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FACTORS AFFECTING INSULATION QUALITY OF LICHEN (*USNEA BARBATA*) AS NEST MATERIAL USED BY THE WOODLAND DORMOUSE

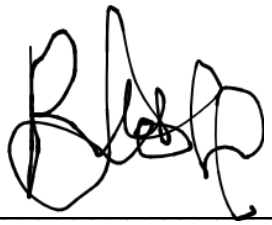
RUTH OLUWAFEM OLAJIDE

A Research Report submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg in partial fulfilment of the requirements for the degree of Master of Science

Johannesburg, September 2020

DECLARATION

I declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



RUTH OLUWAFEMI OLAJIDE

SEPTEMBER, 2020

DEDICATION

In loving memory of my father

Johnson Ezekiel

1952–2017

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LIST OF SYMBOLS AND ABBREVIATIONS

%	–	percent/percentage
^{o}C	–	degree Celsius
+/-	–	plus/minus
Tb	–	bottle temperature (as a proxy for body temperature)
Ta	–	ambient temperature
g	–	gram
n	–	sample size
M	–	mean
SD	–	standard deviation
SE	–	standard error
et al.	–	*et alia* (and others)
BMR	–	basal metabolic rate
Fig.	–	figure
rH	–	relative humidity
ml	–	milliliter

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ABSTRACT

Nest insulation quality is crucial in the selection of nest materials for nest construction by small endotherms that experience heat loss at the critical time of the season when the ambient temperature is low. The primary function of these nests is to increase insulation thereby reducing heat loss to enhance thermoregulatory benefits of the animals and their offspring. The overall aim of my project was to study factors affecting the insulation properties of the old man's beard lichen (*Usnea barbata)* a nesting material found in a majority of artificial nest boxes and tree cavities utilized by African woodland dormice (*Graphiurus murinus)*. I performed laboratory experiments to investigate the cooling rates of nests made from variety of nesting materials, under various ambient temperatures, and the effects of mass and moisture content on those cooling rates. I found that there are differences in the insulation properties of nesting materials. Feathers were the best insulators followed by fresh lichen; dry grass and paper ranked the worst. I also revealed that the quantity of lichen inside the nest had a significant effect on insulation. Heavier nests with more lichen had low cooling rates, thus thermal benefit. I changed the moisture content of the lichen. I found that when lichen nest material is humid, the cooling rates are low, thus high insulation, and wet lichen is the worst at insulation. Variation in ambient temperatures also had significant effects on nest cooling rates, even at high ambient temperatures. I conclude that fresh lichen provides insulation in the nests of woodland dormice. Woodland dormice may highly depend on available, and abundant fresh lichen in the forest because of the insulation and thermal benefits of lichen.

Key words: African woodland dormouse, ambient temperature, *Graphiurus murinus*, insulation, lichen, moisture content, nest

1. INTRODUCTION

Nests can be referred to as structures that are built by animals to live, breed, store eggs and to raise their offsprings (Hansell, 2005). Nest building is driven by a biological urge known as nest instinct which is an innate behaviour that is genetically hardwired in an organism (Demere *et al.*, 2002). While animals are building nests, each individual has several decisions to make: 1) each individual has to choose a suitable area to build a nest (i.e. nest site); 2) each individual has to decide on the type of nesting material to use within the environment they are in; 3) and each individual has to be able to organise the chosen nest material in order to form the shape or design of the nest (Schuetz, 2005). Therefore, nest characteristics are strongly dependent on individual behaviour and ability (Collias and Collias, 1984) and this therefore leads to variations in nest characteristics within or between species. Nests vary with the type of material used, construction, size, shape and location (Demere *et al.*, 2002). Interestingly, nests built by small endotherms are known to be among the most complex nesting structures (Redman *et al.*, 1999), and this was linked to their small size and high heat loss compared to larger animals (Davenport, 1992).

1.1 INSULATION PROPERTIES OF NESTS

Nest insulation can be defined by the flow of heat through the nesting material, whereby well insulated nests have low heat transfer and poor insulated nests have high heat transfer (Heenan, 2013). Nest insulation quality is dependent on a number of factors, which I will review below.

1.1.1 Nest structural characteristics

The thermal properties of a nest may depend on its structural characteristics; this includes the volume, mass, thickness and overall size (Whittow and Berger, 1977; Windsor *et al.*, 2013). Indeed for a number of species, the thicker, heavier and more material used in the construction of a nest, the better the insulation (Møller, 1991; Hilton

et al., 2004; Szentirmai *et al.*, 2005; Pinowski *et al.*, 2006). Empirical examples are shown on penduline tit (*Remiz pendulinus*) nests, whereby height, thickness and volume of nests have a significant influence on insulation (Szentirmai *et al.*, 2005). Tree swallow (*Tachycineta bicolor*) nests that are bigger, cavernous, and have an increased number of feathers are effective at conserving temperatures inside the nest (Windsor *et al.*, 2013). Akresh *et al*. (2017) observed that height and diameter of the nest of yellow warbler (*Setophaga discolor*) influenced the thermal properties of the nest. In addition, American robin (*Turdus migratorius*) nests have deeper nest-cups, larger diameter and thicker nest walls that are linked to nest insulation (Crossman *et al.*, 2011). However, there are disparities between species, whereby the structural characteristics of the nests do not have an influence on nest insulation. For example, empirical studies on white-crowned sparrows (*Zonotrichia leucophrys*) showed that the thickness of the wall had no significant influence on the rate of heat loss inside the nests (Kern, 1984).

1.1.2 Type of nest material

Insulation properties of a nest also depend on the type of materials used to construct the nest. Among the different nesting materials used for nest construction by endotherms are plant material (e.g. leaves, hay, branches, bark and shrubs), as seen in avian nests such as the blue tits (*Cyanistes caeruleus*; Mennerat *et al.*, 2009), European starling (*Sturnus vulgaris*; Gwinner, 1997) and Bonelli's eagle (*Hieraaetus fasciatus*; Ontiveros *et al.*, 2007). The use of green plant material has also been observed in rodent nests such as in the striped mouse (*Rhabdomys pumilio*) as well as in the Karoo bush rat (*Otomys unisulcatus*) (Schradin, 2005). In addition bark was observed to be one of the materials used in the nests of the hazel dormouse (*Muscardinus avellanarius*; Bracewell and Downs, 2017). In primates, the golden brown mouse lemur (*Microcebus ravelobensis*) constructs nests made of leaves (Theron *et al.*, 2010). Nests consisting of lichen are very common in small endotherms. Lichens are composite organisms (Watson, 1929), a combination of fungus and algae (or cyanobacterium) existing in a symbiotic relationship (Brodo *et al.*, 2001). The fungus gives the lichen shape and a protective exterior surface. Lichen is distributed all over the world in rainforests, temperate forests and woodlands (Sharnoff, 2014), and occurs under a wide variety of environmental conditions. Lichen benefits ecosystems and wildlife, by offering moisture and humidity by their ability to retain

water (Wind, 2009). The growth of microorganisms is prevented by some lichen species (Hunneck, 1999). In Northern America, lichen serves as a food resource for many species of birds; such as the spruce grouse (*Canachites canadensis*) (Ellison, 1966) and wild turkey (*Meleagris gallopavo*). Lichen is also useful as nest material, where it functions mainly for insulation and as part of the structural materials used by many animals such as birds, rodents, and bats (Hansell, 1996). The Northern flying squirrels' (*Glaucomys sabrinus*) nests consisted of lichen, and this was associated with thermal benefit, as well as being a potential food source (Hayward and Rosentreter, 1994). Warbles (Parulidae spp.) and vireos (Vireonidae spp.) make use of lichen (*Usnea longissima*) in building their nests. Also, the Madagascar olive headed weaver's nest is made solely from lichen of the genus *Usnea* (Sharnoff and Rosentreter, 1998). The presence of old man's beard lichen (*Usnea barbata*) has also been observed in both nest boxes (Fig. 1.1) and natural cavities (Fig. 1.2) used by the African woodland dormouse (*Graphiurus murinus*) (Madikiza, 2010; Lamani, 2014); however its functional importance has not yet been investigated.



Fig. 1.1. Radio-collared African woodland dormouse (*Graphiurus murinus*) in a nest box containing old man's beard lichen (*Usnea barbata*) as nest material. Photo by E. Do Linh San.



Fig. 1.2. African woodland dormouse nest made of lichen inside a hollow African bushwillow (*Combretum caffrum*) trunk. Photo by E. Do Linh San.

Several small endotherms use feathers to line their nests (Calvello *et al.*, 2006; Liljeström *et al.*, 2009; Ruiz-Castellano *et al.*, 2018). The addition of feathers to nests of animals (Fig. 1.3) proved to be advantageous in terms of insulation properties especially when compared with other types of nest material by Hilton *et al.* (2004). Functional importance of feathers in studies, revealed that nestlings that were in nests that included feathers as lining were larger than nestlings without feathers in nests. This suggests that feathers provide thermal benefits, and as a consequence increased growth in nestlings (Dawson *et al.*, 2011). However, nest material selection may highly depend on availability and on structural design of the material. For example, the

Hawaiian songbirds (*Moho nobilis*) construct nests using wool as nest material only when it is available (van Riper, 1977).



Fig. 1.3. Nest box used by African woodland dormouse containing feathers (at the top) and old man's beard lichen (at the bottom). Photo by E. Do Linh San.

1.1.3 Moisture content

The insulation efficacy of a nest may not only depend on the type of nest material, but also on the thermal characteristics of the nesting material. Therefore, the water content of any nest material influences its ability to conduct heat (thermal conductivity) and on evaporative heat transfer. In other words, nest materials with high moisture content, will have an increased thermal conductivity and thus an increased evaporation rate, leading to lower insulation (Gedeon *et al.*, 2010). Animals have to actively select nest materials that can maintain insulation, even under wet conditions. Rohwer and Law (2010) showed that birds such as yellow warbler (*Dendroica petechia*), from warm and wet enviromental conditions utilised nest materials that absorbed lower amounts of water than birds in colder and drier areas.

Experimental studies on insulatory properties of passerine nests indicated that wetting nest material increased the cooling rates of the nest (Hilton *et al.*, 2004), in addition, even though feathers are known to be good insulators and hydrophobic

(Wainwright *et al.*, 1976; Hilton *et al.*, 2004; Bhushan, 2009), these studies showed that their insulatory properties are greatly reduced when wet. Similar results were observed by Heenan (2013), demonstrating that indeed wetting of nest material increased thermal conductance in nests of tawny crowned honeyeater (*Gliciphilla melanops*). Gedeon *et al*. (2010) experimentally showed that spraying water on the surface of grass (*Festuca pseudovina*) had a negative effect on insulation.

1.1.4 Ambient temperature

Environmental factors such as ambient temperature have an influence on nest construction, and on insulation. Studies on three species of warblers; wood warblers (*Phyllosscopus sibilatrix*), common chiffchaffs (*Phylloscopus collybita*) and willow warblers (*Phylloscopus trochichilus),* showed that there is indeed a positive relationship between insulation of nests and ambient temperatures (Tiainen *et al*., 1983). Cooler ambient temperatures are associated with larger nests (Britt and Deeming, 2011). Mainwaring and Hartley (2008) showed that during the breeding season (when ambient temperatures are high), the amount of nest material used decreased in blue tits. Similar findings were also noted in the great tits (*Parus major*; Deeming *et al.*, 2012). These findings suggest that, animals alter the insulation properties of the nest by reducing the amount of material, in response to changes in ambient temperatures (Mainwaring *et al.*, 2012). The difference in latitudes, has also been shown in several studies to influence insulation of nests. Møller (1984), argued that in species of birds whereby breeding takes place during colder temperatures, it is probable for these species to incorporate feathers into their nests, compared to those species who breed in warmer temperatures. Further support of these findings was shown in common blackbirds (*Turdus merula*) residing in colder areas and higher latitudes of Britain, which constructed nests that had thicker walls, compared to common blackbirds in warmer, higher latitudes (Mainwaring *et al.*, 2014).

1.2 THE BIOLOGY OF DORMICE

Dormice are rodents of the family *Gliridae.* There are at least nine genera and 28–29 extant species of *Gliridae* found in Europe, Africa and Asia (Numone *et al*., 2007; Holden-Musser *et al.*, 2016). There are 15–17 species of dormice in Africa but 14–15 of these species belong to the genus *Graphiurus* (Holden, 2013; Monadjem *et al*.,

2015). These species are usually found in a broad range of habitats which cut across grassland and rocky areas to woodland and forest (Skinner and Chimimba, 2005). The African woodland dormouse (*Graphiurus murinus*) occurs throughout the Ethiopian region and to the Southern parts of Africa and Lesotho. It also streches across Limpopo, North west, Gauteng, Mpumalanga, KwaZulu-Natal and Eastern Cape provinces bordering into Western and Northern Cape, and was also found in Swaziland (Madikiza *et al.*, 2016).

1.2.1 Nesting habits of dormice

Dormice are found in a wide range of habitats, ranging from ancient semi natural woodlands to species rich shrublands (Bright *et al.*, 2006), and construct globular nests in trees (*Muscardinus avellanarius*; Panchetti *et al.*, 2007), rock crevices (*Graphiurus platyops*; Skinner and Chimimba, 2005) and in trees (*Dryomys nitedula*; Pilats *et al.,* 2012), while some use nests abandoned by birds, squirrels and occasionally beehives (Webb and Skinner, 1994). Woodland dormice are mostly found in a variety of woody habitats, and forests (Afromontane or riverine) (Qwede, 2003), highly arboreal and to a lesser extent terrestrial (Lamani, 2014). Their nests are usually found in acacia trees (Skinner and Chimimba, 2005), and locally in rock crevices, wide branches and tree trunks (Lamani, 2011).

1.2.2 Ecology of dormice

Studies by Madikiza *et al*. (2010) and Lamani (2011) showed that woodland dormice use both natural sites and nest boxes for resting and nesting. Resting site ecology plays an essential part in reproduction in species, and there are some ecological factors that influence fitness and use of space such as food availabilty and thermoregulatory capabilities (Lutermann *et al.*, 2010). As suggested by Lutermann *et al.* (2010), thermoregulation provided by resting sites is an advantage to the individual as it provides shelter from inclement weather and guard against fluctuation in ambient temperature and humidity. Nest box utilization by female dormice is crucial for breeding purposes (Madikiza *et al.*, 2010), as the need for a well insulated and protected resting site that provides considerable thermal benefits in order to raise their young is paramount (Lutermann *et al.*, 2010). However, the use of nest boxes changes with season in woodland dormice. The females are reportedly found to use more nest boxes

than the males, which is noticeable during breeding for the female dormice and in mating period for the male dormice (Madikiza, 2010). According to Lamani (2014), woodland dormice usually select areas with thick canopy cover and a high percentage of arboreal connectivity when active at night in order to reduce the risk of predation and to ease movements. They use daily torpor to conserve energy when faced with low temperature or food scarcity (Webb and Skinner, 1995).

1.2.3 Torpor, hibernation, and huddling in dormice

Woodland dormice are known to be competent thermoregulators, that can maintain body temperature between 34^{o}C and 38^{o}C (Whittington-Jones and Brown, 1999), and have been seen adopting behavioral thermoregulatory strategies to offset high thermoregulatory costs such as huddling, nesting, microhabitat selection, increased activity and postural adjustment at low ambient temperatures (Vogt and Lynch, 1982; McDevitt and Andrews, 1994; Fietz *et al.*, 2010). Summer-acclimatized dormice, under laboratory conditions use daily torpor impulsively to save energy when food availability is low (Webb and Skinner, 1996) while the cold-acclimatized woodland dormice hibernate, which in this case are prolonged bouts of torpor (Ellison and Skinner, 1991). Mzilikazi *et al.* (2012), observed that free ranging woodland dormice also undergo multiple-day torpor bouts of up to 8 days during winter with body temperature reaching 2.5^{o}C. Torpid dormice were reportedly found in artificial nest boxes throughout the year and were found torpid when the temperature was below 4^{o}C which corresponds with the beginning of hibernation (Madikiza, 2010). Torpor in woodland dormice is associated with lower food availability and low ambient temperatures and high humidity (Madikiza, 2010). Huddling is also a strategy employed by dormice to increase nest temperature as it has been observed in the nest boxes where dormice huddle to counterbalance high thermoregulatory cost when experiencing low ambient temperatures (Fietz *et al.*, 2010; Madikiza, 2017).

1.3 AIM, OBJECTIVES, QUESTIONS AND HYPOTHESES

The overall aim of this project was to determine the factors affecting the insulation properties of the old man's beard lichen (*Usnea barbata*) as a nesting material for the African woodland dormouse (*Graphiurus murinus)*. This material has indeed been found in most nest boxes and tree cavities utilized by this arboreal rodent species at

the Great Fish River Nature Reserve (Eastern Cape province, South Africa) during previous field studies (Madikiza, 2010; Lamani, 2011).

My objectives were:

- **Objective 1:** To determine the insulation quality of nests in relation to the type of nesting material used.

  Question 1: Do different types of nest material differ in their insulation abilities?

  Hypothesis 1: Nest insulation depends on the material with which a nest is built (Pinowski *et al.*, 2006).

- **Objective 2:** To determine if the moisture content of lichen has an influence on nest insulation.

  Question 2: Does wet lichen insulate better than dry lichen?

  Hypothesis 2: Insulation quality of nest is largely dependent on moisture content (Gedeon *et al.*, 2010).

- **Objective 3:** To determine if the mass of lichen has an influence on nest insulation.

  Question 3: Does the quantity of nest material affect insulation?

  Hypothesis 3: Insulation quality is largely dependent on the mass of the material (Grubbauer and Hoi, 1996).

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

2.1.1 Cooling trials for all experiments

In order to compare the cooling rates, I measured the insulation properties of different nest materials (Experiment 1), different nest mass (Experiment 2), and different moisture content nests (Experiment 3) in a climate-controlled chamber conviron CMP 6010 control system; at 60% rH and maintained at four different ambient temperature regimes (5ºC, 10ºC, 15ºC, 25ºC) for each cooling trial. I chose these ambient temperatures as they were similar to ambient temperatures that regularly occur during winter, autumn, spring, and summer seasons at our study site.

I used 50 ml glass bottles filled with warm water (40°C) and capped with rubber lids. To maintain the water temperature inside the bottles I kept the bottles in a warm water bath of 38.5–39.8ºC just before the start of each trial. The bottles were then removed from the water bath, wiped dry on the outside, and each bottle was positioned in the center of each nest and completely covered with nest material within 1 minute of cooling trials. This experimental setup was designed to simulate a woodland dormouse inside the nest.

