

Milestone report

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Stage 1: Effects of heat stress, and climate change scenarios on reproductive performance of the Australian sheep flock

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Executive Summary

Thermal environment is the largest single stressor affecting the development, growth and reproduction of sheep, especially those managed in the extensive pasture and pastoral based systems typical of Australia. From late spring to early autumn (November to March), cycling and pregnant ewes, as well as working rams, are commonly exposed to ambient temperatures which challenge homeothermy, wellbeing and reproductive function. Based primarily on observed and projected increases in temperature associated with anthropogenic climate change, exposure of the Australian sheep flock to thermal stress will occur more frequently and for longer periods. The purpose of this review is two-fold. First, to determine the impact of heat stress on ewe and ram fertility, as well as the ability of ewes to rear a lamb. Secondly, to identify to what extent the reproductive performance of the national sheep flock is affected by heat stress under current climatic conditions and to what extent it will be affected in the future should temperatures rise by 1°C or 3°C relative to a recent climatological baseline.

Effect of heat stress on sheep reproduction.

It is evident from field-based and hot-room studies that exposing ewes to temperatures of 32.2°C or higher during the mating period adversely affects their fertility. It is also clear from hot-room studies that the impacts of heat stress are most severe when experienced from five days before until five days after oestrus; however, continuing heat stress after this period also impairs pregnancy outcomes. Ovum quality and fertilisation are most vulnerable to heat stress around the time of oestrus, with embryo wastage most affected by heat stress on the day of oestrus until day 5 post-oestrus. Importantly, data from two large, comprehensive field studies, demonstrated that the number of ewes lambing and the number of lambs born per 100 ewes joined were negatively correlated with the number of days per week during the mating period when ambient temperatures were $\geq 32.2^\circ\text{C}$.

Exposing the testes of rams to an increased heat load reduces semen quality, as reflected by decreased motility, number and fertilisation rate as well as increases in abnormal sperm cells. In contrast with ewes, these effects are delayed being observed between 9 and 60 days after heating. Importantly, the effects of heat exposure on spermatogenesis, sperm quality, and hence ram fertility, are closely related to the increase in testicular temperature. There is variation between rams in their capacity to regulate scrotal temperature, with some rams able to maintain scrotal temperatures 2-3°C lower than others. Scrotal thermoregulatory capacity, its variation between rams and the level of heat exposure required to elevate testicular temperature above 39°C, has been little studied under Australian field conditions.

Heat stress for prolonged periods during pregnancy reduces lamb birthweight by 0.6 – 1.4 kg, decreases the proportion of lambs born alive by 30% and causes approximately 25% more lambs to die after birth. This is exemplified from hot room studies, in which ewes were exposed to hot days (ranges: 6-18 h of 35 – 44.4°C and THI of 83.2 to 97.0) and cooler nights (ranges: 6 – 18 h, 16.4 – 35.0°C, THI of 38.3 – 83.5) for prolonged periods (30 to 150 consecutive days) during pregnancy. In addition, ewes also vary in their ability to regulate core temperature under high ambient temperatures, and the negative effects of heat stress on lamb birthweight are less severe in ewes with lower core

temperatures. Heat stress during pregnancy also impairs the growth, reproductive potential, milk production and thermoregulation of offspring, whilst even moderate increases in THI (> 65 – 68) during lactation reduce milk production.

Effect of current and future climate on reproduction of the Australian Sheep Flock.

Australian Gridded Climate Data was used, in conjunction with management data from 26 NRM sites across Australia, to determine the impact of heat stress on reproductive performance. Based on Lindsay et al. (1975), the number of days $\geq 32.2^{\circ}\text{C}$ during the mating period was used to calculate the impacts of heat stress on the number of ewes lambing and lambs born per joined ewe. These values were calculated for each site and multiplied by the number of ewes mated in each site. The number of days pregnant and lactating ewes were exposed to mild (THI > 68), moderate (THI>75) and severe (THI>79) thermal stress was also calculated. This process was conducted for the current climate, as well as a $+1^{\circ}\text{C}$ and $+3^{\circ}\text{C}$ increase in temperature.

Heat stress experienced by ewes joined between spring and early autumn (October to March) significantly decreases the number of lambs born per ewe joined (**Figure 1**). For flocks in Queensland, New South Wales, Victoria, South Australia and Western Australia, the incidence of heat stress experienced under current climate conditions decreased the number of lambs born by 3 – 22% (mean: 9%), with this expected to rise to 4 – 23% (mean: 11) and 5 – 24% (mean: 14%) should temperatures increase by 1°C or 3°C , respectively. In flocks joining during late autumn/winter (April to September), the impacts of heat stress during the joining period are less severe, causing a 0 – 1% (mean: 0.3%), 0 – 2% (mean: 0.5%) and 0 – 3% (mean: 1.6%) decrease in lambs born under current, $+1^{\circ}\text{C}$ and $+3^{\circ}\text{C}$. For these states, and regardless of season of joining, heat stress during the mating period results in an estimated loss of approximately 1.3 million potential lambs under the current climate, with this figure rising to a loss of 1.5 and 2.0 million potential lambs should a $+1^{\circ}\text{C}$ or $+3^{\circ}\text{C}$ in temperature occur.

The impacts of heat stress during gestation on lamb birthweights and post-natal survival are difficult to quantify given the differences between experimental and field conditions. There is considerable variation in the frequency and duration of heat stress events experienced during the gestation of ewes joined between October and March varies (**Figure 2**). For flocks joined in October to March, days of moderate heat stress (THI > 75) account for 9 – 75% (mean: 45%) of the first 50 days of pregnancy, with this increasing to 14 – 82% (mean: 52%) and 26 – 97% (mean: 67%) should temperature increase by 1°C or 3°C , respectively. Of the sites studied, days of moderate heat stress accounted for < 5% of the middle third of pregnancy for five sites, with this reducing to 4 sites should temperature increase by 3°C . However, for the remaining sites days of moderate heat stress accounted for 9 – 67% (mean: 36%), 13 – 74% (mean: 43%) and 25 – 87% (mean: 59%) of the middle third of pregnancy at current, $+1^{\circ}\text{C}$ or $+3^{\circ}\text{C}$, respectively. Predictably, ewes mated during late autumn (April – September) experienced very few days of moderate heat stress during gestation. However, days of mild heat stress (THI > 68) sufficient to impact milk production of dairy sheep and cattle breeds accounted for a reasonable proportion of lactation for spring lambing flocks. Specifically, for the current climate 33% and 58% of the first 50 and second 50 days of lactation had a THI > 68, with these values increasing to 38% and 62% at $+1^{\circ}\text{C}$ and 49% and 72% at $+3^{\circ}\text{C}$, respectively. Whether this heat stress translates to a

reduction in milk production by meat and wool breeds of sheep is not known, but is worthy of future investigation.

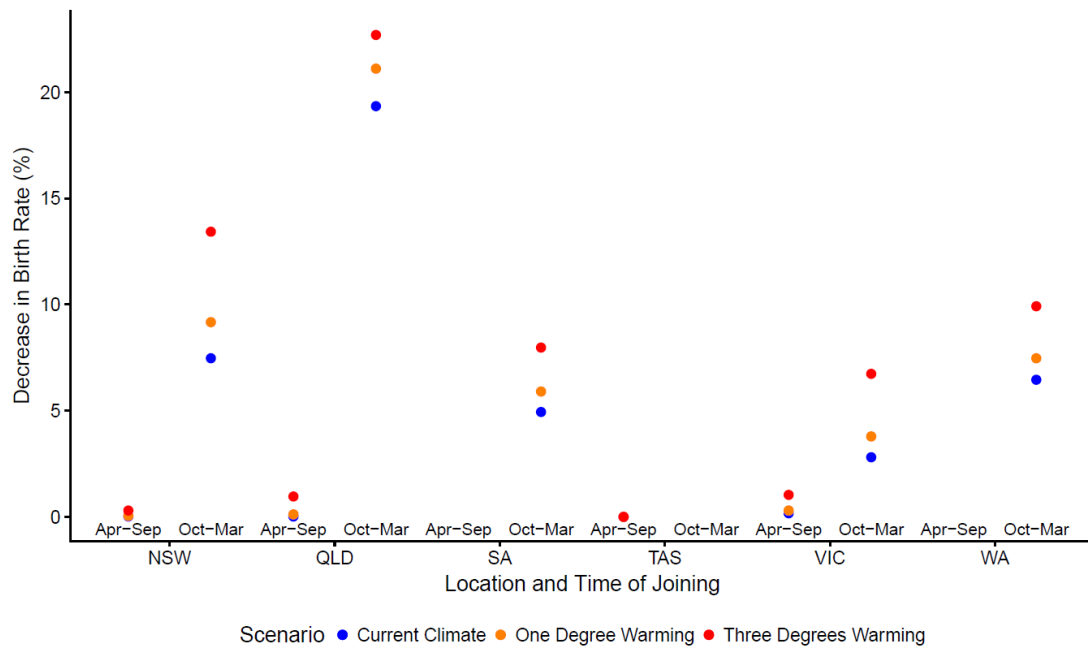


Figure 1. Effect of days > 32.2°C during the week of mating on the number of lambs born per mated ewe for flocks in the 26 selected sites in which joining occurred in spring to early autumn (October – March) or late autumn / winter (April – September).

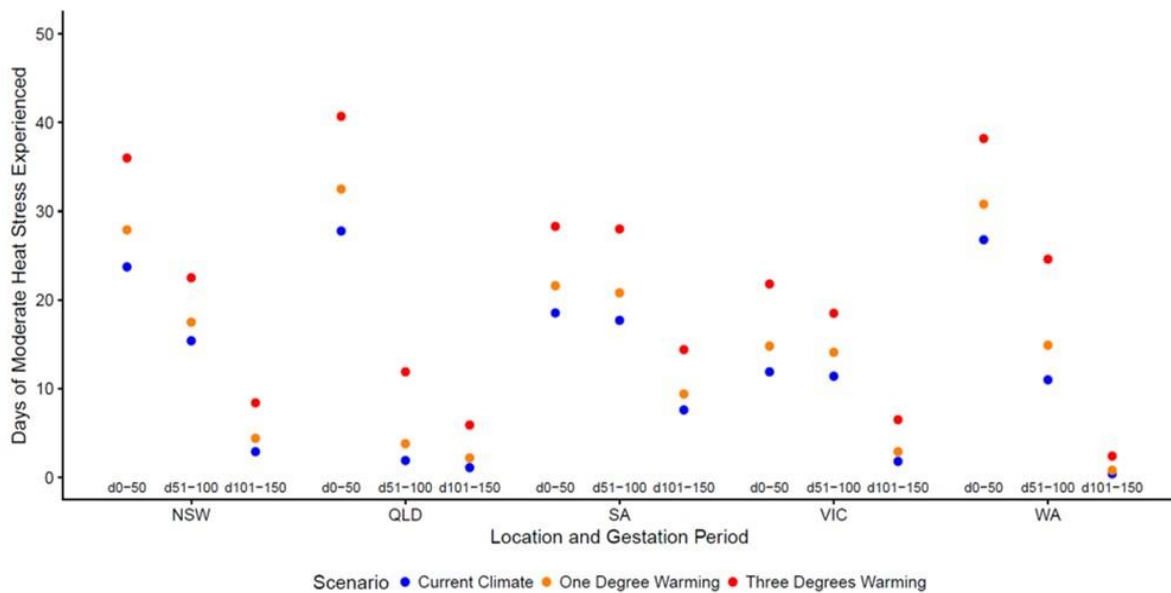


Figure 2. Number of days of moderate heat stress (THI > 75) experienced during d0 – 50, d51 – 100 and d101 – 150 of gestation for ewes joined in spring-early autumn (October – March; data derived from 24 selected sites across Australia).

Conclusions

It is clear that heat stress profoundly impairs sheep reproduction. Although hot-room based studies have allowed the reproductive processes affected by hyperthermia to be identified, a number of limitations mean their relevance to sheep husbandry in Australia is constrained. In most studies, the intensity and duration of both temperature and relative humidity often don't reflect normal grazing situations. Furthermore, no attempt has been made to examine and separate the effects of chronic and acute heat stress, both of which are common place during summer. The effects of heat stress can also be influenced by interactions with other stressors including walking long distances and nutritional deprivation, thus making an assessment of the effects of heat stress *per se* difficult. However, it is clear that thermal stress rather than nutritional stress has the greater impact on reproductive function.

Despite considerable uncertainty associated with the impacts of global warming on reproductive performance, it is clear from the climate data presented, both current and future (+1°C or +3°C warming), that the fertility of the Australian sheep flock is significantly impacted by heat stress during the mating period. This impact of heat stress on ewe fertility and fecundity is particularly profound for flocks which join during spring/summer and early autumn. However, it is estimated based on the sites investigated, and regardless of season of joining, that 1.3 million potential lambs are lost due to heat stress under the current climate. In addition to this, heat stress during gestation is likely to retard conceptus development, particularly for flocks mated in spring/summer, and has the potential to decrease lamb birthweights by 0.6 – 1.4 kg and reduce lamb survival to weaning by 20 – 30%. Should an increase in temperature of between 1°C and 3°C occur, as is likely based on projections from climate models, the number of potential lambs lost will rise to 1.5 and 2.0 million, respectively. This will be associated with a greater incidence of gestation heat stress which will further decrease the weaning rates of the Australian flock.

Based on the outcomes of this review, it is clear that the profitability of the Australian sheep flock is severely impacted by the detrimental impacts of current incidences of heat stress. Further, it is extremely vulnerable to the increases in the frequency and severity of heat stress which will occur should temperatures continue to rise as projected. In light of this, the next steps for this review process will be to model the economic costs of current and future heat stress for the Australian flock and to identify potential strategies to ameliorate and mitigate the risks associated with heat stress.

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1. Introduction

The thermal environment can affect the development, growth and reproduction of all animals making it the largest single stressor affecting the efficiency of animal production systems (Collier et al., 2017). Thermal impacts on the performance and wellbeing of sheep are most profound when temperatures fall below 12°C (lower critical temperature) or rise above 25 – 31°C (upper critical temperature), and it is at these points that thermoregulatory mechanisms are seriously challenged and the ability of sheep to maintain homeothermy is reduced. However, well before these temperatures are reached the physiological and behavioural adaptations required to maintain homeothermy negatively impact non-vital processes, such as reproduction, and impair the welfare and productivity of ewes and rams. The impact of variations in thermal environment are most profound for extensive production systems, such as pasture and rangeland sheep, in which the ability of producers to maintain a stable environment are, essentially, impossible. It is, therefore, extremely common for cycling and pregnant ewes, as well as working rams, to be exposed to ambient temperatures which challenge their productivity and wellbeing during late spring, summer and early autumn (November to March). Based on current climate projections temperatures are projected to increase globally and, as a result, sheep will be exposed to high temperatures and, thus thermal strain, more frequently and for longer periods. In light of this, heat stress has been identified as one of the key vulnerabilities facing Australia's extensive sheep meat and wool production industries (Crimp et al., 2010; Henry et al., 2012; Eldridge and Beecham, 2018).

The impact of exposure to short or long periods of extreme heat, and the resultant 'heat stress', on mating and pregnancy outcomes have been poorly characterised in sheep, especially those managed in the extensive, pasture and pastoral based systems typical of Australia. Therefore, this review has two primary objectives. Firstly, to describe the physiological and behavioural responses of sheep to heat stress, with particular focus on the impact during key stages on the fertility and reproductive performance of ewes and rams managed under Australian conditions. Specifically, the impacts of heat stress around mating and during pregnancy and lactation on ewe reproduction and lamb survival and performance will be discussed, as will the changes in ram fertility which occur under heat stress conditions. Secondly, to identify to what extent the reproductive performance of the national sheep flock is affected by heat stress and, furthermore, to what extent it will be affected should temperatures rise by 1°C or 3°C.

2. Impacts of heat stress on physiology, behaviour and reproduction of sheep

2.1. Thermoregulatory mechanisms of sheep

Sheep can maintain their core temperature within a narrow range (38.3 – 39.9°C) over a relatively wide range of environmental conditions. However, the range of thermal conditions within which the welfare and productivity of agricultural animals, such as ewes and rams, is maximised is relatively narrow. Outside of this narrow range (the zone of thermal comfort; **Figure 3**), the physiology, behaviour and energy expenditure of sheep is altered in order to maintain a stable core temperature (Silanikove, 2000; Al-Dawood, 2017; Johnson, 2018). Due to these modifications, non-essential

processes such as growth, reproduction and milk production can be compromised, and it is at this point that thermal conditions constrain productivity. The upper critical temperature, at which thermoregulatory mechanisms are seriously challenged and begin to involve significant energy expenditure, ranges from 25 to 31°C depending on breed, age and physiological state (Hopkins et al., 1978; Silanikove, 2000; Al-Dawood, 2017). However, the transition from thermal comfort to thermal strain to thermal stress is a continuum, with physiological mechanisms designed to maintain core temperatures activated before the upper critical ambient temperature is reached (**Figure 3**; Silanikove, 2000). It is, therefore, suggested that even at temperatures below an upper critical limit sheep experience hyperthermia with non-essential processes impacted by homeostatic mechanisms. These mechanisms are well understood and have been extensively reviewed (Silanikove, 2000; Marai et al., 2007; Bernabucci et al., 2010; Al-Dawood, 2017), and will, therefore, only be summarised here.

2.2. Physiological responses of sheep to thermal stress

Sheep maintain a stable internal temperature by balancing metabolic heat production and heat storage with heat exchange to, and from, the environment (Silanikove, 2000). As environmental temperature increases, homeostatic mechanisms to increase heat dissipation and decrease metabolic heat production are activated. Transfer of metabolic heat from the sheep's core to the surface increases sensible heat loss (convection, conduction, radiation), and this is achieved by vasodilation of peripheral blood vessels situated close to the surface and in areas with low subcutaneous fat (eg ears, legs) as well as in the upper respiratory tract (Stage 1; **Figure 3**). As a result, the temperature of these areas rises, thus increasing the thermal gradient and promoting heat loss into the environment through either convection or conduction. Internal heat loss is further facilitated by air flow through the nasal passages and upper respiratory tract, as well as elevations in heart rate to increase blood flow to the periphery. The primary method of evaporative cooling available to sheep is respiration. Sheep dissipate 60 – 90% of their heat load by increasing respiration rate whilst only 10% through sweating (Al-Dawood, 2017). As temperatures rise further, heat exchange by evaporation (eg sweating and respiration) becomes increasingly important (Stage 2; **Figure 3**), with the proportion of heat lost via respiration increasing from 20% to 60% when ambient temperature increases from 12 to 35°C (Marai et al., 2007). As environmental temperatures approach and then exceed skin temperature, the thermal gradient between the animal and environment narrows, and then reverses. At this point heat exchange through respiration and, to a lesser extent sweating, becomes the only effective means of cooling (Stage 3; **Figure 3**), with respiration rate increasing 2.6 fold when environmental temperatures increase from 24°C to 40°C (Marai et al., 2007).

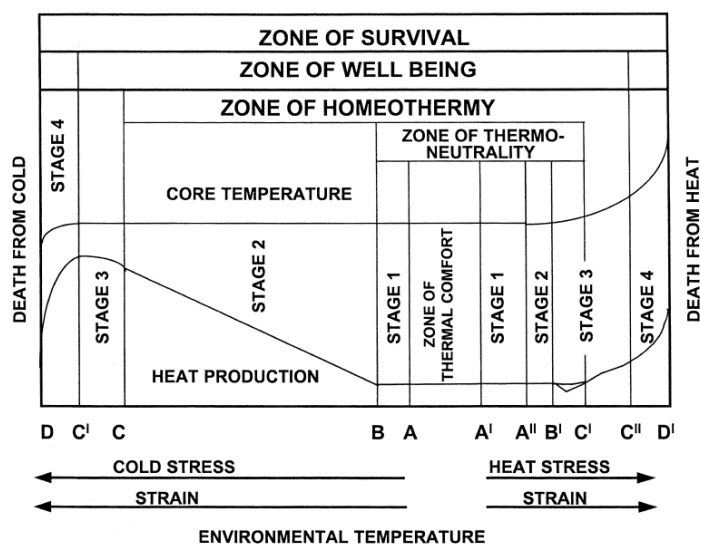


Figure 3. Schematic presentation of the zones of survival, well-being and homeothermy in respect to environmental conditions in ruminants. Taken from Silanikove (2000).

Additional homeostatic mechanisms to maintain a stable core temperature include reduced feed intake to decrease metabolic heat load and increased water intake to compensate for loss through evaporative cooling. Voluntary feed intake of both non-pregnant (Bhattacharya and Hussain, 1974; Dixon et al., 1999; Bernabucci et al., 2009) and pregnant (Shelton and Huston, 1968; Alexander and Williams, 1971; Bell et al., 1989) sheep is reduced in response to high temperatures and humidity. Importantly for pasture and range-land grazed sheep, the heat increment of fibrous diets is particularly high (Silanikove, 2000; Al-Dawood, 2017), and effects of heat stress on appetite increase with fibre content. For example, heat stress decreased food intake of Awassi wethers by 14% when fed a diet containing 25% roughage, but the reduction in food intake was 50% when the diet contained 50% roughage (Bhattacharya and Hussain, 1974). The energy expended to maintain heat loss mechanisms, particularly respiratory evaporation and sweating, increases the maintenance requirements of sheep by 10 – 30% at temperatures of 30 – 40°C (Silanikove, 2000). In addition to increased energy requirements, redistribution of blood supply to the periphery reduces supply to the rumen and gastrointestinal tract, resulting in impaired rumen function and health, including altered nitrogen production, reduced volatile fatty acid absorption and increased risk of rumen disorders (Bernabucci et al., 2010). In other words, sheep under thermal strain have lower food intakes, use the ingested feed less efficiently and have higher energy requirements, resulting in a state of negative energy balance. Further compounding the negative impacts of thermal strain on animal productivity is the increase in metabolic rate which occurs as the intensity of cooling mechanisms increases. At this point, the metabolic heat associated with these cooling mechanisms exceeds heat dissipation and, as a result, body temperature increases and sheep become hyperthermic (Stages 3 and 4, **Figure 3**; Silanikove (2000)).

