

HIBERNATING ABILITY OF *SCOTOPHILUS KUHLII* BY INFRARED CAPACITY

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ABSTRACT

In Indian continent, about 13 orders, 42 families, 180 Genera and 390 species of mammals are present. Of these, 8 orders, 23 families, 45 Genera and 66 species have been recorded from Thar Desert of Rajasthan. Out of 115 species of bats (17 megabats and 99 microbats) reported from India, eight micro bats found in the Thar Desert covering the Shekhawati region (Sikar and Jhunjhunu) of Rajasthan. Hibernation is an adaptive approach in bats that facilitates coping with low ambient temperatures and inadequate food during winter. The over-winter survival of hibernating bats depends on the quantity of energy that animals store prior to hibernation, the rate of depletion of these reserves and the duration of winter. In this study compare the Body temperature (T_{body}) of roosted *Scotophilus kuhlii* during summer and winter using Infra red camera (FLIR C2) in urban environment as historical old haveli of shekhawati region. Period of winter average body temperature is decreased by $9.8 \pm 1.13^{\circ}\text{C}$ and bats with parasite are also decreased by $9.1 \pm 0.95^{\circ}\text{C}$ as compare to summer season. During the winter arousals, bats exhibited movement following T_{body} increase of only $9.8 \pm 1.13^{\circ}\text{C}$, compare to $>30.1 \pm 1.56^{\circ}\text{C}$ increases during normal arousals of *S. kuhlii*. The maximum and minimum values of (T_{body}) in winter with parasites are $21.9 \pm 0.90^{\circ}\text{C}$ and $20.2 \pm 1.10^{\circ}\text{C}$ respectively.

INTRODUCTION

Chiropterans, identifying as bats, are the only factual flying mammals. Bats globally comprise of 1,116 species belong to 202 genera, 18 families. They constitute about a quarter of the entire mammal species and are second to Rodents in phrase of diversity. Thar Desert in Rajasthan has only 6% area of the total area of the country. Within this little area, with hostile climatic environment, about 15.8% (68 out of 428) mammalian species are surviving. In India, about 13 orders, 42 families, 180 Genera and 390 species of mammals are present. Of these, 8 orders, 23 families, 45 Genera and 66 species have been recorded from Thar Desert of Rajasthan. Out of 115 species of bats 17 megabats and 99 microbats are reported from the Thar Desert of India. On the Indian sub-continent, *Scotophilus* represented by two sub-species including *S. heathii* and *S. kuhlii* (Bates and Harrison 1997, Srinivasulu *et al.*, 2010a). *Scotophilus heathii* Horsfield, 1831 is distributed from Afghanistan to South China, including Hainan Island, south to Sri Lanka, Vietnam, Cambodia, Thailand and Burma. The genus *Scotophilus* is represented by 12 species with distribution ranges from South Africa to Indonesia and the Philippines (Simmons 2005). *Scotophilus kuhlii* Leach, 1821 was formerly questioned as *S. heathii* (Tate 1942, Ellerman and Morrison-Scott 1951), however, the taxon was later identified as a distinct species (Peterson

Research Article

1968, Hill and Thonglongya 1972, Corbet and Hill 1992, Koopman 1993, Bates and Harrison 1997, Simmons 2005). The species often mixed with its congeners, representing a complex of several species, and further studies are needed to clarify current taxonomic status (Bates *et al.*, 2008).