Since thermal insulation is the reduction in heat transfer, I therefore expected that the lower the rate at which the water temperature inside the bottles declined, the better will be the insulation. I measured the cooling rates by monitoring the water bottle temperatures as they cooled in each of the nest boxes by attaching *i*-Button® (Model DS1921G Thermocron, Maxim/Dallas *i*-Buttons Products) at the base of each bottle cap. The *i*-Buttons were programmed to record changes in the bottle temperatures inside the nest (Tb) at every 15 minutes intervals, when ambient temperatures (Ta) were at 25°C, 15°C, 10°C, and 5°C, respectively. Data recording of water bottles took 6 hours, providing enough time for each water bottle to cool down to ambient temperatures. After 6 hours the nest boxes were removed from the conviron, *i*-Buttons were detached from the bottles and the data recorded was transferred using an *i*-Button reader for USB port to a computer and analysed using *Softbutton Software*.

This experimental procedure was repeated under all the above stipulated ambient temperatures.

2.1.2 Experiment 1: Insulation properties of nest material (comparison of heat loss in different nesting materials)

In this experiment, I wanted to determine the insulation properties of different nest materials. I first selected four types of nesting material, *Usnea barbata* (lichen), down feathers, dry grass and paper shavings, as some of these materials were found to be the main components of woodland dormice nests in natural populations (Madikiza, 2010), and shaved paper was chosen as the most common nest material provided for rodents under captive environments (Z.J.K. Madikiza *pers. obs*.).

The different nesting materials were then weighed using a digital scale (± 0.1 g) to achieve the desired weight. I used a 60 g mass of each material, as these mass parameters/measurements were found to be similar to the observed size of nests made by woodland dormice in natural populations (Z.J.K. Madikiza *pers. comm*.). I then categorised the nests into different groups based on the type of material. The first group was categorised as lichen nests (n=10), and these were nests made of 60 g freshly harvested lichen. The second group was categorised as down feather nests (n=10), and these were nests made of 60 g down feathers. The third group of nests was categorised as grass nests (n=10), and these were categorised as nests made of 60 g of dry hay. The fourth group was categorised as paper shaving nests (n=10), and these were categorised as nests made of 60 g of paper shavings. I then used 40 custom-built wooden nest boxes, with a dimension of 14.5 x 9.5 x 11.5 cm (l x b x h) to place the different groups of nests inside. In this experiment I also included a control which was categorised as empty nests (n=10), and these were nest boxes that did not have any nest material inside. Therefore, a total number of 50 nest boxes were deployed, 10 nest boxes for each of the nest groups.

2.1.3 Experiment 2: Influence of nest material mass on thermal insulation

To determine if the mass of nest material has an influence on nest insulation, I only selected fresh lichen for this experiment. The lichen was weighed using a digital scale (± 0.1 g) and then categorised into three different groups: 20 g, 40 g and 60 g. I used these mass parameters/measurements since they are similar to the observed size of nests made by woodland dormice inside artificial nest boxes in natural populations

(Z.J.K. Madikiza *pers. comm*.), a total number of 30 nest boxes and 10 nest boxes for each of the nest groups.

2.1.4 Experiment 3: Influence of moisture content of nest material on thermal insulation

In this third experiment, I wanted to investigate the influence of moisture content and conditions of nest material on nest insulation. I first altered lichen nest material moisture content and categorised it into three different phases (Table 2.1). The three phases were related to the different stages of moisture content on a dry-weight bases. The first phase was categorised as fresh lichen (n=10), and this was lichen that was freshly harvested from trees, had a distinct bright green colour and soft condition. The second phase was categorised as dry lichen (n=10), and this was lichen that had 0% moisture content, brown in colour and rough condition. To achieve this, I reduced the moisture content of the lichen material by drying fresh lichen in a laboratory dessicator using dessicant silica gel until there was no moisture left (i.e. 100% solid). The third phase was categorised as wet lichen (n=10), and this was classified as lichen that was damp, with a bright green colour and soft and soggy condition. This was achieved by soaking the lichen for 2 hours in 1000 ml of water prior to experiments. Here too, the experimental procedures followed that of Experiment 1. A total number of 30 nest boxes were deployed, 10 nest boxes for each of the different phases.

Table 2.1. Summary of the physical characteristics used to ascertain type and condition of lichen.

Classes	Physical condition	Mass
Fresh lichen	Freshly harvested, distinct bright green colour and soft in condition.	> 60 g
Dry lichen	Lichen that has 0% moisture content, brown in colour and rough in condition.	> 60 g
Wet lichen	Fresh lichen that was damp, with a bright green colour and soft and soggy in condition.	> 60 g

2.2. STATISTICAL ANALYSES

All statistical analyses were conducted with Excel (Microsoft Inc.) and IBM SPSS Statistics 25.0 (SPSS Inc.) statistical packages. Unless stated otherwise, summary data are reported as mean ± SE. The threshold for statistical significance was set at $p < 0.05$. I tested if data were normally distributed using the (Kolmogorov-Smirnov test). In Experiment 1, to compare the insulation of nests made from different nest materials (lichen, feathers, dry grass, paper shavings, and no material) I used a one way analysis of variance (ANOVA), followed by the Tukey HSD *post hoc* test to compare multiple groups following a highly significant ANOVA test result. In Experiments 2 and 3, the ANOVA test was used to test the influence of a) mass of lichen and b) moisture content of lichen on nest insulation. This was again followed by the Tukey HSD *post hoc* test to compare multiple groups.

3. RESULTS

3.1 INFLUENCE OF DIFFERENT NEST MATERIALS ON NEST INSULATION

At an ambient temperature (Ta) of 5°C, I recorded a statistically significant difference in the temperature of bottles (Tb) between all the nest materials, and this at all time intervals (ANOVA, $p < 0.001$ in all cases). The same differences were obtained at Ta's of 10°C, 15°C and 25°C.

Tukey *post hoc t* tests revealed some significant differences between pairs of treatments (Table 3.1), and I recorded consistent Tb differences between all the nest materials throughout all four temperature regimes and intervals of the experiments (Fig. 3.1 to 3.4).

3.1.1 No material vs. nests with material

Feathers, lichen, grass, and paper shavings provided better nest insulation when compared with nest boxes that had no nest material, and this was throughout all sampling trials.

When I compared feathers with nests that had no material, I found 100% significance in all Ta regimes with average temperature differences between bottles (Tb) ranging from 4.75–9.3°C (Ta = 5°C), 2.95–9.1°C (Ta = 10°C), 2.1–6.9°C (Ta = 15°C) and 3.25–4.15°C (Ta = 25°C), respectively.

When I compared lichen with an empty nest, I found similar results, with significant differences in all Ta regimes with average temperature differences between bottles ranging from 1.8–5.4°C (Ta = 5°C), 1.25–8.40°C (Ta = 10°C), 1.35–4.25°C (Ta = 15°C), and 2.65–3.6°C (Ta = 25°C).

Grass also showed significant differences in temperature of nests, when compared with no nesting material with average temperature differences between bottles ranging from 1.2–2.2°C (Ta = 5°C), 0.3–1.45°C (Ta = 10°C), 1.5–3.75°C (Ta = 15°C) and 1.9–3.1°C (Ta = 25°C), respectively.

Paper shavings showed a significantly better insulation than no material only at Ta's of 10–25°C, with average temperature differences between bottles ranging from 0.4–

7.85°C (Ta = 10°C), 0.35–2°C (Ta = 15°C) and 1–2.15°C (Ta = 25°C), respectively. Interestingly, I did not find any temperature differences at a Ta of 5°C.

3.1.2 Feathers vs. other nest materials

Overall, I found that feathers provided better nest insulation when compared with nest boxes that had lichen, grass and paper shavings. However, these significant differences were not observed in all sampled ambient temperature regimes. Feathers insulated better than lichen only when Ta was at 5°C, 10°C or 15°C, with average temperature differences between bottles ranging from 1.95–4.4°C (Ta = 5°C), 0.05–2.35°C (Ta = 10°C) and 0–3.05°C (Ta = 15°C), respectively. Similar results were observed when comparing feather nests with grass nests, with average temperature differences between bottles ranging from 3.4–7.4°C (Ta = 5°C), 3.25–7.7°C (Ta = 10°C) and 0.6–3.65°C (Ta = 15°C), respectively.

Lastly, I observed that nests with feathers insulated better than nests with paper shavings, and these significant differences were observed at all Ta regimes, with average temperature differences between bottles ranging from 3.85–7.5°C (Ta = 5°C), 0.7–3.1°C (Ta = 10°C), 2.4–5.95°C (Ta = 15°C) and 1.35–3.05°C (Ta = 25°C), respectively.

3.1.3 Lichen vs. other nest materials

Overall, nest boxes with lichen showed better insulation than nest boxes with grass material, and this was significant only at Ta's of 5°C, 10°C, and 15°C, with average temperature differences between bottles ranging from 0.4–3.45°C (Ta = 5°C), 1.5–7.05°C (Ta = 10°C), and 1.4–1.85°C (Ta = 15°C), respectively.

In addition, nest boxes with lichen showed better insulation than nest boxes with paper shavings, and this was significant at Ta's of 5°C, 15°C and 25°C with average temperature differences between bottles ranging from 1.05–3.45°C (Ta = 5°C), 0.5–4°C (Ta = 15°C), and 0.75–2.4°C (Ta = 25°C), respectively.

3.1.4 Grass vs other nest materials

I recorded that grass was better at providing insulation than paper shavings, but this significance was only recorded when ambient temperatures were at 15°C, with average temperature differences between bottles ranging from 0.9–2.65°C.

3.1.5 Paper shavings vs other nest materials

I found that paper shavings performed better than grass at insulating nests at a Ta of 10°C only, with an average temperature difference between bottles ranging from 0.7–6.5°C.

Table 3.1. Average bottle temperature (Tb) differences between groups of nest material used (lichen, grass, feather, paper shavings and no material) at four different ambient temperature (Ta) regimes. Statistically significant differences (Tukey *post hoc t* tests) between nest material categories are indicated (***: p < 0.001; **: p < 0.01; *: p < 0.05; NS: not significant).

	Average Tb differences at:			
Comparisons	**Ta = 5°C**	**Ta = 10°C**	**Ta = 15°C**	**Ta = 25°C**
Tb (Feathers) > Tb (No material)	7.2°C 100% ***	6.8°C 100% ***	4.0°C 100% ***	3.6°C 100% ***
Tb (Lichen) > Tb (No material)	3.5°C 100% ***	5.8°C 100% ***	3.0°C 96% ***	3.2°C 100% ***
Tb (Grass) > Tb (No material)	1.6°C 29.2% *	1.0°C 50% *	2.0°C 100% ***	2.4°C 100% ***
Tb (Paper) > Tb (No material)	1.3°C 0% NS	5.2°C 96% **	0.2°C 4.4% **	1.4°C 29.3% *
Tb (Feathers) > Tb (Lichen)	3.6°C 96% **	1.0°C 42% *	1.1°C 29.2% *	0.3°C 0% NS
Tb (Feathers) > Tb (Grass)	5.6°C 100% ***	5.7°C 100% ***	1.9°C 96% **	1.2°C 0% NS
Tb (Feathers) > Tb (Paper)	5.8°C 100% **	1.5°C 67% *	3.9°C 100% ***	2.1°C 100% ***
Tb (Lichen) > Tb (Grass)	2.0°C 63% *	4.8°C 96% **	0.8°C 42% *	0.9°C 0% NS
Tb (Lichen) > Tb (Paper)	2.2°C 92% ***	0.5°C 0% NS	2.8°C 92% **	1.8°C 46% *
Tb (Grass) > Tb (Paper)	0.3°C 0% NS	4.2°C 92% ***	2.0°C 96% *	1.0°C 0% NS

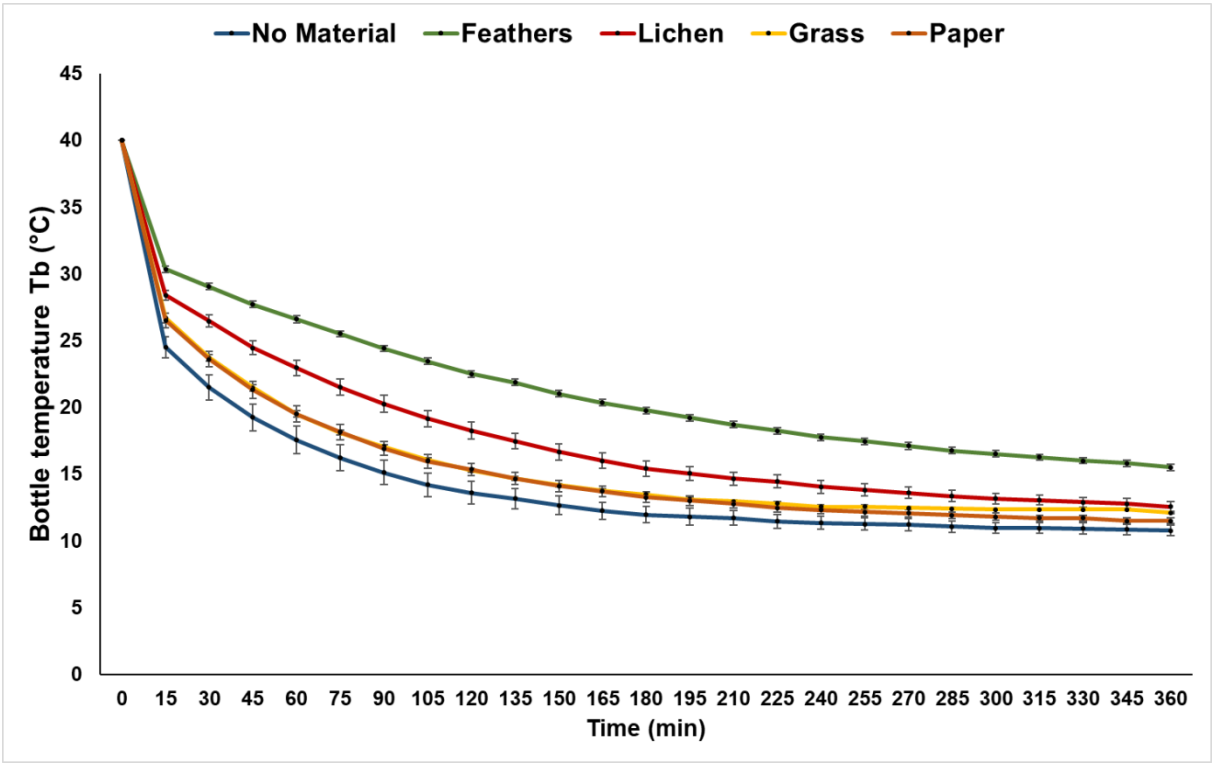


Fig. 3.1. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing different types of nest materials at an ambient temperature of 5°C.

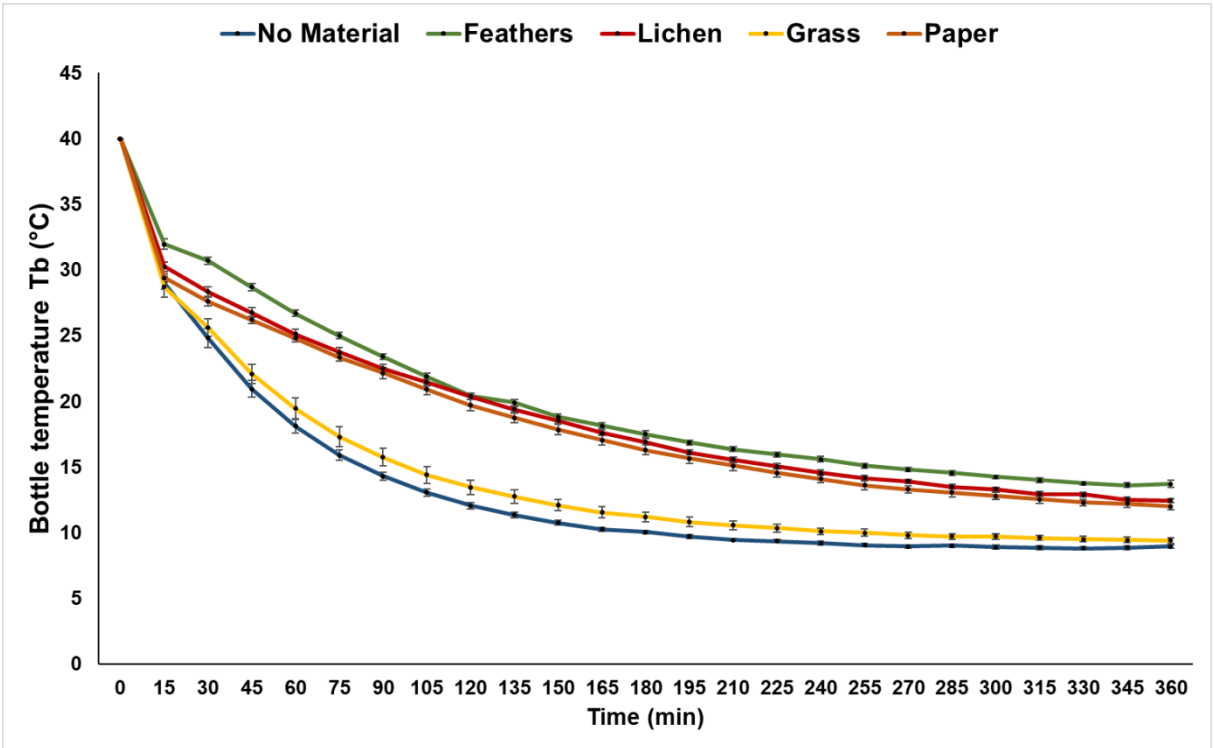


Fig. 3.2. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing different types of materials at an ambient temperature of 10°C.

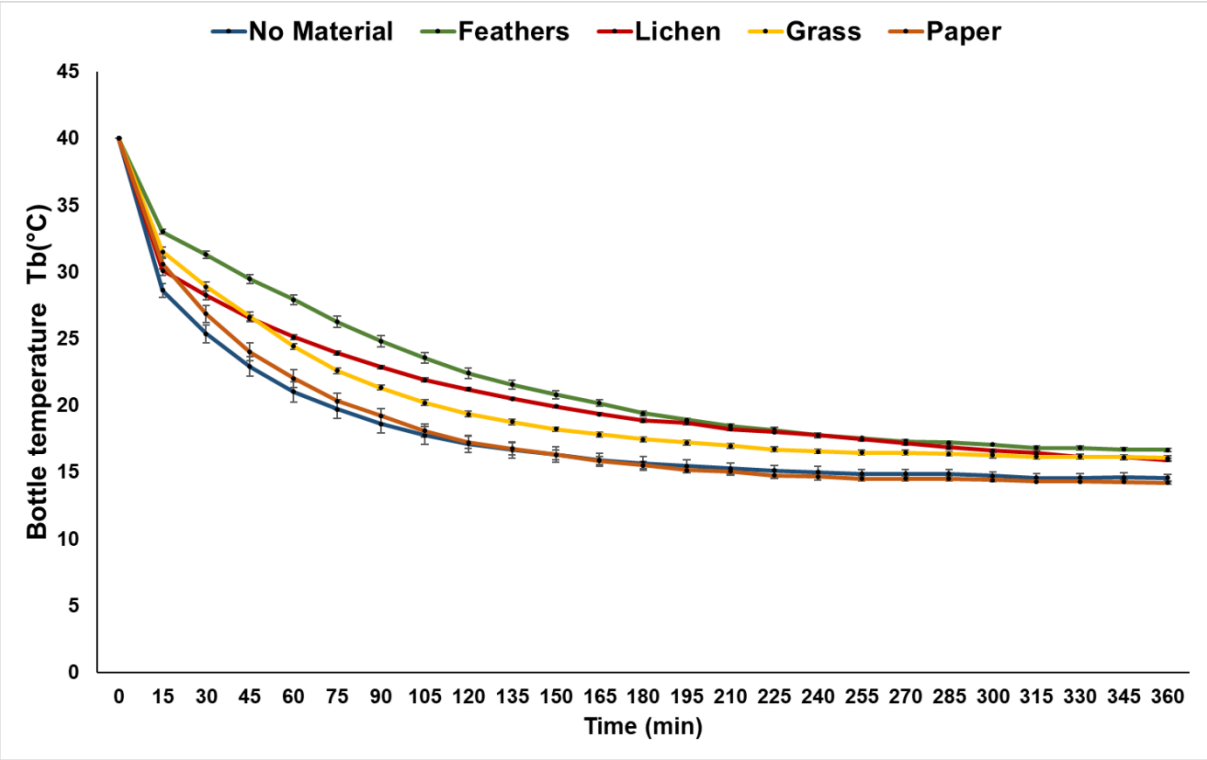


Fig. 3.3. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing different types of materials at an ambient temperature of 15°C.