Importantly, prolonged (approximately three weeks) daily exposure of sheep to high temperatures and humidity levels stimulates physiological and/or behavioural changes designed to reduce sensitivity to thermal strain, and help to maintain a more stable core temperature (acclimation; Alexander et al. (1987) and Bernabucci et al. (2010)). As sheep acclimate to thermal strain there is a progressive

increase in blood flow to the extremities, and a corresponding decreased blood flow to internal organs, including the reproductive tract, rumen and intestines. The endocrine status of the sheep also changes to increase their tolerance of high thermal loads, including a slowdown in metabolic rate and, potentially, a reprioritisation of nutrient use away from non-essential process. These endocrine changes include reduced levels of growth hormone (GH), insulin-like growth factor-I (IGF-I), glucocorticoids and thyroid hormone levels, as well as increased prolactin levels. The impact of these changes on metabolic and reproductive function remain to be fully elucidated. However, reduced thyroid hormone levels decrease metabolic rate and, thus, metabolic heat production, and elevated prolactin levels in thermally stressed animals appear to improve heat dissipation, by promoting evaporative cooling mechanisms and maintaining water balance (Bernabucci et al., 2010; Alamer, 2011). Reductions in GH, IGF-I and glucocorticoids alter post-absorptive energy metabolism, such that the ability of heat stressed animals to divert energy stores to reproductive processes is likely to be reduced (Bernabucci et al., 2010).

Many of the negative impacts of heat stress on ewe and ram physiology described later (**Sections 2.6 and 2.8**) can be attributed to reduced energy intake and availability, as well as the negative energy balance associated with thermal strain. However, redirection of blood flow to the periphery and upper respiratory tract in response to acute and prolonged heat stress effectively reduces blood supply to the reproductive organs, profoundly affecting nutrient and endocrine support for oocyte, embryo and conceptus development as well as milk production. Furthermore, alterations in the somatotrophic axis appear to alter post-absorptive energy metabolism in a manner that decreases energy availability for reproduction and milk production, with reduced GH production also likely to negatively impact conceptus development. However, only a relatively minor portion of the negative effects of heat stress on reproduction and lactation can be attributed to alterations in nutrient intake (Silanikove, 2000; Bernabucci et al., 2010; Johnson, 2018) with the remainder attributed to the physiological and endocrine changes associated with homeothermy and acclimation.

2.3. Foraging behaviour, activity and mating behaviour in sheep under thermal strain

Sheep, like all non-sessile animals, move through time and space to find critical resources such as food and mates and to avoid risks, including predators and unsuitable environmental conditions, for instance high ambient temperature (Nathan et al., 2008; Kays et al., 2015). Sheep have evolved a number of behavioural responses to reduce heat load and aid in temperature dissipation. These include; alterations in body position to minimise surface area exposure to solar radiation and / or promote heat dissipation via the skin; decreased activity levels to reduce metabolic rate and increased use of shade to avoid solar radiation (reviewed by Al-Dawood, 2017).

Understanding how sheep change their use of the landscape (spatial change) and their activity patterns (temporal change) in response to environmental conditions would provide valuable information regarding coping strategies to heat stress conditions. Furthermore, understanding the consequences of these behavioural changes for reproductive success will provide important insight into whether these coping strategies are adaptive. Based on data derived from GPS collars, during hot ($\geq 26.0^{\circ}\text{C}$) compared with cold ($\leq 23.2^{\circ}\text{C}$) days, ewes travelled more slowly (0.48 versus 0.72 km/h), and

kept closer to water points (2.73 km versus 3.74 km) (Thomas et al., 2008). Increasing the use of areas that provide shade can mitigate the energy costs of physiological thermoregulation during periods with high ambient temperatures (Al-Dawood, 2017), and Thomas et al. (2008) suggested that the observed spatial preference may be associated with the proximity to water, and/or shade. Leu et al (in review) combined detailed habitat knowledge with GPS derived measures of sheep space use and movement. On days when the maximum daily temperature exceeded 40°C, and the minimum daily temperature remained above 25°C, sheep in the Australian arid rangelands spent more time in shaded tree patches and at the water point (**Figure 4**) compared with periods with more moderate temperatures (Leu et al., In review). Individuals were also less active (distance travelled) during the hot midday period, but more active during other parts of the day (Leu et al., In review). These findings are consistent with behavioural observations of sheep confined in yards. For instance, Sherwin and Johnson (1987) reported that shade-use increased with rising ambient temperature, which was further modulated by an individual's position in the social hierarchy, with dominant animals spending longer periods in the shade. However, Sherwin and Johnson (1989) could not confirm this relationship between dominance hierarchy and shade use in free-ranging sheep, potentially due to the lack of dominance in free-ranging compared with confined sheep. Interestingly, earlier studies have not always found support for a relationship between high ambient temperatures and shade seeking behaviour in sheep. For instance, wethers in irrigated paddocks showed very little shade seeking behaviour at hot temperatures (Sherwin and Johnson, 1989). This counter intuitive result may be explained by the hydration status of the sheep. Evaporative cooling behaviour (increased respiration rate) is influenced by an animal's hydration status and affects body temperature (Angilletta et al., 2010). Body temperature is elevated in dehydrated animals. The animals in this study were likely well hydrated occupying a regularly irrigated paddock and were not hyperthermic despite remaining in the sun (Sherwin and Johnson, 1989).

While seeking shade is a spatial response to high ambient temperatures, animals can also change their activity times to periods of the day with more suitable ambient conditions. For instance, several desert ungulates forage at night to avoid high temperatures (Cain III et al., 2006). More broadly, Levy et al. (2019) suggested that diurnally active mammals may shift their activity to night time to mitigate the costs of heat stress and compensate for the effects of climate change. Adding to the complexity, the time of foraging can also directly influence body temperature. For example, postpartum ewes that only foraged in the afternoon developed higher body temperatures than those that foraged the same amount of food in the morning, or both morning and afternoon (Godfrey et al., 2013). This could make these ewes more susceptible to heat stress, and result in a feedback loop between foraging time and the need for behavioural thermoregulation.

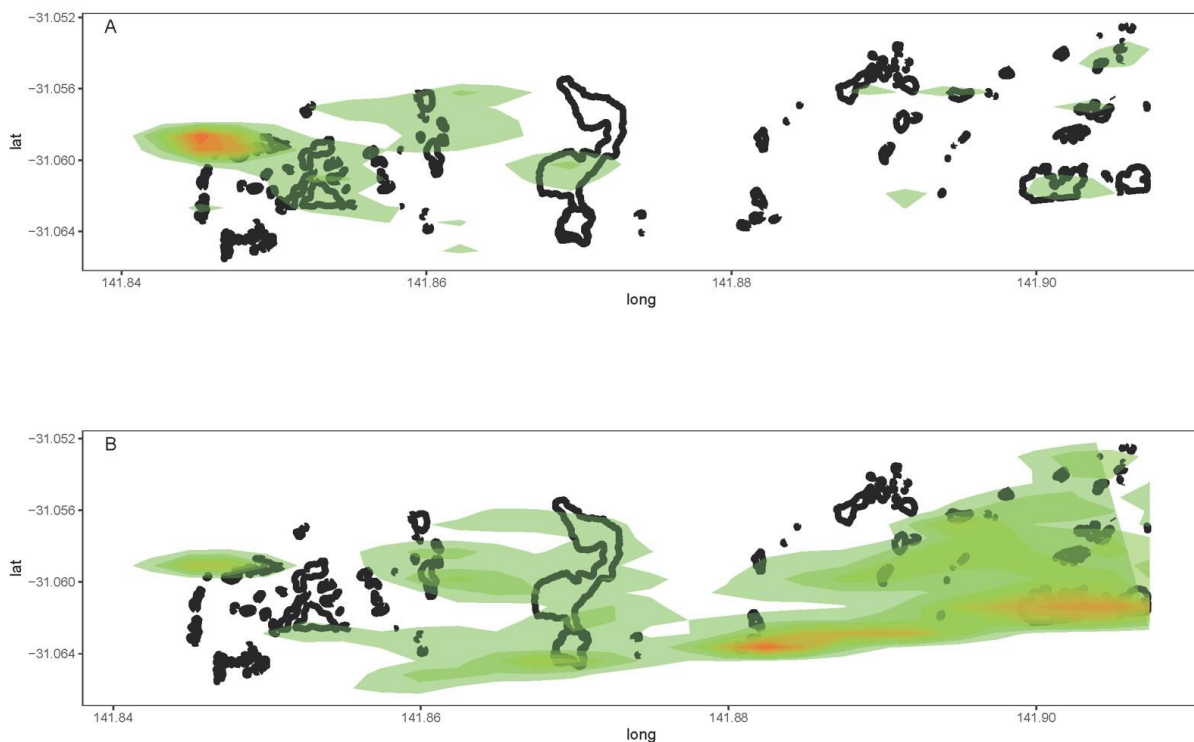


Figure 4. Space use of 48 ewes in Northern New South Wales. **A)** days with hot ambient temperatures (maximum daily temperature > 40°C, and minimum daily temperature > 25°C). **B)** days with more typical temperatures (maximum daily temperature < 35°C) for the time of the year (mid-December to early March). Areas that provide shade (trees) are outlined in black. Colours indicate the Intensity of use for each area, increasing from green to red.

Thermal conditions that influence when and where animals are active can be costly in regard to energy intake. This is the case if heat load constrains foraging time (Owen-Smith, 1998) or if foraging is shifted to areas that provide shade and/or proximity to water sources but are lower in feed quality. This illustrates a trade-off between thermoregulation and foraging. Individual differences in internal state and heat tolerance would influence this trade-off and could explain the observed variation among individuals in their shade seeking behaviour (Johnson, 1987; Sherwin and Johnson, 1987). For instance, shade-seeking behaviour in free-ranging sheep increases with body condition. Leu et al. (In review) showed that individuals that spent more time in the shade during hot parts of the day had better body condition at the end of the study. This may suggest that shade seeking does not constrain foraging time and hence is not costly. However, the mechanistic link that drives this relationship is not well understood. One possible explanation could be that individuals in better body condition have higher fasting heat production and hence a higher basal metabolic rate (Graham, 1969; Shinde et al., 2007), which increases their body temperature and hence requirement to seek shade to thermoregulate. Consistent with this hypothesis, sheep in poorer body condition lose body heat more rapidly at the end of the day, compared with sheep in better body condition (Parer, 1963). This may allow them to have higher body temperatures during the day (Parer, 1963), and hence seek shade less frequently. Alternatively and with reversed causality, individuals with lower shade seeking behaviour experience greater heat stress, which typically leads to reduced feed intake and consequently body

condition (Abdalla et al., 1993; Maurya et al., 2018). For instance, wethers exposed to 38°C for seven consecutive days reduced their feed intake by up to 22%, resulting in a 5% decrease in bodyweight during a period of 11 days (seven days of heat stress, plus two days of thermoneutral temperatures both before and afterwards; Alhidary et al. (2012)). Furthermore, heat stress also increases energy use for homeothermic mechanisms (Belhadj Slimen et al., 2016), which would exacerbate the effect on body condition.

There is limited information describing the effects of increased temperatures on mating behaviour in sheep. A review by (Fowler, 1984) indicated that mating activity is generally lower during the hours before and after mid-day presumably due to higher temperatures. The author commented that flock dispersion increased when rams and ewes seek shade during periods of high temperatures reducing contact between sexes. In addition, there is some indication that breed may influence mating behaviour of rams when exposed to high temperatures (near 43°C) with Dorset Horn and Border Leicester rams less active than Merino rams (Lindsay, 1969).

It is evident from the research reviewed that sheep in yards or under free-range conditions, can compensate, to some degree, the effects of heat stress by changing their behaviour. This includes changes to activity on both a spatial and temporal scale, assuming the needed resources (shade patches, water) are available. However, how these behavioural changes affect reproductive output, and hence farm productivity, remains to be investigated.

2.4. Reproductive responses of ewes and rams to heat stress

For the purposes of this section of the review, the impacts of heat stress on ewe reproduction have been divided into three broad periods. One, the period around mating which focusses on oestrus expression and duration, fertilisation, and oocyte and embryo wastage. Two, the period of pregnancy, which focusses on conceptus development, birthweight, survival and post-natal performance of the lamb as well as subsequent lactation performance of the ewe. Three, the period of lactation and the impacts of heat stress during lactation on milk production. The effects of exposure to heat stress on ram reproduction are also considered.

Numerous studies on the effects of heat stress on sheep reproduction have been conducted. However, a number of common limitations mean that the relevance of their findings to future sheep husbandry in Australia is difficult to determine. Of particular importance are the following:

- Most studies have involved hot-rooms where the temperature and relative humidity applied and its duration (hours per day and/or number of consecutive days) often don't reflect normal grazing situations
- The effects of heat stress can be influenced by interactions with other stressors including nutritional deprivation and walking long distances thus making an assessment of the effects of heat stress per se difficult.
- No attempt has been made to examine and separate the effects of chronic and acute heat stress both of which are common place during Australian summers.

2.5. Heat stress around mating and reproductive performance of ewes in the field

Seasonal variation in pasture conditions together with changes in day length ensure that most flocks in Australia are joined between November and March (Smith, 1964; Lindsay et al., 1975), the period when temperatures are at their highest. Mean daily maximum temperatures during this period regularly approach or exceed 35°C in both southern and tropical regions. It has long been established that there is a link between heat stress and reproductive wastage in these regions (Smith, 1962; Lindsay et al., 1975; Kleemann and Walker, 2005). The studies of Lindsay et al. (1975) and Kleemann and Walker (2005) were extensive in nature, involving 53 and 68 flocks, respectively, and it is from these studies that the effects of heat stress on reproductive performance in the field, and under Australian conditions, can best be assessed.

In both studies, ewe fertility (number of ewes lambing per 100 ewes joined) was negatively correlated with the number of days per week during the mating period when ambient temperatures were $\geq 32.0^{\circ}\text{C}$. Additionally, Lindsay et al. (1975) obtained a negative correlation between heat stress during mating and the number of lambs born per 100 ewes joined. The equations developed by Lindsay et al. (1975) are used later in this document to establish the effect of heat stress around joining on the number of lambs born and the number of ewes lambing in **Section 3.3.7** and **3.3.8**. Of particular interest in the latter study was the finding of a negative correlation between the number of lambs born per 100 ewes joined and daily maximum temperatures in the three weeks after mating indicating an on-going effect of heat stress on reproductive wastage

A positive association between the proportion of ewes returning to oestrus/service, indicative of fertilisation failure, and the number of days $\geq 32.0^{\circ}\text{C}$ during the mating period was detected by Kleemann and Walker (2005). Further evidence that heat stress affects fertilisation rates in the field is provided by data from over 150 flocks in Spain, in which ewes underwent artificial insemination (AI) (Santolaria et al., 2014; Palacios and Abecia, 2015). In these studies, pregnancy rates declined significantly when maximum daily temperatures were $\geq 30^{\circ}\text{C}$ for two days before insemination. On the other hand, there is no evidence that the incidence of oestrus in the field is affected by heat stress (Yeates, 1956; Kleemann and Walker, 2005) even though oestrous activity in Merino ewes in hotter regions of Australia can be irregular (Smith, 1962; Lamond et al., 1963; Smith, 1964), and ewe and ram behaviour is impaired during heat stress (Fowler, 1984). Furthermore, the effect of heat stress on ovulation rate is equivocal – evidence of a negative association was detected by Kleemann and Walker (2005) but no association was found by Lindsay et al. (1975).

Both Lindsay et al. (1975) and Kleemann and Walker (2005) concluded that high temperatures during the mating period adversely impact embryo survival and it is likely that this loss continues for several weeks after fertilisation. This information, together with that of Santolaria et al. (2014) and Palacios and Abecia (2015), indicate that under field conditions heat stress immediately prior to, during and shortly after oestrus severely impairs mating outcomes, as well as lambing and pregnancy outcomes.

2.6. Effects of heat stress around mating on components of ewe reproduction

Numerous hot-room studies have examined the effects of heat stress on the components of reproduction but, as mentioned, care in interpretation is needed because of the difficulties in simulating field conditions.

2.6.1. Oestrous cycle

Parameters of the cycle that may be affected by heat stress are the number of ewes in oestrus (incidence), the inter-oestrous period (length of cycle) and the duration of standing oestrus.

The incidence of oestrus is affected by both the timing and intensity of heat stress (**Table 5-1**). When heat stress was applied from -d5 to d0 (d0 = day of oestrus), the percentage of Merino ewes in oestrus was reduced (Sawyer et al., 1979; Sawyer, 1979a). In these studies, approximately 25 – 30% of ewes failed to display oestrus following heat stress. This effect only occurred when heat stress was applied in the -d5 to d0 period. On the other hand, heat stress applied either -d5 to d5 or -d35 to d0 with diurnal relief had no effect on the incidence of oestrus (Rich and Alliston, 1970; Indu et al., 2015). In a meta-analysis of published data, (Romo-Barron et al., 2019) concluded that thermo-neutral ewes were five times more likely to express oestrus than were ewes stressed for short periods but, somewhat paradoxically, when ewes were exposed to heat stress for longer periods, expression of oestrus was more likely than in control ewes.

The length of the cycle can be increased by heat stress (**Table 5-1**), and in a recent meta-analysis, Romo-Barron et al. (2019) found that heat stress increased the length of the cycle by an average of 0.57 days. When heat stress was applied -d5 to d0, mean cycle length was increased by 1 day (Sawyer, 1979a) and 1.7 days (Sawyer et al., 1979). Dutt et al. (1959) also reported that, when heat stress was applied five days before oestrus, cycle length was longer in shorn ($16.8 \pm 1.20d$) and unshorn sheep ($17.1 \pm 0.28d$) compared with control sheep ($16.2 \pm 1.13d$). However, there is no evidence that heat stress applied outside this five-day period or applied in a diurnal manner, irrespective of its duration, affects cycle length (Sawyer et al., 1979; Indu et al., 2015).

Consensus from the literature is that heat stress reduces the duration of oestrus (**Table 5-1**), with (Romo-Barron et al., 2019) reporting decreases in the length of oestrus by an average of 7.09h. However, the timing of heat stress relative to the day of oestrus (d0) affects this response. Heat stress applied either -d5 to d0 or -d3 to d0 reduced the duration of oestrus (by approximately 5 - 9h) but there was no comparable effect when heat stress was applied between -d7 to -d4 (Sawyer et al., 1979). These authors also observed a seemingly less intense oestrus in ewes exposed to heat stress. Heat stress applied 35 days before oestrus has also been recorded to reduce the duration of oestrus by approximately 6 – 8h in Indian native breeds (Naqvi et al., 2004; Indu et al., 2015). On the other hand, Rich and Alliston (1970) reported a non-significant increase (6.5h) in duration when heat treatment was applied five days before oestrus although cycles were artificially synchronised.

Sawyer (1979a) concluded that the effects of heat stress are greatest when ewes are exposed to heat stress during the five days before oestrus with all three parameters (incidence, length of cycle and

duration of oestrus) being affected. If exposure is for three days, the response is limited to a reduction in the duration of oestrus. Underlying mechanisms for these effects are not understood but heat stress during the final stages of follicle maturation affect circulating hormone levels. These changes include a reduction in LH secretion (Schillo et al., 1978; Wise et al., 1988) as well as a reduction in the number of LH pulses (Ozawa et al., 2005) which reduces stimulation of follicle growth and development. Not surprisingly, the concentrations of oestradiol and aromatase activity in dominant follicles are lower in heat stressed ewes (Badinga et al., 1993; Ozawa et al., 2005) in association with lower circulating oestradiol concentrations (Wolfenson et al., 1995; Wolfenson et al., 1997; Wilson et al., 1998a; Wilson et al., 1998b). These changes could impact both the incidence of oestrus as well as the duration of oestrus. Changes in the length of the cycle might result from altered rates of progesterone production and/or clearance. To this end, several studies (Alliston et al., 1961; Sawyer et al., 1979; Hill and Alliston, 1981; Sheikheldin et al., 1988) have reported higher levels of circulating progesterone during the luteal phase in ewes exposed to hyperthermia compared with control ewes. Alterations in progesterone production and/or clearance may also result in asynchrony between embryonic development and the uterine environment resulting in impaired implantation rates.

2.6.2. Follicle growth and oocyte quality

Most of what is known of the effects of heat stress on follicle dynamics has been obtained from studies in dairy cows exposed to seasonal variations in ambient temperature (Badinga et al., 1993; Wolfenson et al., 1997; Trout et al., 1998; Roth et al., 2000; Wolfenson et al., 2000; Hansen, 2009). These studies indicate that seasonal heat stress affects follicle growth and development in multiple ways. These include enhancement in the number of large follicles in the first follicle wave, an increase in the number of small and medium follicles, suppression of the dominant follicle of both the first and second waves, an earlier emergence of the dominant follicle of the second wave as well as a decline in both aromatase activity and plasma concentrations of oestradiol. These changes are likely to operate through either changes to the function of follicular cells and/or changes in the secretion of gonadotrophins (Hansen, 2009). In the field, these changes in follicle dynamics are implicated in reduced rates of embryo survival and increased rates of infertility (Wiebold, 1988; Ryan et al., 1993; Sartori et al., 2002) but the extent to which these findings can be extrapolated to the sheep with its lower metabolic demands is debatable.

Despite numerous studies on the effects of seasonal heat stress on ovarian function in the dairy cow, studies on the effects of acute heat stress are largely limited to observations on fertility and embryo development. High temperatures 10 days before oestrus were associated with low fertility (Al-Katanani et al., 1999) whereas a 10h exposure from the beginning of oestrus increased the incidence of abnormal embryos (Putney et al., 1989). Similarly, embryo development was impaired in cows exposed to heat stress one day after oestrus but exposure thereafter (day 3, 5 or 7) had no effect (Ealy et al., 1993), a result very similar to that obtained in sheep (Dutt, 1964; Thwaites, 1971). That ovarian function is adversely affected by short term heat stress is supported by *in vitro* studies in cattle. Heat stress applied for 20 to 26 days reduced steroid production from follicle cells (Roth et al., 2001) whilst heat stress for 12h induced early activation of primordial follicles, reduced the rate of oocyte nuclear maturation and disrupted steroid production (Paes et al., 2016). Comparable impaired nuclear

maturation of oocytes *in vitro* has been reported in other studies (Nabenishi et al., 2012; Cebrian-Serrano et al., 2013; Maya-Soriano et al., 2013).

One study in the goat (Ozawa et al., 2005) has examined the effects of acute heat stress on follicle dynamics during synchronised oestrous cycles. Heat stress (36°C, 70% relative humidity; *rh*) applied from -48h to 0h relative to the last prostaglandin injection (i.e. approximately 24 – 72h before onset of oestrus) delayed recruitment of follicles for ovulation by 24h, and tended to delay the timing of ovulation. These changes in follicle dynamics occurred in conjunction with lower concentrations of oestradiol in plasma and follicular fluid, and lower aromatase activity and LH receptor levels.