Hibernation and Arousal Capacity in Bats

Hibernation is an energy-saving strategy that is strongly influenced by the ambient conditions in a cave. When a bat is hibernating, low ambient temperatures lead to a decrease body temperature and metabolic rate. South Asia is characterized by harsh winter conditions, when feeding opportunities decline and low ambient temperatures there is increase in heat loss and thus it increases the thermoregulatory costs of bats. Because they are small flying animals, micro bats are not able to accumulate large fat reserves to maintain a constant body temperature. They have two other ways of coping with this problem: hibernation and migration (Davis, 1970; Fleming & Eby, 2003). Most European temperate zone bats are 'resident', usually selecting underground hibernation sites, such as fortifications, natural and man-made caves and ice cellars (Mitchell-Jones *et al.*, 2007). During hibernation, the bats reduce the difference between body and ambient temperature to an absolute minimum (*i.e.* become torpid) acting like heterotherms. This technique allows them to survive the winter with just a small amount of body fat (on average a maximum of 25% of their body weight). Bats hibernate from autumn to spring although the exact period varies between the species. This period is characterized by prolonged bouts of torpor, punctuated with periodic arousals, on average every two weeks, during which the bats return to endothermy (e.g. Daan & Wichers 1968, Brack & Twente 1985, Harmata 1985, 1987). According to Daan (1972), species of the genus *Myotis* such as the whiskered bat *M. mystacinus*, the Daubenton's bat *M. daubentonii* and the pond bat *M. dasycneme* wake up on average 9.0-13.3 times during a winter. These arousal frequencies are much lower than those given by Dunbar & Tomasi (2006) for the eastern red bat *Lasiurus borealis* in Canada, where a maximum of 39 arousals was recorded at an ambient temperature of 8⁰C. Also the Greater Horseshoe Bats *Rhinolophus ferrumequinum* and the common Pipistrelle bat *Pipistrellus pipistrellus*, both European bat species, arouse more frequently than the three *Myotis* species (Avery 1985, Park *et al.*, 2000), probably due to their ability to forage during the winter. Bats arouse more readily in warm conditions than in cold conditions (e.g. Ransome 1971, Park *et al.*, 2000, Humphries *et al.*, 2002, Boyles *et al.*, 2007), a phenomenon also known in other mammals (e.g. Geiser & Kenagy 1988, Ortmann & Heldmaier 2000). In laboratory experiments, researchers (Thomas *et al.*, 1990) found that the little brown bat *Myotis lucifugus* uses approximately 84% of its winter fat reserve to cover the cost of arousals, whereas only 16% is needed to maintain the low metabolic rate. The energy expenditure during 80 days of hibernation is equivalent to one hour of flight (Kokurewicz 2004). During the warming phase of arousals, the heat generated (mainly by burning fat in brown adipose tissue and muscle shivering) causes the body temperature to rise from near ambient to euthermic levels (Smalley 1963, Hayward & Ball 1966). The whole process takes an average of 30 minutes (Barclay *et al.*, 1996, Lee *et al.*, 2002). A bat can stay aroused from hibernation torpor for 15 hours or longer (Twente & Twente 1987, Speakman *et al.*, 1991, Park *et al.*, 2000) with animals tending to remain endothermic longer at higher ambient temperatures (Harmata, 1985). Researchers have assumed that these periods of arousal are necessary for bats to eliminate accumulated metabolites, urine, escape from predators or to adjust

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to changes in the temperature of their hibernacula (e.g. Prendergast *et al.*, 2002, Humphries *et al.*, 2003; Davydov, 2004). Other activities undertaken during these arousals include drinking and copulation (McCracken & Wilkinson 2000; Kokurewicz, 2004; Boyles *et al.*, 2006). Arousals are sometimes externally induced by disturbance resulting from exposure to air currents (Pflitsch & Piasecki, 2003), heat, light, sounds and physical contact (Davis 1970, Speakman *et al.*, 1991, Thomas 1995, Thomas & Geiser 1997, Johnson *et al.*, 1998). The annual bat census of all known underground hibernacula (Mitchell-Jones *et al.*, 2007) undertaken in order to monitor bat population can potentially be a source of such disturbance. The census is performed between 15 December and 15 February. Bat observers use torches, binoculars and sometimes mirrors (to look behind corners) to search for bats (e.g. Mitchell-Jones & McLeish 1999, Smirnov *et al.*, 2007). In the European census, bats are never handled for identification and most bat observers try to avoid creating non-tactile stimuli. However, during these censuses, bats are sometimes found to show signs of arousal. During a field experiment in a hibernacula, Thomas (1995) concluded that visits by bat observers caused a dramatic increase in the flight activity of bats, beginning within 30 minutes of the visit, peaking 1.0-7.5 hours later, and remaining significantly above baseline level for up to 8.5 hours. The increased level of activity is not just caused by bat observers, but also indirectly by the bats themselves. One arousing bat can start a cascade of arousals from other bats in the same hibernacula. The first bat may wake up a second bat, by sound or tactile stimuli (for example an active bat attempting to reinsert itself into a hibernating cluster or male bats trying to mate with the hibernating females).