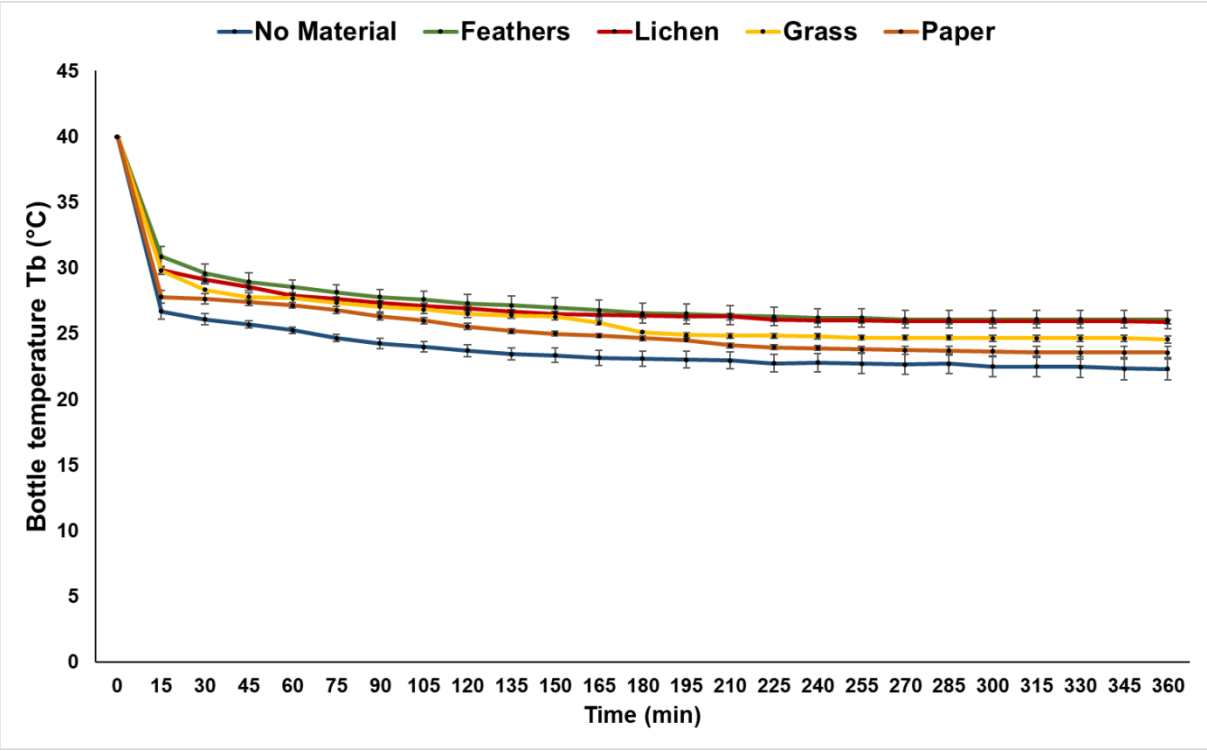


Fig. 3.4. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing different types of materials at an ambient temperature of 25°C.

3.2 INFLUENCE OF MOISTURE ON NEST INSULATION

Firstly, my results showed that there were statistical differences in the temperatures of the bottles at all the different ambient temperatures, and during all the time intervals (Anova, $p < 0.001$).

When I performed the Tukey *post hoc t* tests, my results further revealed that there are some significant differences between pairs of treatments (Table 3.2), and I recorded consistent differences in all the different phases of moisture content of nest material, and this throughout all four temperature regimes, and time intervals of the experiment (Fig. 3.5 to 3.8).

3.2.1 Fresh lichen vs. all other moisture contents of lichen

I first found significant differences in insulation properties of different lichens with different moisture content, and this throughout all ambient temperatures. When I compared fresh lichen with all the other different moisture contents at all Ta regimes. I found that fresh lichen provided better insulation on the bottles than dry lichen, with average temperature differences ranging from 0.25–3.7°C (Ta = 5°C), 2.05–5.05°C (Ta = 10°C), 2.95–6.8°C (Ta = 15°C), 0.65–2.65°C (Ta = 25°C), respectively.

I also found that fresh lichen provided better insulation on the bottles than wet lichen. This was evident throughout all four ambient temperatures, with average temperature differences ranging from 0.85–5.15°C (Ta = 5°C), 3.7–7.7°C (Ta = 10°C), 0.55–4.85°C (Ta = 15°C), and 0.8–4.7°C (Ta = 25°C), respectively.

3.2.2 Dry lichen vs. all other moisture contents of lichen

I then compared dry lichen with wet lichen; dry lichen proved to have better insulation than wet lichen, but this was only at Ta's of 5°C, 10°C, and 25°C, with average temperature differences between bottles ranging from 0.6–1.65°C (Ta = 5°C), 1.35°–3.05°C (Ta = 10°C), and 0.15°–2.4°C (Ta = 25°C), respectively.

I recorded significant differences between wet lichen and dry lichen. I found that wet lichen had better insulation than dry lichen, but only at a Ta of 15°C, with a mean difference temperature ranging from 0.35–1.65°C.

Table 3.2. Average bottle temperature (Tb) differences between between groups of nest boxes containing lichen with different moisture content (dry, fresh, and wet) at four different ambient temperature (Ta) regimes. Statistically significant differences (Tukey *post hoc t* tests) between lichen moisture content groups are indicated (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; NS: not significant).

	Average Tb differences at:			
Comparisons	**Ta = 5°C**	**Ta = 10°C**	**Ta = 15°C**	**Ta = 25°C**
Tb (Dry) vs. Tb (Fresh)	2.28°C 88% *	3.36°C 96% **	4.85°C 100% **	2.10°C 88% **
Tb (Dry) vs. Tb (Wet)	1.20°C 64% **	1.96°C 100% **	1.54°C 84% **	1.34°C 84% **
Tb (Fresh) vs. Tb (Wet)	3.48°C 92% **	5.81°C 100% **	3.31°C 92% **	3.44°C 92% NS

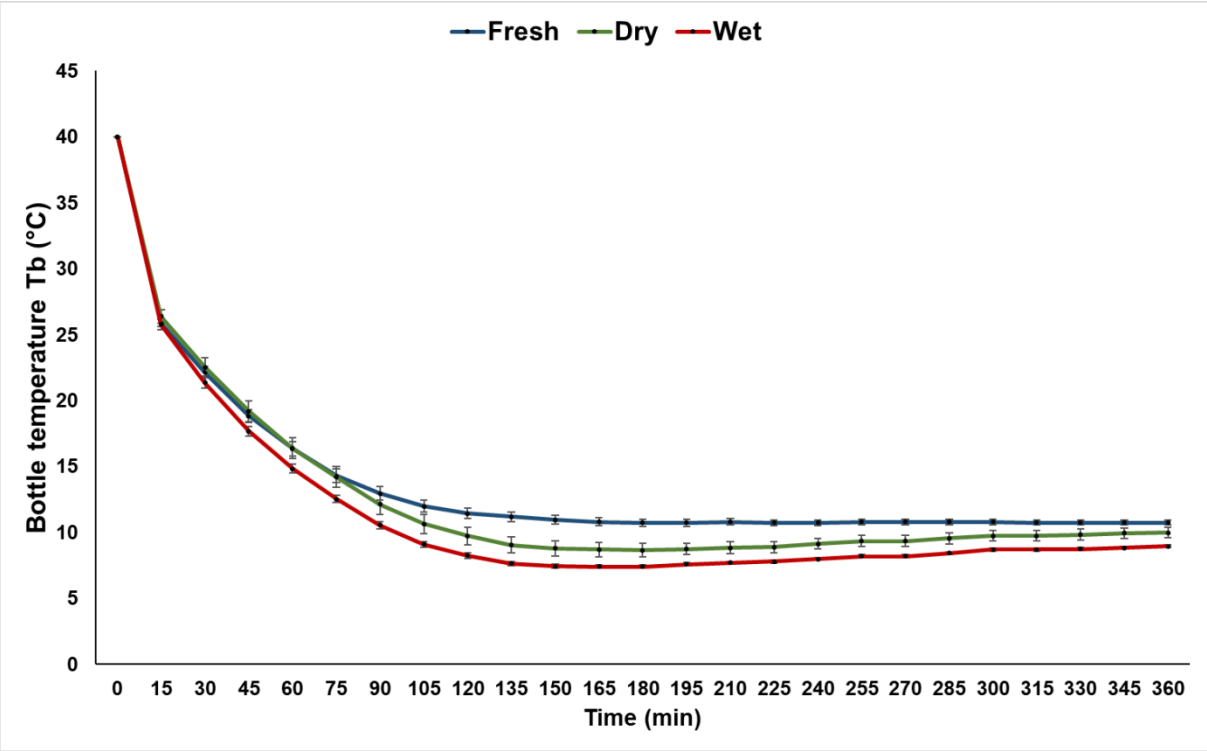


Fig. 3.5. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing lichen with different moisture content at 5°C.

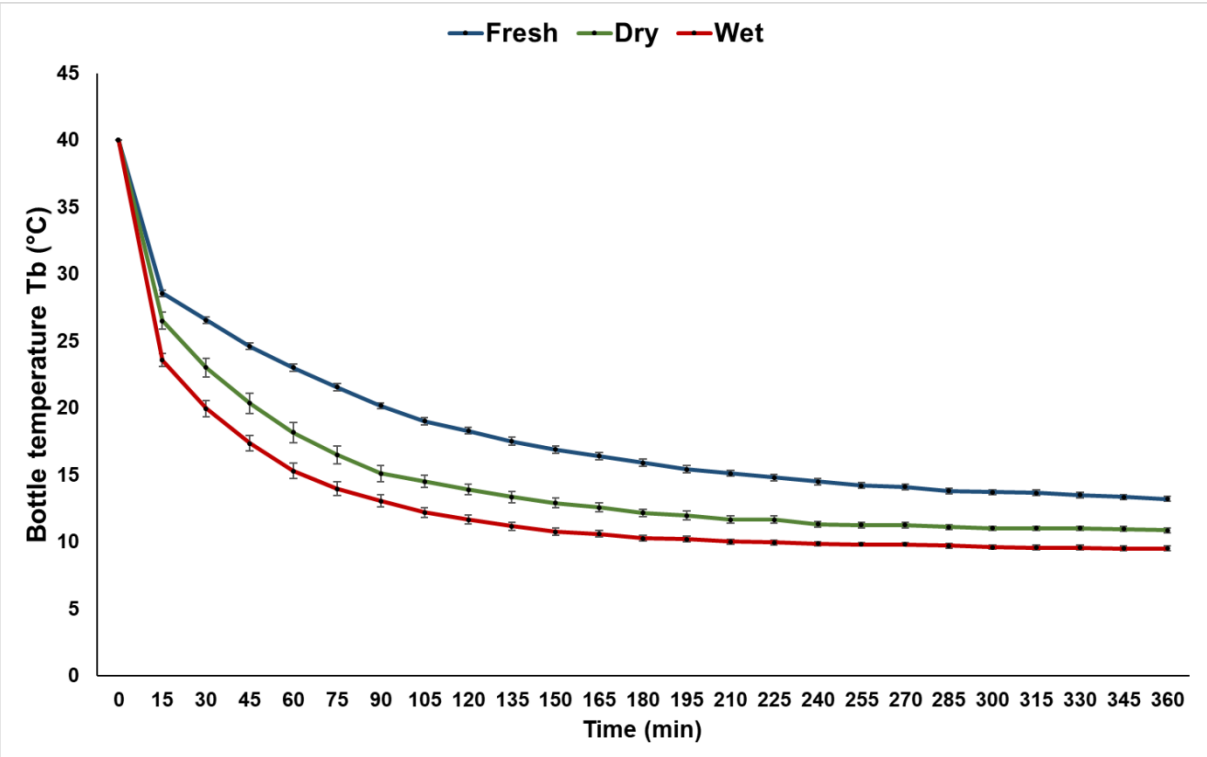


Fig. 3.6. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing lichen with different moisture content at 10°C.

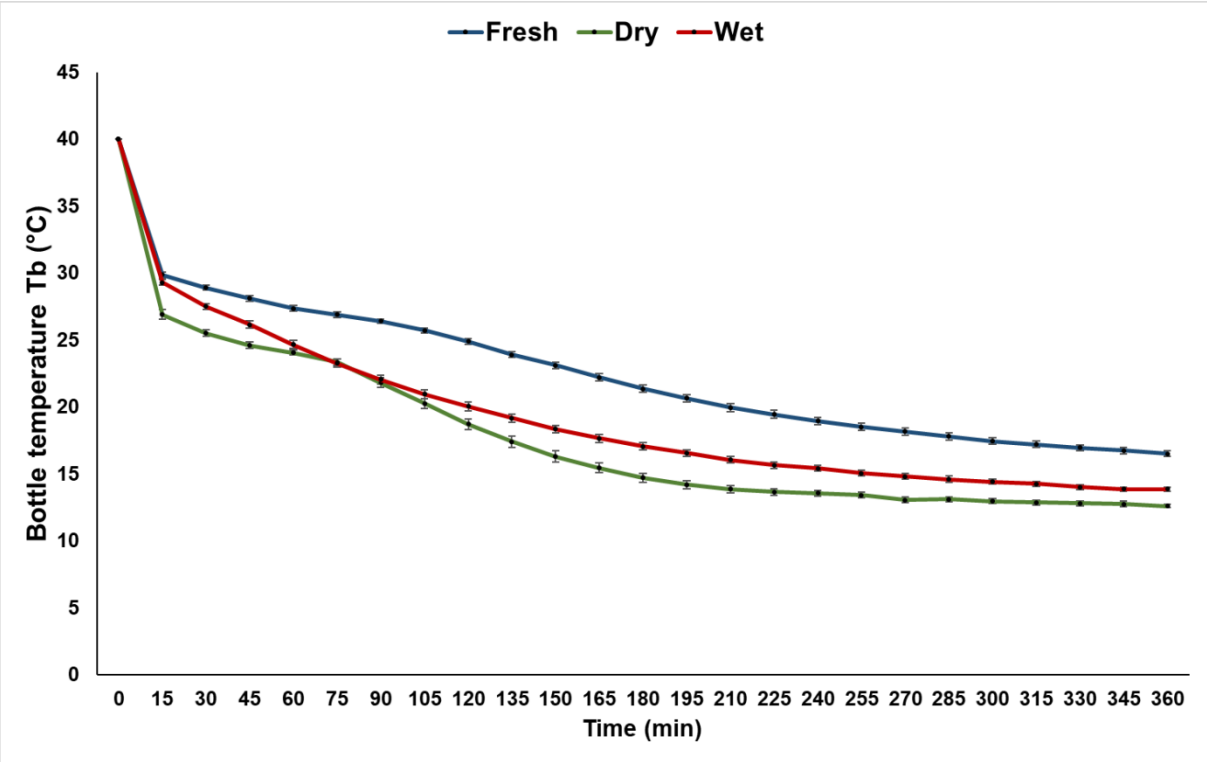


Fig. 3.7. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing lichen with different moisture content at 15°C.

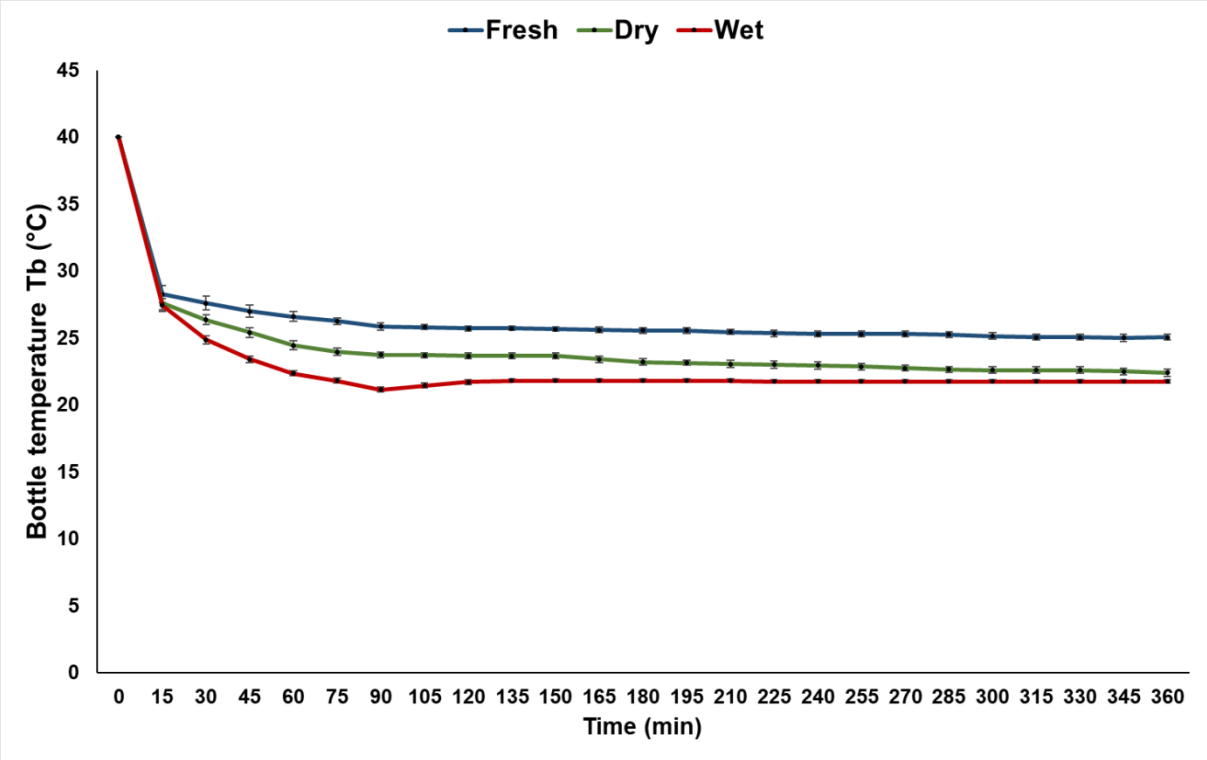


Fig. 3.8. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing lichen with different moisture content 25°C

3.3 INFLUENCE OF MASS ON NEST INSULATION

My results showed that there were statistical differences in insulation properties across the different masses of lichen (20 g, 40 g and 60 g) and this throughout all the time intervals (Anova, $p < 0.001$). Tukey *post hoc t* tests revealed significant differences between pairs of treatments (Table 3.3), and I recorded consistent differences between all the different masses of lichen throughout all four temperature regimes and at different time intervals of the experiments (Fig. 3.9 to 3.12).