2.6.3. Ovulation rate

Ovulation rate appears not to be affected by heat stress, a finding that is surprising given the effects of hyperthermia on follicle dynamics in the cow. When heat stress was applied to sheep before oestrus, only small reductions in ovulation rate were reported (Dutt et al., 1959; Ryle, 1961; Dutt, 1964; Rich and Alliston, 1970; Sawyer, 1979b). Similarly, in a study of superovulated ewes (Naqvi et al., 2004), the mean ovulation rate did not differ between control (8.14±1.27) and diurnal heat-stressed ewes (6.71±1.12). These findings are in general agreement with those observed in field studies (Lindsay et al., 1975; Kleemann and Walker, 2005).

2.6.4. Fertilisation

Heat stress applied either shortly before the onset of oestrus or during early oestrus reduces fertilisation rate (**Table 5-2**; Dutt et al., 1959; Alliston and Ulberg, 1961; Dutt, 1963; Dutt, 1964). The magnitude of fertilisation failure was greater when heat stress was applied -d5 to d0 (d0 = day of oestrus) (Dutt et al., 1959; Dutt, 1964) compared with either -d3 to d0 or d0 (Alliston and Ulberg, 1961; Dutt, 1963; 1964). Failure rates in these studies doubled from approximately 25% to 50% as treatment was extended from the d0 to -d5. Losses were also greater in unshorn sheep (51.9%) compared with shorn sheep (28.6%; Dutt et al. (1959)). On the other hand, significant differences were not recorded when heat stress was applied in association with diurnal relief (Rich and Alliston, 1970; Naqvi et al., 2004).

In several studies, heat stress applied before and/or during oestrus induced a marked increase in the percentage of abnormal ova including cytoplasmic vacuoles and globules, ruptured oolemma and cracked zonae pellucida (**Table 5-3**; Dutt et al., 1959; Alliston et al., 1961; Dutt, 1963; Dutt, 1964). In one other similar study where ewes were exposed to diurnal relief from heat stress (Alliston and Ulberg, 1961), the increases in the percentage of abnormal ova was significant. In all studies, the percentage of abnormal ova may have been artificially high due to the age of the ova at the time of collection (three or seven days after mating).

Aberrant maturation of oocytes during or following hyperthermia is the most likely cause of fertilisation failure. In the mouse, heat stress disrupts nuclear maturation to metaphase II and causes retention of the first polar body (Baumgartner and Chrisman, 1981a; 1981b). Heat stress of bovine oocytes *in vitro* (Lenz et al., 1983; Payton et al., 2004; Roth and Hansen, 2005; Wang et al., 2009)

affected both nuclear and cytoplasmic maturation. Similarly, with ovine oocytes, heat stress resulted in a significant decrease in the percentage of oocytes progressing past M1 (Gharibzadeh et al., 2015; Ahmadi et al., 2019). Interestingly, in the study of Ahmadi et al. (2019), oocytes collected in summer were more resistant to thermal stress during *in vitro* maturation than were oocytes collected in winter. This delay in oocyte maturation is likely a consequence of elevated concentrations of reactive oxygen species (ROS), which are increased in embryos and/or oviducts following heat stress in mice (Matsuzuka et al., 2005a; Ozawa et al., 2005) and cattle (Sakatani et al., 2008). In the sheep, ROS levels increased significantly in oocyte – cumulus complexes when matured *in vitro* at 42°C compared with 39°C (Barakat et al., 2016). An associated reduction in the intracellular concentration of glutathione (Ozawa et al., 2002) and the ameliorating effects of antioxidant treatments on oocyte meiotic maturation and developmental competence (Matsuzuka et al., 2005b; Sakatani et al., 2008; Barakat et al., 2016) support the likely involvement of ROS in heat induced effects on oocyte maturation.

2.6.5. Ovum and Embryo wastage

Applying heat stress before and during oestrus or shortly after oestrus increased both ovum wastage and early embryo loss (**Table 5-4**). Limited data indicates that the effects of heat stress are most severe when applied on the day of oestrus. In the study of Dutt (1964), embryo mortality figures of 100% (12.2% in control sheep) were obtained irrespective of whether heat stress was applied between –d5 to d0 or on d0 alone, indicating that heat stress before oestrus is far less important than during oestrus. In other studies that examined the effects of heat stress leading up to and including d0 (Dutt et al., 1959; Dutt, 1963; Thwaites, 1969), embryo mortality rates of 100% were similarly obtained.

Embryo loss also occurs when heat stress is applied after oestrus, with the extent of embryo mortality declining as the time between oestrus and the start of heat stress increases (**Table 5-4**). Thwaites (1971) found that ovum wastage was greatest when heat treatment commenced on d1 but that heat stress commencing even a day later did not significantly increase losses compared with controls (Figure 5). An examination of the effects of heat stress commencing on days 0, 1, 3, 5 and 8 and continuing to d24 (19-24 days of heat exposure) found that estimates of embryo mortality decreased as time between oestrus and start of heat stress induction increased (**Figure 5**; Dutt 1963; 1964).

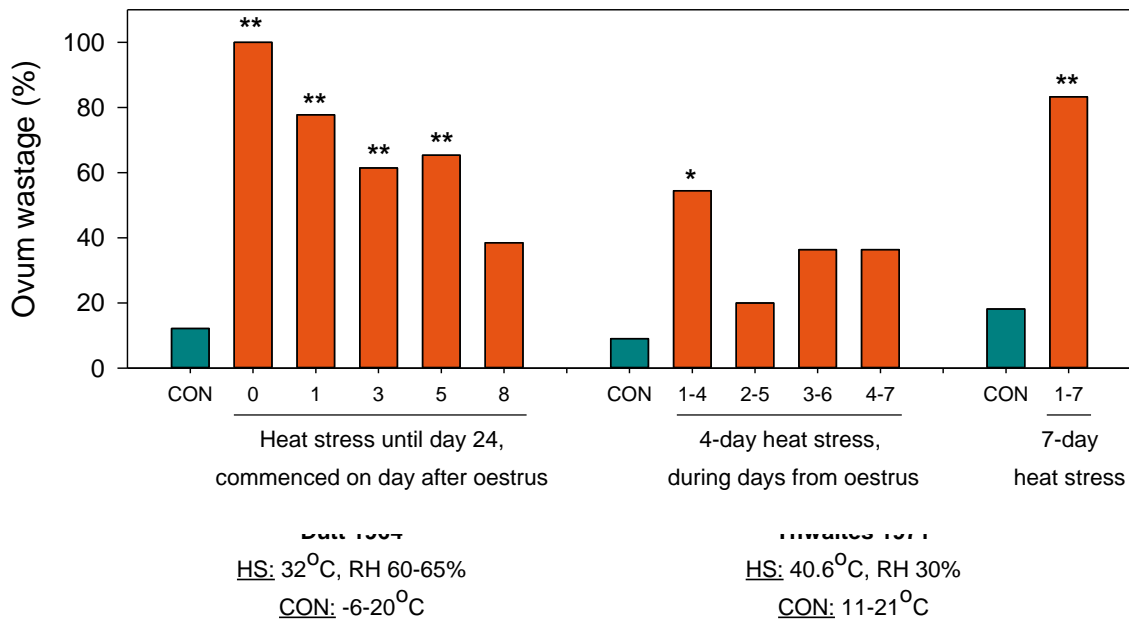


Figure 5. Embryo loss is greatest when heat stress starts close to oestrus. Ewes were housed in non-heat-stressed conditions (teal bars) or hyperthermic conditions (orange bars). Abbreviations: CON, control group, HS, heat-stressed group, RH, relative humidity. Significant differences from controls are indicated by symbols; *, P<0.05; **, P<0.01. Data are from Dutt (1964) and Thwaites (1971).

Thwaites (1967) examined the effect of heat stress for the first 20 days of pregnancy and found all ova/embryos were lost in Merino and Southdown ewes. Return-to-service records indicate that most of this loss (75%) occurred before the time of maternal recognition (day 12) but that there was some later loss attributed to heat stress. In a subsequent study, Thwaites (1969) conducted two experiments applying heat stress for the first 15 days of pregnancy. Heat stress for 8 hours each day did not significantly increase ovum wastage in the first year of experiments, but continuous heat stress in the second year caused an average loss of ova/embryos of 83%, compared with <20% in control ewes, with unshorn ewes far more susceptible to continuous heat stress than were shorn ewes (**Figure 6**). The author concluded that appreciable embryo mortality in the field, as a consequence of heat stress, is likely only under severe heat wave conditions.

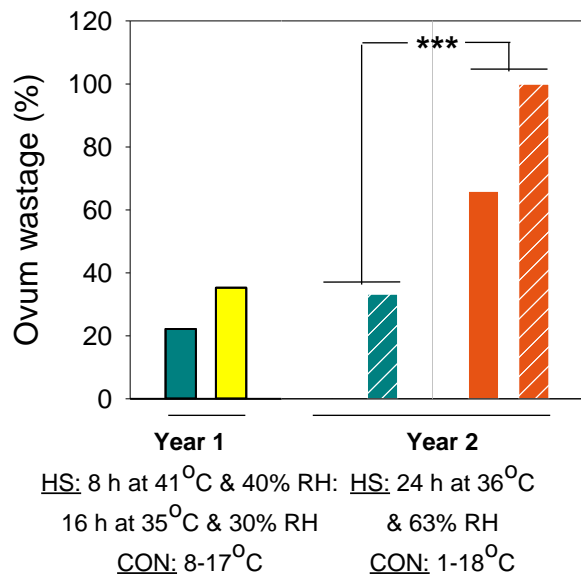


Figure 6 Ovum wastage is increased by continuous heat stress (year 2) but not by diurnal patterns allowing heat loss overnight (year 1). From d1 to 15 of pregnancy, ewes were housed in non-heat-stressed conditions (teal bars) or in diurnal (yellow bars) or continuous (orange bars) hyperthermic conditions. For year 2, solid bars indicate shorn ewes (0.5 cm fleece length) with broken bars indicating unshorn ewes (5 cm fleece length). Abbreviations: CON, control group, HS, heat-stressed group, RH, relative humidity. Significant effects of continuous heat stress are indicated by symbols; ***, $P < 0.001$. Data are from Thwaites (1969)

Meta-analyses (Romo-Barron et al., 2019) indicate that ewes exposed to short term heat stress are 12.4 times more likely to experience embryo mortality or to have unfertilised ova compared with control ewes. This figure increases to 26.3 with moderate periods of heat stress. Whilst the cause(s) of embryo mortality following heat stress are not known, it is likely that compromised oocyte maturation due to heat stress not only affects the processes of fertilisation but also has carry-over effects on the survival of embryos. Staigmiller and Moor (1984) reported that immature oocytes can be fertilised and undergo cleavage without developing to the blastocyst stage. Additionally, the production of ROS in the oviducts (Matsuzuka et al., 2005a), is also likely to increase mortality whilst embryos are still in this region, and is consistent with the greatest effects of heat stress on embryo wastage occurring in the days immediately following oestrus (Dutt, 1963; 1964; Woody and Ulberg, 1964; Thwaites, 1971). That heat stress adversely affects the oviduct environment is further evident in the study of Dutt et al. (1959) who found fewer sperm in the oviducts of ewes exposed to heat stress in association with an increase in the percentage of ova without sperm.

2.6.6. Pregnancy rates

Pregnancy rates are a function of the rates of fertilisation and ova/embryo wastage. Again, it is apparent that heat stress applied on either d0 and/or d1 after oestrus has a marked effect on pregnancy rate (

Table 5-5). For example, heat stress applied on –d5 to d0 or d0 alone resulted in 0.0% pregnancies compared with 87.5% in control animals (Dutt, 1964). Similarly, heat stress on d0 + d1 resulted in a 10% pregnancy rate compared with 85.0% in control ewes (Dutt, 1963). Significant reductions in pregnancy rates have also been obtained with hyperthermia applied after d1 (Dutt, 1963; 1964) but the reductions were significantly less than obtained on either d0 and/or d1. Several other studies (Ryle, 1961; Rich and Alliston, 1970; Sawyer et al., 1979; Sawyer, 1979a; 1979b) reported reduced pregnancy rates in response to hyperthermia but low numbers prevented the detection of significant differences. Overall, meta-analyses indicate that heat-stressed ewes are 2.4 times less likely to get pregnant than thermo-neutral ewes (Romo-Barron et al., 2019).

2.7. Interaction of heat stress around mating with other environmental stressors

Most studies on the effects of heat stress on ewe reproductive performance have used hot-rooms where the effects of heat stress can be examined with minimal involvement of confounding factors. However, the potential for other stressors to have an influence in the field is substantial. Firstly, animals exposed to acute heat stress can experience reduced feed intake (Smith et al., 1966; Thwaites, 1967; Ozawa et al., 2005). Secondly, as pointed out by Sejian et al. (2012), ewes that are exposed to heat stress in the field, can not only be exposed to nutritional deprivation and loss of appetite but also experience the stress of having to walk long distances to access both food and water. During the hotter periods of the year, the food available is typically dry and highly fibrous, dietary characteristics known to increase internal heat load (Silanikove, 2000). These secondary stressors can produce responses that potentially confound and/or exacerbate the effects of heat stress *per se* on reproductive performance.

There is very little information on how these stressors interact. Spies et al. (1965) examined the effects of forced exercise (30 or 60 min daily) and ambient heat stress applied immediately before and after oestrus. Exercise stress and heat stress independently reduced fertilisation rates ($P < 0.05$) with the worst result being obtained when the two stressors were combined. Most embryo mortality occurred within three days of mating, a finding that agrees with other findings (Dutt et al., 1959; Dutt, 1963; 1964; Thwaites, 1971). Other studies (Sejian et al., 2011; Kumar et al., 2019) have found that combined stressors (heat and nutrition) adversely affect reproductive performance but in neither study did the design allow for the separation of individual effects. Sejian et al. (2011) examined the effects of thermal stress and nutritional stress (30% intake) over two oestrous cycles and found that combined stress significantly ($P < 0.05$) reduced duration of oestrus and increased cycle length as well as oestradiol and progesterone concentrations. Sejian et al. (2012) subsequently examined the effects of ambient heat stress combined with both a restricted diet (30% intake) and walking 14 km per day on the reproductive performance of Malpura sheep in India. All parameters examined were adversely affected by this combined stress – the percentage of ewes that came into oestrus was reduced from 66.7% to 41.7%, the duration of oestrus was reduced from 32h to 14.4h, conception rates were reduced from 83.3% to 50.0% ($P > 0.05$) and steroid levels were significantly altered.

Sejian et al. (2011) concluded that sheep are better able to adapt to nutritional stress than to heat stress. This conclusion is supported by observations on numerous biochemical parameters (Hb, PCV,

and concentrations of glucose, cholesterol, phosphatase, cortisol, thyroxine and insulin) used to measure the adaptive capability of ewes (Sejian et al., 2010). These latter authors found that nutritional stress had a less significant effect on these parameters than thermal stress but, notably, when these two stressors were combined, the impact was severe.

2.8. Effects of heat stress on ram reproduction

In most mammals with scrotal testes, including sheep, testicular temperature must be maintained below core body temperature for optimal production of motile and morphologically normal spermatozoa (Setchell, 1998). Intra-testicular temperatures of 33-35°C are reported in the ram at ambient temperatures of 20-30°C, representing a rectal-testicular temperature gradient of ~4-6°C (Waites and Moule, 1961; Waites and Setchell, 1964; Fowler, 1968a; Coulter et al., 1988). Multiple studies have described the negative effects of elevated testicular temperature on semen production in the ram. Similarly, physiological mechanisms contributing to thermoregulation of the ovine testis are well-described. However, as with studies of heat stress on ewe reproduction, most studies have been conducted using climate-controlled rooms, or for the male, through scrotal insulation or heating. Thus, how the heat load applied in these studies compares with that experienced by sheep maintained under Australian grazing and natural mating conditions is often difficult to determine.

2.8.1. Regulation of testicular temperature

In sheep, as in many mammals, the testes are located external to the body within the scrotum. Scrotal responses to temperature have a primary role in thermoregulation of the ovine testes, providing a regulatory pathway that is largely independent of regulation of whole body temperature (Setchell, 1978; Maloney and Mitchell, 1996). Scrotal surface area and distance of the testes from the body are regulated by the tunica dartos muscle, a smooth muscle layer beneath the scrotal skin, and the cremaster muscle (Waites, 1991). Positive correlations between degree of scrotal retraction to the body and subcutaneous scrotal temperature are evident in sheep exposed to short-term heat (40°C) or cold (6°C) conditions (Maloney and Mitchell, 1996). Apocrine sweat glands in the scrotal skin release sweat for evaporative cooling when scrotal temperature increases (Waites and Voglmayr, 1962; Waites and Voglmayr, 1963), and this is facilitated by the increase in scrotal surface area (Fowler and Waites, 1971). When scrotal skin temperature is experimentally elevated to 35-37°C in Merino rams, scrotal sweat glands synchronously discharge up to 10 times per hour, with concomitant short-term decreases in scrotal skin temperature of up to 2.6°C (Waites and Voglmayr, 1962; Waites and Voglmayr, 1963). Tunica dartos muscle and sweat gland responses to elevated temperature are mediated through cutaneous scrotal thermoreceptors (Waites and Voglmayr, 1962; Waites and Voglmayr, 1963; Maloney and Mitchell, 1996; Gibson et al., 2002). Additionally, heating the scrotum above 36°C increases respiration rate of the woolled ram, a response that is also regulated by scrotal thermoreceptors (Waites, 1961; Waites, 1962; Maloney et al., 2003). Finally, the pampiniform plexus within the spermatic cord provides countercurrent heat exchange between arterial blood entering the scrotum and the cooler venous blood exiting, under normothermic conditions (Waites and Moule, 1961), and the degree of scrotal retraction may contribute to the efficiency of heat transfer within the plexus (Maloney and Mitchell, 1996).

When capacity of these thermoregulatory mechanisms is exceeded, as occurs in many experimental protocols used to assess effects of hyperthermia on testicular function, significant increases in scrotal and testicular temperature can result. Scrotal insulation through application of an insulating bag to the scrotum, and scrotal heating, using a temperature controlled chamber, are two approaches used to assess heat effects. Scrotal insulation for 8 to 24h elevates scrotal temperature by $\sim 2^{\circ}\text{C}$ (Mieusset et al., 1991). Scrotal heating allows for a more controlled increase in testicular temperature, and this approach has been used in some studies to elevate scrotal or testicular temperature by $\sim 6\text{-}8^{\circ}\text{C}$ (Waites and Voglmayr, 1963; Fowler, 1968b; Fowler and Setchell, 1971). Other studies use climate-controlled rooms, thus aiming to assess the effects of whole body temperature increase on testicular thermoregulation. For example, Fowler and Kennedy (1968) placed Merino rams in a climate room for 3h with increasing temperatures from 20 to 45°C , at 17 mm Hg, with 45h between tests. Both scrotal and testicular temperatures were elevated by $\sim 4\text{-}5^{\circ}\text{C}$ when exposed to 45°C for 3 h, compared with 20°C for 3h, while variation in rectal temperature in response to this heat load was less than 1°C . Rectal-testicular temperature gradient was reduced to $\sim 1.5^{\circ}\text{C}$ by 3h exposure to 35°C , and abolished by 3h exposure to 45°C in these studies (Fowler and Kennedy, 1968). When these experiments were repeated with temperature exposure starting at 45°C and decreasing, a greater elevation in testicular temperature occurred at 45°C , suggesting that rams were less able to regulate testicular temperature in response to a rapid elevation to a high temperature (Fowler, 1968a). How these findings compare with temperature effects on scrotal or testicular temperature under field conditions has been little studied. Brown (1971) reported subcutaneous scrotal temperatures of 33 to 37.5°C over 5 days, $\sim 4\text{-}5^{\circ}\text{C}$ below rectal temperature, in three rams under grazing conditions in central western NSW, where maximum daily air temperatures ranged from 32.5 to 35°C . Setchell (1998) similarly describes preliminary findings that air temperature and scrotal temperature are linearly related in rams in the Australian summer, but with loss of the abdominal-scrotal temperature gradient when air temperature exceeds 40°C .

2.8.2. Effects of heat stress on spermatogenesis

Multiple studies have applied local or whole-body heating to assess the effects of elevated testicular temperature on ram semen production and quality. However, few studies have systematically assessed temperature effects on semen quality under Australian field conditions. Some variation in semen quality between seasons has been summarised in Egyptian breeds and conditions (Marai et al., 2008). In Australia, no consistent trend was observed in semen characteristics in rams studied for 18 months in 1960-61 (Fowler, 1965). However, individual rams did vary, with 4 of 11 rams showing evidence of seminal degeneration during the summer months (Fowler, 1965). Of note, (Lindsay et al., 1975) observed no relationship between the number of ewes lambing and mean maximum temperature in the three weeks preceding mating. This contrasts with their findings, discussed earlier, of significant associations of reproductive outcomes with maximum temperature (Lindsay et al., 1975) around the time of mating, and suggests a minimal contribution of semen quality to variation in pregnancy outcomes in their extensive field studies.

Despite this, experimental studies provide clear evidence for an effect of heat exposure on semen production and quality (**Table 6-1**). Significant variation exists between studies in duration of heat exposure, temperatures applied, the increase in testicular temperature induced and the timing at

which outcome measures are assessed. Most studies report a reduction in semen quality; evident through a decreased percentage of motile sperm and an increase in the proportion of abnormal sperm. Rathore Rathore (1969; 1970a) reported increased proportions of pyriform spermatozoa and mid-piece abnormalities when rams were heated to 40.5°C, 45% humidity for 8 h per day, for 2 or 4 days, with abnormal sperm apparent from 9 days after heating. Levels of abnormal sperm peaked at >30%, 18 days after heat treatment in these studies. Acrosome abnormality was also observed, but only when rams were heated for 4 or 5 days (Rathore, 1970b). Increased proportions of abnormal sperm are reported across a range of heat exposures, including scrotal insulation for 30 h (Kastelic et al., 2017), and hot room exposure at 41°C for 9 or 13.5h (Smith, 1971), or 32°C for 4 days (Howarth, 1969), with effects peaking at 18-24 days after treatment and persisting until 30-35 days. Spermatozoa take up to 14 days to transit the ovine epididymis (Ortavant 1958, cited by Smith, 1971; Rathore, 1969) suggesting that abnormalities appearing at 9-10 days post-heating may originate in the epididymis. The continued presence of sperm abnormalities until 30 days after heat stress suggests that heat stress can also induce damage during the transition of spermatids to spermatozoa (Rathore, 1969; 1970a; 1970b).

