean mT_b of the wild free-ranging *N. p. albus* decreased from 37.9-38.1 ºC in autumn to 35.9-36.5 ºC in winter, and that of the fasted enclosure-housed animals decreased from 36.4-37.6 ºC in early winter to 35.9-36.7 ºC in late winter. This reduction in mT_b is similar to findings for *N. p. ussuriensis* in Finland, in which T_b of feral animals decreased from 37.8-38.3 ºC to 35.9-36.5ºC and T_b of fasted farmed animals decreased from 37.6 ºC to 36.1 ºC (Mustonen et al. 2007b; Nieminen et al. 2005).

In the American badger (*Taxidae taxus*), which has a body weight similar to *N. p. albus*, resting HR is well correlated with metabolic rate (Harlow 1981). In a typical bout of torpor, the decrease in T_b in the American badger is 9 ºC; together with decreased HR, this corresponds to a 27 % reduction in energy expenditure. In the raccoon dog, the theoretical energy benefit from a decrease in T_b of 1-2 ºC during winter denning is less than 10 %, although it has been documented that the metabolic rate of raccoon dogs could be reduced by as much as 25 % in the winter (Heptner et al. 1974). In the two enclosure-housed animals in this study, resting mHR was reduced by 18-36 % when T_b was at its lowest (between 06:00-10:00 h daily), but these decreases in HR were only briefly sustained (5-30 min) before HR and T_b transiently increased and then slowly fell back towards the minimum HR and T_b. These measurements suggest that the resting metabolic rate of *N. p. albus* can be temporarily reduced by 18-36 %, which supports earlier observations.

Behavioral strategies, in addition to physiological specializations, can be employed to minimize loss of heat to the environment. In small mammals of a similar size to the raccoon dog, such as the alpine marmot (*Marmota marmota*; Arnold 1988) and the striped skunk (*Mephitis mephitis*; Hwang et al. 2007), communal denning during winter is associated with considerable energy savings for each individual animal. In Honshu, Japan, raccoon dogs (*N. p. viverrinus*) often live in groups (Ikeda et al. 1979; Ward and Wurster-Hill 1989) and we found that *N. p. albus* also denned communally during winter. In this study, WTF1 utilized an extensive den system with eight other raccoon dogs. While WTF2 and WTM3 denned together, they also shared their den with another male raccoon dog. This communal denning behavior probably affords an energy advantage during winter. Among our enclosure-housed animals, the observation that mT_b of ZTF03, which was housed singly, was lower than that of ZTM07, which was housed in a pair, supports this speculation.

Wild *N. p. albus* accumulated body fat in autumn, and those body fat stores were substantially decreased by the end of winter. Weight loss and reduction in the percentage of body fat were very similar between the wild free-ranging and the fasted enclosure-housed animals, implying that the wild animals were in negative energy balance during winter denning. In bears (*Ursus americanus, U. arctos arctos*) and badgers (*Meles meles*, *Taxidea taxus*), both of which have decreased T_b during winter, body weight has been observed to decrease by 20-30 % from autumn to spring (Craighead et al. 1974; Harlow 1981; Hissa et al. 1994; Tanaka 2006). In the *N. p. albus* studied, body weight decreased by 45 % (wild) and 33 % (enclosure-housed) from autumn to spring suggesting a higher rate of weight loss. Despite fasting and the depletion of body fat during winter, as previously reported in wild and fasted farmed raccoon dogs in Finland (Mustonen et al. 2007a, c), the circulating glucose, total protein and triglyceride levels were similar in both wild free-ranging and enclosure-housed animals between autumn and spring. Also consistent with animals in Finland (Mustonen et al. 2004, 2007a), the serum urea nitrogen level was significantly lower in *N. p. albus* in spring, indicating a reduction of protein catabolism. Protein conservation with utilization of fat is a metabolic strategy also used by denning American black and European brown bears (Hissa et al. 1994; Nelson et al. 1984). The collective expression of this metabolic strategy in these two different mammalian genera suggests that protein conservation may be a common strategy for animals with passive overwintering. In contrast to these serum components, cholesterol levels in our autumn animals showed some differences from the Finnish cohort. As commonly observed in carnivores, the serum

HDL-cholesterol level in *N. p. ussuriensis* is usually much higher than LDL-cholesterol level (Mustonen et al. 2007a), but both cholesterol levels in our autumn animals were similar in the present study. Though this might be because of the difference in their genetic backgrounds, we speculate that it is probably caused by the food provided at the zoo. Animals in our study were mainly given chicken with cholesterol-rich eggs and could have obtained food from cache place before sampling; both of these factors could have increased the serum cholesterol levels and changed the LDL- and HDL-cholesterol balance (Dubois et al. 1994; Julien et al. 1984). The high ration of HDL/LDL cholesterol level in enclosure-housed spring animals, which were completely fasted when blood was sampled, would support this speculation.