Bats lose 2-3.2% of their body weight during one arousal (Daan 1972, Boyles & Brack 2009), so additional, externally induced; arousals are an unwelcome burden on their energy budget. These arousals may mean that the bats will not have adequate fat reserves to sustain them through the winter. During a bat census, not all non-tactile stimuli can be avoided. Bat observers are a source of sound, light and heat. The light from a torch is often directed at the bat, especially to identify bats hidden deep in a crevice. We assume this can cause an arousal stimulus. Besides light, a torch also produces heat, another potential arousal stimulus. In this paper, we present an experiment aimed at determining if commonly used torches can raise the temperature in crevices by more than a threshold value and provide an arousal stimulus to bats hidden in crevices. As we did not want to repeat arousal experiments, we used the threshold value for arousal found by Speakman *et al.*, (1991). During a study performed in a respirometry chamber, Speakman *et al.*, (1991) found bats responded very strongly to a temperature increase of 58⁰C. The experiment did not permit drawing any further conclusions, such as a sex-specific response rate, the effect of signal inhibition after repeated stimuli or the respond rate during different hibernation temperatures.

Vampire bats are receptive to power densities (a measure of emitted energy) greater than 50 $\mu\text{W}/\text{cm}^2$ at distances between 13 and 16 cm (a power density of $1.8 \times 10^{-4} \text{W}/\text{cm}^2$ corresponds to 50 °C). This was first determined by quantifying the temperature at which vampire bats could not behaviorally distinguish between heat emitting and room temperature SUs. A positive linear relationship exists between the energy-threshold of heat detection and distance from stimuli. Through mathematical calculations, at a distance of 8 cm, vampire bats should be able to detect humans who emit radiation of 80 $\mu\text{W}/\text{cm}^2$ (Kürten *et al.*, 1982) Temperature threshold measurements were directly measured by stimulating nerve fibers of thermoreceptors in the

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nose-leaf and upper lip with a water-circulated brass thermode and recording the impulses/second at every 5 °C shift in temperature from 10 to 40 °C. These receptors have a threshold of 28 °C and a maximum temperature response to 40 °C, beyond which there was either no firing or an irregular firing pattern (Kürten, 1984 and Schäfer *et al.*, 1988). This threshold is 8 °C lower compared to those of warm receptor in other species of mammals, which implies extreme sensitivity to heat. After stimulation of these receptors, there is a transient increase in impulse activity which quickly decays due to adaptation and thus strengthens temporal acuity (Schäfer *et al.*, 1988).