3.3.1 Lichen material: 40 g vs. 20 g mass

When I compared the insulation properties of nests with 40 g lichen vs. nests with 20 g lichen, I found that 40 g nest material was better at providing insulation than 20 g nest material, throughout all Ta regimes, with average temperature differences between bottles ranging from 0.86–4.8°C (Ta = 5°C), 0.8–3.65°C (Ta = 10°C), 1.15–3.65°C (Ta = 15°C), and 0.9–1.5°C (Ta = 25°C), respectively. At 25°C, the significant differences between the two masses were only recorded after 130 minutes of the experiment.

3.3.2 Lichen material: 60 g vs. 40 g and 20 g mass

Overall, I found that 60 g quantity of nest material insulates the bottles better than 40 g and 20 g of nest material quantity. Nest boxes with 60 g lichen showed better insulation than nest boxes with 20 g lichen, and this was significant throughout all Ta regimes, with average temperature differences between bottles ranging from 3.15–7.35°C (Ta = 5°C), 1.7–4°C (Ta = 10°C), 2–5.3°C (Ta = 15°C), and 0.2–1.2°C (Ta = 25°C) respectively.

I found that 60 g of lichen material provided better nest insulation when compared to 40 g lichen material, and this was the case throughout all Ta regimes, with average temperature differences between bottles ranging from 0.75–3.2°C (Ta = 5°C), 0.3–1°C (Ta = 10°C), 0.8–1.7°C (Ta = 15°C), and 0.4–1.35°C (Ta = 25°C), respectively.

However, even though I recorded significant differences in all four Ta regimes, I found nest boxes with 60 g nest material insulating the bottles better, and this throughout all time intervals (i.e. the experimental time adopted for the experiment which was at 15 minutes intervals for 6 hours). But at Ta's of 10°C and 25°C, I only

noted significant differences in the last hour of the experiment and in the first two hours of the experiment.

Table 3.3. Average bottle temperature (Tb) differences between nesst containing different amounts of lichen material (20 g, 40 g, 60 g) at four different ambient temperature (Ta) regimes. Statistically significant differences (Tukey *post hoc t* tests) between lichen mass groups are indicated (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; NS: not significant).

	Average Tb differences at:			
Comparisons	**Ta = 5°C**	**Ta = 10°C**	**Ta = 15°C**	**Ta = 25°C**
Tb (40 g) vs. Tb (20 g)	3.31°C 95.8% *	2.05°C 91.7% **	2.43°C 100% **	0.54°C 79.2% *
Tb (60 g) vs. Tb (20 g)	5.05°C 100% **	2.70°C 100% **	3.71°C 100% **	0.90°C 79.2% *
Tb (60 g) vs. Tb (40 g)	1.74°C 100% *	0.64°C 20.8% *	1.30°C 91.7% *	0.34°C 37.5% *

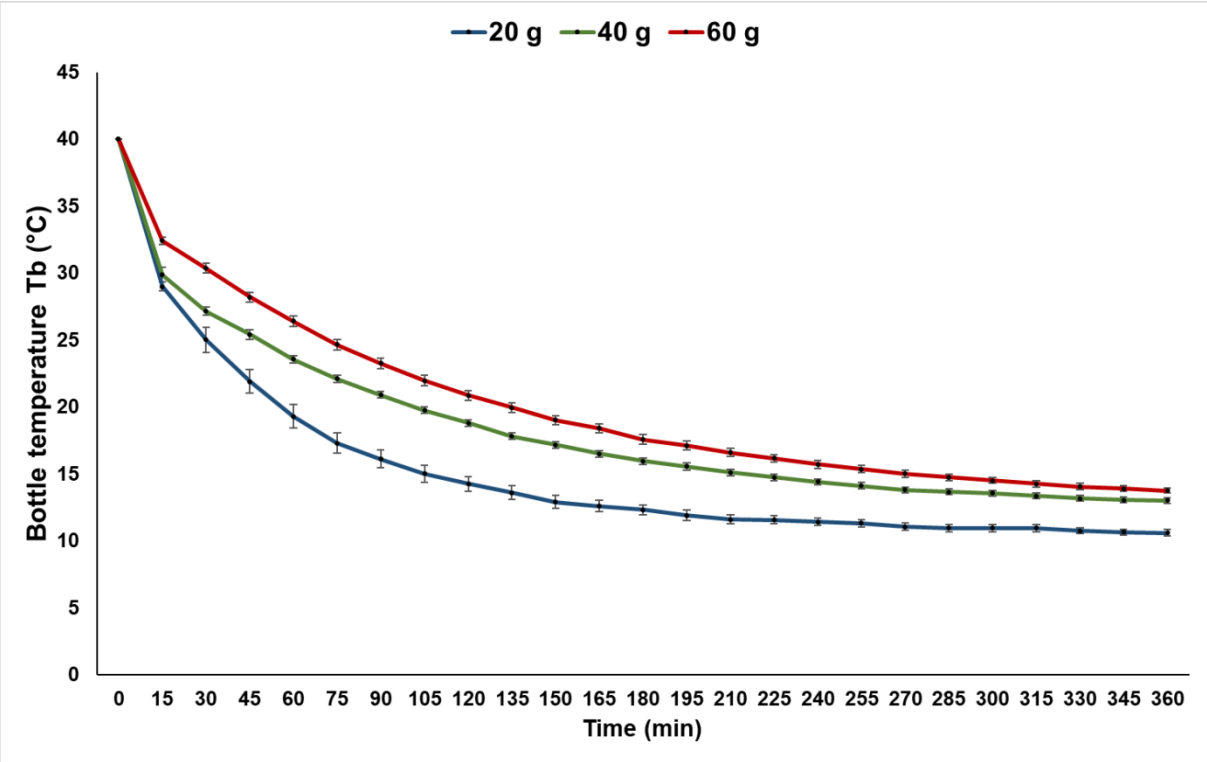


Fig. 3.9. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing different masses of lichen at 5°C.

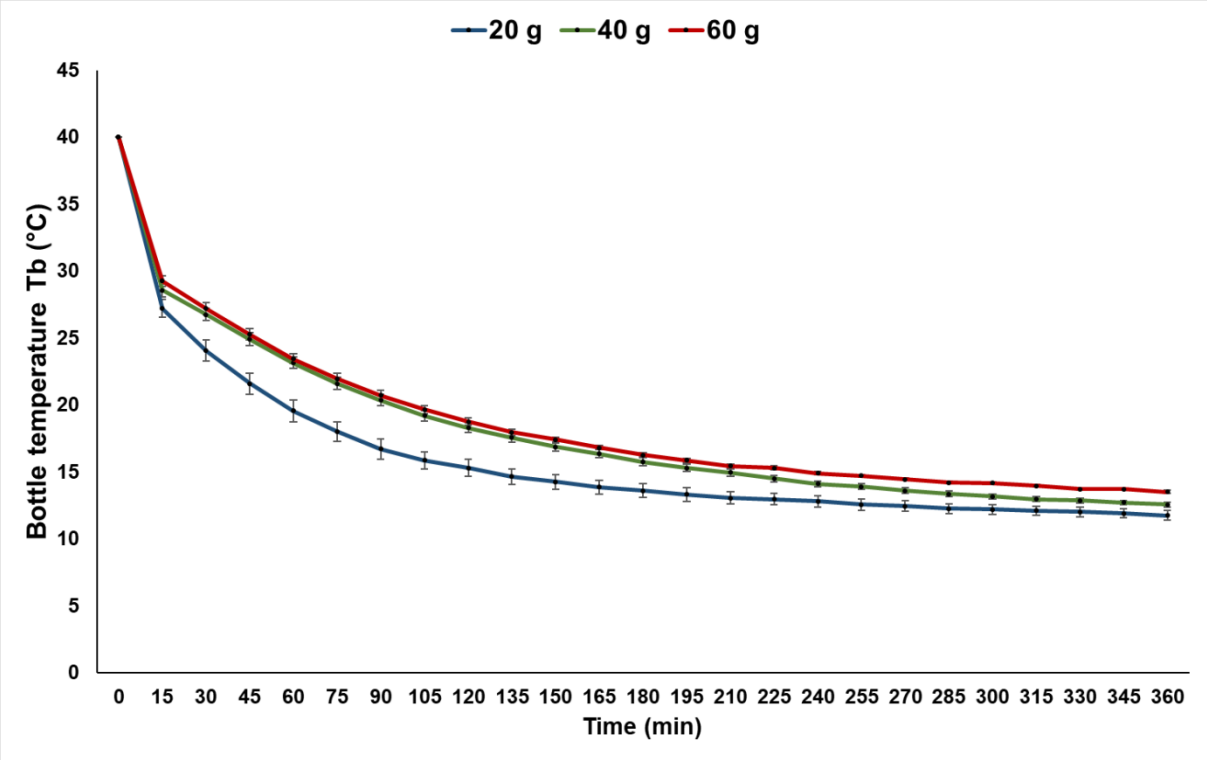


Fig. 3.10. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing different masses of lichen at 10°C.

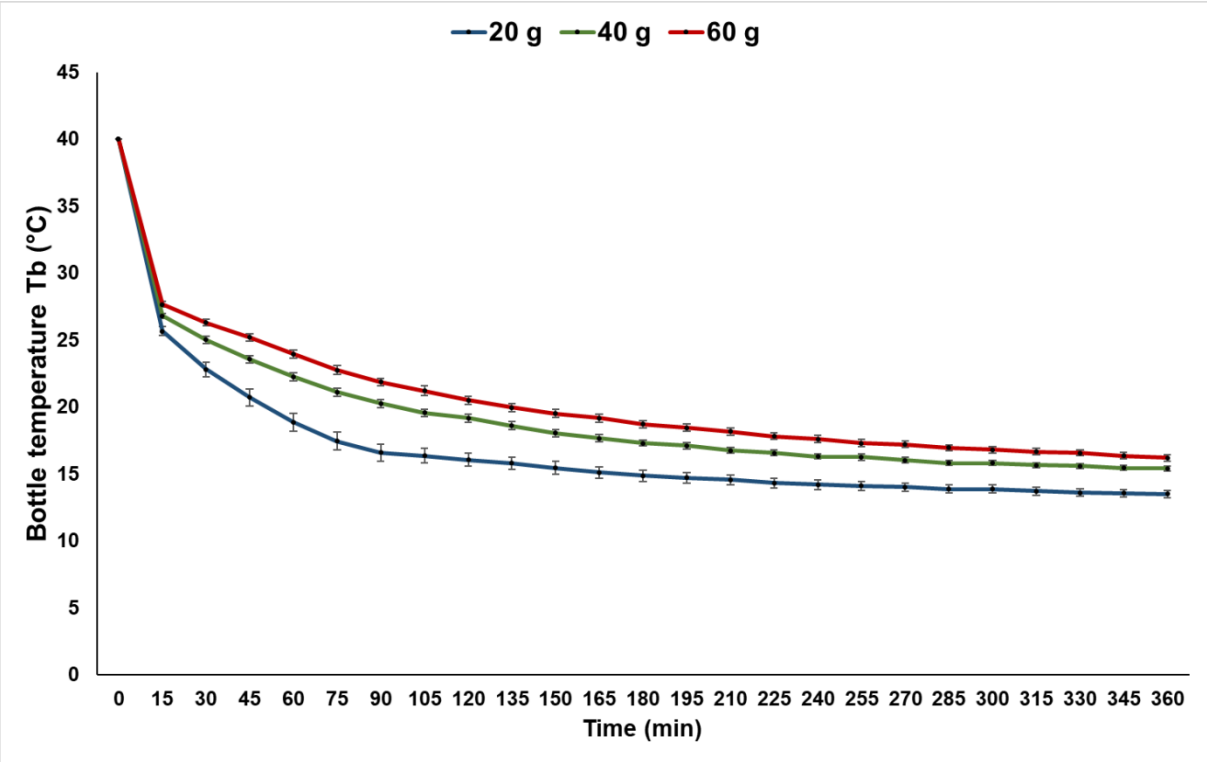


Fig. 3.11. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing different masses of lichen at 15°C.

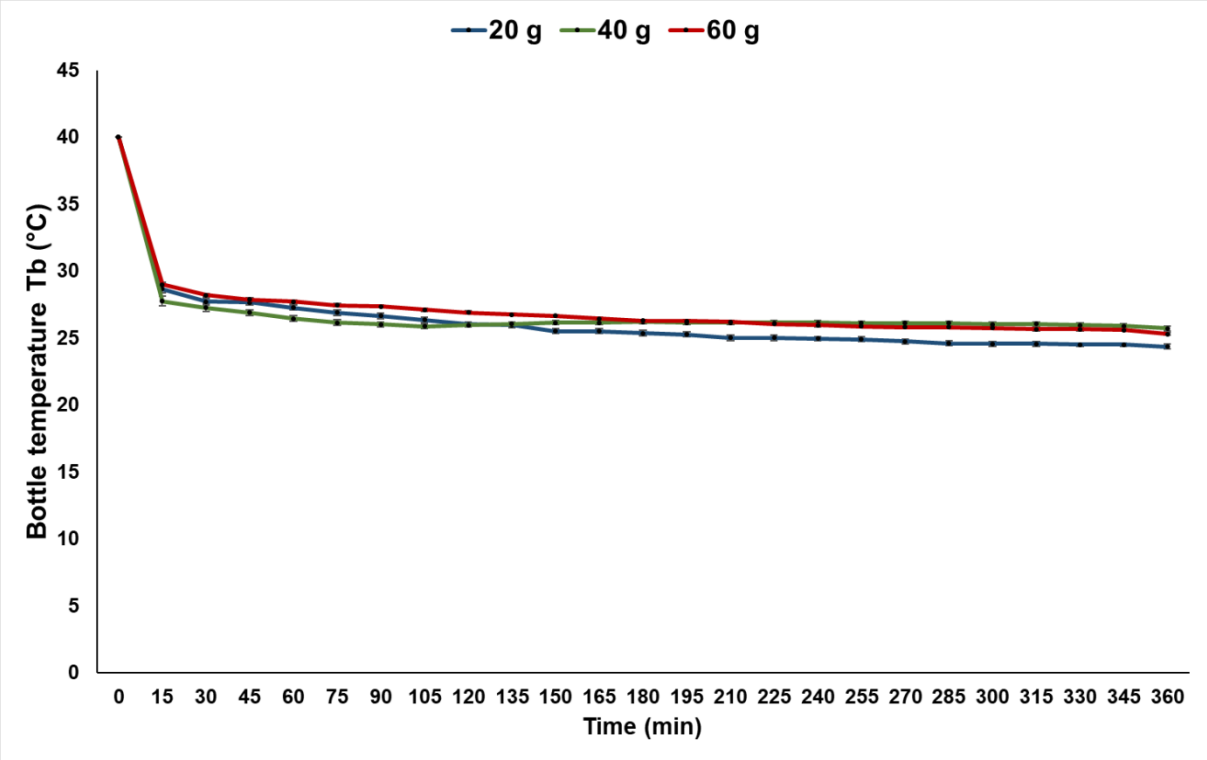


Fig. 3.12. Rate of heat loss of water bottles (Tb given as mean ± SE; n=10) in nest boxes containing different masses of lichen at 25°C.

4. DISCUSSION

4.1 INFLUENCE OF DIFFERENT NEST MATERIALS ON NEST INSULATION

The aim of my experiment was to assess the insulation properties of nests in relation to the type of nesting material used by woodland dormice, I hypothesized that the type of nest material with which a nest is built will determine its insulation quality (Pinowski *et al*., 2006) (Hypothesis 1). My results revealed that there were differences in the level of insulation provided by the different nest materials, and thus my hypothesis was supported. Feathers ranked the best insulator followed by fresh lichen and then dry grass; paper shavings had the lowest insulation out of all the nest materials. This means that different nest materials possibly used by animals, have different thermal abilities, and these differences could have a profound effect on thermal properties of the nest, and an impact on the rates of heat loss in animals inhabiting these nests. The insulation of a nest is dependent on the type of material used in its construction, which in turn provides a thermal benefit to the animal (Calder, 1971; Harris *et al.*, 1997; Hilton *et al.*, 2004; De Zwaam and Martin, 2018). A study on a natural population of woodland dormouse, observed the presence of lichen and feathers inside their nests (Madikiza, 2010). It is thus possible that the use of these nest materials by woodland dormice is not necessarily linked to availability of these resources alone, but might also be attributed to the insulation properties of these materials. Therefore, dormice might be actively choosing lichen and feathers for thermal benefits. Several studies on rodents and birds such as in the European ground squirrel (*Spermophilus citellus*) (Gedeon *et al*., 2010), mice (*Mus musculus*) (Gaskill *et al.*, 2013), blue tit (Mainwaring *et al.*, 2016), great tit (Mertens, 1977), have shown that the type of nest material incorporated in the nests of animals has an influence on the insulation properties of the nest.

My results further indicate that there are significant differences in insulation between nests with nest material and nests without nest material (empty nests), and this was evident throughout all temperature regimes. This means that a nest lined with nesting material significantly improves the microclimate of the nest, thus providing thermal benefits to animals. These thermal benefits may be crucial for endothermic small mammals that use nests or cavities for breeding or nesting, where protection against extreme environmental temperatures is essential (Griffiths *et al.*, 2018; Rowland *et al.*,

2017). Similar findings were reported for the marsh tit (*Poecile palustris*) (Maziarz, 2019), Syrian woodpecker (*Dendrocopos syriacus*) (Mersten-Katz *et al.*, 2012), great tit (Maziarz and Wesolowski, 2013) whereby cavities and nest boxes with nest materials provided better insulation than empty cavities or nest boxes.