In conclusion, *N. p. albus* in Hokkaido shares similar adaptations with another subspecies of raccoon dogs living in cold environments in that they exhibit a profound fattening in autumn and decrease their winter energy expenditure by voluntarily choosing prolonged, communal denning. Transient decrease in HR of approximately 30 % and a decrease in mT_b from 38 ºC in autumn to 35.9-36.7 ºC in winter, while retaining a clear circadian rhythm of T_b contribute to a slight decrease in daily energy expenditure. Together these behavioral and physiological adaptations prove to be an effective overwintering strategy for *N. p. albus* in Hokkaido, and represent an intermediate survival strategy between mammals that remain cenothermic and those that utilize extreme hypothermia during winter.

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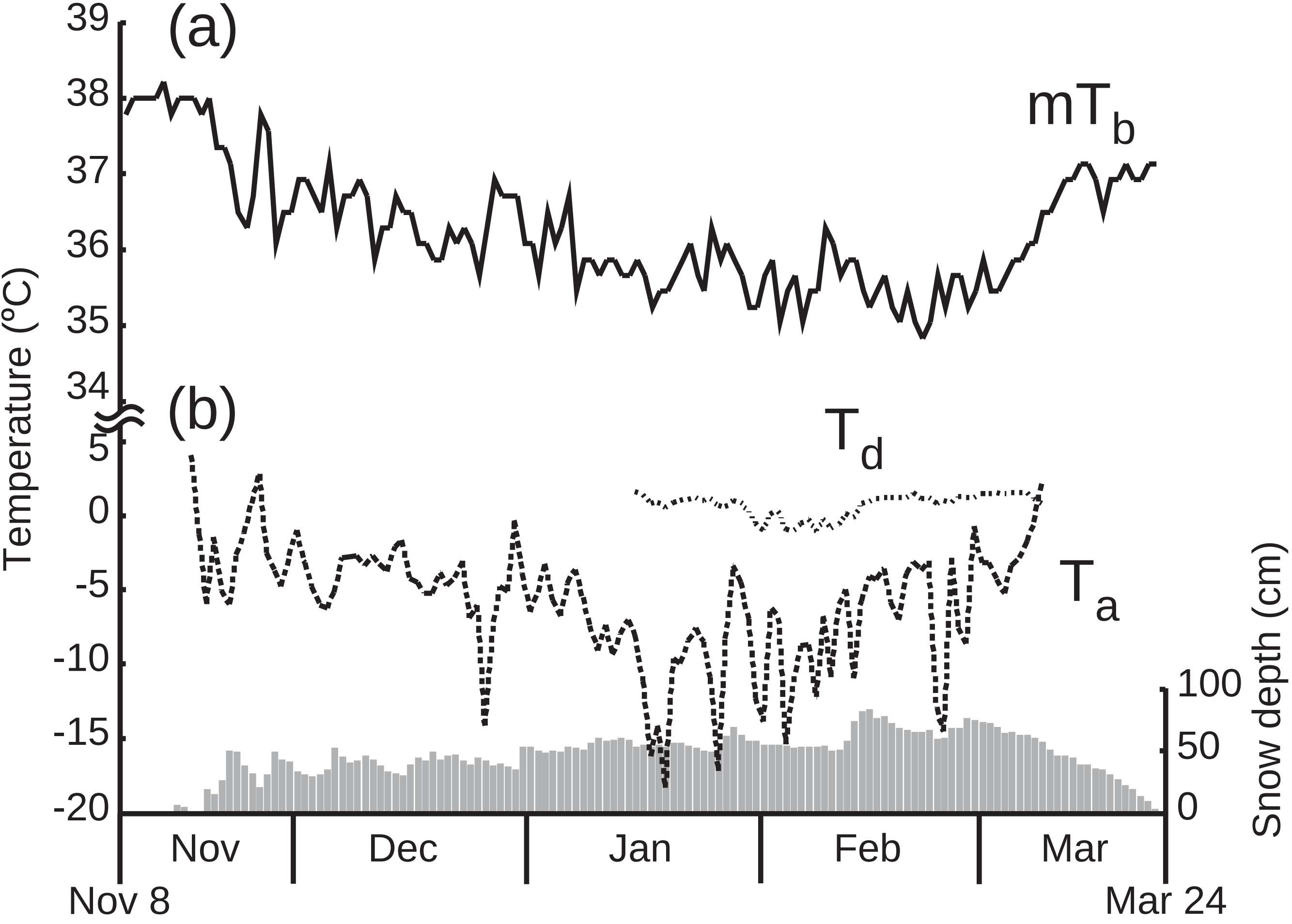
Figure legends