Molecular mechanism of infrared detection

A family of TRP (transient receptor potential) channels, including TRPV1 (transient receptor potential vanilloid) and TRPA1 (transient receptor potential cation channel A1), is important in thermal and pain detection. (Gracheva *et al.*, 2010) TRPV1 channels are activated by capsaicin (a chemical which can be extracted from chili peppers), noxious temperature ranges (>43 °C), membrane-derived lipids, low pH, and voltage changes. (Rosenbaum *et al.*, 2007) Activation of TRPV1 by capsaicin results in calcium and sodium influx, and functionally allows for detection of painful thermal stimuli. (Rosenbaum *et al.*, 2007) TRPV1 may also act as a molecular thermometer in response to temperatures greater than 43 °C. The result is an inward calcium and sodium current similar to capsaicin-evoked currents. TRPV1 channels may also have voltage-sensitive properties responsible for its activation. (Matta *et al.*, 2007) Phosphorylation and mutations, especially at the C-terminus (carboxylic acid end of primary amino acid sequence), can alter the threshold temperature of heat-activation. (Rosenbaum *et al.*, 2007) The specific mechanism behind heat-activation of TRPV1 channels has yet to be deciphered. Regional migration involves bats migrating to and from a central hibernaculum where they spend the winter months in a state of hibernation. This type of movement is common among temperate species (Griffin 1970; Fleming and Eby 2003; Hutterer *et al.*, 2005) and is the best documented form of bat migration. During the summer months, females typically form maternity colonies where pups are born and reared while males remain solitary. In late summer, bats return to hibernacula (typically caves and abandoned mines) where mating occurs in a behaviour known as swarming (Fenton 1969; Parsons *et al.*, 2003; Piksa 2008; McGuire *et al.*, 2009). The bats then hibernate through the winter, emerge in the spring when females become pregnant, and males and females migrate back to their separate summer quarters. In micro bat have large deposit of adipose tissue, particularly around the neck and between the shoulder blades and originally these considered as a hibernating gland, because the size of adipose tissue deposits was observed to increase and decrease seasonally in hibernating mammals. Adipose tissue long thought to serve primarily thermogenic function.

(1) *Hibernation period (mid-November–early March)*: Bats demonstrate very low or almost no activity and departure from the cave are very rare. Interval of lethargy in these bats is most often caused by (i) changes in ambient conditions outside cave, (ii) changes in the physiological condition of the hibernating bat (e.g. dehydration), or (iii) direct disturbance (Speakman & Race, 1989 and Thomas, 1995). During hibernation, average temperature and daily temperature range (i.e. the difference between daily maximum and minimum temperatures) are key factors predicting the general level of flight activity (Ransome, 1990, Park *et al.*, 1992 and Berková & Zukal, 2010). As temperature increases, so the percentage of nights with bat activity also

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increases. Similarly, an increase in temperature fluctuation during the day will also result in bat arousals and increased flight activity. Note, however, that bat activity at the cave entrance has been recorded at temperatures as low as -13.2°C (cf. (Boyles *et al.*, 2006). Daily recordings were positive at maximum daily temperature exceeding 6.2°C , when some bat species are able to forage (Ransome, 1990). The activity within defined temperature groups (Berková & Zukal, 2010) was significantly lower during deep hibernation period than during late hibernation. Opinions on the level of activity desynchronization at sunset and loss of nocturnality during hibernation differ and the results of research are inconsistent, some supporting desynchronization and others not (e.g. (Park *et al.*, 1992, Thomas *et al.*, 1993, Nagel *et al.*, 1997 and Hope & Jones, 2012). Berková & Zukal, 2010 study indicate that activity at the cave entrance is synchronized with sunset, even in winter, and that a concentration of activity occurs between 3 and 3.5 h after sunset.

(2) *Late hibernation period (March–mid-April)*: with intensive departure activity during the first quarter of the night. Movement activity inside the cave is relatively high and the bats are probably already preparing themselves for departure from the hibernaculum (Zukal *et al.*, 2005). Flight activity is positively affected by average daily temperature, and negatively so by minimum temperature during the preceding day. Bats react very quickly to temperature changes from day to day, with activity decreasing or increasing if temperatures drop or rise by more than 2°C . Such rapid changes in activity level become feasible as the bats move towards the hibernaculum entrance, enabling them to register fluctuations in ambient temperature (Glove & Altringham, 2008 and Zukal *et al.*, 2005) and, as a consequence, potential changes in insect abundance. Bats are capable of foraging at very low temperatures, e.g. Daubenton's bat *Myotis daubentonii* at temperatures as low as -3.3°C (Ciechanowski *et al.*, 2007). In some species, the activity increases during late hibernation period, presumably, as food availability is already higher and foraging effectively compensates for any energy loss (Dunbar *et al.*, 2007).