Effects on sperm motility are also evident from 14-24 days following the heat insult in many studies. For example, semen samples collected following 21 days of scrotal insulation for 16h per day contain ~20% motile sperm (Mieusset et al., 1992; Arman et al., 2006). Lesser effects are observed when scrotal insulation is applied for 8h per day, with a mean motility of 58% across 160 days of scrotal insulation, compared with 73% in controls (Mieusset et al., 1991). Heating to 32°C, 65%rh for four days reduced motile sperm from 80% to <10% at two weeks post-treatment (Howarth, 1969). Sustained effects on motility are also reported in some studies, with three days at ~35°C reducing motility score from 15-35 days post-heating in a line of Merino rams with a high skin fold score (Fowler and Dun, 1966). Effects of heat exposure on sperm concentration are more variable between studies, and can be influenced by the frequency of semen collection for follow-up assessment. Waites and Ortavant (1968) histologically assessed damage following 150 min scrotal heating at 41°C and detected degeneration of pachytene spermatocytes and partial loss of round spermatids, commencing 12h post-treatment. Mitosis of B-spermatogonia was also impacted, indicating that earlier stages of germ cell development can be affected. Cell loss at the spermatocyte and spermatid stages would be expected to influence content of the ejaculate from ~35 days post-exposure (Howarth, 1969; Braden and Mattner, 1970). Consistent with this, sperm numbers in the ejaculate are decreased from 25 – 30 days after scrotal heating to 40.5°C, and don't return to pre-treatment levels until days 50 to 60 (Braden and Mattner, 1970). In contrast, no effect of scrotal insulation for 8h per day for 162 days was observed on sperm concentration, however, scrotal insulation for 16h per day reduced concentration from day 100, and when insulation was continuous for 24h for 30 days, concentration fell from day 12 of treatment and showed a marked decrease, to near zero levels, between days 19-58 (Mieusset et al., 1991).

Some studies have attempted to relate sperm quality measures to the induced temperature increase, by considering the association of a mean composite semen score (based on motility, morphology and concentration measures) with scrotal temperature achieved during heating. For example, Smith (1971) exposed rams to 4, 6, 9 or 13.5h at 41°C. Minimal effects were observed after 4h, but 9h and 13.5h at

41°C increased the proportion of abnormal sperm and reduced motility. Across all treatments a negative correlation was observed between the mean semen composite score and maximum subcutaneous scrotal temperature (Smith, 1971). Moule and Waites (1963) also report a positive correlation of maximum scrotal temperature and semen damage score (difference between seminal scores pre-treatment and days 13-52 post-heating). These authors also observed significant variation between rams (**Table 6-1**), with some rams showing significant seminal degeneration following two 6h exposure to 40.5°C, at differing humidity, while others exhibited limited effects. Rams with lower levels of heat-induced seminal effects were able to maintain scrotal temperatures 2-3°C lower than affected rams during heating.

2.8.3. Effects of heat stress during spermatogenesis on fertility and pregnancy rates

Some studies have extended these observations of reduced semen quality following exposure of rams to heated rooms or scrotal heating to assessment of fertilisation and pregnancy rates. Similar to studies of semen quality, these studies vary in the timing and degree of heat exposure. Howarth (1969) exposed rams to 32°C, and 65% *rh*, for four days. Fertilisation (assessed at 30h) and embryonic survival (at day 34) did not differ in ewes mated to rams one week post-heating. In contrast, fertilisation rates were low when rams were mated two weeks post-heating, and no fertilised embryos were detected at 3 weeks post-heating. In another study, ewes mated to rams that had been heated to 40.5°C, 45% humidity for 8h daily for 1, 2, 3 or 4 days, 10-27 days prior to mating, had fertilisation rates at 6-70h post-mating of 66%, 42%, 23%, 6%, compared with 93% for a control ram (Rathore, 1970c). These rates reflect the increased percentages of abnormal spermatozoa observed with increasing duration of heating (Rathore, 1969; 1970a; 1970b). Their follow-up studies assessed pregnancy outcomes from rams exposed to 40.5°C for 8h for 2 or 4 days, 10-16 days prior to mating. Heating of rams reduced pregnancy rates, with 3/20 ewes lambing following mating to rams exposed to two days of heat stress, and 0/20 ewes pregnant following mating to rams exposed to four days of heat stress compared with 12/20 ewes pregnant after mating to unheated control rams. While fertilisation rates were reduced, some ewes apparently pregnant at day 23 (non-return to oestrus) were identified as non-pregnant at day 40 (2 d ram: 6 ewes, 4 d ram: 3 ewes, control: 2 ewes), suggesting that embryonic loss may also contribute to the reduced pregnancy rates in heat-exposed rams (Rathore, 1969).

Others have studied the effects of scrotal heating on fertilisation and pregnancy. Braden and Mattner (1970) heated the scrotum of two Merino rams in a chamber with 41-44°C water for 1.5-2h and assessed mating outcomes over 60 days. Ejaculates from scrotal-heated rams contained increased proportions of dead or abnormal spermatozoa from approximately 15 until 50 days after heating and reduced spermatozoa numbers from around day 25 (**Table 6-1**). Scrotal-heated rams were infertile between 36 to 47 days post-heating. Some suppression of fertility was also evident from 15 to 35 days post-heating, with pregnancy rates varying from 13-70%, across rams, time points, and experiments. In contrast, 4h scrotal heating with 39.5°C water in two rams, had no effect on fertilisation or pregnancy rates. An association between the thermal load applied to the testes and later pregnancy rates was also reported by Fowler (1968b). When the intra-testicular temperature of Merino rams was heated to 38-42°C for 2h, an increase of ~6-10°C, pregnancy rates of 40-45% were obtained when ewes were artificially inseminated with semen collected between 14 and 35 days post-heating, compared

with pregnancy rates of 69% using semen from control rams. The most marked effects occurred between 14 and 21 days after heating, with pregnancy rates of 30-38%. In contrast, scrotal heating of an additional group of rams to an intra-testicular temperature of 38-40°C, an increase of ~5-7°C, for 2h had minimal effects on pregnancy rates. No assessment of whether losses were due to fertilisation failure or embryonic loss was performed. Calculation of a corrected heat load for all rams, using area under the curve of testicular temperature, demonstrated a significant negative association between heat load and pregnancy rate (Fowler, 1968b) with this association confirmed in a subsequent study (Fowler and Setchell, 1971).

Results of later studies using intermittent scrotal insulation contrast with earlier reports of an effect of heat exposure on fertilisation. Mieuxset et al. (1991; 1992) performed intra-uterine insemination in 636 ewes with frozen semen from Merino rams submitted to scrotal insulation, to elevate scrotal temperature by ~2°C, for 16h per day for 4, 15, or 21 days. Pregnancy rates did not differ significantly at day 17, suggesting minimal effects on fertilisation. However, 65 days post insemination, pregnancy rates were reduced when semen was used from rams heated for 4, 15 or 21 days, and embryonic mortality from day 17 to 65 was increased (79, 79, 93%, respectively) compared with insemination with semen from control rams collected on equivalent days (55, 59, 66%). Embryo losses following insemination with control semen were high (55-66%), suggesting potential issues with the frozen semen and/or insemination; however, higher losses from heat treated rams indicate an additional impact of heat exposure. Semen samples collected at day 21 had reduced motility and increased dead spermatozoa; however, minimal differences in semen quality measures were evident at days 4 and 15. Effects on embryonic mortality following insemination with samples collected four days post-heating suggest effects on epididymal sperm that alter the capacity of the spermatozoa to produce a viable embryo, but not capacity to fertilise the oocyte. Increased DNA fragmentation index and increased susceptibility of DNA to denaturation are reported following scrotal insulation for 24 or 48h (McDonald et al., 2007). However, these effects appeared 21 days post-insulation, thus occurring before sperm entered the epididymis. As Mieuxset et al. (1991, 1992) used frozen-thawed semen, it is possible that scrotal insulation increased the sensitivity of sperm to freezing induced damage. In support of this, a greater fall in percent motile sperm was reported following freeze-thawing of semen from rams that were insulated for 16h per day for 21 days, compared with controls (Arman et al. 2006). A preliminary report suggests that ovine sperm collected during, or within two weeks following, a 21-35 day period of scrotal insulation for 8 or 16h per day had reduced ability to fertilise oocytes *in vitro*, and embryos produced had a higher rate of degeneration and delayed development (Setchell, 1994). The potential for embryonic effects following fertilisation with heat-affected sperm is also suggested by rodent studies, where exposure of male mice to whole body or scrotal heating reduces developmental competence of embryos *in vitro* and *in vivo* (Zhu and Setchell, 2004; Zhu et al., 2004; Paul et al., 2008).

While these studies suggest effects of ram heat exposure on the fertilisation and/or embryonic development, the field based studies of Lindsay et al. (1975) and Kleemann and Walker (2005) observed no relationship between number of ewes lambing or lambs born and mean maximum temperature in the three weeks preceding mating, suggesting a minimal contribution of the ram to variation in lambing performance. The number of days in a week above 32.2°C exceeded four on at least two occasions during the study of Lindsay et al. (1975). Howarth (1969) reported significant

effects on fertility 2-3 weeks after exposing rams to 32°C for four days; however, heat treatment was continuous with 65%*rh*, highlighting the potential for variation between climate room studies and field conditions.

2.8.4. Mechanisms of heat stress effects on spermatogenesis and other factors

Exposure of the testes to elevated temperature can affect spermatogenesis through germ cell apoptosis and perturbations of sperm maturation, including induction of structural and functional abnormalities and DNA damage (Waites and Ortavant, 1968; Rathore, 1969; 1970a; 1970b; Setchell, 1998; Druery et al., 2007). Effects vary with cell stage, with pachytene spermatocytes and round spermatids identified as particularly susceptible to heat-induced loss and damage (Waites and Ortavant, 1968; Pérez-Crespo et al., 2008; Houston et al., 2018), and consequences of later exposures including structural and functional abnormalities (Rathore, 1969; 1970a; 1970b). Hypoxia has been suggested as a possible mechanism through which hyperthermia affects testes function and induces germ cell damage (Waites and Setchell, 1964; Setchell, 1994). An early study indicated that when ram testes were heated to 39°C, blood flow did not increase to meet the increase in metabolic and oxygen demands, leading to hypoxia (Waites and Setchell, 1964). However, this contrasts with recent reports of increased testicular blood flow and oxygen extraction in anaesthetised rams with sequential increases in testicular temperature to 40°C (Rizzoto et al., 2018). Spermatozoa are vulnerable to oxidative stress induced damage, including lipid peroxidation of sperm membranes and DNA damage (Aitken et al., 2016), and rodent models of scrotal heat stress demonstrate induction of markers of oxidative stress (Paul et al., 2009; Houston et al., 2018). The effects of heat stress on testosterone levels vary between studies, with 72h scrotal insulation in White Dorper rams (Alves et al., 2016) and 6h per day at 42°C, 55%*rh* in Malpura rams in India ((Maurya et al., 2016), associated with reduced testosterone levels, while 6h per day exposure of Garole x Malpura rams to temperatures increasing from 38°C to 44°C did not affect testosterone concentration (De et al., 2017). In Merino rams, testosterone levels were not affected by constant scrotal insulation for 7d, but levels were reduced following 14 days of insulation (Byers and Glover, 1984). In general, Leydig cells are thought to be less susceptible to heat-induced perturbation when compared with the spermatogenic cells (Setchell, 1998; Hansen, 2009).

Testis function can also be affected by nutrition (Guan and Martin, 2017), and as discussed earlier heat stress reduces appetite and feed intake. In Malpura rams, 42°C, 55%*rh* for 6 h per day, reduced semen mass motility and concentration, averaged across 45 days, while nutritional restriction to 30% *ad lib* intake over the same time period had minimal effects on these measures, when compared with control rams (Maurya et al., 2016). Semen quality in rams exposed to both heat and nutritional stress did not differ from those exposed to heat stress alone (Maurya et al., 2016), indicating thermal stress as a more significant stressor for sperm production. Specific nutrient deficiencies, including Vitamin E and selenium, may also influence the susceptibility to heat-stress induced effects on spermatogenesis (Liu et al., 2014), with one study suggesting improved semen characteristics when Awassi rams were supplemented during the hot season (Ali et al., 2009).

In cattle, testicular thermoregulatory capacity varies between *B. indicus* and *B. taurus* bulls (Brito et al., 2004). In sheep, a reduction in semen motility occurred when Border Leicester and Merino rams were exposed to 38°C under hot-room conditions, while sperm motility in Dorset Horn rams was not affected until the temperature reached 43°C, suggesting variation between breeds in effects of heat on semen quality (Lindsay, 1969). While some studies have considered variation between breeds in adaptability of rams to tropical, sub-tropical or hot arid conditions in other countries (Marai et al., 2007; Moura et al., 2019), effects of breed on adaptation to Australian conditions have been less studied. In Merinos, studies in the 1960s demonstrated greater susceptibility to heat-induced infertility in rams selected for a high degree of skin fold (Folds plus), when compared with low skin folds (Folds minus) (Fowler and Dun, 1966). This was associated with altered scrotal and testicular thermoregulatory capacity, including differences in testicular blood flow, scrotal surface area, sweat gland size and density, and capacity for fluid loss when temperature was elevated (Fowler and Dun, 1966; Fowler, 1968a; Fowler and Kennedy, 1968; Fowler and Setchell, 1971; Fowler and Waites, 1971), highlighting the capacity for variation in scrotal thermoregulatory mechanisms

2.9. Impact of heat stress during pregnancy on lamb birthweight and survival

2.9.1. Birthweight

Exposing ewes to elevated ambient temperatures throughout sustained periods of pregnancy impairs fetal growth, severely reducing the weights of fetuses near term (at 132 – 141 d of gestation, gD, term 145-150 gD) and of new born lambs (

Table 2-1). This suggests that increasing duration of heat stress during pregnancy causes a greater reduction in fetal growth, with the possible exception of heat stress that persists throughout the entire pregnancy. An increasing impact of longer durations of heat stress is supported by data from the single study in which effects of differing durations of maternal heat stress have been compared. Galan et al. (1999) exposed ewes to relatively severe heat stress conditions (18 h at 40°C: 6 h at 35) starting at gD35, for either 55 or 80 days, before returning them to thermoneutral conditions. Fetal weights in late gestation (~gD130) were reduced by 45% in ewes exposed to heat for 55 days, and 74% in ewes exposed to heat for 80 days. Given the reduced fertility of ewes exposed to heat around mating, it is possible that the smaller reduction in lamb birthweight in ewes exposed to heat throughout pregnancy reflects a “survivor effect”, so only the best-adapted ewes conceive. In addition to larger decreases in birthweight as an increasing proportion of pregnancy is subjected to heat exposure, the reductions in birthweight are more severe when heat remains elevated throughout the day with no diurnal relief (**Figure 7**).

Table 2-1. Maternal hyperthermia during pregnancy reduces newborn/late fetal and placental weights in sheep

Pregnancy outcome	Timing of hyperthermia							
	No. Ewes	Mating to term	No. Ewes	Middle 1/3 of pregnancy	No. Ewes	Last 1/3 of pregnancy	No. Ewes	Last 2/3 of pregnancy
[§] Lamb / fetal weight								
Control, kg	26	3.67	10	3.57	27	4.27	121	3.96
Heated, kg	25	3.03	10	2.67	25	2.97	131	2.55
Difference from Control								
kg		-0.58		-0.91		-1.30		-1.41
%		-15.9		-25.3		-30.5		-36.0
[#] Placental Weight								
Control, g					8	413	34	487
Heated, g					6	390	28	190
Difference from Control								
g						-23		-297
%						-5.5		-61.0

Weighted means calculated from studies in which hyperthermic conditions were induced using approximate circadian rhythms, and measures were taken from either new born lambs, or fetuses within 15 days of predicted birth day. § Data derived from the following 15 studies: (Yeates, 1953; Yeates, 1956; Yeates, 1958; Shelton and Huston, 1968; Alexander and Williams, 1971; Cartwright and Thwaites, 1976; Brown et al., 1977; Bell et al., 1987; Bell et al., 1989; Early et al., 1991; Thureen et al., 1992; Abdalla et al., 1993; McCrabb et al., 1993). # Data derived from the following 5 studies: (Yeates, 1953; Bell et al., 1987; Bell et al., 1989; Early et al., 1991; Thureen et al., 1992).

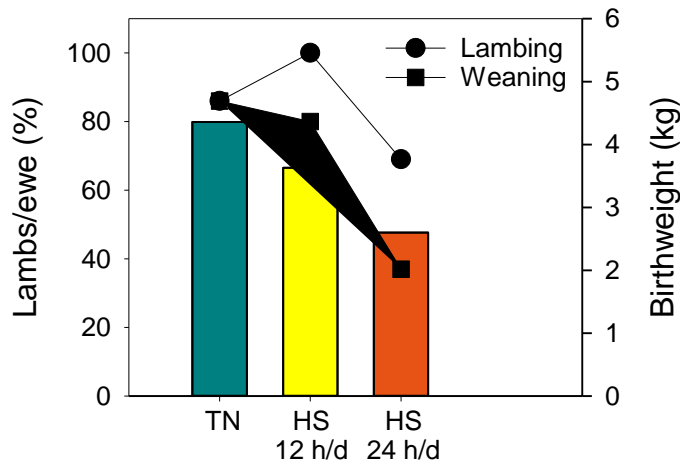


Figure 7 Lambing and weaning percentages and birthweight decrease with increasing duration of heat stress (HS; 32°C). Ewes were maintained under thermoneutral (TN) conditions (n=21) or at 32°C for either 12 or 24 h/d during the last two thirds of pregnancy. Data from Shelton and Huston (1968).

Although heat exposure throughout pregnancy consistently reduces birthweight, effects are variable, and direct comparison between studies is complicated by variation in the extent and duration of the hyperthermic conditions (**Table 7-1**) breed of sheep and the timing at which measures were collected. In the majority of the studies pregnant ewes were housed in climate controlled chambers, and a circadian rhythm of temperature was enforced with a hotter day (ranges: 6-18 h, 35 – 44.4°C and THI of 83.2 to 97.0) and cooler night (ranges: 6 – 18 h, 16.4 – 35.0°C, THI of 38.3 – 83.5). Interestingly, the impact of hyperthermia during pregnancy on fetal growth appears to be unaffected by litter size, with similar reductions in birth weight of singleton (↓1.16 kg) and twin lambs (↓0.99 kg) in response to maternal heat stress through the last two thirds of pregnancy (**Figure 8**; Shelton and Huston (1968)).

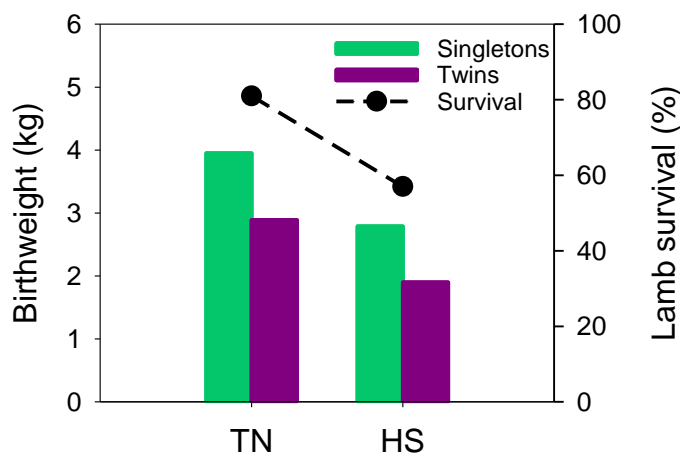


Figure 8 Effect of heat stress (HS) compared with thermoneutral (TN) conditions during pregnancy on birthweight and survival of singleton and twin lambs. Data from Shelton and Huston (1968).

2.9.2. Lamb Survival

Birthweight is one of the main risk factors contributing to neonatal loss of lambs (Yapi et al., 1990; Fogarty et al., 2000; Geenty et al., 2014), with mortalities higher for lambs outside the ideal birthweight range of 4.0–6.0 kg. Low birthweight lambs typically have disproportionately lower brown adipose stores, expend their glycogen and lipid stores more rapidly post-birth, take longer to stand and suckle, and ingest less colostrum during the critical 24 hour period after birth (Mellor and Cockburn, 1986; Scales et al., 1986; Dwyer, 2003; Refshauge et al., 2016). Lambs weighing less than 4 kg are, therefore, more susceptible to starvation-mismothering, hypothermia and, ultimately death (Scales et al., 1986). Only two studies have reported the impact of pregnancy hyperthermia on lamb viability and post-natal survival. Consistent with these adverse effects of low birthweight on survival, maternal hyperthermia throughout the last two thirds of pregnancy reduced survival by >25% (Figure 7; Shelton Shelton and Huston (1968)). The effects of the proportion of pregnancy affected by heat stress (number of days or months) on survival have not been reported. However, the impacts of elevated temperature during the last two thirds of pregnancy (32°C) were more severe when hyperthermic conditions were maintained for 24 rather than 12 hours per day (100% survival in TN conditions cf. 80% in 12 h/day HS and 55% in 24 h/day HS, **Figure 8**; Shelton and Huston (1968)). At least some of the impact of maternal hyperthermia on lamb mortality occurs due to peripartum death. In Targhee x Suffolk cross-bred ewes, the proportion of lambs born alive fell from 97% in range-housed ewes to 67% in ewes housed under 16h 38°C: 8h 28-32°C conditions (Brown et al., 1977). Since most studies have not reported survival, we have used a model of the relationship between birthweight and lamb survival, based on records from 24,699 lambs across eight sites in Southern Australia (Geenty et al., 2014), to predict the impact of maternal hyperthermia during pregnancy on survival. Using this model, the mean reported 1.32 kg reduction in birthweight in ewes exposed to hyperthermia for at least a third of pregnancy is predicted to reduce survival by 26% (**Figure 9**). Depending on the duration of exposure, maternal hyperthermia is predicted to reduce survival by 12.5% (after HS throughout pregnancy), 19% (after HS only in middle third of pregnancy), 23% (after HS only in last third of pregnancy), and 28.5% (after HS throughout last two thirds of pregnancy).