Figure 1.

Minimum daily body temperature (mT_b) (a; solid line), mean local ambient temperature (T_a) (b; dashed line) and mean den temperature (T_d) (b; dotted line) of a wild-free ranging female *N. p. albus* (WTF1), and snow accumulation in the study area (b; solid bar) from autumn 2007 to spring 2008.

Figure 2.

One-week body temperature (T_b; black line) and heart rate (HR; gray line) fluctuations of an enclosure-housed male *N. p. albus* (ZTM07) during the early (a) and late (b) fasting period. Black bars represent the dark period (18:00-6:00).


(a)
(b)
mT_b
T_d
T_a
Temperature (°C)
39
38
37
36
35
34
5
0
-5
-10
-15
-20
Snow depth (cm)
100
50
0
Nov
Dec
Jan
Feb
Mar
Nov 8
Mar 24

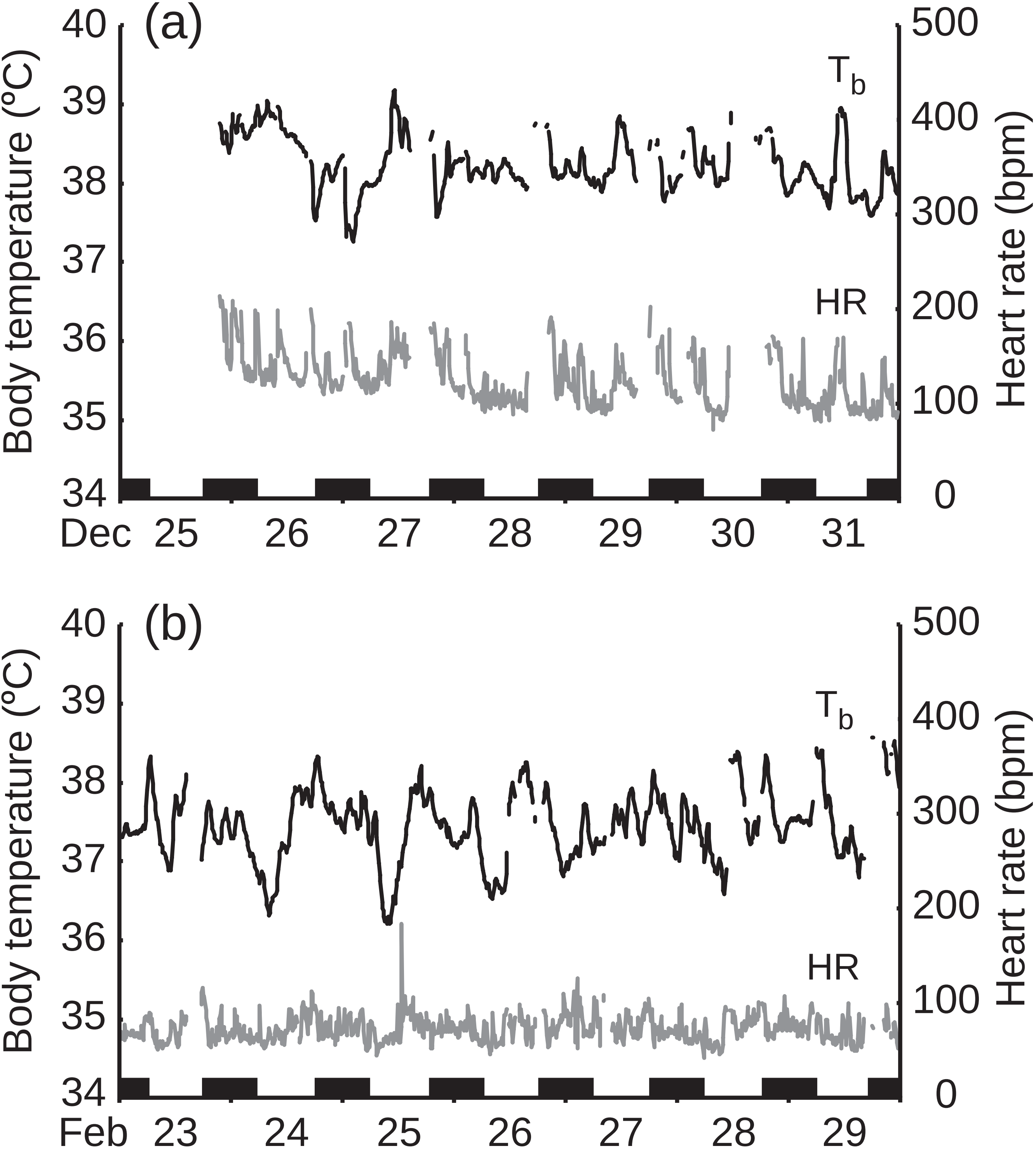

(a)
Body temperature (°C)
40
39
38
37
36
35
34
Heart rate (bpm)
500
400
300
200
100
0
T$_b$
HR
Dec
25
26
27
28
29
30
31
(b)
Body temperature (°C)
40
39
38
37
36
35
34
Heart rate (bpm)
500
400
300
200
100
0
T$_b$
HR
Feb
23
24
25
26
27
28
29

Table 1.

Body temperature and heart rate of two enclosure-housed *N. p. albus* (ZTF03 and ZTM07) in early fasting period and late fasting period when the lowest T_b was recorded. (mT_b = minimum daily body temperature; mHR = minimum daily heart rate. Values are means ± SD.)

	Early fasting period (Dec 25-31)		Late fasting period (Feb 23-29)		