(3) *Spring migration (mid-April–early June)*: a period of relatively high activity. At this time, the cave may serve as a transitional roost during the spring migration and, from around May, as a temporary roost for males as females already start to form summer colonies. Emergence activity in the first quarter of the night is high, and a small number of bats may re-enter the cave in the last part of the night. Average daily temperature and average daily atmospheric pressure at this time has a significant positive influence on overall flight activity. The degree of variability in activity explained by such climatic factors is the lowest during this period, however, suggesting that either temperature is no longer a limiting factor, or that endogenous rhythms have a strong influence on departure from the hibernaculum (Degn *et al.*, 1995, Harrje *et al.*, 1995, Simon *et al.*, 1999 and Berková & Zukal, 2010). However, the use of underground roosts including caves in the spring may be species specific; it may differ by region, and can also depend on roost structure (Perry, 2013, Park *et al.*, 1999, Skiba *et al.*, 1987).

(4) *Summer period (mid-June–end of July)*: During this period, the cave is used only sporadically, though the bats visiting the roost stay the whole night, i.e. they enter before midnight and leave after midnight. This type of activity suggests that, during this period, the cave may be being used as a night roost between peaks in foraging activity or as a transitional day roost (Degn *et al.*, 1995 and Park *et al.*, 1999). At this time, the cave entrance is visited almost exclusively by males (Bauerová & Zima, 1988, Whitaker & Rissler, 1992) as adult females

Research Article

occupy maternity roosts during lactation and return to these between foraging bouts, night roosts being used sporadically and for brief periods (Anthony *et al.*, 1981 and Lučan & Hanák, 2011).

(5) *Flight activity*: Flight activity at the night roost entrance is influenced by fluctuation in ambient temperature, rather than any absolute temperature threshold, the higher the difference between maximum and minimum daily temperature, the higher the activity level. This corresponds with a model proposing that activity changes in temperate insectivorous bats reflect changes in insect activity (Erkert, 1982), i.e. if day-insect abundance is high due to warmer nights, bat foraging activity may continue overnight with no visits registered at the cave entrance (low activity). On the other hand, when nights are cooler and the daily temperatures range is higher, bats will tend to spend more time in the night roost. Foraging activity is highest at dusk and just before dawn, after which the bats return to the day roost (Anthony *et al.*, 1981). This model is also supported by the influence of rainfall, with flight activity at the cave entrance increasing as rainfall increases whether the nights are warm or cold.

(6) *Autumn migration or swarming period (late July–mid-November)*: This period is typified by very high general activity and an increasing number of bats entering the cave. With the break-up of the summer breeding colonies, activity at the cave entrance gradually increases as adult females and juveniles arrive (Řehák, 1994 and Horáček, 1978), often in small groups of 2–12. The majority of bats does not roost in the cave and probably arrive after the first foraging period; hence, peak activity tends to occur around midnight. Activity around the cave entrances in autumn probably enables juveniles to recognize potential hibernacula and to meet individuals of the opposite sex, which live separately during summer (Rivers *et al.*, 2006). Activity level is positively related to average daily temperature, atmospheric pressure and rainfall. Thus, when nights are warm and insect activity is high (high atmospheric pressure), the bats will quickly catch enough prey and will search for the cave entrances (swarming sites) in order to mate or obtain shelter if it be raining (Parsons *et al.*, 2003, Berková & Zukal, 2010).

MATERIALS AND METHODS

In desert ecosystem, for the period of extreme summer between March and June, a highest diurnal temperature change is seen. The over-winter survival of hibernating bats depends on the quantity of energy that animals store prior to hibernation, the rate of depletion of these reserves and the duration of winter. The turn down in metabolic rate (MR) and body temperature (T_{body}) of a bat during hibernation enables a significant reduction of usage of nutrients and water, enhancing the probability of survival. Presumably, critical processes or functions that must be periodically restored at normothermic T_{body} for the organism's survival necessitate these arousals. Observations were recorded on January 21, 2016 to 20 December 2017 from Nawalgarh, Jhunjhunu, Rajasthan (27° 51' 5.80" N & 75° 16' 25.82" E) in Seth G B Podar College, Nawalgarh. For collection and comparison of different body part temperature FLIR C2 compact Thermal Imaging System was used. The FLIR C2 is pocket-sized thermal camera designed for hidden heat patterns of the body signal energy waste and structural defects. The C2's must-have features including MSX® real time image enhancement, high sensitivity with fully radiometric imagery. The mechanism of temperature perception is not known and there is no indication of an "intermediate substance" being involved between the sensory receptor cell