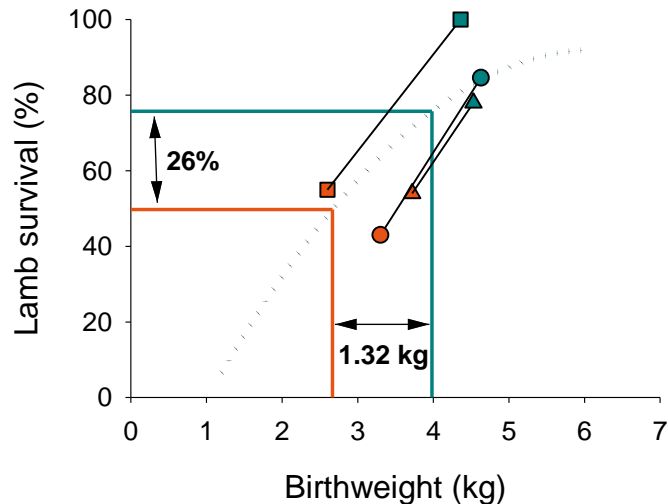


Figure 9 Predicted and reported impacts of maternal hyperthermia on lamb survival. Dotted line shows survival as a function of birthweight from Geenty et al. (2014). Paired symbols indicate birthweight and survival data from normothermic (21-27°C, blue symbols) and hyperthermic pregnancies (continuous at 32°C, orange symbols) within groups of comparably-fed ewes from (Shelton and Huston, 1968). Drop lines show weighted mean birthweights derived from seven published studies in sheep and predicted survival for lambs from normothermic (blue line) and hyperthermic (orange line) pregnancies. Heat stress was imposed during the middle third of pregnancy (Alexander and Williams, 1968), the final two-thirds of pregnancy (Shelton and Huston, 1968; Cartwright and Thwaites, 1976), the last third of pregnancy (Yeates, 1953; Alexander and Williams, 1971; Brown et al., 1977) or the entirety of pregnancy (Yeates, 1956; Yeates, 1958).

2.9.3. Mechanisms for effects of heat stress during pregnancy on fetal growth:

It is likely that several mechanisms contribute to the reduced birthweight of lambs in ewes that experience ongoing heat stress throughout pregnancy. These impact fetal growth from mid-gestation, with normal fetal weight at gD55, but reduced abdominal circumference evident from gD70 and reductions in long bone lengths and skull width emerging at gD80 and gD90, respectively (Barbera et al., 1995). By late gestation, fetal sizes in HS ewes were 2 standard deviations or more below those of fetuses gestated by thermoneutral ewes (Barbera et al., 1995). Consistent with an increasing severity of restriction with advancing gestation, newborn lambs and late gestation fetuses from heat-stressed ewes have altered morphology, including the brain sparing effects (Thureen et al., 1992; Regnault et al., 1999; Regnault et al., 2002; De Vrijer et al., 2004; De Vrijer et al., 2006), that are also seen following intrauterine growth-restriction (IUGR) in humans (Miller et al., 2016) and in experimentally-induced IUGR in fetal and newborn sheep (Robinson et al., 1979; Cock and Harding, 1997; De Blasio et al., 2007; Carr et al., 2012). Reduced fetal growth in heat-stressed ewes appears to be primarily due to impaired placental development and function, with relatively minor effects of maternal food intake. Elevated maternal temperatures may also directly suppress fetal metabolism by restricting fetal ability to shed heat.

2.9.3.1. Nutrition

Nutrition during pregnancy is a key determinant of placental and fetal growth (Symonds et al., 2010; Herring et al., 2018), and undernutrition during the last two-thirds of pregnancy can impair placental and fetal growth (Vonnahme et al., 2013). Non-pregnant sheep reduce their voluntary feed intake when exposed to high ambient temperatures and humidity levels and, under experimental conditions, hyperthermia reduced the voluntary feed intake of pregnant ewes by 6 – 34% (Shelton and Huston, 1968; Alexander and Williams, 1971; Bell et al., 1989). However, most studies suggest the negative effects of heat stress on placental and fetal growth are largely independent of nutrient intake (Shelton and Huston, 1968; Alexander and Williams, 1971; Brown et al., 1977; Bell et al., 1989). Specifically, despite the effect of heat stress on food intake, when the feed intake of ewes housed in thermoneutral conditions was matched to that of heat-stressed ewes, the effects of heat stress on birthweight were far greater than those of the reduced feed intake alone. In comparison with *ad libitum*-fed ewes housed under thermoneutral conditions, birthweight was not significantly reduced (average ↓156g) in pair-fed ewes, but was substantially reduced (average ↓1850g) in ewes housed under hyperthermic conditions (**Figure 10**; Shelton and Huston (1968); Cartwright and Thwaites (1976); Brown et al. (1977)). Similarly, reduced fetal weights (24-27% ↓ at gD90-93) and (23 – 53% ↓ at gD134), are seen in ewes housed in hyperthermic conditions relative to thermoneutral ewes pair-fed to the hyperthermic group (Regnault et al., 1999; De Vrijer et al., 2004; De Vrijer et al., 2006). It is therefore clear that reduced maternal feed intake is not the primary cause of heat stress-induced reductions in birthweight.

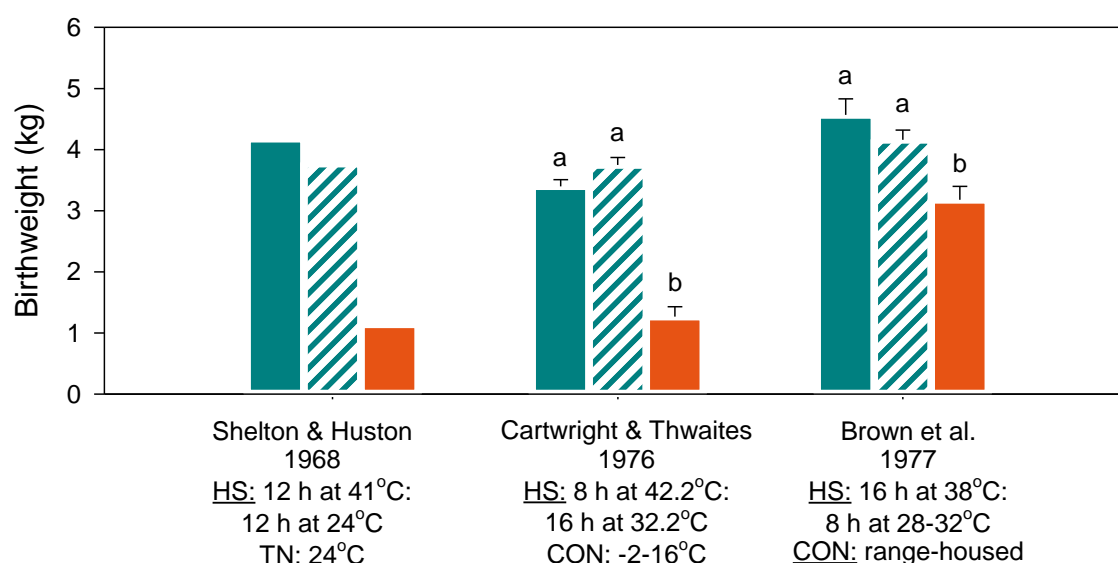


Figure 10 Reduced feed intake is not the major mechanism for reduced birthweight in heat-stressed ewes. Ewes were housed in non-heat-stressed conditions (blue bars) and either fed *ad libitum* (solid bars) or pair-fed (striped bars) to ewes housed in hyperthermic conditions. Data are means, error bars indicate SEM, and significant differences are indicated by differing superscripts where stated in the source. Data are derived from (Shelton and Huston, 1968; Cartwright and Thwaites, 1976; Brown et al., 1977).

2.9.3.2. Placental growth and function

Maternal hyperthermia impairs placental development and placental function, depending on the stage/s of pregnancy at which ewes are exposed. Exposure during early-mid pregnancy does not reduce either placental or fetal weight two weeks later. At these early stages of pregnancy, there appear to be compensatory placental adaptations to maintain fetal growth. For example, when hyperthermia is induced from about day 35 of pregnancy, the ratio of fetal to placental weight 15-20 days later is approximately 40% higher than in control ewes and fetal weight is not different from that of control ewes (Regnault et al., 1999; De Vrijer et al., 2006). Increased expression of growth factors within the placenta at gD55, specifically insulin-like growth factors, placental growth factor and vascular endothelial growth factor (VEGF) suggest candidate mechanisms for initial compensatory mechanisms (Regnault et al., 2002; De Vrijer et al., 2006). Conversely, decreased expression of the VEGF receptor (VEGFR1) might impair angiogenic responses to VEGF (Regnault et al., 2002). Measures of vascularity in the placenta throughout pregnancy are needed to determine whether reduced angiogenesis contributes to later impairments in placental function. After 55 days of heat stress, fetal weights are reduced by 24-27%, with similar decreases in placental weight (33-36%), although the latter were non-significant (Regnault et al., 1999; De Vrijer et al., 2006). Consistent with impaired placental growth evident by mid-gestation, maternal circulating placental lactogen is reduced from gD62 and circulating progesterone is reduced from gD72 (Regnault et al., 1999), when the placenta replaces the ovary (corpus luteum) as the main source of progesterone (Bassett et al., 1969). Placental effects of hyperthermia commencing later in pregnancy are less well characterised. The 30% reduction in fetal weight that occurs despite unchanged placental weight after maternal hyperthermia is imposed throughout the last third of pregnancy (

Table 2-1) implies that this exposure substantially impacts placental function.

Table 2-1. Maternal hyperthermia during pregnancy reduces newborn/late fetal and placental weights in sheep

Pregnancy outcome	Timing of hyperthermia							
	No. Ewes	Mating to term	No. Ewes	Middle 1/3 of pregnancy	No. Ewes	Last 1/3 of pregnancy	No. Ewes	Last 2/3 of pregnancy
[§] Lamb / fetal weight								
Control, kg	26	3.67	10	3.57	27	4.27	121	3.96
Heated, kg	25	3.03	10	2.67	25	2.97	131	2.55
Difference from Control								
kg		-0.58		-0.91		-1.30		-1.41
%		-15.9		-25.3		-30.5		-36.0
[#] Placental Weight								
Control, g					8	413	34	487
Heated, g					6	390	28	190
Difference from Control								
g						-23		-297
%						-5.5		-61.0

Weighted means calculated from studies in which hyperthermic conditions were induced using approximate circadian rhythms, and measures were taken from either new born lambs, or fetuses within 15 days of predicted birth day. § Data derived from the following 15 studies: (Yeates, 1953; Yeates, 1956; Yeates, 1958; Shelton and Huston, 1968; Alexander and Williams, 1971; Cartwright and Thwaites, 1976; Brown et al., 1977; Bell et al., 1987; Bell et al., 1989; Early et al., 1991; Thureen et al., 1992; Abdalla et al., 1993; McCrabb et al., 1993). # Data derived from the following 5 studies: (Yeates, 1953; Bell et al., 1987; Bell et al., 1989; Early et al., 1991; Thureen et al., 1992).

Placental consequences of chronic maternal hyperthermia that persist from early through to late pregnancy have been characterised in detail at late pregnancy, where substantial impairment of both placental weight and function are evident. Reduced placental weights at late pregnancy reflect smaller rather than fewer cotyledons (Bell et al., 1987), with evidence for inhibition of placental cell division resulting in fewer placental cells (Early et al., 1991). Decreased maternal circulating concentrations of placental lactogen from mid gestation further suggest impairments in development of binucleated trophoblast cells, which produce this hormone. Both active and passive transport across the placenta from maternal to fetal circulations is markedly impaired at mid-late pregnancy following heat stress imposed from early pregnancy. In late gestation (gD135), placental clearance of ethanol, a measure of placental diffusion capacity, is reduced by 47% in ewes exposed to 38-40°C for 9h/d and 30-32°C for 15 h/d between gD 45 and 120 (Bell et al., 1987). Similarly, reductions of 47-52% were obtained in ewes maintained under more severe hyperthermia (40°C for 18 h/d and 35°C for 6 h/d) from ~gD40 until gD120-gD125 (Thureen et al., 1992; Regnault et al., 2003). This reduction in diffusion across the placenta is proportional to fetal and placental weights (Thureen et al., 1992; Regnault et al., 2003).

Oxygen extraction from maternal blood by the placenta is also lower after chronic hyperthermia, and although uterine oxygen delivery is proportional to fetal weight, lower umbilical blood flows result in fetal hypoxia, with 21-42% reductions in fetal arterial O₂ saturation in late gestation (Bell et al., 1987; Thureen et al., 1992; Regnault et al., 2003). Reduced umbilical blood flow is associated with elevated markers of resistance to blood flow, which becomes apparent in longitudinal ultrasound studies by ~gD80-90 (Galan et al., 1998), and implicates resistance to blood flow within the placental bed. Placental transport of glucose is likewise impaired in heat-stressed ewes, resulting in a 37% reduction of fetal plasma glucose (Thureen et al., 1992). Placental uptake and transfer of the branched-chain amino acid analogue ACP, which is actively transported via system L-amino acid transporters, is also impaired in ewes after chronic heat stress through most of pregnancy (De Vrijer et al., 2004).

Importantly, although the impacts are less severe than those of continuing heat stress, effects of heat stress in early-mid pregnancy persist even when ewes return to thermoneutral conditions in mid-pregnancy. In ewes exposed to 50 days of heat stress, fetal and placental weights at gD80 were reduced by 10% and 29%, respectively (McCrabb et al., 1993). Although ewes were then returned to thermoneutral conditions, fetal and placental weights at gD140 were still 26% and 42% lower, respectively, than those of ewes maintained in thermoneutral conditions throughout pregnancy (McCrabb et al., 1993). Based on these data, it is evident that the negative impacts of maternal heat stress on fetal and placental growth and development are not recovered when ambient temperatures are lowered. This implies that hyperthermic conditions occurring ONLY during the middle third of pregnancy, as is likely when ewes are joined in spring or early summer, are likely to reduce lamb birthweights.

2.9.3.3. Temperature

The chronic hyperthermic conditions reported in these studies increased ewe core temperature by between 0.6 and 2.0°C (Alexander and Williams, 1968; Cartwright and Thwaites, 1976; Brown et al., 1977; Brown and Harrison, 1981; Bell et al., 1989; Vatnick et al., 1991; Thureen et al., 1992; Regnault et al., 1999; Regnault et al., 2002; De Vrijer et al., 2006). Ewe rectal temperatures were between 40 – 41°C after six hours at 44°C (THI 97; Alexander and Williams (1968)), 40.2 and 40.5°C after 9 hours at 40°C (THI 90; Bell et al. (1989)), and still elevated after 15 hours at 30°C (THI 77; Bell et al. (1989)). The metabolically active fetus produces heat, 85% of which is lost via the circulatory systems of the umbilical cord and placenta (Gilbert et al., 1985). The remaining 15% dissipates across the amniotic fluid and into the uterine wall (Gilbert et al., 1985), a process which is facilitated by the fact that fetal temperatures exceed those of the dam. Under thermoneutral conditions the difference between fetal and maternal temperature (feto-maternal temperature gradient) is approximately 0.4 – 0.7°C, depending on the stage of pregnancy (Oakes et al., 1976; Laburn et al., 1992; Laburn et al., 2002). Ewe core temperature and fetal temperature both rise in response to short periods (1 – 4 hours) of heat exposure; however, at progressively higher ambient temperatures the feto-maternal gradient decreases. Four hours of exposure to 35°C (THI 83) decreased the feto-maternal temperature gradient but still allowed maintenance of a positive gradient from fetus to dam (Laburn et al., 1992). However, after 2.5 hours at 40°C (THI 92) fetal and maternal temperatures became equal (Laburn et al., 2002) and 1 hour of 42°C (THI 96) resulted in a negative feto-maternal gradient (Oakes et al., 1976). This

reduction in the feto-maternal gradient has been attributed to changes in uterine and umbilical blood flow in response to heat stress (Oakes et al., 1976; Laburn et al., 1992; Andrianakis and Walker, 1994; Laburn et al., 2002). Alterations in blood flow are part of the ewe's thermoregulatory response to elevated temperature (Bell and Hales, 1985). Fetal capacity to dissipate heat is positively related to the rate of blood flow away from the placenta and uterus as well as umbilical blood flow (Oakes et al., 1976; Laburn et al., 1992; Laburn et al., 2002). Under moderate heat stress, when the maternal temperature increase is less than 2°C, and when hyperthermia is induced gradually, uterine blood flow increases (Andrianakis and Walker, 1994) effectively increasing fetal heat loss via the circulation. In contrast, one hour of severe heat stress (42°C, THI 96) significantly reduced both uterine and umbilical blood flow (Oakes et al., 1976), with uterine blood flow reduced approximately 78 minutes following exposure to 40°C (Brown and Harrison, 1981). It seems likely that accumulation of heat in the fetus will result in suppression of fetal metabolic rate to reduce heat generation, and that this may contribute to impaired fetal growth, particularly under conditions of extreme or rapid increases in heat.

Interestingly, changes in core temperature in response to elevated temperature vary between ewes Hopkins (Hopkins et al., 1980; McCrabb and Bortolussi, 1996) presumably reflecting differences in their tolerance, and thermoregulatory response, to temperature elevations. There is a significant, negative relationship between ewe rectal temperature during periods of high temperature and both lamb birthweight (Alexander and Williams, 1971; Hopkins et al., 1980) and placental weight (McCrabb and Bortolussi, 1996). Depending on the year, lamb birthweight decreased by 1.42 to 1.59 kg per °C increase in ewe rectal temperature in response to high ambient temperature, and ewes which reared a lamb had lower rectal temperatures than those which failed to do so (Hopkins et al., 1980).

2.9.4. Progeny performance and thermotolerance

The negative impact of exposure to heat stress *in utero* on lamb birthweight and, potentially, post-natal survival are clear. However, to the best of our knowledge the impacts of *in utero* heat stress on post-natal growth, body composition, wool growth, reproductive potential and thermoregulation have received little attention in sheep. In contrast, it is evident that exposing dairy cows to heat stress during late pregnancy alters the physiology and performance of their progeny, both as neonates and as adults (Tao et al., 2018). As is the case in sheep, exposing pregnant cows to sustained periods of hyperthermia reduced uterine and umbilical blood flow (Reynolds et al., 1985), with heat stress during the middle (Reynolds et al., 1985) and end (Tao et al., 2012) of pregnancy reducing birthweight (Reynolds et al., 1985; Tao et al., 2012; Monteiro et al., 2016). Heat stress during the last 50 days of pregnancy reduced the efficiency of immunoglobulin absorption and impaired passive immunity in dairy calves (Tao et al., 2012; Monteiro et al., 2016). Heifers experiencing *in utero* heat stress for the 46 days prior to birth required more services per pregnancy (Monteiro et al., 2016), and produced less milk during their first lactation (Monteiro et al., 2016; Skibieli et al., 2018), due to alterations in mammary gland morphology (Skibieli et al., 2018). Interestingly, there is also preliminary evidence of lower core temperature (Ahmed et al., 2017; Skibieli et al., 2018) and improved thermo-tolerance (Ahmed et al., 2017) in adults cows that had experienced *in utero* heat stress for the 50 days prior to birth (Ahmed et al., 2017). In contrast to cattle, however, *in utero* heat stress impairs rather than improves progeny

thermotolerance in pigs, increasing the post-natal core temperature set point, and reducing their ability to thermoregulate in response to elevated ambient temperature and humidity (Johnson et al., 2013; Johnson et al., 2015). It is also evident from a number of studies in pigs that *in utero* heat stress can impair reproductive development of male and female progeny, increase fat and decrease lean deposition, and impair milk yield (reviewed by Johnson and Baumgard (2019)). Based on the literature available, and discussed above, it is clear that *in utero* heat stress has the potential to alter progeny phenotype sufficiently to alter their productivity, and ability to cope with extreme thermal environment. To the best of our knowledge, the impact of *in utero* heat stress on post-natal physiology, performance and thermotolerance has not been investigated in sheep.

2.9.5. Subsequent lactation

The impact of pregnancy heat stress on mammary function and, thus, colostrum and milk production and pre-weaning lamb growth has not been investigated in sheep. In dairy cattle, heat stress (THI ~ 75) during the last 50 days of pregnancy compromises mammary development, such that alveoli number are reduced (Dado-Senn et al., 2019), mammary cell proliferation is decreased (Tao et al., 2011) and colostrum and milk production are lower (4 – 5 kg, reduction; Tao et al., 2011; Dado-Senn et al., 2019). These negative effects of heat stress during pregnancy on mammary development and milk production have been attributed to altered placental hormone production and reduced dry matter intake (Dahl et al., 2017). As previously discussed, heat stress alters endocrine function of the sheep placenta and reduces feed intake of pregnant ewes, and there is good evidence that both factors impair mammary development and subsequent milk production. Placental lactogen promotes mammary development and milk production in sheep (Akers, 1985) and the decreased placental lactogen evident by mid-pregnancy in heat-stressed ewes (Regnault et al., 1999) is therefore likely to impair mammary development. In the absence of heat stress, a 40% reduction in feed intake during the last two thirds of pregnancy decreased mammary weight and colostrum production by 20% and 43%, respectively (Swanson et al., 2008), and a 50% reduction in feed intake during the last 42 days of pregnancy reduced the volume of colostrum and milk produced by 68% and 33%, respectively (Tygesen et al., 2008). It is, therefore, possible that heat stress induced reductions in voluntary feed intake may also impair mammary development and colostrum production.

2.10. Impact of heat stress during lactation

Although the majority of the Australian sheep flock is managed so that lambing occurs when temperatures are cooler, for spring lambing flocks the later stages of lactation coincide with elevations in temperature and THI (**Table 3-8** to **Table 3-10**). The sensitivity of ewes to thermal stress is higher in lactation compared with late pregnancy, reflecting the high metabolic, and thus heat, load associated with milk production (Abdalla et al., 1993). Despite this, data describing the impact of elevated temperature and humidity on lactation is sparse for wool and meat producing sheep breeds. In contrast, it is evident that thermal stress during lactation decreases milk production of dairy breeds of cow (Tao et al., 2018) and sheep (Sevi and Caroprese, 2012). Dairy cows exposed to heat stress during lactation (THI > 68) produce 25 to 40% less milk, primarily due to heat induced reductions in feed intake, alterations in metabolism and impaired mammary gland development (Tao et al., 2018). In dairy sheep, milk production appears to be optimal at mean daily temperatures of 10 – 22°C (Ramón

et al., 2016), with modest variations in temperature sufficient to reduce milk yield. Milk volume reduced by 0.1 – 0.3 g/d per °C increase above 22°C (Ramón et al., 2016), with milk volume 15%, 20% and 20% lower, respectively, when maximum temperatures exceeded 21 – 24°C and THI increased from 60 – 65 to 72 – 75 (reviewed by Sevi and Caroprese (2012)). Interestingly, for every 1°C drop below 10°C, milk volume decreased by 0.2 – 0.6 g/d (Ramón et al., 2016), which may have implications for ewes lambing and lactating during the colder parts of the year. Lactating dairy goats are also affected by thermal stress, eating 29% less feed, drinking 41% more water and producing 8% less milk when exposed to thermal stress (THI 75 – 83) for 32 days (Contreras-Jodar et al., 2018). It is apparent that milk production of cows, sheep and goats is sensitive to thermal stress. Based on this, it is, therefore, suggested that rising temperatures during spring may impair the lactation performance of a proportion of the Australian sheep flock, and thus pre-weaning growth of their lambs.