	Mean mT_b (ºC)	Mean mHR (bpm)	Mean mT_b (ºC)	Mean mHR (bpm)	% decrease of HR (%)
ZTF03	36.4 ± 0.1	72.4 ± 3.1	35.9 ± 0.3	59.4 ± 7.1	18.0
ZTM07	37.6 ± 0.2	89.8 ± 14.7	36.7 ± 0.3	57.8 ± 3.6	35.7

Table 2.

Body composition and blood biochemistry of wild free-ranging and enclosure-housed *N. p. albus* before and after winter. Values are means ± SD.

	Wild free-ranging (n = 3)		Enclosure-housed (n = 3[a])	
	Autumn (Oct)	Spring (Mar-Apr)	Autumn (Oct-Nov)	Spring (Mar)
Body weight (kg)	6.89 ± 1.52	3.73 ± 0.61[c]	6.72 ± 0.49	4.74 ± 0.45[c]
Body fat percentage (%)	30.5 ± 5.5 (n = 2[b])	15.3 ± 0.5	35.5 ± 0.5 (n = 2[b])	23.5 ± 2.5 (n = 2[b])
Glucose (mg/dL)	108.0 ± 20.1	77.3 ± 2.1	91.3 ± 23.2	84.3 ± 12.8
Total protein (g/dL)	7.4 ± 0.7	6.6 ± 0.1	7.9 ± 0.9	7.1 ± 0.7
Triglyceride (mg/dL)	63.7 ± 6.3	65.0 ± 16.1	55.7 ± 4.2	51.0 ± 3.7
Urea Nitrogen (mg/dL)	17.0 ± 1.4	9.7 ± 1.2[c]	16.9 ± 5.2	9.2 ± 1.1
Total cholesterol (mg/dL)	370.7 ± 172.2	286.7 ± 63.3	387.0 ± 137.7	170.3 ± 15.5
HDL cholesterol (mg/dL)	154.7 ± 8.0	117.1 ± 22.5	167.5 ± 23.0	110.7 ± 21.2
LDL cholesterol (mg/dL)	149.9 ± 138.5	94.4 ± 49.6	168.4 ± 122.2	15.6 ± 5.0

[a] One enclosure housed female in 2003 was excluded from the analysis. [b] Body fat percentage was measured in two wild free-ranging animals in autumn 2007, and in two enclosure-housed animals in 2007. [c] $P < 0.05$ vs. Autumn.