Figure 1: Study site



Figure 2: Characteristics features of *Scotophilus kuhlii*

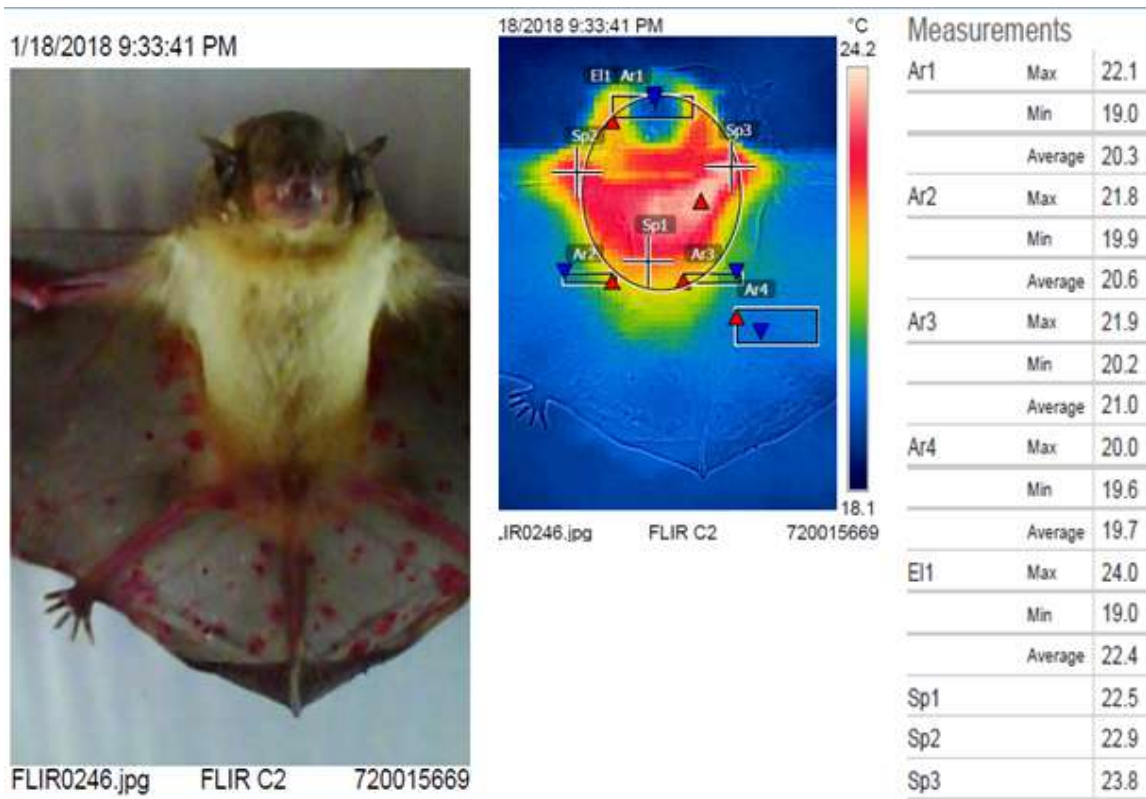
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and the heat waves. Anatomically the thermal sense organs have simple sensory cells and are usually localized by observation of the reactions of bats to temperature and the way their behaviour modifies on amputation of various parts of body of small mammals.

RESULTS AND DISCUSSION

Scotophilus kuhlii Leach, 1821: The external body, cranial and bacular measurements of the captured specimens were compared with the available literature. The forearm length (n=3) was 49.40 ± 3.03 mm, the greatest length of the skull (n=3) was 18.98 ± 0.61 mm. The mean frequency of maximum energy was 56.9 ± 3.6 kHz, the mean start and end call frequencies were 103.5 ± 12.3 kHz and 50.6 ± 1.0 kHz.