3. Current and projected exposure of the Australian flock to heat stress

The literature reviewed and discussed previously demonstrate a clear, negative impact of heat stress on ewe and ram fertility, as well as lamb survival and post-natal growth and performance. The primary function of this section is to determine the impact of the current and future Australian climate on reproductive performance of the Australian sheep flock.

3.1. Thermal Heat Index as an appropriate measure of stress in extensive livestock

According to Hahn et al. (2009), there have been more than 100 thermal indices for humans and animals developed over the last 150 years. As shown in (Error! Reference source not found.), these indices range from the use of a critical ambient temperature to indices such as Thermal Humidity Index (THI) that consider temperature and humidity. More complex indices that take into account temperature, humidity, radiation and wind are available, but these are commonly used in more intensive livestock systems, in which routine daily observation of animals is possible. Examples from Australia include feedlot cattle (Meat & Livestock Australia, 2006; Gaughan et al., 2008), dairy (Dunshea et al., 2013) and live export of sheep (Caulfield et al., 2014; Phillips, 2016). Some of the more complex indices have constants and adjustments that are specific to the sex breed and age of the animal (Gaughan et al., 2008). Internationally, the heat balance of intensive livestock has received much more attention than extensive livestock systems (Silanikove, 2000).

For extensive sheep production, THI is selected for further analysis as it represents a compromise between robustness and complexity. The index is robust as current and future measures of temperature and humidity are easy to access and relatively reliable. Although the index is a simple addition of temperature and a fraction of humidity, taking humidity into account includes the major pathway of heat dissipation.

THI was calculated using the following equation:

$$THI = t_{max} + 0.36t_{dew} + 41.2$$

where t_{max} is the daily maximum temperature, and t_{dew} is the daily dew point temperature, calculated from Clausius-Clapeyron equation as follows:

The daily dew point temperature t_{dew} was determined from the 9am and 3pm recordings of mean vapour pressure. Vapour pressure is a direct measure of the moisture in the air, and thus an indicator of specific humidity. The daily dew point was calculated following estimation of the Clausius-Clapeyron equation.

$$t_{dew} = \frac{234.5 * \ln \frac{vph}{6.112}}{17.67 - \ln \frac{vph}{6.112}}$$

where vph is the daily vapour pressure calculated as the average of the 9am and 3pm vapour pressure recordings. Vapour pressure is a direct measure of the moisture in the air, and thus an indicator of specific humidity.

Although wind and radiation can also be important aspects of the energy balance, their relative importance depends on the position of the paddock in the landscape, access to shade trees, and animal behaviour. Thus, obtaining precise measurements of these variables is often problematic. In contrast, not only is THI robust, it is widely used in animal physiology literature and thresholds are understood by many in the sheep industry. Moreover, after consulting the livestock industries, the Australian Bureau of Meteorology is currently issuing a fortnightly forecast of THI as an experimental product as part of the Rural R&D for Profit project *Forewarned is Forearmed* funded by the Commonwealth Government and managed by Meat & Livestock Australia.

Table 3-1. Summary of commonly used indices for calculating heat stress

Index	Variables used	Benefits	Limitations for this study on extensive sheep	Reference
Critical Temperature	T_{max}	- Readily available and easy to understand and communicate	- Does not take into account night temperature, humidity, radiation or wind.	Lindsey et al. (1975), Kleemann and Walker (2005)
Excess Heat Factor (EHF)	T_{max}, T_{min}	- Data is readily available - Relatively easy to understand - Used by BoM for human heat wave alerts	- Does not account for humidity, radiation or wind.	Nairn and Fawcett, (2015)
Temperature Humidity Index (THI)	T_{max}, T_{dew}	- Includes potential heat loss from evaporative cooling - Data is readily available - Thresholds for extensive livestock are defined in literature	- Does not account for wind and radiation	Silanikove (2000), Hahn et al. (2009), Thom (1958) Dunshea (2013) Berry et al. (1964)
THI adjusted	T_{max}, T_{dew}, ws, sr	- Includes potential heat loss from evaporative cooling, wind and shade	- ws and sr are not widely available - most thresholds in the literature refer to the non-adjusted THI	Mader et al. (2002)
Dairy heat load index (DHLI)	T_a, rh, ws	- Includes potential heat loss from evaporative cooling, and wind	- ws is not widely available	Dunshea et al (2013)
Wet bulb temperature	T_w	- Reflects the maximum cooling of a wet body in a strong wind - Data is readily available	- Thresholds defined for extreme human heat stress but few thresholds for livestock.	Sherwood and Huber (2010), Caulfield et al. (2014)
Wet bulb globe temperature (WBGT)	Measured as T_g, T_w T_g can be estimated T_a, T_w, ws & sr .	- International standard for human work, health and safety. - Takes into account full heat balance (temperature, humidity, radiation and wind).	- Direct measurement of T_g (globe temperature) is expensive and rarely recorded. Estimates require ws and sr which are not widely available.	Budd, (2008)
Heat load index (HLI)	T_g, rh, ws	- Standard in Australian cattle feedlot industry - Based on WBGT	- T_g and ws are not widely available	MLA 2006, Gaughan et al. 2008

T_a – ambient temperature/dry-bulb temperature at a point in time; T_{max} – daily maximum temperature; T_{min} – daily minimum temperature; T_w – Wet-bulb temperature; T_{dew} – Dew point temperature; rh – Relative humidity (note: rh, T_{dew}, T_w are all measures of the moisture content of the air); ws – Wind speed; sr – Solar radiation; T_g – temperature of standard black globe that takes into account radiation and wind.

3.2. Description of Current and Future Climate

As discussed in **Section** Error! Reference source not found., the primary drivers of heat stress are temperature and humidity. Investigating the current and projected exposure of the Australian flock to heat stress requires an understanding of both the current climate conditions for these drivers, and how they are likely to change into the future. In this section, a description of the historical period of climate used as a baseline is given, as well as a description of current conditions, and likely future change in temperature and humidity.

3.2.1. Meteorological data used to inform analysis and establishing a climate baseline

Throughout this report, the Australian Gridded Climate Data (AGDC) is used as a data set to evaluate current climate across Australia. This dataset provides daily historical records of climate variables at a 5km resolution as part of the Australian water availability project (AWAP), and has been obtained by interpolating from historical gauged weather records. The climate variables that are used in the THI calculation of heat stress are specific vapour pressure (hPa) at 9am, $vph09$, specific vapour pressure (hPa) at 3pm, $vph15$, and the daily maximum temperature ($^{\circ}\text{C}$), t_{max} . The daily minimum temperature ($^{\circ}\text{C}$), and daily rainfall (mm) were also obtained to report on current climate conditions across Australia. The AGDC data were obtained through TERN AusCover. TERN is Australia's land-based ecosystem observatory delivering data streams to enable environmental research and management, and is a part of Australia's National Collaborative Research Infrastructure Strategy.

The baseline period adopted in this report was 1986-2005, to be consistent with the period used by the Climate Change in Australia data set (CSIRO and Bureau of Meteorology, 2015), and this period is henceforth referred to as representing the 'current' climate. It is important to note that the global average temperature over the period from 1986-2005 is 0.61°C warmer than the 1850-1900 pre-industrial baseline (IPCC, 2013). This difference will therefore need to be taken into account when assessing results of change scenarios presented in this report relative to a pre-industrial baseline.

3.2.2. Description of baseline climate for key meteorological variables

In order to understand possible future exposure to heat stress, it is necessary to understand how the key drivers currently vary both seasonally and spatially across Australia. **Figure 11** shows the historical seasonal averages of maximum daily temperature across Australia, for the baseline period (1986-2005). The daily maximum temperature is strongly heterogeneous across Australia, with a range of 30°C between the warmest and coolest regions. The contours of temperature change largely follow the latitude, indicating that distance from the equator is a key factor in determining temperature, although orography (e.g. the Great Dividing Range), proximity to coastline and various micro-meteorological factors also play an important role in determining temperature.

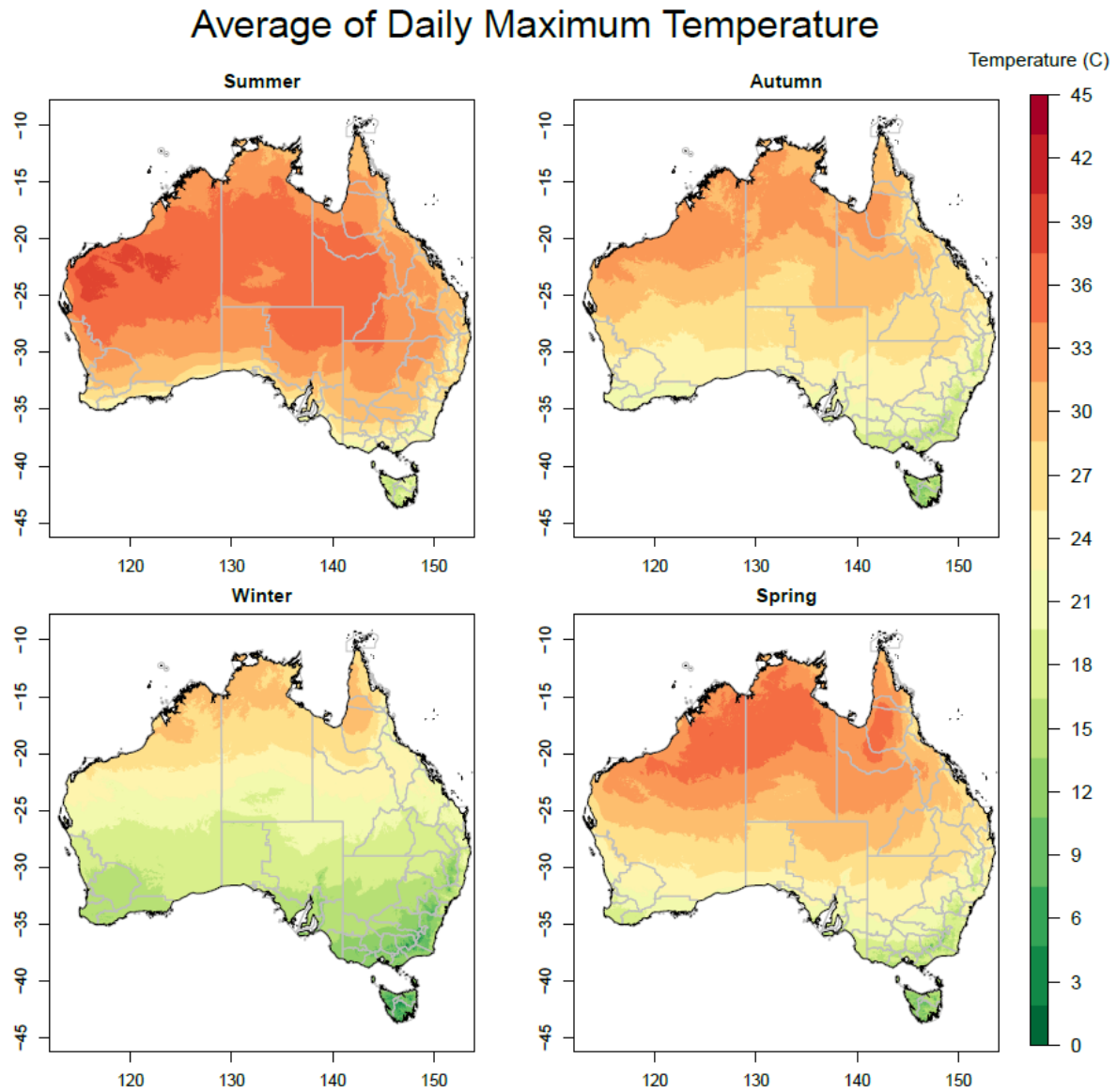


Figure 11. Seasonal averages of maximum temperature in Australia.

Figure 12 shows the average of the 9am and 3pm vapour pressure across Australia. The vapour pressure is lowest in the middle of Australia, in part because the air is furthest from the oceans and there are no open water surfaces (**Figure 12**). Vapour pressure does not vary as strongly as temperature across Australia, although the largest pressure still occurs in the tropical regions in the north most coastal areas due to the wetter climate. Throughout the year, the vapour pressure is lowest in the winter and spring, and is often constrained by temperature as cooler air cannot hold as much moisture.

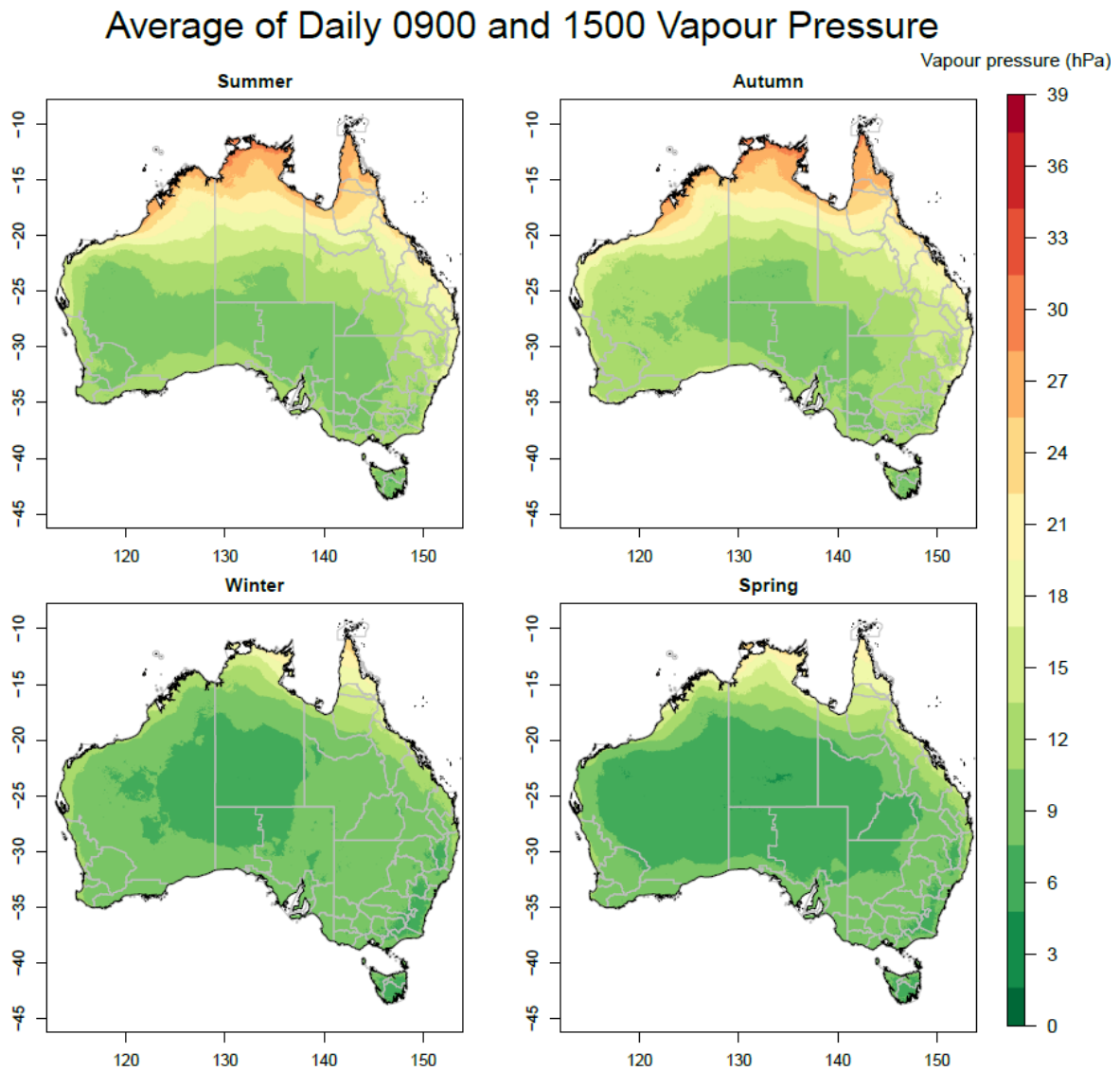


Figure 12 - Seasonal averages of vapour pressure in Australia.

From these two recorded climate variables, the current THI levels can be calculated (given by the equation in **Section** Error! Reference source not found.. **Figure 13** shows the variation of THI across Australia for each season. When considering the contours of **Figure 12** and **Figure 13**, the THI can be seen to be mostly dominated by temperature, which is apparent from the relative weighting of temperature and dew point in the equation of THI. Both temperature and humidity vary a lot across Australia, hence it is important that a localised approach is used to understand the impact.

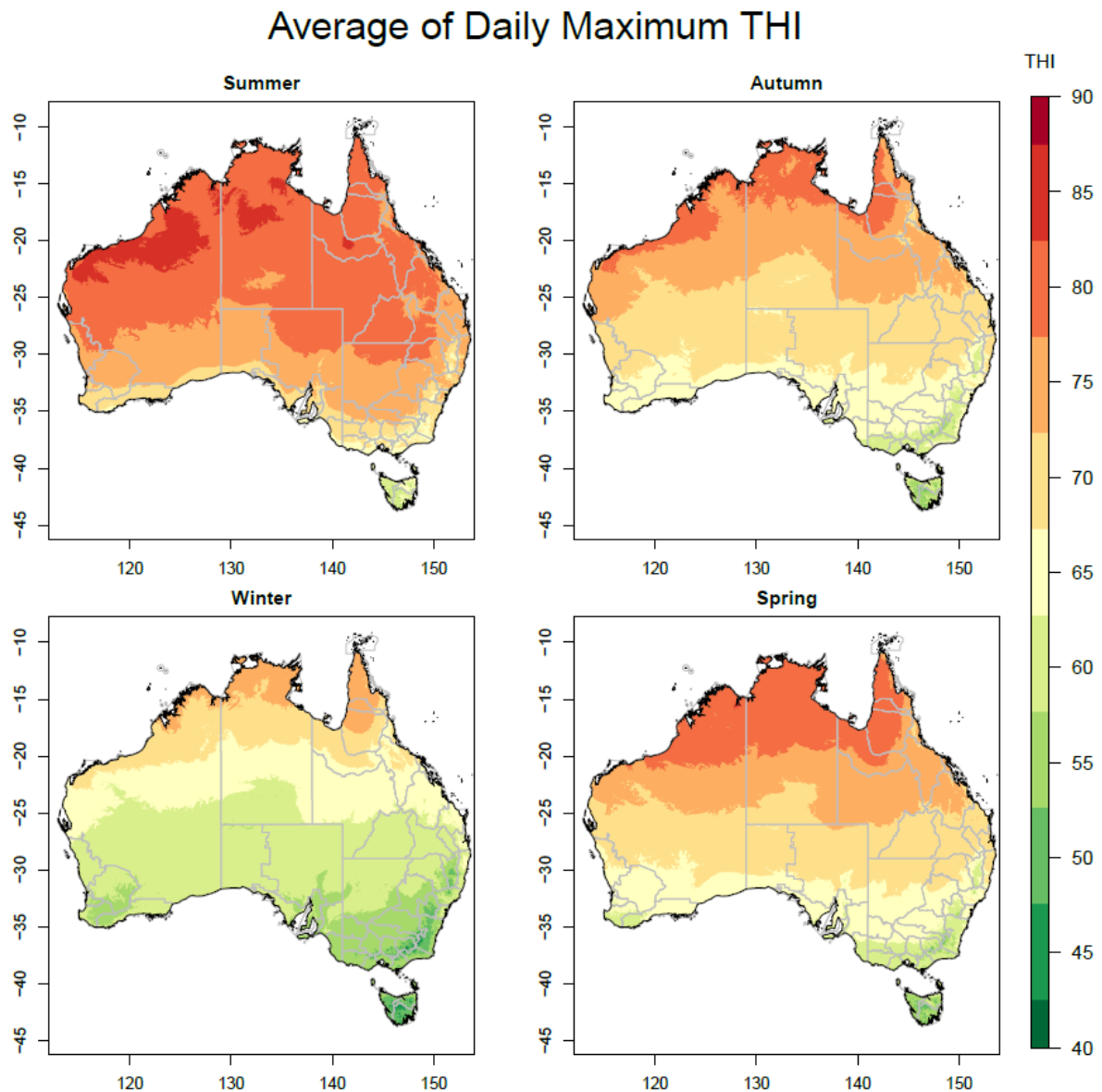


Figure 13 - Seasonal averages of THI in Australia.

3.2.3. Future climate

To understand possible changes to heat stress in the future, the climate projections from the Climate Change in Australia report are used (CSIRO and Bureau of Meteorology, 2015). Projections are not provided directly for measures of heat stress indices like THI, but they are available for the key drivers: temperature and humidity. Regarding the specific variables in the historical data set, projected changes to maximum temperature are available; however, changes to specific vapour pressure are not. The measure of humidity that is reported on by Climate Change in Australia is relative humidity, which is the ratio of the specific vapour pressure compared to the saturation vapour pressure given the temperature.

The climate projections detail potential changes to these variables until the end of the century, however, there is large uncertainty associated with the assessment. This is due to two key reasons:

- Uncertainty surrounding possible greenhouse gas emissions.* Climate Change in Australia project how the climate might change in response to four representative concentration pathways (RCPs), known as RCP2.6, RCP4.5, RCP6.0 and RCP8.5. The pathways describe plausible emissions scenarios and the resulting greenhouse gases up to the end of the century, where RCP2.6 is a low emissions scenario and RCP8.5 is a high emissions scenario. While no RCP is deemed more likely than another, the lower emissions scenarios require rapid decreases in emissions to be achieved. It can be seen from **Figure 14** that the pathways largely agree about emissions leading up to mid-century, but they diverge substantially towards the end of the century.