In my study, nest boxes with feathers showed significantly higher thermal insulation when compared to nest boxes with lichen, grass and paper shavings, and this was evident at all ambient temperature regimes. My findings support the experimental finding by Hilton *et al.* (2004), where feathers proved to have a higher level of thermal insulation when compared with a range of other nest materials. Similar findings were also reported in tree swallows (*Tachycineta bicolor*) (Lombardo *et al.*, 1995), long-tailed tits (*Aegithalos caudatus*) (McGowan *et al.*, 2004), and in Chilean swallows (*Tachycineta meyeni*) (Liljesthrom *et al.*, 2009). A highly insulated nest helps maintain an appropriate micro climate by regulating the nest temperature, thus providing thermal benefits, and as a consequence contributing towards growth and reproductive performance of the animal (Møller, 1991; Winkler, 1993; Lombardo *et al.*, 1995; Dawson *et al.*, 2005). In birds, nestlings in nests that were lined with feathers, were significantly larger than nestlings that had feathers removed from their nests (Dawson *et al*., 2011). The presence of feathers in the nests of dormice, confirms that dormice possibly use feathers to insulate their nests, even though nests with feathers were not found to be common (Madikiza, 2010). This could be attributed to the availability and abundance of feathers in dormice habitats.

I compared the insulation properties of lichen to grass and paper shavings, and my results showed that lichen is a better insulator than grass and paper shavings. The ability of lichen to insulate better could be attributed to its morphological characteristics. Lichen depends solely on the environment for its water content, and this is due to its poikilohydric nature (Van Zuijlen *et al.*, 2020). In addition, the amount of water absorbed by lichen highly depends on its morphological structures (i.e. the thallus). Old-man's beard lichen found in the nests of woodland dormice has typically long strands of thin thalli, thus lower water holding capacity (Gauslaa, 2014; Phinney *et al.*, 2018), leading to lower thermal conductivity, and as a result increased insulation. Hence lichen can buffer against variations in ambient temperatures of nests (White and Kinney, 1974; Schmid, 1998; Speakman *et al.*, 1999; Kelser and Haig, 2005). The use of lichen is very common in nests of small mammals, e.g. in northern flying squirrels (Hayward and Rosentreter, 1994), southern flying squirrels (*Glaucomys*

volans) (Prange and Nelson, 2006), bats (Poissant *et al*., 2010) and in several species of birds (Hansell, 1996; Sharnoff and Rosentreter, 1998), and has been attributed to insulation.

Indeed, lichen is the most utilized material in the nest of dormice (Madikiza, 2010). The high insulation properties of lichen could explain this active preference by woodland dormice. However, one could argue that woodland dormice are simply selecting this material because of its abundance and availability in the forest; since lichen is abundant throughout the year with no seasonal occurrences or influences (Nash, 2008). But, I still maintain or argue that the prevalence of lichen in dormice nests cannot only be explained by its availability in the environment, but also by its thermal benefits.

My results showed that grass had the lowest insulation. A similar finding was noted by Hilton *et al.* (2004), whereby grass was a poor insulator in comparison with feathers and fur. The poor insulation of dry grass could help elucidate and strengthen the reason why dormice actively select lichen and feathers instead of grass in the forest.

4.2. INFLUENCE OF MOISTURE CONTENT ON NEST INSULATION

The insulation quality of a nest is largely dependent on the moisture content of its component materials (Gedeon *et al.*, 2010). The aim of this experiment was to determine if the moisture content of lichen affects insulation. My initial findings showed that overall there were significant differences in insulation properties of dry, fresh and wet lichen, and these differences were evident throughout all ambient temperatures regimes. This indicates that physical characteristics of nest material such as moisture content indeed have an influence on the thermal properties of nests. The effects of moisture on the thermal properties of nest material have been well reported in the European ground squirrel (Gedeon *et al.*, 2010), in tawny crowned honeyeater (*Gliriphilla melanops*) (Heenan, 2013), and in wood ant (*Formica polyctena*) (Frouz, 2000), thus supporting my initial findings.

In detail, my results indicated that the insulation efficacy of fresh lichen nests was greater in comparison to nests built from dry lichen and wet lichen. Wetting the lichen in my study significantly decreased insulation. The amount of water that was soaked up by the lichen seems to have had an effect on its insulation properties. This could be attributed to the hydrophilic nature and, amorphous fungal wall structures called

lichenin, which have the ability to retain water in the thallus (Honegger & Haisch, 2001). My results are supported by Reid *et al.* (2002), who showed that wetting feathers and moss greatly reduced the insulatory efficacy of these two nesting materials. Indeed, an increase in water content of a material can reduce its insulatory properties (Brandt, 1980; Hilton *et al.*, 2004; Bozikova and Hlavac, 2005; Gedeon *et al.*, 2010; Heenan, 2013; Kadochova and Frouz, 2014; Deeming and Campion, 2018).

I further noted that, nests with fresh lichen maintained a higher temperature especially during low ambient temperatures. Thus, the insulation efficacy of the nest is also affected by ambient temperatures (i.e. ambient temperature influences the insulation of nests and the thermal efficacy of the material). However, temperature and precipitation hence referred to as climate act in combination indicating that the response to one variable depends upon the level of the other (Heenan, 2013). Similar findings were noted by Gedeon *et al.* (2010), that fresh fescue insulated the nests better than dry or very wet fescue, and the insulation increased at cooler temperatures. In addition, Heenan (2013) showed that nests constructed in warm climates but at two extremes of rainfall shows a pronounced decrease in insulation for nests built in areas with high rainfall compared to areas with low rainfall. Indeed, during cold stressful ambient temperatures, nests must be able to provide insulation for small mammals. Woodland dormice use tree hollows as nesting sites, the use of these tree hollows increases in winter, with a significant nest fidelity (Lamani, 2014). Therefore, it is possible to speculate that the use of fresh lichen in nests could be attributed to the insulation properties offered by fresh lichen especially under cold ambient temperatures. This could be valuable for dormice during the winter season when the temperature gradient between the body and the environment is at its highest.

4.3 INFLUENCE OF NEST MATERIAL MASS ON NEST INSULATION

I hypothesised that, the insulation quality of nest is largely dependent on the mass of nest materials (Grubbauer and Hoi, 1996). My aim was then to determine if the mass of lichen had an influence on nest insulation. My initial results demonstrated that the amount of lichen inside a nest had an influence on the insulation properties. This suggests that nest characteristics (viz. mass) play an important role in insulation efficacy of nests. My results are further supported by findings in prairie warbler (*Setophaga discolor*) (Akresh *et al*., 2017), penduline tit (Szentirmai *et al.*, 2005), white-

crowned sparrow (*Zonotrichia leucophrys*) (Kern, 1984) and in common blackbirds (Mainwaring *et al.*, 2014), whereby nest characteristics had an influence on the thermal properties of the nest.

In detail, my results revealed that the rate of heat loss was significantly lower in nests that had more lichen. Indeed, as Heenan (2013) stated, well insulated nests have lower heat transfer and poorly insulated nests have higher heat transfer. Therefore my results are in agreement with my proposed hypothesis. This means that the insulation of a nest is dependent, at the very least on the quantity of nest material (lichen) inside the nest box. I can conclude that lichen maybe a good insulator, as per my first experimental results however, the amount of lichen also contributes to increasing insulation even further. Similar findings have been reported in yellow warbler (*Setophaga petechia)* (Rohmer and Law, 2010), acorn woodpecker (*Melanerpes formicivorus*) (Hooge *et al.*, 1999), and in male short-tailed field vole (*Microtis agrestis*) (Redman *et al.*, 1999), where heavier nests lost heat at a slower rate than smaller nests.

Furthermore, in my study nest insulation efficacy (and therefore the rate of heat loss) varied with ambient temperature. The lower the ambient temperature, the higher the difference in temperature between the nest and ambient temperature. Nests with more lichen maintained a higher temperature even at decreasing ambient temperatures. This means that the lower the ambient temperature, the more lichen added, the better the insulation. Pooled together, my results suggest that at very low ambient temperatures, with a threshold lying between 5 and 15°C, this is when nest insulation is more critical and considered effective. This indicates that there exist ambient temperature thresholds under which nest insulation is even more effective. Therefore an increase in the amounts of lichen inside the nests of dormice might play an important role in buffering against unfavourable low ambient temperatures. Several studies have indeed shown that there is a correlation between ambient temperatures and insulation properties of nests in several species of rodents and birds, whereby cold ambient temperatures are linked to heavier nests. Prime examples of such a relationship are shown in mice and rats that increase the quantities of nesting material during lower ambient temperatures, thus increasing nest insulation (Rajendram *et al.*, 1987). Similar observations were recorded in birds such as, blue tit (Britt and Deeming, 2011), great tit (Deeming *et al.*, 2012), long-tailed tits (*Aegithalos caudatus*) (McGowan

et al., 2004), warbler (*Parulidae spp.*) (Crossman *et al.*, 2011), as well as in the common blackbirds (Mainwaring *et al.*, 2014).

Pooled together, this means that nest building in woodland dormice serves as an important behavioural strategy in conserving heat, and in addition the use of lichen to build the nests, as well as the nest characteristics (in my case study, the mass and the moisture of lichen) proved to play a very important role in increasing thermal insulation of dormice nests, both strategies could possibly be important in decreasing energy expenditure especially during low ambient temperatures (Muul, 1968; Stapp, 1992; Merrit *et al.*, 2001; Tompkins, 2003).

5. SUMMARY

The overall aim of my project was to study factors affecting the insulation properties of the old man's beard lichen (*Usnea barbata)*, a nesting material found in many nest boxes and tree cavities utilized by African woodland dormice (*Graphiurus murinus)*. My study has helped provide knowledge on the functional importance of lichen in woodland dormice nests, as well as on the importance of specific habitat characteristics that enhance small mammal survival.

This study first elucidates on the thermal properties of lichen and various other nest materials used by animals. My study indicated that there were thermal benefits linked to lichen as well as other nesting materials. However, this differed with the type of material. Indeed, the type of nest material with which a nest is built determines its insulation quality (Pinowski *et al*., 2006). In my study the insulation properties of lichen were highly superior to the insulation properties of dry grass and paper shavings, but significantly lower than those of feathers. I further provided knowledge on how nest material characteristics such as the amount as well as the moisture content of lichen could have an impact on the insulation properties of the nest. I revealed that the amount of lichen had a significant effect on insulation, the heavier the nest material the higher the insulation quality. The condition of the nest material influences insulation efficacy. Fresh (humid) lichen had a highly significant effect on insulation efficacy than dry or wet lichen. My results indicated that there were acquired thermoregulatory benefits in nest constructions. The presence of nest material and the characteristics of nest material increased temperature inside the nest, and this was extremely significant at the lower temperature regimes.

Future studies should experimentally investigate the motivation to build nests, as well as the preference for lichen material in nest construction. In addition, my studies were conducted under controlled laboratory environments, future studies should replicate these studies, or investigate these patterns under natural environments using live animals.

I conclude that fresh lichen provides insulation in the nests of woodland dormice. Woodland dormice may highly depend on available, and abundant fresh lichen in the forest because of the insulation and thermal benefits this material has to offer. My

conclusions require further studies on other populations of dormice, to assess if my conclusions are applicable in other populations, and consequently elucidate on the evolution of nest building in the species.

BIBLIOGRAPHY

Akresh, M.E., Ardia, D.R. and King, D.I. (2017). Effect of nest characteristics on thermal properties, clutch size and reproductive performance for an open-cup nesting songbird. *Avian Biology Research* 10(2): 107–118.

Bhushan, B. (2009). Biomimetic: lessons from nature – an overview. *Philosophical Transactions of the Royal Society of London, Series A* 397: 1445–1486.

Bozikova, M. and Hlavac, P. (2005). Thermo-physical parameters of chosen biological materials. Pp. 25–26 in Horabik, J. and Zdunek, A., editors. *The 4th International Conference of Young Scientists*, *Biophys Spring 2005*. Book of Abstracts. Institute of Agrophysics, Polish Academy of Sciences, Lublin, Poland and Czeck University of Agriculture, Prague, Czech Republic, 15–16 May 2005, Lublin, Poland.

Bracewell, M. and Downs, N.C. (2017). Hazel dormouse (*Muscadinus avellanarius*) nest preferences and collection distances, in southern England. *Mammal Communications* 3: 1–10. London: Mammal Society.

Brandt, C.J. (1980). The thermal diffusivity of the organic material of a mound of *Formica polyctena* forest in relation to the thermoregulation of brood (Hymenoptera, Formicidae). *Netherlands Journal of Zoology* 30(2): 326–344.

Bright, P., Morris, P. and Mitchell, J.T. (2006). *Dormouse Conservation Handbook.* London: Mammal Society.

Britt, J. and Deeming, D.C. (2011). First egg date and air temperature affect nest construction in Blue Tits *Cyanistes caeruleus* but not in Great Tits *Parus major*. *Bird Study* 58: 78–79.

Brodo, I. M., Sharnoff, S.D. and Sharnoff, S. (2001). *Lichen of North America.* New Haven: Yale University Press.

Calder, W.A. (1971). Temperature relationships and nesting of the calliope hummingbird. *Condor* 73: 314.

Calvello, S., Trejo, A. and Ojeda, V. (2006). Botanical composition and structure of hummingbird nest in different habitats from Northwestern Patagonia (Argentina). *Journal of Natural History* 40: 589–603.

Collias, N. E. and Collias, E.C. (1984). *Nest Building and Bird Behaviour.* Princeton: Princeton Univeristy Press.

Crossman, C.A., Rohwer, V.G. and Martin, P.R. (2011). Variation in the structure of bird nests between Manitoba and Southeastern Ontario. *PLoS ONE* 6(4): e19086.

Dawson, R.D., Lawrie, C.C. and O'Brien, E.L. (2005). The importance of microclimate variation in determining size, growth and survival of avian offspring: experimental evidence from a cavity nesting passerine. *Oecologia* 144: 499–507.

Dawson, R.D., O'Brien, E.L. and Mlynowski, T.J. (2011). The price of insulation: cost and benefits of feather delivery to nests for male tree swallows *Tachycineta bicolor. Journal of Avian Biology* 42: 102.

Davenport, J. (1992). *Animal Life at Low Temperature.* London: Chapman and Hall.

De Zwaam, D.R. and Martin, K. (2018). Substrate and structure of ground nests have fitness consequences for an Alpine songbird. *Ibis* 160: 790–804.

Deeming, D.C. and Campion, E. (2018). Simulated rainfall reduces the insulative properties of bird nests. *Acta Ornithologica* 53(1): 91–97.

Deeming, D.M., Mainwaring, M., Hartley, I.R. and Reynolds, S.J. (2012). Local temperature and not latitude determines the design of blue tit and great tits nests. *Avian Biology Research* 5(4): 203–208.

Demere, T., Hollingsworth, B. D. and Unit, P. (2002). *Nests and Nest Building Animals.* San Diego: San Diego Natural History Museum (archived).

Ellison, L. (1966). Seasonal foods and chemical analysis of winter diet of Alaskan spruce goose. *Journal of Wildlife Management* 30(4): 729–735.

Ellison, G.T.H. and Skinner, J.D. (1991). Thermoregulation and torpor in African woodland dormice *Graphiurus murinus*, following cold acclimation. *Zeitschrift für Säugetierkunde* 56: 41–47.

Fietz, J., Klose, S.M. and Kalko, E.K.V. (2010). Behavioral and physiological consequences of male reproductive trade-offs in edible dormouse *Glis glis. Naturwissenschften* 97: 883–890.

Frouz, J. (2000). The effect of nest moisture on daily temperature regime in the nest of *Formica polyctena* wood ants. *Insectes Sociaux* 47: 229–235.

Gaskill, B.N., Gordon, C.J., Pajor, E.A., Lucas, J.R., Davis, J.K. and Garner, J.P. (2013). Impact of nesting material on mouse body temperature and physiology. *Physiology & Behav*ior 110–111: 89–95.

Gauslaa, Y. (2014). Rain, dew and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *The Lichenologist* 46(1): 1–16.

Gedeon, C.I., Marko, G., Nemeth, I., Nyitrai, V. and Altbacker, V. (2010). Nest material selection affects nest insulation quality for the European ground squirrel (*Spermophilus citellus*). *Journal of Mammalogy* 91(3): 636–641.

Griffiths, S., Lentini, P., Semens, K. and Watson, S. (2018). Chainsaw-carved cavities better mimic the thermal properties of natural tree hollows than nest boxes and long hollows. *Forests* 9(5): 235.

Grubbauer, P. and Hoi, H. (1996). Female penduline tits (*Remiz pendulinus*) choosing high quality nests benefit by decreased incubation effort and increased hatching success. *Ecoscience* 3: 274–279.

Gwinner, H. (1997). The function of green plants in nest of European Starling (*Sturnus vulgaris*). *Behavior* 134: 337–351.

Hansell, M.H. (1996). The function of lichen flakes and white spider cocoon on the outer surface of birds' nest. *Journal of Natural History* 30(2): 303–307.

Hansell, M.H. (2005). *Animal Architecture.* Oxford: Oxford Univeristy Press.

Harris, M.P., Wanless, S., Barton T.R., and Elston, D.A. (1997). Nest site characteristics, duration of use and breeding success in the guillemot (*Uria aalge*). *Ibis* (Lond. 1859) 139: 468–476.

Hayward, D.G., and Rosentreter, R. (1994). Lichen as nesting material for Northern flying squirrels in the northern Rocky Mountains. *Journal of Mammalogy,* 75(3):663–673.

Heenan, C.B. (2013). An overview of the factors influencing the morphology and thermal properties of avian nests. *Avian Biology Research* 6:104–118.

Hilton, G.M., Hansell, M.H., Ruxton, G.D., Reid, J.M. and Monaghan, P. (2004). Using artificial nests to test the importance of nesting material and nest shelter for incubation energetics. *The Auk,* 121(3): 777–787.

Holden, M.E. (2013). Family Gliridae. Pages 102–104 *in* Happold, D., editor. *Mammals of Africa. Volume III: Rodents, Hares and Rabbits*. London: Bloomsbury Publishing.

Holden-Musser, M.E., Juškaitis, R. and Musser, G.M. (2016). Family Gliridae (Dormice). Pages 838–889 *in* Wilson, D.E., Lacher, T.E. Jr., Mittermeier, R.A., editors. *Handbook of the Mammals of the World. Vol. 6. Lagomorphs and Rodents I.* Barcelona: Lynx Edicions.

Honegger, R. and Haisch, A. (2001). Immunocytochemical location of the (1→3) (1→4)-Beta-glucan lichenin in the lichen forming ascomycete *Cetraria islandica* ("Icelandic moss"). *New Phytologist* 150: 739–46.

Hooge, P.N., Stanback, M.T. and Koenig, W.D. (1999). Nest site selection in the Acorn woodpecker. *Auk* 116: 45–54.

Hunneck, S. (1999). The significance of lichen and their metabolites. Naturwissenschaften 86: 559–570.

Kadochova, S. and Frouz, J. (2014). Thermoregulation strategies in ants in comparison to other social insects with a focus on red wood ants (*Formica rufa group*). *F1000 Research* 2: 280.