In this study compare the Body temperature (T_{body}) of roosted *Scotophilus kuhlii* during summer and winter using Infra red camera (FLIR C2) in urban environment as historical old haveli of shekhawati region. Period of winter average body temperature is decreased by $9.8 \pm 1.13^\circ\text{C}$ and bats with parasite are also decreased by $9.1 \pm 0.95^\circ\text{C}$ as compare to summer season. During the winter arousals, bats exhibited movement following T_{body} increase of only $9.8 \pm 1.13^\circ\text{C}$, compare to $>30.1 \pm 1.56^\circ\text{C}$ increases during normal arousals of *S. kuhlii*. The maximum and minimum values of (T_{body}) in winter with parasites are $21.9 \pm 0.90^\circ\text{C}$ and $20.2 \pm 1.10^\circ\text{C}$ respectively.

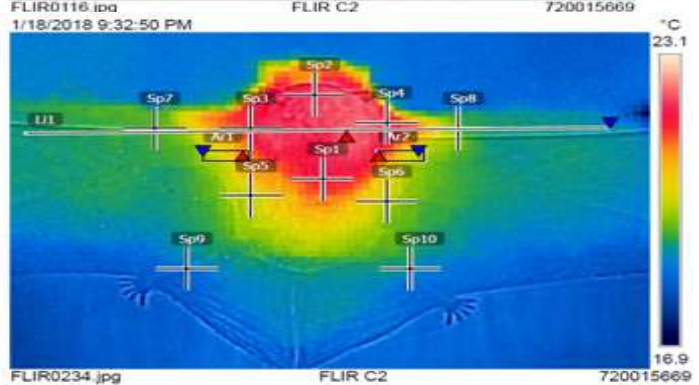
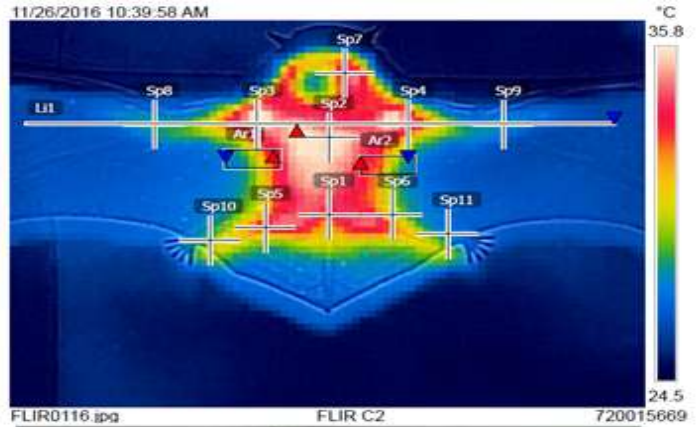


Summer

Measurements			°C
Ar1	Max	33.8	
	Min	27.8	
	Average	30.3	
Ar2	Max	33.7	
	Min	27.8	
	Average	30.9	
Sp1		33.5	
Sp2		35.8	
Sp3		35.2	
Sp4		33.7	
Sp5		32.8	
Sp6		30.6	
Sp7		34.6	
Sp8		28.7	
Sp9		27.9	
Sp10		29.8	
Sp11		27.8	
Li1	Max	35.4	
	Min	26.4	
	Average	30.1	

Winter

Measurements			°C
Ar1	Max	21.0	
	Min	19.8	
	Average	20.4	
Ar2	Max	21.6	
	Min	19.9	
	Average	20.6	
Sp1		21.7	
Sp2		22.1	
Sp3		21.7	
Sp4		22.1	
Sp5		20.0	
Sp6		19.9	
Sp7		19.4	
Sp8		19.3	
Sp9		18.4	
Sp10		18.8	
Li1	Max	22.4	
	Min	18.9	
	Average	20.3	



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