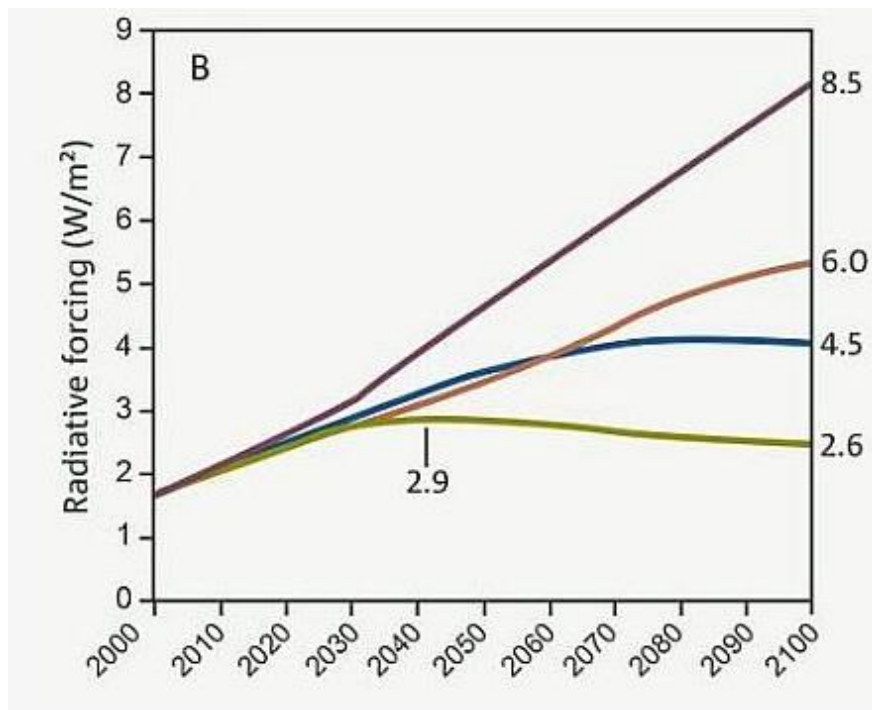


Figure 14. Radiative forcing for the different RCPs. The numbers on the right show the final radiative forcing at 2100 and give each scenario its name (8.5, 6.0, 4.5 and 2.6 W/m²). Source CSIRO and Bureau of Meteorology (2015).

- Uncertainty amongst the models that project changes in climate.* In order to generate projections of change to atmospheric variables such as temperature and humidity for a given RCP, an ensemble of climate models are used. The end result is that for an assumption about greenhouse gas levels (given by an RCP), there is still uncertainty in how the individual climate variables will respond. Interestingly, this uncertainty can be less than expected for an analysis of heat stress, as it relies on joint-variables temperature and humidity. Because these two variables are physically related, changes in the two are not fully independent, and it has been observed that the climate models that project a greater warming in temperature also show a stronger reduction in relative humidity. As a result there can be less uncertainty in projected changes to heat stress (Fischer and Knutti, 2012; Brouillet and Joussaume, 2019) compared to a range of other climate variables.

Figure 15 and **Figure 16** show the projected changes in daily maximum temperature and relative humidity in Australia, respectively. Four Natural Resource and Management regions (NRM) clusters are shown: the Monsoonal north, Central slopes, Murray basin and southern and south-western flatlands. RCP2.6, RCP4.5 and RCP8.5 are shown, with the spread in climate model results indicated by the boxplots.

It can be seen that projected changes in maximum temperature range from a one degree warming to a four-degree warming by the end of the century, relative to the 1986-2005 baseline. The effect of change is broadly the same across the year, showing little seasonal variation. The increases are slightly larger for the Murray basin and Central slopes regions.

Projected changes in relative humidity are much smaller and less consistent. The median change is a slight decrease, but some models predict an increase. The largest projected changes are at the end of the century (in the order of 2% and 4%), occurring in winter and spring. While no information on specific humidity is given, temperature and humidity are physically related. Given a projected warming of temperature, the air will be able to hold more water under these conditions, as given by the Clausius-Clapeyron relationship. If relative humidity is to remain unchanged or decrease by only a small amount, it means that the specific vapour pressure will increase as temperature does (Willett et al., 2007). Therefore, although projections for specific vapour pressure are not provided by CSIRO, the conclusions from combining **Figure 15** and **Figure 16** are at specific vapour will increase at close to the rate predicted by the Clausius-Clapeyron relationship assuming near-constant relative humidity.

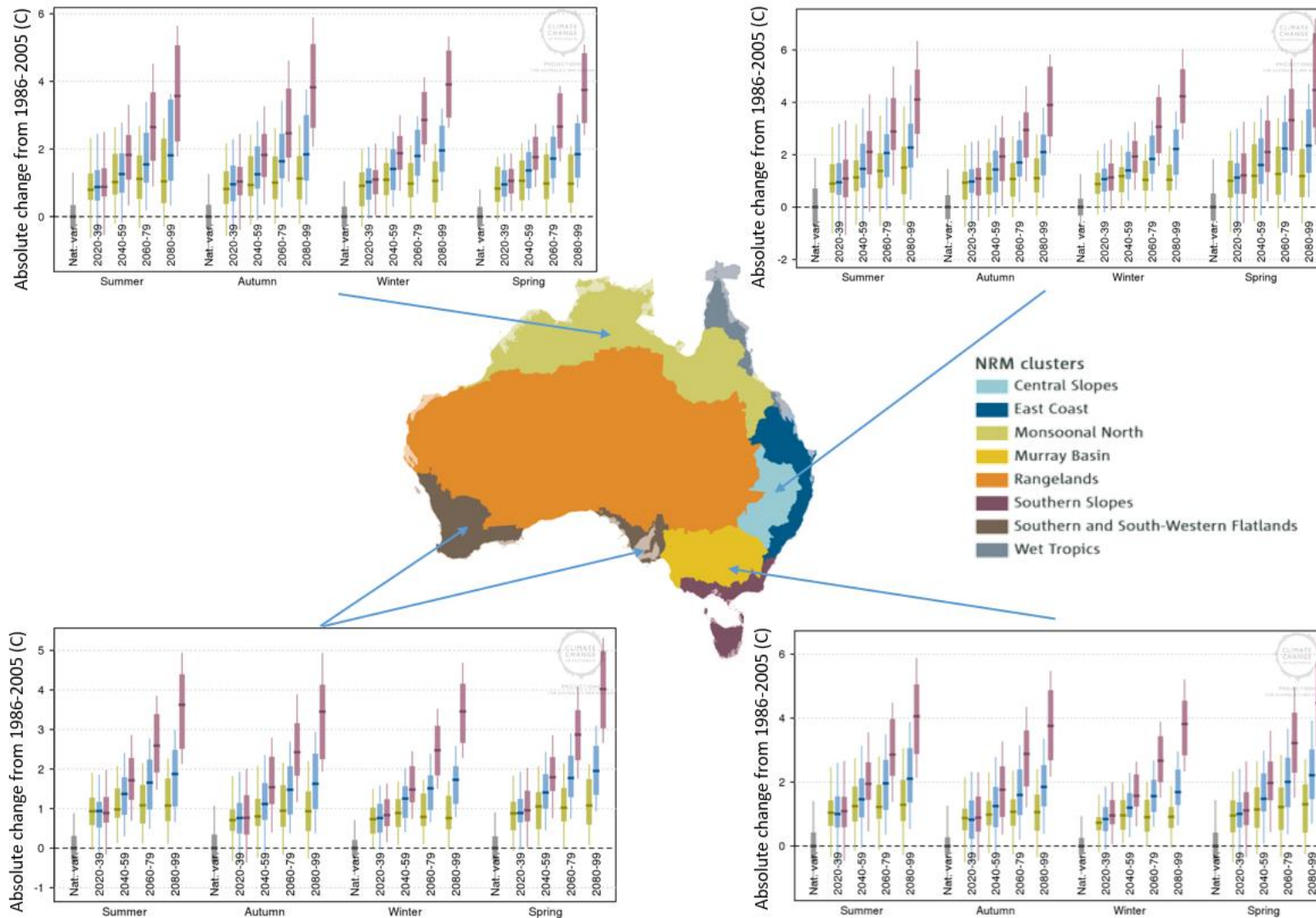


Figure 15 – Projected changes to maximum temperature for four NRM regions in Australia. Projections are shown under RCP2.6 (green), RCP4.5 (blue) and RCP8.5 (purple) relative to a historical period. Adapted from CSIRO and Bureau of Meteorology (2015).

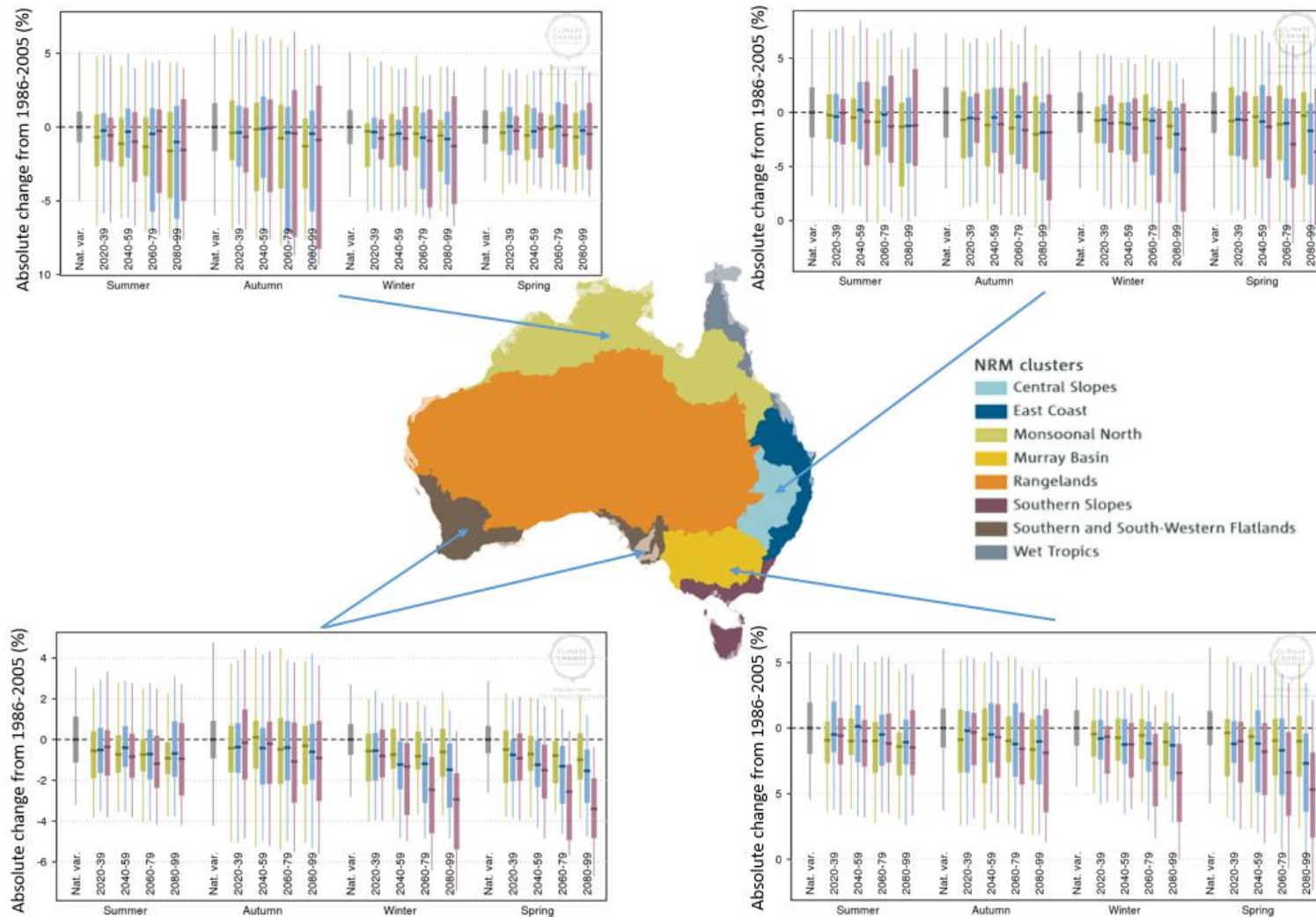


Figure 16 - Projected changes to relative humidity for four NRM regions in Australia. Projections are shown under RCP2.6 (green), RCP4.5 (blue) and RCP8.5 (purple) relative to a historical period. Adapted from CSIRO and Bureau of Meteorology (2015).

3.3. Current and future exposure of the Australian flock to heat stress

3.3.1. Developing exploratory climate scenarios

The current exposure to heat stress measured via the THI is provided in this section with reasonable confidence, given the high quality of observed climate data in Australia. As discussed in Section 3.2.3, the projected changes in climate are much more uncertain. Although the projections could be modelled directly, there would be a very wide bound of confidence on the findings, particularly through to the end of the century. Instead, the approach taken is to develop a small number of representative climate 'scenarios'. Scenarios are defined here as hypothetical future changes, but without an indication of the probability of occurrence. The scenarios proposed here are developed relative to current conditions (a baseline of 1986-2005). Importantly, the climate scenarios do not represent a point in time, but rather they describe a plausible set of future climate conditions that can be used to identify the potential impacts on sheep reproductive performance. This way, should that change in climate occur, the effects of that exposure will be known and can be prepared for.

As has been discussed, temperature and saturation pressure are physically related to one another. This is given by the Clausius-Clapeyron relationship, which described how the air can hold more moisture as it warms. To explore the impact of future changes in these variables on the heat stress of livestock in Australia, it is necessary to explore plausible changes to both temperature and humidity together, so that the scenarios used for the remaining analysis are internally consistent.

3.3.2. Temperature

To explore the effect of changing temperature on heat stress, two scenarios are formed from the Climate Change in Australia projections (CSIRO and Bureau of Meteorology, 2015):

- The first scenario is a 1°C Celsius warming of current temperature relative to the 1986-2005 baseline. As can be seen from Figure 5, this warming level is reached under all RCPs as soon as mid-century. Relative to pre-industrial climate, this is equivalent to a 1.6°C warming in temperature (Section 3.2.3). To place these results into context, at the 2015 Paris agreement in 2015 governments agreed to limit global warming to 2°C degrees relative to a pre-industrial period, with a goal of 1.5°C.
- The second scenario is a 3°C warming compared with current temperature. This warming level is a more extreme case of possible change, but still falls below the median of the highest emission end-of-century RCP8.5 scenario as provided by the CSIRO Climate Change in Australia projections.

3.3.3. Humidity

The measure of humidity most commonly reported on, and available from the climate change in Australia projections, is relative humidity. Using these projections, the following changes to relative humidity are indicated:

- It is projected that there will be little to no change in relative humidity by the middle of the century (under all RCPs).

- By the end of the century, changes in relative humidity across Australia are projected to occur, but the amount varies based on the time of year. In summer and autumn, there is little change projection, typically less than a 1% decrease. In winter and spring, the relative humidity is projected to decrease by 2-4%, depending on the local government region.

The historical data set used in this report provides measures of vapour pressure, which is also used in the calculation of the THI index used in this report (Hahn et al., 2009). Change in vapour pressure, or specific humidity, is less widely reported on by climate change projections. However, an increase in specific humidity is strongly implied given a change in temperature and constant relative humidity (given by the Clausius-Clapeyron equation) (IPCC, 2013). As the air temperature increases, the saturation point of the air is higher, and so for the relative humidity to stay constant the specific humidity must increase.

Using an estimation of the daily saturation point (Dingman, 2015), increases in maximum temperature of 1 and 3 degrees correspond to increases in vapour pressure of ~6% and 18% (based on 30°C) respectively. This is consistent with the findings on how specific vapour pressure will change in literature (Willett et al., 2007; Sherwood and Huber, 2010) and the IPCC indicating an increase of around 5% by mid-century (IPCC, 2013). Therefore, there are likely to be significant increases to specific humidity even under scenarios of small changes in relative humidity.

Given the uncertainties in the projections of relative humidity, particularly at the end of the century (**Figure 17**), the sensitivity of the THI calculations to humidity assumptions in the developed climate scenarios is examined. The following figure shows the current annual THI for the Bendigo airport BoM weather station, and THI in the above defined scenario with 3°C warming and no change in relative humidity, 2% decrease in relative humidity, and a 4% decrease in relative humidity.

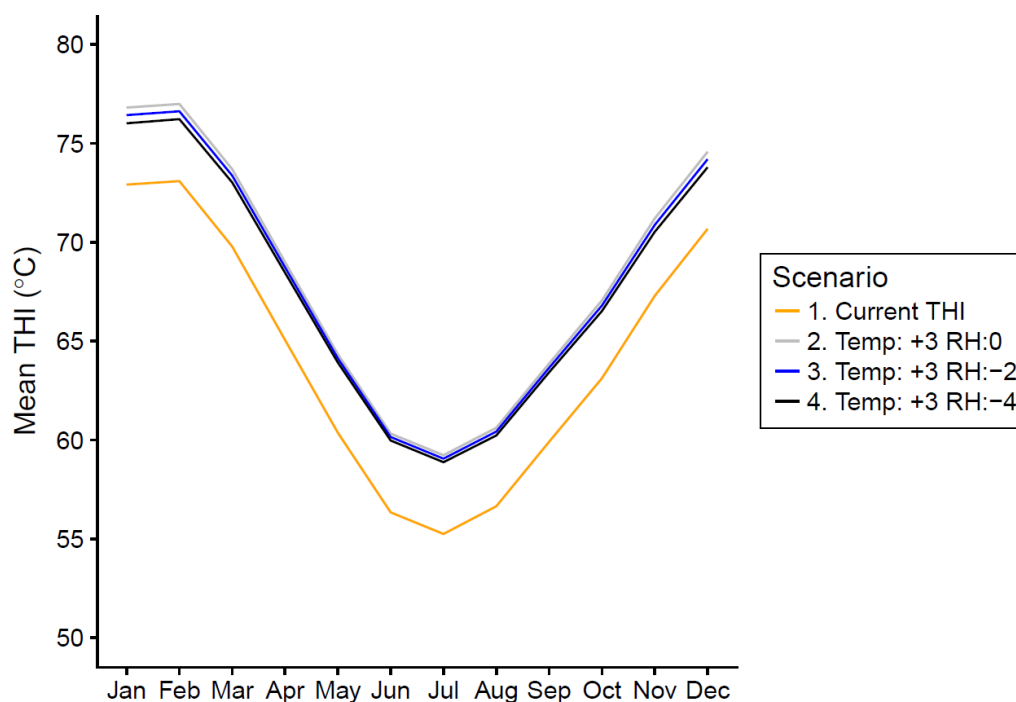


Figure 17. Impacts of relative humidity assumptions on THI

The changes in THI due to relative humidity assumptions are most pronounced in summer, when the THI is highest; however, the projected changes to relative humidity are lowest in this time period, and are largest in winter and spring when there is very little effect.

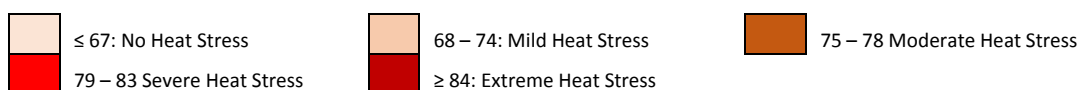
3.3.4. Selected exploratory scenarios

Based on the Climate Change in Australia projections, and a sensitivity analysis on relative humidity, the following scenarios will be used to explore future heat stress, in addition to the baseline of current climate, and using the THI threshold provided in Table 3-2. Categories of livestock weather safety index associated with THI Values (Table 3-2):

- 1°C increase in temperature, 0% change in relative humidity (implies ~6% increase to specific humidity)
- 3°C increase in temperature, 0% change in relative humidity (implies ~18% increase to specific humidity)

Table 3-2. Categories of livestock weather safety index associated with THI Values

Temp °C	Relative Humidity (%)																			
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
21	64	64	64	65	65	65	66	66	66	67	67	67	68	68	68	69	69	69	70	70
22	65	65	65	66	66	67	67	67	68	68	69	69	69	70	70	70	71	71	72	72
23	66	66	67	67	67	68	68	69	69	70	70	70	71	71	72	72	73	73	74	74
24	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	74	75	75	76	76
26	68	68	69	69	70	70	71	71	72	73	73	74	74	75	75	76	76	77	77	78
27	69	69	70	70	71	72	72	73	73	74	75	75	76	76	77	78	78	79	79	80
28	69	70	71	71	72	73	73	74	75	75	76	77	77	78	79	79	80	81	81	82
29	70	71	72	73	73	74	75	75	76	77	78	78	79	80	80	81	82	83	83	84
30	71	72	73	74	74	75	76	77	78	78	79	80	81	81	82	83	84	84	85	86
31	72	73	74	75	76	76	77	78	79	80	81	81	82	83	84	85	86	86	87	88
32	73	74	75	76	77	78	79	79	80	81	82	83	84	85	86	86	87	88	89	90
33	74	75	76	77	78	79	80	81	82	83	84	85	85	86	87	88	89	90	91	92
34	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94
36	76	77	78	79	80	81	82	83	85	86	87	88	89	90	91	92	93	94	95	96
37	77	78	79	80	82	83	84	85	86	87	88	89	90	91	93	94	95	96	97	98
38	78	79	80	82	83	84	85	86	87	88	90	91	92	93	94	95	97	98	99	100
39	79	80	81	83	84	85	86	87	89	90	91	92	94	95	96	97	98	100	101	102
40	80	81	82	84	85	86	88	89	90	91	93	94	95	96	98	99	100	101	103	104
41	81	82	84	85	86	88	89	90	91	93	94	95	97	98	99	101	102	103	105	106
42	82	83	85	86	87	89	90	92	93	94	96	97	98	100	101	103	104	105	107	108
43	83	84	86	87	89	90	91	93	94	96	97	99	100	101	103	104	106	107	109	110



Modified from Associated Livestock Weather Safety Index, Livestock Conservation Inc (1970).

3.3.5. Exposure to current and future climate

When analysing specific BoM weather station sites, all climate data comes from the closest data point in the Australian Gridded Climate Data dataset. There are three types of site-specific climate analysis presented in this report. The first details the known effects of heat stress during early (joining/d0 to 50), mid- (d51-100) and late-gestation (d101-150), as documented by Lindsay et al. (1975) and summarises the effects on the selected sites. The second details the heat stress experienced during early and late-lactation (d 0-50 and 50-100, respectively). The third is an in-depth analysis of how the climate variables and heat stress indices vary annually for each site (**Section 8**).

3.3.6. The identification and selection of key sheep producing sites across Australia

Twenty-six sites across Australia were selected within 23 Natural Resource Management (NRM) regions which represent the main sheep producing regions across Australia (**Figure 18**). For each of these sites, a BoM weather station was selected (**Table 8-1**) from which climate data was obtained and changes in THI scenarios were developed.