Kelser, D. and Haig, S.M. (2005). Microclimate and nest-site selection in Micronesian Kingfishers. *Pacific Science* 59(4): 499–508.

Kern, M.D. (1984). Racial differences of nest of white-crowned sparrows. *Condor* 86: 455–466.

Lamani, S. (2011). *Resting site ecology of the woodland dormouse Graphiurus murinus at the Great Fish River Reserve, Eastern Cape.* B.Sc. Honours Thesis. University of Fort Hare, Alice, South Africa.

Lamani, S. (2014). *Diet and microhabitat use of the woodland dormouse Graphiurus murinus at the Great Fish River Reserve, Eastern Cape.* M.Sc. Thesis. University of Fort Hare, Alice, South Africa.

Liljesthrom, M., Schiavini, A. and Reboreda, J.C. (2009). Chilean swallows (*Tachycineta meyeni)* adjust the number of feathers added to the nest with time of breeding. *Wilson Journal of Ornithology* 121(4): 783–788.

Lombardo, M.P., Bosman, R.M., Faro, C.A., Houtteman, S.G. and Kluisza, T.S. (1995). Effect of feathers as nest insulation on incubation behaviour and reproductive performance of tree swallow (*Tachycineta bicolor*). *Auk* 112(4): 973–981.

Lutermann, H., Verburgt, L. and Rendigs, A. (2010). Resting and nesting in a small mammal: sleeping sites as a limiting resource for female grey mouse lemurs. *Animal Behaviour* 79: 1211–1219.

Madikiza, Z.J.K. (2010). *Population biology aspects of the socio-spatial organisation of the woodland dormouse Graphiurus murinus (Desmaret 1822) in the Great Fish River Reserve, South Africa.* M.Sc. Thesis. University of Fort Hare, Alice, South Africa.

Madikiza, Z.J.K. (2017). Sociality in the African woodland dormouse. Ph.D. Thesis. University of the Witwatersrand, Johannesburg, South Africa.

Madikiza, Z.J.K., Bertolino, S., Baxter, R. and Do Linh San, E. (2010). Nest box use by woodland dormice (*Graphiurus murinus*): the influence of life cycle and nest placement. *European Journal of Wildlife Research,* 56: 1–10.

Madikiza, Z.J.K., Lamani, S.F., Baxter, R.M., Kryštufek, B., MacFadyen, D. and Do Linh San, E. (2016). A conservation assessment of *Graphiurus murinus*. *In* Child, M.F., Roxburgh, L., Do Linh San, E., Raimondo, D. and Davies-Mostert, H.T., editors. *The Red List of mammals of South Africa, Swaziland and Lesotho*. Pretoria and Johannesburg: South African National Biodiversity Institute and Endangered Wildlife Trust.

Mainwaring, M.C. and Hartley, I.R. (2008). Seasonal adjustments in nest cup lining in blue tits *Cyanistes caeruleus*. *Ardea* 96: 278–282.

Mainwaring, M.C., Hartley, I.R., Bearhop, S., Brulez, K., du Feu, C.R., Murphy, G., Plummer, K.E., Webber, S.L., Reynolds, S.J. and Deeming, D.C. (2012). Latitudinal variation in blue and great tit nest characteristics indicate environmental adjustment. *Journal of Biogeography* 39: 1669–1677.

Mainwaring, M.C., Deeming, D.C., Jones, C.I. and Hartley, I.R. (2014). Adaptive latitudinal variation in common blackbird *Turdus merula* nest characteristics. *Ecology and Evolution* 4: 841–851.

Mainwaring, M.C., Wolfenden, A., Read, J.E., Robson, J.M.A., Tomlinson, C.J. and Hartley, I.R. (2016). Feathering the nest: the effects of feather supplementation to blue tit nests. *Avian Biology Research* 9: 89–95.

Maziarz, M. (2019). Breeding birds actively modify the initial microclimate of occupied tree cavities. *International Journal of Biometeorology* 63: 247–257.

Maziarz, M. and Wesolowski, T. (2013). Microclimate of tree cavities used by great tits *(Parus major*) in a primeval forest. *Avian Biology Research* 6: 47–56.

McDevitt, R.M. and Andrews, J.F. (1994). The importance of nest utilization as a behavioral thermoregulatory strategy in *Sorex munitus* the pygmy shrew. *Journal of Thermal Biology* 19: 97–102.

McGowan, A., Sharp, S.P. and Hatchwell, B.J. (2004). The structure and function of nests of Long-tailed Tits *Aegithalos caudatus. Functional Ecology* 18: 578–583.

Mennerat, A., Perret, P. and Lambrechts, M.M. (2009). Local individual preference for nest material in a Passerine bird. *PLoS ONE* 4(4), e5104.

Merrit, J.F., Zegers, D.A. and Rose, L.R. (2001). Seasonal thermogenesis of southern flying squirrels (*Glaucomys volans*). *Journal of Mammalogy* 73: 914–920.

Mersten-Katz, C., Barnea, A., Yom-Tov, Y. and Ar, A. (2012). The woodpecker's activity microenvironment: advantageous or resting? *Avian Biol. Res.* 5: 227–237.

Mertens, J.A.L. (1977). Thermal conditions for successful breeding in Great tits (*Parus major L*.). II. Thermal properties of nests and nest boxes their implications for the range of temperature tolerance of Great Tit broods. *Oecologia* 28: 57–65.

Møller, A.P. (1991). The effect of feather nest lining on reproduction in the swallow (*Hirundo rustica*). *Ornis Scandinavica* 22: 396–400.

Monadjem, A., Taylor, P.J., Denys, C. and Cotterill, F.F.D. (2015). *Rodents of Sub-Saharan Africa: A Biogeographic and Taxonomic Synthesis.* Berlin: De Gruyter.

Muul, I. (1968). Behavioral and physiological influences on the distribution of flying squirrel (*Glaucomys volans*). *Miscellaneous Publications of the Museum of Zoology, University of Michigan* 134: 1–66.

Mzilikazi, N., Madikiza, Z., Oelkrug, R. and Baxter, R.M. (2012). Hibernation in free ranging African woodland dormice *Graphiurus murinus*. Pages 41–50 in Ruf, T. Bieber, C., Arnold, W. and Milliesi, E, editors. *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations.* Berlin: Springer.

Nash, T.H.III (2008). *Lichen Biology.* 2nd edn. London: Cambridge University Press.

Numone, M., Yasuda, S.P., Sato, J.J. and Vogel, P. (2007). Phylogenetic relationships and divergence times among dormice (Rodentia, Gliridae) based on three nuclear genes. *Zoologica Scripta* 36: 537–547.

Ontiveros, D., Caro, J. and Pleguezuelos, J.M. (2007). Green plant material versus ectoparasites in nests of Bonelli's eagle. *Journal of Zoology* 274: 99–104.

Panchetti, F., Sorace, A., Amori, G. and Carpaneto, G.M. (2007). Nest site preference of common dormouse (*Muscadinus avellanarius*) in two different habitat types of central Italy. *Italian Journal of Zoology* 74: 363–369.

Phinney, N.H., Solhaug, K.A. and Gauslaa, Y. (2018). Rapid resurrection of chlorolichens in humid air: specific thallus mass drives rehydration and reactivation kinetics. *Environmental and Experimental Botany* 148: 189–191.

Pilats, V., Pilate, D., Ornicans, A. and Karklins, A. (2012). Microhabitat utilization by forest dormice (*Dryomys nitedula*) in boreo-nemoral forest preliminary results. *Peckiana* 8: 77–85.

Pinowski, J., Haman, A., Jerzak, L., Pinowska, B., Barkowska, M., Grodzki, A. and Haman, K. (2006). The thermal properties of some nests of the Eurasian tree sparrow, Passer montanus. *Journal of Thermal Biology* 31: 573–581.

Poissant, J.A., Broders, H.G. and Quinn, G.M. (2010). Use of lichen as a roosting substrate by *Perimyotis subflavus*, the tricolored bats in Scotia. *Ecoscience* 17: 372–378.

Prange, S. and Nelson, D.H. (2006). Use of fungal rhizomorphs as nesting materials by southern flying squirrels (*Glaucomys volans*). *Southeastern Naturalist* 5: 355–360.

Qwede, K. (2003). *Observation on two populations of woodland dormouse Graphiurus murinus, in the Eastern Cape South Africa.* B.Sc. Honours Thesis. University of Fort Hare, Alice, South Africa.

Rajendram, E.A., Brain, P.F., Parmgiani, S. and Mainardi, M. (1987). Effects of ambient temperature on nest construction in four species of laboratory rodents. *Bolletino di Zoologia* 54: 75–81.

Redman, P., Selman, C. and Speakman, J.R. (1999). Male short-tailed field voles (*Microtus agrestis*) build better insulated nests then females. *Journal of Comparative Physiology B* 169, 581–587.

Reid, J.M., Cresswell, W., Holts, S., Mellanby, R.J., Whitfield, D.P. and Ruxton, G.D. (2002). Nest scrape design and clutch heat loss in pectoral sandpipers (*Calidris melanotos*). *Functional Ecology* 16: 305–312.

Rohwer, V.G. and Law, J.S.Y. (2010). Geographic variation in nest of yellow warblers breeding in Churchill, Manitoba and Elgin, Ontario. *Condor* 112: 596–604.

Rowland, J.A., Briscoe, N.J. and Handasyde, K.A. (2017). Comparing the thermal suitability of nest boxes and tree-hollows for the conservation management of arboreal marsupials. *Biological Conservation* 209: 341–348.

Ruiz-Castellano, C., Tomas, G., Ruiz-Rodriguez, M. and Soler, J. (2018). Nest material preference by spotless starlings. *Behavioural Ecology* 29: 137–144.

Schmid, J.R. (1998). Marine turtle populations on the west central coast of Florida: Results of tagging studies at the cedar keys, Florida. *Fishery Bulletin-National Oceanic and Atmospheric Administration* 96: 589–602.

Schradin, C. (2005). Nest site competition in two diurnal rodents from the succulent Karoo of South Africa. *Journal of Mammalogy* 84: 757–762.

Schuetz, J.G. (2005). Common waxbills use carnivore scat to reduce the risk of nest predation. *Behavioural Ecology* 16: 133–137.

Sharnoff, S. (2014). *Field Guide to California Lichens.* New Haven: Yale University Press.

Sharnoff, S. and Rosentreter, R. (1998). *Lichen Used by Wildlife in North America.* Boise: Bureau of Land Management.

Skinner, J. and Chimimba, C. (2005). *The Mammals of the Southern African Subregion.* 3rd edn. Cambridge: Cambridge University Press.

Speakman, J., Selman, C. and Redman, P. (1999). Male short tailed field voles *(Microtus agrestis)* build better insulated nest than females. *Journal of Comparative Physiology* 169: 581–587.

Stapp, P. (1992). Energetic influences on the life history of *Glaucomys volans*. *Journal of Mammalogy* 73: 914–920.

Szentirmai, L., Szekely, T. and Liker, A. (2005). The influence of nest size on heat loss of Penduline tit eggs. *Acta Zoologica Academiae Scientiarum Hungaricae* 51: 59–66.

Theron, S., Quietzch, F. and Radespiel, U. (2010). Leaf nest use and construction in the golden-brown mouse lemurs (*Microcebus ravelobensis*) in the Ankarafantsika National Park. *American Journal of Primatology* 72: 48–55.

Tiainen, J., Hanski, I.K. and Mehtala, J. (1983). Insulation of nests and the northern limits of three Phylloscopus warblers in Finland. *Ornis Scandinavica* 14: 149–153

Tompkins, B.J. (2003). The insulative properties and energetics benefits of the nesting material used by the southern flying squirrel, *Glaucomys volans*. MSc. Thesis. Wake Forest University, Winston-Salem, North Carolina.

van Riper, C. III. (1977). The use of sheep wool in nest construction by Hawaiian birds. *Auk* 94: 646–651.

Van Zuijlen, K., Roos, R.E., Klanderud, K., Lang, S. and Asplund, J. (2020). Mat forming lichens affect microclimate and litter decomposition by different mechanism. *Fungal Ecology* 44, 100905.

Vogt, F.D. and Lynch, G.R. (1982). Influence of ambient temperature, nest availability, huddling and daily torpor on energy expenditure in the white-footed mouse *Peromyscus leucopus. Physiological zoology* 55: 56–63.

Wainwright, S.A., Biggs, W.D., Currey, J.D. and Gosline, J.M. (1976). *Mechanical Design in Organisms.* London: Edward Arnold. Reprint: 1982. Princeton: Princeton University Press.

Watson, W. (1929). The classification of lichens. *The New Phytologist* 28: 85–116.

Webb, P. and Skinner, J. (1994). The dormouse *(Myoxidae)* of Southern Africa. *Hysrix, the Italian Journal of Mammalogy* 6: 287–293.

Webb, P.I. and Skinner, J.D. (1996). Summer torpor in African woodland dormice *Graphiurus murinus* (Myoxidae: Graphiurinae). *Journal of Comparative Physiology B* 166: 325–330.

White, F.N. and Kinney, J.L. (1974). Avian incubation. *Science* 189: 107–115.

Whittington-Jones, C.A. and Brown, C.R. (1999). Thermoregulatory capabilities of the woodland dormouse *Graphiurus murinus. South African Journal of Zoology* 34: 34–38.

Whittow, G.C. and Berger, A.J. (1977). Heat loss from the nests of the Hawaiian honeycreeper “Amakihi”. *The Wilson Bulletin* 89: 480–483.

Wind, D. (2009). Lichen, an Ecosystem Wonder. www.davesgarden.com/guides/articles/views/2407.

Windsor, R.L., Fegely, J.L. and Ardia, D.R. (2013). The effects of nest size and insulation on thermal properties of Tree Swallow nest. *Journal of Avian Biology* 44: 305–310.

Winkler, D.W. (1993). Use and importance of feathers as nest lining in tree swallows (*Tachycineta bicolor*). *Auk* 110: 29–36.

ANNEXURE

Table A1. Nest material type datasets showing the rate of heat loss from the different nest materials at every 15 minutes across four ambient temperature (Ta) regimes tested. AVE = average, SE = standard error, NM = No material, F = Feathers, L = Lichen, G = Grass, P = Paper shavings.

Ta	Nest materials		Time (min)																								
			0	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240	255	270	285	300	315	330	345	360
5ºC	AVE	NM	40.0	26.70	23.70	21.45	19.50	18.10	17.05	16.05	15.25	14.65	14.15	13.75	13.45	13.10	12.95	12.80	12.55	12.55	12.45	12.40	12.35	12.35	12.35	12.35	12.10
		F	40.0	30.35	29.05	27.70	26.60	25.50	24.40	23.45	22.50	21.85	21.00	20.35	19.75	19.20	18.70	18.25	17.75	17.45	17.10	16.75	16.50	16.25	16.00	15.80	15.50
		L	40.0	28.40	26.45	24.45	22.95	21.50	20.25	19.15	18.25	17.45	16.65	16.00	15.40	15.05	14.65	14.45	14.05	13.80	13.60	13.35	13.15	13.05	12.90	12.75	12.55
		G	40.0	24.50	21.50	19.25	17.55	16.20	15.10	14.20	13.60	13.15	12.65	12.25	11.95	11.80	11.70	11.45	11.35	11.25	11.20	11.05	10.95	10.95	10.90	10.85	10.75
		P	40.0	26.50	23.60	21.30	19.50	18.15	16.90	15.95	15.35	14.65	14.10	13.70	13.25	13.00	12.75	12.45	12.30	12.15	12.05	11.90	11.80	11.70	11.70	11.50	11.50
	SE	NM	0	0.36	0.27	0.22	0.22	0.17	0.15	0.15	0.15	0.15	0.15	0.15	0.08	0.14	0.08	0.11	0.13	0.13	0.08	0.1	0.10	0.10	0.10	0.10	0.1
		F	0	0.24	0.24	0.23	0.25	0.21	0.22	0.24	0.24	0.24	0.24	0.24	0.23	0.26	0.26	0.23	0.23	0.25	0.26	0.23	0.23	0.22	0.23	0.23	0.23
		L	0	0.37	0.45	0.52	0.59	0.61	0.63	0.62	0.63	0.56	0.61	0.58	0.55	0.50	0.50	0.47	0.47	0.44	0.40	0.43	0.41	0.39	0.36	0.40	0.39
		G	0	0.79	0.93	1.00	1.02	0.97	0.90	0.87	0.83	0.76	0.71	0.64	0.60	0.61	0.55	0.49	0.51	0.44	0.44	0.45	0.41	0.41	0.42	0.39	0.40
		P	0	0.54	0.59	0.62	0.60	0.58	0.52	0.50	0.45	0.45	0.42	0.38	0.38	0.34	0.32	0.33	0.28	0.28	0.26	0.26	0.26	0.22	0.22	0.23	0.23
10ºC	AVE	NM	40.0	29.00	24.80	20.95	18.10	15.90	14.30	13.05	12.05	11.35	10.75	10.25	10.05	9.70	9.45	9.35	9.20	9.05	8.95	9.00	8.90	8.85	8.80	8.85	8.95
		F	40.0	29.40	27.60	26.20	24.80	23.35	22.35	21.45	20.45	19.65	18.80	18.15	17.50	16.85	16.35	15.95	15.60	15.10	14.80	14.55	14.25	14.00	13.75	13.60	13.70
		L	40.0	31.95	30.70	28.70	26.70	25.00	23.40	21.90	20.65	19.50	18.55	17.60	16.90	16.10	15.55	15.05	14.55	14.15	13.90	13.50	13.30	12.95	12.90	12.50	12.45
		G	40.0	28.70	25.60	22.10	19.45	17.30	15.75	14.40	13.45	12.75	12.10	11.55	11.20	10.80	10.55	10.35	10.10	10.00	9.80	9.70	9.70	9.60	9.50	9.45	9.40
		P	40.0	30.25	28.35	26.70	24.95	23.55	22.15	20.90	19.70	18.75	17.85	17.05	16.30	15.65	15.10	14.55	14.10	13.60	13.30	13.05	12.80	12.55	12.30	12.20	12.00
	SE	NM	0	0.49	0.73	0.64	0.50	0.40	0.32	0.29	0.25	0.21	0.17	0.17	0.11	0.16	0.11	0.13	0.13	0.11	0.11	0.12	0.14	0.13	0.13	0.15	0.13
		F	0	0.30	0.31	0.28	0.26	0.26	0.26	0.26	0.26	0.22	0.26	0.22	0.23	0.19	0.19	0.17	0.19	0.19	0.16	0.18	0.13	0.18	0.13	0.17	0.27
		L	0	0.39	0.28	0.27	0.24	0.24	0.23	0.23	0.22	0.25	0.25	0.20	0.23	0.17	0.20	0.20	0.20	0.18	0.16	0.19	0.16	0.15	0.16	0.19	0.15
		G	0	0.75	0.66	0.74	0.78	0.75	0.68	0.64	0.55	0.50	0.44	0.42	0.37	0.35	0.32	0.28	0.23	0.27	0.24	0.22	0.22	0.19	0.21	0.20	0.19
		P	0	0.35	0.35	0.38	0.41	0.40	0.40	0.38	0.42	0.38	0.38	0.39	0.36	0.38	0.36	0.34	0.32	0.32	0.29	0.32	0.24	0.30	0.24	0.26	0.28
15ºC	AVE	NM	40.0	31.50	28.90	26.65	24.40	22.60	21.30	20.20	19.35	18.75	18.20	17.80	17.45	17.20	16.95	16.70	16.55	16.45	16.45	16.35	16.25	16.15	16.15	16.10	16.05
		F	40.0	33.00	31.30	29.45	27.90	26.25	24.80	23.55	22.40	21.50	20.70	20.00	19.45	18.90	18.30	18.00	17.60	17.30	17.00	16.70	16.45	16.25	16.00	15.95	15.80
		L	40.0	30.10	28.25	26.50	25.10	23.90	22.85	21.90	21.20	20.50	19.90	19.30	19.00	18.65	18.20	18.00	17.75	17.55	17.30	17.20	17.05	16.80	16.80	16.70	16.65
		G	40.0	28.60	25.35	22.90	21.00	19.70	18.60	17.75	17.10	16.65	16.30	15.90	15.65	15.45	15.25	15.10	15.00	14.85	14.85	14.85	14.70	14.55	14.55	14.60	14.55
		P	40.0	30.60	26.85	24.00	22.00	20.30	19.20	18.10	17.20	16.75	16.30	15.85	15.50	15.15	15.05	14.75	14.65	14.50	14.50	14.50	14.40	14.30	14.30	14.25	14.20
	SE	NM	0	0.37	0.33	0.34	0.23	0.20	0.18	0.21	0.18	0.20	0.16	0.2	0.17	0.2	0.17	0.2	0.17	0.17	0.17	0.15	0.18	0.16	0.16	0.17	0.17
		F	0	0.19	0.27	0.31	0.38	0.41	0.42	0.39	0.37	0.38	0.38	0.34	0.33	0.28	0.24	0.25	0.20	0.2	0.16	0.16	0.15	0.17	0.12	0.11	0.11
		L	0	0.40	0.33	0.25	0.20	0.16	0.13	0.14	0.13	0.10	0.1	0.08	0.12	0.10	0.08	0.10	0.11	0.08	0.13	0.11	0.08	0.13	0.13	0.11	0.10
		G	0	0.54	0.66	0.73	0.73	0.71	0.67	0.67	0.64	0.59	0.56	0.49	0.51	0.48	0.44	0.4	0.40	0.35	0.35	0.35	0.32	0.32	0.32	0.32	0.28
		P	0	0.4	0.63	0.68	0.68	0.62	0.57	0.48	0.46	0.44	0.36	0.30	0.27	0.23	0.26	0.22	0.23	0.16	0.16	0.13	0.14	0.11	0.11	0.11	0.11

Table A1. Continued.

Ta	Nest materials		Time (min)																							
25°C	AVE	NM	40.0	26.70	26.10	25.70	25.25	24.65	24.25	24.00	23.70	23.45	23.35	23.15	23.10	23.00	22.95	22.75	22.80	22.70	22.65	22.70	22.50	22.50	22.45	22.35	22.30
		F	40.0	30.85	29.60	28.95	28.55	28.15	27.80	27.60	27.30	27.15	27.00	26.80	26.55	26.45	26.30	26.20	26.00	26.00	25.90	25.85	25.85	25.60	25.55	25.50	25.45
		L	40.0	29.50	29.10	28.50	27.60	26.80	26.30	26.00	25.55	25.20	25.00	24.85	24.65	24.35	24.35	24.30	24.15	24.00	24.00	23.95	23.90	23.90	23.90	23.85	23.75
		G	40.0	29.60	28.10	27.40	27.15	26.95	26.90	26.75	26.75	26.55	26.50	26.45	26.40	26.30	26.30	26.10	26.00	26.00	25.95	25.95	25.95	25.95	25.95	25.95	25.90
		P	40.0	28.55	28.55	28.65	28.65	28.45	28.30	28.25	28.10	28.05	27.60	26.75	25.60	24.65	23.65	22.70	22.00	21.30	20.65	20.15	19.70	19.25	18.90	18.65	19.45
	SE	NM	0	0.59	0.42	0.24	0.22	0.27	0.37	0.40	0.44	0.47	0.54	0.58	0.57	0.64	0.63	0.68	0.69	0.75	0.74	0.75	0.80	0.80	0.79	0.85	0.85
		F	0	0.82	0.72	0.65	0.54	0.55	0.56	0.64	0.66	0.69	0.73	0.76	0.74	0.78	0.81	0.81	0.84	0.84	0.83	0.88	0.88	0.85	0.90	0.90	0.89
		L	0	0.40	0.29	0.25	0.24	0.24	0.23	0.24	0.21	0.16	0.19	0.16	0.16	0.16	0.16	0.15	0.15	0.13	0.12	0.11	0.14	0.14	0.14	0.13	0.13
		G	0	0.30	0.27	0.24	0.22	0.25	0.20	0.21	0.21	0.24	0.22	0.25	0.25	0.24	0.24	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.20	0.17	0.16
		P	0	0.42	0.34	0.28	0.24	0.22	0.18	0.17	0.16	0.13	0.17	0.21	0.24	0.23	0.23	0.27	0.27	0.27	0.23	0.23	0.24	0.23	0.19	0.23	0.18

Table A2. Lichen moisture content datasets showing the rate of heat loss of the different groups of lichen moisture nests at every 15 minutes across four ambient temperature (Ta) regimes tested. AVE = average, SE = standard error.

Ta	Moisture content		Time (min)																								
			0	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240	255	270	285	300	315	330	345	360
5°C	AVE	Fresh	40.0	25.90	22.15	18.85	16.35	14.30	12.95	12.00	11.45	11.20	10.95	10.80	10.75	10.75	10.80	10.75	10.75	10.80	10.80	10.80	10.80	10.75	10.75	10.75	10.75
		Dry	40.0	26.40	22.55	19.20	16.40	14.20	12.15	10.65	9.75	9.05	8.80	8.70	8.65	8.75	8.85	8.90	9.15	9.35	9.35	9.55	9.75	9.75	9.85	9.95	10.00
		Wet	40.0	26.65	23.80	21.25	18.95	17.00	15.50	14.15	13.40	12.75	12.25	11.95	11.70	11.60	11.50	11.50	11.45	11.35	11.35	11.35	11.45	11.40	11.45	11.40	11.40
	SE	Fresh	0	0.30	0.43	0.48	0.54	0.51	0.51	0.43	0.38	0.35	0.32	0.30	0.28	0.28	0.28	0.21	0.21	0.2	0.2	0.2	0.2	0.18	0.18	0.18	0.18
		Dry	0	0.52	0.70	0.78	0.81	0.77	0.78	0.74	0.65	0.62	0.55	0.52	0.50	0.42	0.42	0.42	0.41	0.42	0.42	0.40	0.38	0.38	0.42	0.39	0.38
		Wet	0	0.29	0.38	0.44	0.45	0.40	0.40	0.38	0.34	0.26	0.27	0.21	0.26	0.23	0.19	0.19	0.18	0.16	0.16	0.16	0.15	0.16	0.15	0.16	0.16
10°C	AVE	Fresh	40.0	28.55	26.55	24.60	23.00	21.55	20.15	19.00	18.30	17.50	16.90	16.40	15.90	15.40	15.10	14.80	14.50	14.20	14.10	13.80	13.70	13.65	13.50	13.35	13.20
		Dry	40.0	26.50	23.00	20.35	18.15	16.50	15.10	14.50	13.90	13.35	12.90	12.55	12.15	11.95	11.65	11.65	11.30	11.25	11.25	11.10	11.00	11.00	11.00	10.95	10.85
		Wet	40.0	26.00	24.00	22.25	20.65	19.30	18.15	17.10	16.25	15.45	14.90	14.40	13.95	13.45	13.10	12.70	12.60	12.20	12.20	11.90	11.80	11.70	11.60	11.45	11.35
	SE	Fresh	0	0.24	0.22	0.24	0.24	0.25	0.23	0.27	0.26	0.28	0.26	0.26	0.26	0.26	0.20	0.23	0.23	0.22	0.20	0.2	0.18	0.21	0.21	0.18	0.18
		Dry	0	0.63	0.71	0.75	0.73	0.66	0.6	0.47	0.4	0.41	0.37	0.32	0.27	0.32	0.27	0.27	0.23	0.22	0.22	0.19	0.16	0.16	0.16	0.18	0.18
		Wet	0	0.37	0.34	0.40	0.37	0.40	0.40	0.39	0.38	0.36	0.36	0.36	0.32	0.32	0.30	0.27	0.25	0.27	0.27	0.22	0.22	0.22	0.20	0.18	0.22
15°C	AVE	Fresh	40.0	29.85	28.90	28.10	27.35	26.90	26.40	25.70	24.90	23.90	23.10	22.20	21.35	20.65	19.95	19.45	18.95	18.50	18.15	17.80	17.45	17.20	16.95	16.75	16.50
		Dry	40.0	26.90	25.50	24.60	24.05	23.35	21.80	20.25	18.70	17.40	16.30	15.45	14.70	14.20	13.85	13.65	13.55	13.40	13.05	13.10	12.95	12.85	12.80	12.75	12.60
		Wet	40.0	29.30	27.50	26.15	24.65	23.25	22.05	20.95	20.05	19.15	18.35	17.65	17.05	16.55	16.05	15.65	15.40	15.05	14.80	14.60	14.40	14.25	14.00	13.85	13.85
	SE	Fresh	0	0.23	0.17	0.19	0.19	0.20	0.16	0.16	0.20	0.23	0.25	0.28	0.28	0.25	0.30	0.30	0.27	0.27	0.25	0.27	0.26	0.26	0.21	0.23	0.22
		Dry	0	0.35	0.24	0.22	0.18	0.24	0.35	0.36	0.39	0.40	0.39	0.36	0.35	0.3	0.26	0.23	0.21	0.20	0.21	0.19	0.17	0.18	0.18	0.20	0.14
		Wet	0	0.18	0.22	0.24	0.29	0.30	0.33	0.32	0.33	0.30	0.27	0.30	0.26	0.26	0.26	0.23	0.20	0.21	0.2	0.22	0.17	0.17	0.19	0.15	0.15
25°C	AVE	Fresh	40.0	28.25	27.60	27.00	26.60	26.25	25.85	25.80	25.70	25.70	25.65	25.60	25.55	25.55	25.45	25.35	25.30	25.30	25.30	25.25	25.15	25.05	25.05	25.00	25.05
		Dry	40.0	27.60	26.35	25.40	24.45	23.90	23.55	23.60	23.75	23.70	23.65	23.40	23.20	23.15	23.05	23.00	22.95	22.85	22.75	22.65	22.60	22.60	22.60	22.50	22.40
		Wet	40.0	28.05	26.50	25.55	24.75	24.15	23.60	23.30	23.20	23.25	23.25	23.25	23.35	23.30	23.20	23.20	23.20	23.20	23.15	23.00	22.85	22.95	22.85	22.85	22.85
	SE	Fresh	0	0.63	0.50	0.42	0.33	0.26	0.24	0.18	0.18	0.15	0.15	0.221	0.18	0.22	0.18	0.23	0.2	0.2	0.2	0.21	0.22	0.22	0.22	0.24	0.22
		Dry	0	0.52	0.38	0.36	0.32	0.28	0.22	0.20	0.18	0.2	0.211	0.25	0.23	0.21	0.25	0.24	0.26	0.25	0.22	0.21	0.22	0.22	0.22	0.24	0.25
		Wet	0	0.24	0.18	0.21	0.23	0.27	0.24	0.22	0.22	0.23	0.238	0.23	0.22	0.21	0.18	0.18	0.18	0.18	0.22	0.23	0.25	0.22	0.25	0.25	0.25

Table A3. Lichen mass datasets showing the rate of heat loss from the different groups of lichen mass nests at every 15 minutes across four ambient temperature (Ta) regimes tested. AVE = average, SE = standard error.

Ta		Mass	Time (min)																							
			0	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240	255	270	285	300	315	330	345	360
5°C	AVE	20 g	40.0	29.00	25.00	21.90	19.30	17.30	16.10	15.00	14.25	13.60	12.90	12.60	12.30	11.90	11.60	11.55	11.40	11.30	11.05	10.95	10.95	10.95	10.75	10.65	10.60
		40 g	40.0	29.86	27.15	25.40	23.55	22.10	20.90	19.75	18.80	17.80	17.15	16.50	15.95	15.55	15.10	14.75	14.40	14.10	13.80	13.65	13.55	13.35	13.15	13.05	13.00
		60 g	40.0	32.40	30.35	28.20	26.40	24.65	23.25	21.95	20.85	19.95	19.00	18.40	17.55	17.10	16.60	16.15	15.70	15.35	15.00	14.75	14.50	14.25	14.05	13.90	13.75
	SE	20 g	0	0.34	0.93	0.9	0.86	0.78	0.66	0.64	0.55	0.53	0.47	0.40	0.38	0.37	0.33	0.31	0.27	0.29	0.27	0.26	0.26	0.26	0.21	0.22	0.22
		40 g	0	0.55	0.29	0.35	0.28	0.27	0.24	0.23	0.24	0.24	0.24	0.24	0.24	0.25	0.23	0.23	0.23	0.23	0.21	0.21	0.22	0.19	0.21	0.22	0.21
		60 g	0	0.28	0.36	0.37	0.37	0.40	0.40	0.40	0.38	0.37	0.36	0.35	0.36	0.32	0.32	0.28	0.30	0.25	0.26	0.23	0.22	0.23	0.22	0.20	0.18
10°C	AVE	20 g	40.0	27.20	24.05	21.60	19.55	18.00	16.70	15.85	15.30	14.65	14.25	13.85	13.60	13.30	13.05	12.95	12.80	12.55	12.45	12.25	12.20	12.10	12.00	11.90	11.75
		40 g	40.0	28.55	26.75	24.90	23.15	21.60	20.35	19.20	18.30	17.55	16.85	16.35	15.75	15.30	14.95	14.50	14.10	13.90	13.60	13.35	13.15	12.95	12.85	12.70	12.55
		60 g	40.0	29.25	27.20	25.25	23.45	21.95	20.70	19.65	18.75	17.95	17.40	16.80	16.25	15.85	15.45	15.30	14.90	14.70	14.45	14.20	14.15	13.95	13.70	13.70	13.50
	SE	20 g	0	0.68	0.77	0.78	0.80	0.74	0.77	0.64	0.62	0.58	0.56	0.52	0.50	0.49	0.44	0.41	0.42	0.41	0.39	0.36	0.36	0.35	0.35	0.32	0.34
		40 g	0	0.46	0.47	0.47	0.42	0.43	0.43	0.38	0.36	0.33	0.30	0.30	0.29	0.3	0.26	0.23	0.22	0.22	0.22	0.21	0.19	0.18	0.16	0.16	0.18
		60 g	0	0.41	0.46	0.42	0.36	0.39	0.37	0.31	0.27	0.24	0.19	0.15	0.15	0.15	0.13	0.13	0.12	0.08	0.11	0.08	0.10	0.08	0.08	0.08	0.10
15°C	AVE	20 g	40.0	25.65	22.80	20.70	18.85	17.45	16.60	16.35	16.05	15.80	15.45	15.10	14.85	14.70	14.55	14.30	14.20	14.10	14.00	13.85	13.85	13.70	13.60	13.55	13.50
		40 g	40.0	26.80	25.00	23.55	22.25	21.10	20.25	19.55	19.15	18.60	18.05	17.65	17.30	17.10	16.75	16.55	16.30	16.25	16.00	15.80	15.80	15.65	15.60	15.45	15.40
		60 g	40.0	27.65	26.30	25.20	23.95	22.75	21.85	21.20	20.50	19.95	19.50	19.15	18.70	18.45	18.15	17.80	17.60	17.30	17.20	16.95	16.80	16.65	16.55	16.35	16.20
	SE	20 g	0	0.34	0.55	0.64	0.64	0.67	0.63	0.54	0.47	0.46	0.46	0.41	0.40	0.38	0.36	0.35	0.35	0.33	0.31	0.30	0.30	0.3	0.29	0.29	0.26
		40 g	0	0.2	0.26	0.28	0.30	0.30	0.30	0.28	0.28	0.27	0.27	0.26	0.23	0.23	0.22	0.21	0.18	0.22	0.21	0.18	0.18	0.18	0.17	0.17	0.17
		60 g	0	0.21	0.24	0.28	0.30	0.32	0.29	0.35	0.29	0.29	0.28	0.27	0.27	0.29	0.27	0.24	0.27	0.24	0.26	0.22	0.24	0.24	0.21	0.25	0.23
25°C	AVE	20 g	40.0	28.65	27.70	27.65	27.25	26.90	26.65	26.35	26.00	25.95	25.50	25.50	25.35	25.25	25.00	25.00	24.95	24.90	24.75	24.60	24.55	24.55	24.50	24.50	24.35
		40 g	40.0	27.75	27.25	26.90	26.45	26.15	26.00	25.85	25.95	26.05	26.15	26.15	26.20	26.15	26.15	26.15	26.15	26.10	26.10	26.10	26.00	26.05	25.95	25.90	25.70
		60 g	40.0	29.00	28.20	27.85	27.70	27.45	27.35	27.10	26.90	26.75	26.65	26.45	26.30	26.25	26.20	26.05	25.95	25.85	25.80	25.80	25.75	25.65	25.65	25.60	25.30
	SE	20 g	0	0.25	0.23	0.19	0.18	0.20	0.22	0.21	0.19	0.20	0.19	0.19	0.22	0.18	0.19	0.19	0.15	0.16	0.18	0.17	0.17	0.17	0.12	0.12	0.18
		40 g	0	0.34	0.30	0.20	0.18	0.18	0.16	0.13	0.11	0.13	0.13	0.13	0.11	0.13	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.15	0.15	0.14	0.16
		60 g	0	0.16	0.13	0.15	0.13	0.11	0.10	0.12	0.12	0.11	0.10	0.11	0.08	0.11	0.11	0.11	0.11	0.10	0.08	0.08	0.08	0.10	0.10	0.1	0.08

Table A4. Values of water bottle temperature (Tb) differences in nest boxes containing different material types tested for insulation across four ambient temperature (Ta) regimes. AVE = average, R = range, NM = No material, F = Feathers, L = Lichen, G = Grass, P = Paper shavings.

Material types and comparisons		Ta (°C)	Water bottle temperature (Tb) differences																							AVE	R
NM	NM	5	-5.85	-7.55	-8.45	-9.05	-9.3	-9.3	-9.25	-8.9	-8.7	-8.35	-8.1	-7.8	-7.4	-7	-6.8	-6.4	-6.2	-5.9	-5.7	-5.55	-5.3	-5.1	-4.95	-4.75	**-7.15**	**4.55**
	vs.	10	-2.95	-5.9	-7.75	-8.6	-9.1	-9.1	-8.85	-8.35	-8.55	-8.05	-7.9	-7.45	-7.15	-6.9	-6.6	-6.4	-6.05	-5.85	-5.55	-5.35	-5.15	-4.95	-4.75	-4.75	**-6.75**	**6.15**
	F	15	-4.4	-5.95	-6.55	-6.9	-6.55	-6.2	-5.8	-5.3	-4.9	-4.5	-4.25	-3.75	-3.45	-3.2	-3.05	-2.75	-2.7	-2.45	-2.35	-2.35	-2.25	-2.25	-2.1	-2.1	**-4.00**	**4.8**
		25	-4.15	-3.5	-3.25	-3.3	-3.5	-3.55	-3.6	-3.6	-3.7	-3.65	-3.65	-3.45	-3.5	-3.45	-3.55	-3.4	-3.5	-3.45	-3.4	-3.6	-3.6	-3.65	-3.75	-3.75	**-3.56**	**0.9**
	NM	5	-3.9	-4.95	-5.2	-5.4	-5.3	-5.15	-4.95	-4.65	-4.3	-4	-3.75	-3.45	-3.25	-2.95	-3	-2.7	-2.55	-2.4	-2.3	-2	-2.1	-2	-1.9	-1.8	**-3.49**	**3.6**
	vs.	10	-1.25	-3.55	-5.8	-7	-7.85	-8.2	-8.4	-8.3	-8.05	-7.8	-7.35	-6.85	-6.4	-6.1	-5.7	-5.35	-5.1	-4.95	-4.5	-4.4	-4.1	-4.1	-3.65	-3.5	**-5.76**	**7.15**
	L	15	-1.5	-2.9	-3.6	-4.1	-4.2	-4.25	-4.15	-4.1	-3.85	-3.6	-3.45	-3.2	-3.25	-2.95	-2.9	-2.75	-2.6	-2.3	-2	-1.9	-1.85	-1.6	-1.5	-1.35	**-2.91**	**2.9**
		25	-3.15	-3	-2.85	-2.65	-3	-3.1	-3.1	-3.2	-3.2	-3.15	-3.3	-3.3	-3.3	-3.35	-3.35	-3.2	-3.3	-3.3	-3.25	-3.45	-3.45	-3.5	-3.6	-3.6	**-3.23**	**0.95**
	NM	5	-2.2	-2.2	-2.2	-1.95	-1.9	-1.95	-1.85	-1.65	-1.5	-1.5	-1.5	-1.5	-1.3	-1.25	-1.35	-1.2	-1.3	-1.25	-1.35	-1.4	-1.4	-1.45	-1.5	-1.35	**-1.58**	**1**
	vs.	10	0.3	-0.8	-1.15	-1.35	-1.4	-1.45	-1.35	-1.4	-1.4	-1.35	-1.3	-1.15	-1.1	-1.1	-1	-0.9	-0.95	-0.85	-0.7	-0.8	-0.75	-0.7	-0.6	-0.45	**-0.98**	**1.75**
	F	15	-2.9	-3.55	-3.75	-3.4	-2.9	-2.7	-2.45	-2.25	-2.1	-1.9	-1.9	-1.8	-1.75	-1.7	-1.6	-1.55	-1.6	-1.6	-1.5	-1.55	-1.6	-1.6	-1.5	-1.5	**-2.11**	**2.25**
		25	-3.1	-2.25	-2.1	-2.45	-2.7	-2.8	-2.85	-2.8	-2.95	-2.95	-2.65	-2	-1.9	-1.9	-2.1	-2	-2	-2.05	-2	-2.15	-2.15	-2.2	-2.3	-2.25	**-2.35**	**1.2**
	NM	5	-2	-2.1	-2.05	-1.95	-1.95	-1.8	-1.75	-1.75	-1.5	-1.45	-1.45	-1.3	-1.2	-1.05	-1	-0.95	-0.9	-0.85	-0.85	-0.85	-0.75	-0.8	-0.65	-0.75	**-1.31**	**1.45**
	vs.	10	-0.4	-2.8	-5.25	-6.7	-7.45	-7.85	-7.85	-7.65	-7.4	-7.1	-6.8	-6.25	-5.95	-5.65	-5.2	-4.9	-4.55	-4.35	-4.05	-3.9	-3.7	-3.5	-3.35	-3.05	**-5.23**	**7.45**
	P	15	-2	-1.5	-1.1	-1	-0.6	-0.6	-0.35	-0.1	-0.1	0	0.05	0.15	0.3	0.2	0.35	0.35	0.35	0.35	0.35	0.3	0.25	0.25	0.35	0.35	**-0.14**	**2.35**
		25	-1.1	-1.55	-1.7	-1.9	-2.15	-2.05	-2	-1.85	-1.75	-1.65	-1.7	-1.55	-1.5	-1.15	-1.2	-1.1	-1.1	-1.1	-1	-1.15	-1.1	-1.1	-1.2	-1.25	**-1.45**	**1.15**
F	F	5	1.95	2.6	3.25	3.65	4	4.15	4.3	4.25	4.4	4.35	4.35	4.35	4.15	4.05	3.8	3.7	3.65	3.5	3.4	3.35	3.2	3.1	3.05	2.95	**3.64**	**2.45**
	vs.	10	1.7	2.35	1.95	1.6	1.25	0.9	0.45	0.05	0.5	0.25	0.55	0.6	0.75	0.8	0.9	1.05	0.95	0.9	1.05	0.95	1.05	0.85	1.1	1.25	**0.98**	**2.3**
	L	15	2.9	3.05	2.95	2.8	2.35	1.95	1.65	1.2	1.05	0.9	0.8	0.55	0.2	0.25	0.15	0	0.1	0.15	0.35	0.45	0.4	0.65	0.6	0.75	**1.09**	**3.05**
		25	1	0.5	0.4	0.65	0.5	0.45	0.5	0.4	0.5	0.5	0.35	0.15	0.2	0.1	0.2	0.2	0.2	0.15	0.15	0.15	0.15	0,15	0.15	0.15	**0.32**	**0.9**
	F	5	3.65	5.35	6.25	7.1	7.4	7.35	7.4	7.25	7.2	6.85	6.6	6.3	6.1	5.75	5.45	5.2	4.9	4.65	4.35	4.15	3.9	3.65	3.45	3.4	**5.56**	**4**
	vs.	10	3.25	5.1	6.6	7.25	7.7	7.65	7.5	6.95	7.15	6.7	6.6	6.3	6.05	5.8	5.6	5.5	5.1	5	4.85	4.55	4.4	4.25	4.15	4.3	**5.76**	**4.45**
	G	15	1.5	2.4	2.8	3.5	3.65	3.5	3.35	3.05	2.8	2.6	2.35	1.95	1.7	1.5	1.45	1.2	1.1	0.85	0.85	0.8	0.65	0.65	0.6	0.6	**1.89**	**3.05**
		25	1.05	1.25	1.15	0.85	0.8	0.75	0.75	0.8	0.75	0.7	1	1.45	1.6	1.55	1.45	1.4	1.5	1.4	1.4	1.45	1.45	1.45	1.45	1.5	**1.20**	**0.9**
	F	5	3.85	5.45	6.4	7.1	7.35	7.5	7.5	7.15	7.2	6.9	6.65	6.5	6.2	5.95	5.8	5.45	5.3	5.05	4.85	4.7	4.55	4.3	4.3	4	**5.83**	**3.65**
	vs.	10	2.55	3.1	2.5	1.9	1.65	1.25	1	0.7	1.15	0.95	1.1	1.2	1.2	1.25	1.4	1.5	1.5	1.5	1.5	1.45	1.45	1.45	1.4	1.7	**1.51**	**2.4**
	P	15	2.4	4.45	5.45	5.9	5.95	5.6	5.45	5.2	4.8	4.5	4.3	3.9	3.75	3.4	3.4	3.1	3.05	2.8	2.7	2.65	2.5	2.5	2.45	2.45	**3.86**	**3.55**
		25	3.05	1.95	1.55	1.4	1.35	1.5	1.6	1.75	1.95	2	1.95	1.9	2	2.3	2.35	2.3	2.4	2.35	2.4	2.45	2.5	2.55	2.55	2.5	**2.10**	**1.7**

Table A4. Continued.

Material types and comparisons		Ta (°C)	Water bottle temperature (Tb) differences																							AVE	R
L	L	5	1.7	2.75	3	3.45	3.4	3.2	3.1	3	2.8	2.5	2.25	1.95	1.95	1.7	1.65	1.5	1.25	1.15	0.95	0.8	0.7	0.55	0.4	0.45	**1.92**	**3.05**
	vs.	10	1.55	2.75	4.65	5.65	6.45	6.75	7.05	6.9	6.65	6.45	6.05	5.7	5.3	5	4.7	4.45	4.15	4.1	3.8	3.6	3.35	3.4	3.05	3.05	**4.77**	**5.5**
	G	15	-1.4	-0.65	-0.15	0.7	1.3	1.55	1.7	1.85	1.75	1.7	1.55	1.4	1.5	1.25	1.3	1.2	1	0.7	0.5	0.35	0.25	0	0	-0.15	**0.8**	**3.25**
		25	0.05	0.75	0.75	0.2	0.3	0.3	0.25	0.4	0.25	0.2	0.65	1.3	1.4	1.45	1.25	1.2	1.3	1.25	1.25	1.3	1.3	1.3	1.3	1.35	**0.87**	**1.4**
	L	5	1.9	2.85	3.15	3.45	3.35	3.35	3.2	2.9	2.8	2.55	2.3	2.15	2.05	1.9	2	1.75	1.65	1.55	1.45	1.35	1.35	1.2	1.25	1.05	**2.18**	**2.4**
	vs.	10	0.85	0.75	0.55	0.3	0.4	0.35	0.55	0.65	0.65	0.7	0.55	0.6	0.45	0.45	0.5	0.45	0.55	0.6	0.45	0.5	0.4	0.6	0.3	0.45	**0.52**	**0.55**
	P	15	-0.5	1.4	2.5	3.1	3.6	3.65	3.8	4	3.75	3.6	3.5	3.35	3.55	3.15	3.25	3.1	2.95	2.65	2.35	2.2	2.1	1.85	1.85	1.7	**2.76**	**4.5**
		25	2.05	1.45	1.15	0.75	0.85	1.05	1.1	1.35	1.45	1.5	1.6	1.75	1.8	2.2	2.15	2.1	2.2	2.2	2.25	2.3	2.35	2.4	2.4	2.35	**1.78**	**1.65**
G	G	5	0.2	0.1	0.15	0	-0.05	0.15	0.1	-0.1	0	0.05	0.05	0.2	0.1	0.2	0.35	0.25	0.4	0.4	0.5	0.55	0.65	0.65	0.85	0.6	**0.26**	**0.95**
	vs.	10	-0.7	-2	-4.1	-5.35	-6.05	-6.4	-6.5	-6.25	-6	-5.75	-5.5	-5.1	-4.85	-4.55	-4.2	-4	-3.6	-3.5	-3.35	-3.1	-2.95	-2.8	-2.75	-2.6	**-4.24**	**5.8**
	P	15	0.9	2.05	2.65	2.4	2.3	2.1	2.1	2.15	2	1.9	1.95	1.95	2.05	1.9	1.95	1.9	1.95	1.95	1.85	1.85	1.85	1.85	1.85	1.85	**1.96**	**1.75**
		25	2	0.7	0.4	0.55	0.55	0.75	0.85	0.95	1.2	1.3	0.95	0.45	0.4	0.75	0.9	0.9	0.9	0.95	1	1	1.05	1.1	1.1	1	**0.90**	**1.6**

Table A5. Values for water bottle temperature (Tb) differences in nest boxes containing different masses (M) of lichen tested for insulation across four ambient temperature (Ta) regimes. AVE = average, R = range.

M	Ta (°C)	Water bottle temperature (Tb) differences																								AVE	R
60 g	**5**	2.54	3.2	2.8	2.85	2.55	2.35	2.2	2.05	2.15	1.85	1.9	1.6	1.55	1.5	1.4	1.3	1.25	1.2	1.1	0.95	0.9	0.9	0.85	0.75	**1.73**	**2.45**
vs.	**10**	0.7	0.45	0.35	0.3	0.35	0.35	0.45	0.45	0.4	0.55	0.45	0.5	0.55	0.5	0.8	0.8	0.8	0.85	0.85	1	1	0.85	1	0.95	**0.63**	**0.7**
40 g	**15**	0.85	1.3	1.65	1.7	1.65	1.6	1.65	1.35	1.35	1.45	1.5	1.4	1.35	1.4	1.25	1.3	1.05	1.2	1.15	1	1	0.95	0.9	0.8	**1.28**	**0.9**
	25	1.25	0.95	0.95	1.25	1.3	1.35	1.25	0.95	0.7	0.5	0.3	0.1	0.1	0.05	-0.1	-0.2	-0.25	-0.3	-0.3	-0.25	-0.4	-0.3	-0.3	-0.4	**0.34**	**1.75**
60 g	**5**	3.4	5.35	6.3	7.1	7.35	7.15	6.95	6.6	6.35	6.1	5.8	5.25	5.2	5	4.6	4.3	4.05	3.95	3.8	3.55	3.3	3.3	3.25	3.15	**5.04**	**4.2**
vs.	**10**	2.05	3.15	3.65	3.9	3.95	4	3.8	3.45	3.3	3.15	2.95	2.65	2.55	2.4	2.35	2.1	2.15	2	1.95	1.95	1.85	1.7	1.8	1.75	**2.68**	**2.3**
20 g	**15**	2	3.5	4.5	5.1	5.3	5.25	4.85	4.45	4.15	4.05	4.05	3.85	3.75	3.6	3.5	3.4	3.2	3.2	3.1	2.95	2.95	2.95	2.8	2.7	**3.71**	**3.3**
	25	0.35	0.5	0.2	0.45	0.55	0.7	0.75	0.9	0.8	1.15	0.95	0.95	1	1.2	1.05	1	0.95	1.05	1.2	1.2	1.1	1.15	1.1	0.95	**0.88**	**1**
40 g	**5**	0.86	2.15	3.5	4.25	4.8	4.8	4.75	4.55	4.2	4.25	3.9	3.65	3.65	3.5	3.2	3	2.8	2.75	2.7	2.6	2.4	2.4	2.4	2.4	**3.31**	**3.94**
vs.	**10**	1.35	2.7	3.3	3.6	3.6	3.65	3.35	3	2.9	2.6	2.5	2.15	2	1.9	1.55	1.3	1.35	1.15	1.1	0.95	0.85	0.85	0.8	0.8	**2.05**	**2.85**
20 g	**15**	1.15	2.2	2.85	3.4	3.65	3.65	3.2	3.1	2.8	2.6	2.55	2.45	2.4	2.2	2.25	2.1	2.15	2	1.95	1.95	1.95	2	1.9	1.9	**2.43**	**2.5**
	25	-0.9	-0.45	-0.75	-0.8	-0.75	-0.65	-0.5	-0.05	0.1	0.65	0.65	0.85	0.9	1.15	1.15	1.2	1.2	1.35	1.5	1.45	1.5	1.45	1.4	1.35	**0.54**	**2.4**

Table A6. Values for water bottle temperature (Tb) differences in nest boxes containing mass of lichen tested for insulation across four ambient temperature (Ta) regimes. AVE = average, R = range.

Cp.	Ta (°C)	Water bottle temperature (Tb) differences																									AVE	R
F	5	0.45	0.85	2.45	3.55	4.1	4.45	4.95	5.05	5.15	5.1	4.8	4.55	4.3	4	3.8	3.7	3.45	3.15	3.15	2.9	2.75	2.7	2.7	2.55	2.45	**3.48**	**4.70**
vs.	10	1.9	5	6.6	7.25	7.7	7.6	7.1	6.8	6.65	6.35	6.15	5.8	5.65	5.2	5.1	4.85	4.65	4.4	4.3	4.1	4.1	4.1	3.95	3.85	3.7	**5.31**	**5.80**
W	15	0.25	0.55	1.4	1.95	2.7	3.65	4.35	4.75	4.85	4.75	4.75	4.55	4.3	4.1	3.9	3.8	3.55	3.45	3.35	3.2	3.05	2.95	2.95	2.9	2.65	**3.31**	**4.60**
	25	0.05	0.8	2.75	3.6	4.25	4.45	4.7	4.35	4	3.9	3.85	3.8	3.75	3.75	3.65	3.6	3.55	3.55	3.55	3.5	3.4	3.3	3.3	3.25	3.3	**3.44**	**4.65**
W	5	0.35	0.6	1.2	1.5	1.55	1.65	1.6	1.55	1.5	1.4	1.35	1.3	1.25	1.15	1.15	1.1	1.15	1.15	1.15	1.1	1.05	1.05	1.1	1.1	1.05	**1.20**	**1.30**
vs.	10	1.75	2.95	3.05	3	2.85	2.55	2.05	2.3	2.25	2.2	2.15	1.95	1.9	1.75	1.65	1.7	1.45	1.45	1.45	1.4	1.4	1.45	1.45	1.45	1.35	**1.96**	**1.70**
D	15	-1.8	-2.4	-2	-1.55	-0.6	-0.1	-0.25	-0.7	-1.35	-1.75	-2.05	-2.2	-2.35	-2.35	-2.2	-2	-1.85	-1.65	-1.75	-1.5	-1.45	-1.4	-1.2	-1.1	-1.25	**-1.54**	**2.50**
	25	0.2	0.15	1.5	2	2.1	2.1	2.4	2.15	2.05	1.9	1.85	1.6	1.4	1.35	1.25	1.25	1.2	1.1	1	0.9	0.85	0.85	0.85	0.75	0.65	**1.34**	**2.25**
F	5	0.1	0.25	1.25	2.05	2.55	2.8	3.35	3.5	3.65	3.7	3.45	3.25	3.05	2.85	2.65	2.6	2.3	2	2	1.8	1.7	1.65	1.6	1.45	1.4	**2.28**	**3.60**
vs.	10	0.15	2.05	3.55	4.25	4.85	5.05	5.05	4.5	4.4	4.15	4	3.85	3.75	3.45	3.45	3.15	3.2	2.95	2.85	2.7	2.7	2.65	2.5	2.4	2,.35	**3.36**	**4.90**
D	15	2.05	2.95	3.4	3.5	3.3	3.55	4.6	5.45	6.2	6.5	6.8	6.75	6.65	6.45	6.1	5.8	5.4	5.1	5.1	4.7	4.5	4.35	4.15	4	3.9	**4.85**	**4.75**
	25	-0.15	0.65	1.25	1.6	2.15	2.35	2.3	2.2	1.95	2	2	2.2	2.35	2.4	2.4	2.35	2.35	2.45	2.55	2.6	2.55	2.45	2.45	2.5	2.65	**2.10**	**2.80**