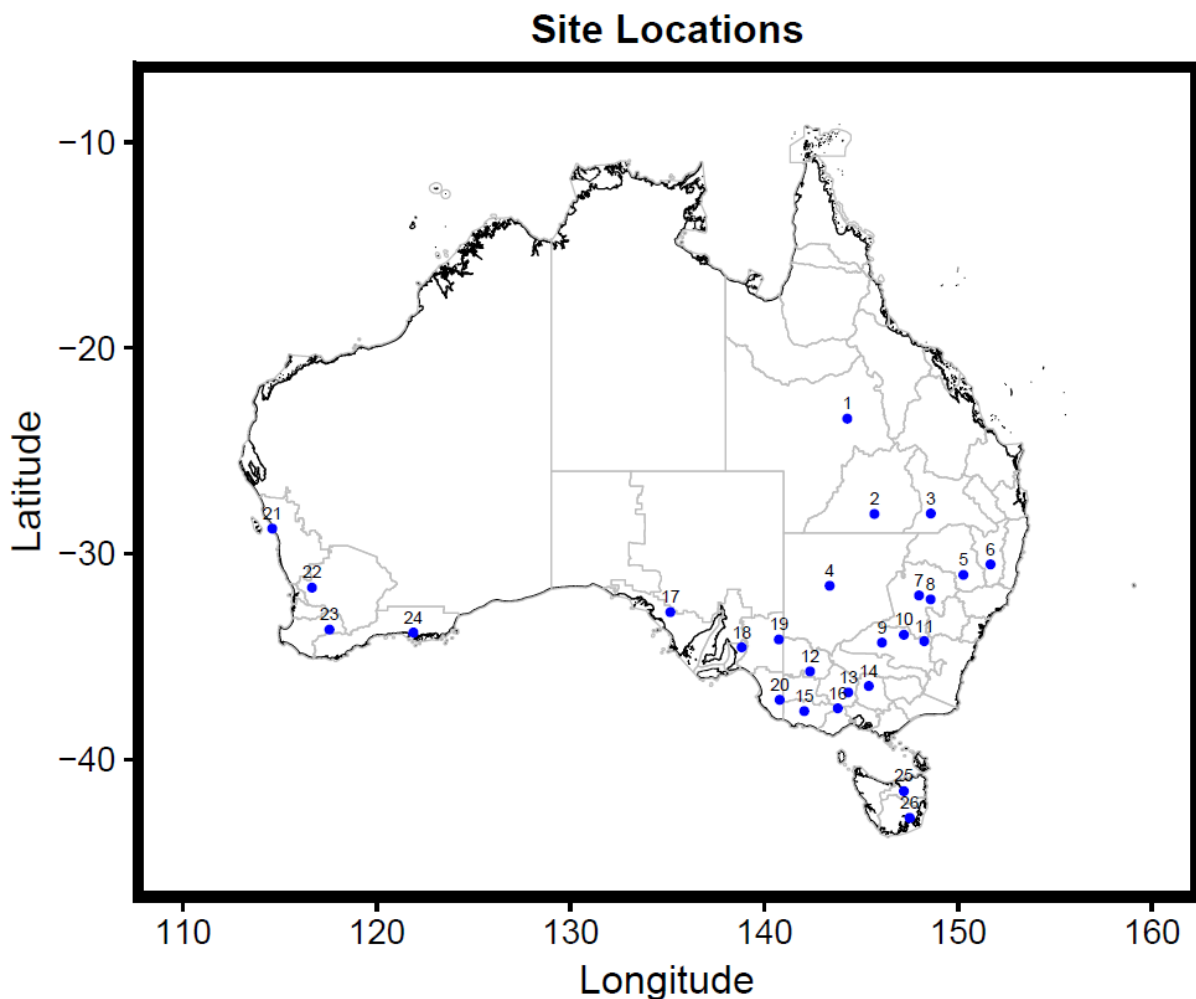


Figure 18. Map of BoM weather stations selected within the sheep producing regions across Australia

The 26 sites listed in **Table 3-3** were selected based on: (1) the overall sheep population across the NRM regions, and (2) sites that were currently or likely to be affected by high THI or increased number of hot days into the future. The remaining three sites were selected to reflect extremes in THI occurrences; Longreach in the Desert Channels region (north QLD) which currently experiences extreme high THI, and Launceston and Hobart in TAS which are largely unaffected by heat stress. For each selected site, sheep information was obtained from ABARES 2015-2016 census data for the Local Government Area (The Australian Bureau of Statistics, 2017), and updated sheep populations (ewes mated to produce lambs, and total lambs marked) per state was obtained from ABARES 2017-2018 data (The Australian Bureau of Statistics, 2019). Lambing percentages were calculated using the number of lambs marked against the number of ewes mated to produce lambs.

Locations within large NRM regions were not selected for specific heat stress climate change scenarios as the climate conditions within a single region were variable, and a single location would not have provided an accurate representation of the effect of THI on sheep production within that region. For example, the Rangelands in WA covers 85% of the state (2.2 million km²) and only consists of > 200k sheep. Similarly, SA Arid Lands makes up half the state of SA and shares borders with NSW, QLD and NT. While there are >800k sheep over 400 000 km², this one region has a variety of different ecosystems including sandy deserts, stony plains, as well as the Gawler, Flinders and Olary ranges. In both cases, one or two locations within a large area would not have provided an accurate representation of the effects of THI on sheep production within the region. While some sites/NRM regions were not included in the specific climate change projections presented in **Table 3-3** to **Table 3-10**, the seasonal effects for the decreased percentage of ewes joining due to heat stress (**Figure 22** to **Figure 25**), the decreased percentage of lambs born due to heat stress events during joining (**Figure 26** to **Figure 29**), and the seasonal difference in the number of days at moderate and severe THI thresholds (**Figure 30** to **Figure 37**) are presented on a national scale, and data can be extrapolated for these excluded regions.

3.3.7. Key reproductive events and breeding management calendars

Breeding management calendars were developed for each site using data extrapolated from Croker et al. (2009) as well as information provided by regional livestock consultants, industry partners and commercial producers. In the Croker et al. (2009) report, lambing times for each state's statistical division was calculated for 10, 50 and 90% ewes lambing, as well as the range of months that lambing occurred. Using a modified methodology here, the statistical regions for each of the sites in the current report were updated to match the current 2017-2018 ABS divisions, and key reproductive events established based on the information provided for the 2005 statistical division to match the current division. For example, in 2007, seven statistical divisions represented sheep producers in NSW, whereas the 2017-2018 census data reports 13 sheep-producing statistical divisions across 13 NRM regions within NSW.

Table 3-3. Key/major sheep producing sites/regions across Australia. Number of animals represented as thousand.

State	Map #	Site Information		Key Reproduction (month)			Site estimates				
		Location	NRM Region	Joining	Lambing	Range*	Total (n)	Ewes (n)	State % ¹	Marked lambs (n)	Lambing (%)
QLD	1	Longreach	Desert Channels	Mar	Aug	7	146.8	54.6	6.2	30.9	57
	2	Cunnamulla	South West	Mar	Aug	6	76.2	44.7	5.1	22.2	50
	3	St George	Q. Murray Darling Basin	May	Oct	8	360.7	154.6	17.6	138.8	90
<i>Total number of ewes mated to produce lambs within QLD</i>							878.3				
NSW	4	Wilcannia	Western	Feb	Jul	7	548.1	288.2	24.1	242.3	84
	5	Gunnedah	North West	Apr	Sep	6	50.5	31.9	2.7	33.8	106
	6	Armidale	Northern Tablelands	Apr	Sep	4	553.4	298.7	25.0	278.2	93
	7	Trangie	Central West	May	Oct	7	301.5	149.1	12.5	144.5	97
	8	Dubbo	Central West	Mar	Aug	7	870.1	401.8	33.6	410.7	102
	9	Griffith	Riverina	Dec	Apr	6	43.8	20.7	1.7	20.1	97
	10	West Wyalong	Riverina	Dec	Apr	6	608.5	283.1	23.7	269.7	95
	11	Young	Riverina	Mar	Aug	5	1 597.3	800.7	67.0	802.9	100
<i>Total number of ewes mated to produce lambs within NSW</i>							1 194.7				
VIC	12	Walpeup	Mallee	Apr	Sep	4	203.1	84.1	1.2	86.5	103
	13	Bendigo	North Central	Mar	Aug	6	229.8	108.5	1.6	100.1	92
	14	Shepparton	Goulburn Broken	Dec	Apr	5	117.4	58.7	0.9	64.1	109
	15	Hamilton	North Central	Apr	Sep	6	2 041.2	1 068.5	15.8	1 069.5	100
	16	Ballarat	Corangamite	May	Oct	6	85.9	37.5	0.6	40.7	109
<i>Total number of ewes mated to produce lambs within VIC</i>							6 748.1				
SA	17	Minnipa	Eyre Peninsula	Dec	Apr	5	168.3	70.9	1.3	64.5	91
	18	Rosedale	Adelaide/Mt Lofty Ranges	Dec	Apr	4	91.5	41.7	0.8	39.3	94
	19	Renmark	SA. Murray Darling Basin	Feb	Jul	4	208.2	96.4	1.8	92.7	96
	20	Struan	South East	Dec	Apr	4	1 018.1	502.7	9.2	552.3	110
<i>Total number of ewes mated to produce lambs within SA</i>							5 455.3				
WA	21	Geraldton	Northern Agricultural	Feb	Jul	4	179.9	79.7	1.1	66.3	83
	22	Northam	Wheatbelt	Feb	Jul	4	155.6	75.4	1.0	70.1	93
	23	Katanning	South West	Feb	Jul	4	214.4	103.4	1.4	94.6	92
	24	Esperance	South Coast	Feb	Jul	4	623.5	302.7	4.2	276.1	91
<i>Total number of ewes mated to produce lambs within WA</i>							7 199.9				
TAS	25	Launceston	North	Apr	Sep	3	4.7	19.7	1.7	22.1	112
	26	Hobart	South	May	Oct	3	2.9	1.1	0.1	343	30
<i>Total number of ewes mated to produce lambs within TAS</i>							1 129.5				

*Reproduction range determined by the number of months that 10 and 90% of ewes had lambs. Data extrapolated from Croker et al. (2009)

¹The site's contribution to the total number of ewes mated to produce lambs within the state

The joining and lambing periods reported in **Table 3-3** were estimated based on the month 50% of ewes had lambs, then back-dated 150 days to account for gestation to determine estimated joining month. The range of lambing, and/or joining period, was determined by the month where 10 and 90% ewes had lambs. For example, in Armidale (statistical division 130 in 2005-2007), peak lambing was reported to occur during September. Therefore, we estimate that the peak joining period is April. However, lambing was observed/recorded over four months, which suggests that for this site, joining periods are between February and May, and would be subject to individual producer management strategies.

The weaning periods estimated in the current report represents the youngest lambs to be weaned based on the month 90% ewes had lambs. Therefore, the weaning dates depicted on the current management calendars are indicative of a time when all breeding ewes should be dry from the selected ‘weaning’ month to the beginning of the ‘joining’ month.

3.3.8. Maximum temperature and decreases to birth rates/lambs lost

3.3.8.1. Data calculation and summary for each site

To demonstrate the effects of heat stress, site specific plots were developed for an 18-month calendar to visually demonstrate periods of pregnancy and lactation (**Section 8**). Monthly average t_{max} and t_{min} rainfall and temperature data for the 20-year period was obtained from the BoM. In the following paragraphs an explanation of these calendars for one site (West Wyalong) will be described as an example. **Figure 19** depicts West Wyalong, a climate with hot, dry summers and cool winters. Breeding management practices were overlaid based on peak lambing month reported by Croker et al. (2009). For West Wyalong, peak lambing occurs during winter when rainfall within the region is at its highest, which would coincide with pasture availability throughout lactation through to weaning. In the Riverina region, lambing occurs over a five to six month period, which indicates joining occurs from late spring through summer.



Figure 19. Monthly average temperature and rainfall for West Wyalong, NSW

The measurement of heat stress used by Lindsay et al. (1975) and Kleemann and Walker (2005) was the number of days in a week that the maximum temperature exceeded 32.2°C. For each site, a month when joining mostly occurred was determined, and the average number of days per week that t_{max} exceeded 32.2°C was calculated. This was first calculated at the daily time step – for the week centred on each day, then averaged across each month for all 20 years. **Figure 20** shows the mean weekly count of days with t_{max} above 32.2°C for West Wyalong. The mean number of days over 32.2°C peak during joining and early pregnancy.

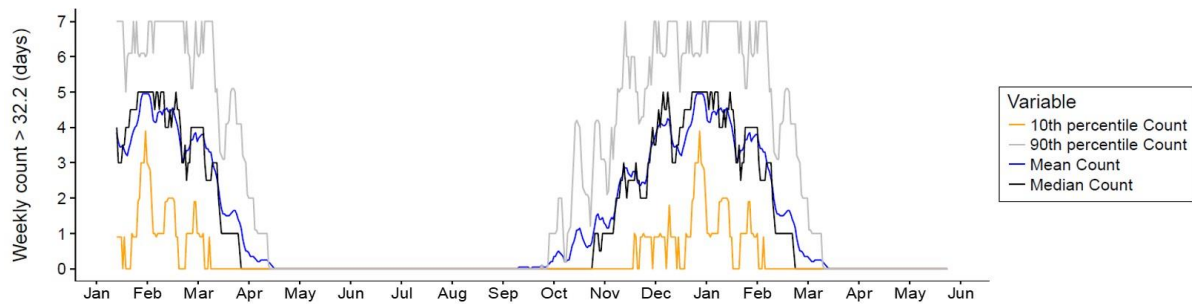


Figure 20. The mean, median, 10th and 90th percentile of the weekly number of days above 32.2°C at West Wyalong, NSW.

To determine the effects of heat stress for each of the selected sites, the average count of days with t_{max} above 32.2°C was used with the following two equations from Lindsay et al. (1975) to calculate the number of ewes lambing per 100 ewes joined (Y_1) and the number of lambs born per 100 ewes joined.

$$Y_1 = 61.6 + 0.3X_1 + 2.5X_2 - 2.7X_3$$

$$Y_2 = 52.6 + 0.6X_1 + 3.7X_2 - 3.5X_3$$

where X_1 is body weight (kg), X_2 is age (yrs) and X_3 is the mean days per week with t_{max} above 32.2°C.

The mean number of days per week that t_{max} exceeded 32.2°C was calculated and the daily time step was calculated for the week by centering on each day then averaging across each month for all 20 years. Considering only the heat stress component of these findings, multiplying the days per week with t_{max} above 32.2°C by 2.7 gives the percentage decrease in number of ewes lambing, and multiplying by 3.5 gives the percentage decrease in the number of lambs born. The average count of days with t_{max} above 32.2°C for each month was used to determine the effects on the number of ewes lambing and lambs born should joining occur in that month. The percentage decrease for both variables was then multiplied by the annual number of ewes mated to produce lambs to estimate the number of ewes and lambs affected (**Table 3-4**).

The current heat stress effects for West Wyalong shows that the mean THI, and frequency above moderate and severe THI thresholds, increases throughout joining and early pregnancy (**Figure 21**). Under current climate conditions, the frequency of moderate heat stress increases from November, with the frequency of severe heat stress events increasing approximately a month later.

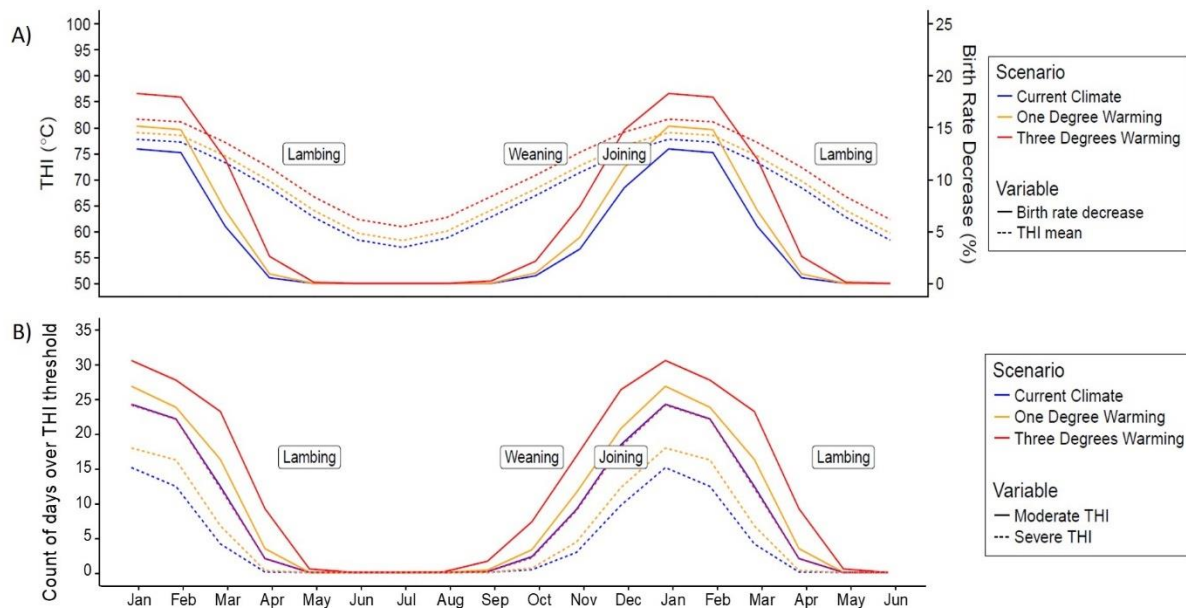


Figure 21. The THI measure and thresholds frequency for West Wyalong over 18-months for the three climate scenarios. **A)** The monthly mean THI (°C) and associated decrease in birth rates (%). **B)** The frequency and duration of moderate and severe THI events.

The current frequency and duration of days over 32.2 °C during the joining period in West Wyalong results in over 20,000 ewes (7%) unsuccessfully mated, leading to a 9% decrease in lambs born, which equates to a loss of 26,000 lambs (Table 3-4). For the two projected climate scenarios, the number of ewes unsuccessfully mated will increase to 9 and 11% at +1°C and +3°C warming, respectively, with the number of potential lambs lost increasing to 11 and 15%.

Calendars for each of the 26 sites are available in **Section 0**.

3.3.8.2. Heat stress during mating and decreases in birth rates/lambs lost

The effect of days over 32.2°C (heat stress) during the month when 50% of ewes are joined on the number of ewes lambing and the number of ewes born per mated ewes are presented in Table 3-4. In addition, plots for the percentage of ewes (Figure 22 to Figure 25) lambing and the percentage of lambs lost (Figure 26 to Figure 29) demonstrate the effects heat stress has on both variables on a national scale. A summary of the effects of heat stress by state and for flocks joining in spring through early autumn (October to March) or Late autumn / winter (April to September) is as follows:

- **Queensland.**
 - For flocks mating in spring through to early autumn, the average decrease in the number of ewes lambing at current, +1°C and +3°C climates was 15% (n = 7,400 ewes), 16% (8,050 ewes) and 17.5% (8,650 ewes), respectively, with the average decrease in the number of lambs born calculated to be 19% (9,600 lambs), 20.9% (10,500 lambs), and 22.5% (11,300 lambs).
 - For flocks mating in late autumn through winter, the decrease in the number of ewes lambing at current, +1°C and +3°C climates was 0%, 0% and 1% (n = 1,100 ewes)

respectively, with the decrease in the number of lambs born calculated to be 0%, 0.1% (n = 200 lambs), and 1% (n = 1,500 lambs).

- **New South Wales**

- For flocks mating in spring through to early autumn, the average decrease in the number of ewes lambing at current, +1°C and +3°C climates was 7% (20,700 ewes), 8.2% (25,380 ewes) and 11.4% (37,180 ewes), respectively, with the average decrease in the number of lambs born calculated to be 9% (26,800 lambs), 10.8% (32,900 lambs) and 14.8% (48,180 lambs)
- For flocks mating in late autumn through winter, the average decrease in the number of ewes lambing at current, +1°C and +3°C climates was 0%, 0.3% (n = 67 ewes) and 1% (367 ewes) respectively, with the average decrease in the number of lambs born calculated to be 0%, 0.23% (67 lambs) and 1% (467 lambs).

- **Victoria**

- For flocks mating in spring through to early autumn, the average decrease in the number of ewes lambing at current, +1°C and +3°C climates was 2% (2,100 ewes), 3% (2,900 ewes) and 5% (5,500 ewes) respectively, with the average decrease in the number of lambs born calculated to be 3% (2,700 lambs), 3.5% (3,800 lambs) and 7% (7,200 lambs).
- For flocks mating in late autumn through winter, the average decrease in the number of ewes lambing at current, +1°C and +3°C climates was 1.25% (950 ewes), 1.5% (1,450 ewes) and 3.25% (3,575 ewes) respectively, with the average decrease in the number of lambs born calculated to be 1.5% (1,250 lambs), 2.2% (1,875 lambs) and 4% (4,625 lambs).

- **South Australia**

- For flocks mating in spring through to early autumn, the average decrease in the number of ewes lambing at current, +1°C and +3°C climates was 5.3% (6,775 ewes), 6.3% (8,125 ewes) and 8.3% (10,925 ewes) respectively, with the average decrease in the number of lambs born calculated to be 7.3% (8,775 lambs), 8.3% (10,500 lambs) and 10.8% (14,175 lambs)
- Flocks were not mated in late autumn through winter in the areas studied

- **Western Australia**

- For flocks mating in spring through to early autumn, the decrease in the number of ewes lambing at current, +1°C and +3°C climates was 6.8% (7,000 ewes), 8% (8,075 ewes), and 10.5% (10,725 ewes) respectively, with the decrease in the number of lambs born calculated to be 9% (9,075 lambs), 10.2% (10,450 lambs) and 13% (13,925 lambs).
- Flocks were not mated in late autumn through winter in the areas studied

- **Tasmania**

- There were no reductions in either the number of ewes lambing or the number of lambs born.

Combining the data from the sites investigated in Queensland, New South Wales, Victoria, South Australia and Western Australia, for flocks joined during spring through early autumn, heat stress at

joining is responsible for a 2 – 17% decrease in the number of ewes lambing and a 4 - 19% decrease in the number of lambs born under current climatic conditions. This increases to a 4 – 19% decrease in ewes lambing and a 5 – 24% decrease in the number of lambs born under a +3°C increase in temperatures. In flocks which join during late autumn or during winter, the impacts of heat stress during the joining period are less severe, with the maximum decrease in ewes lambing and lambs born approximately 1% under current conditions and increasing to 3% should temperatures increase by 3°C.

3.3.9. Mild, moderate and severe THI during pregnancy and lactation

To determine the effects of heat stress during the key reproductive stages, the number of days above the mild (THI 68: **Table 3-5** and **Table 3-8**), moderate (THI 75: **Table 3-6** and **Table 3-9**) and severe (THI 79: **Table 3-7** and **Table 3-10**) thresholds were calculated. Note that an assumption here, is that the timing of the daily maximum temperature being different from the mean of the vapour pressure does not influence the accuracy significantly. With the daily THI values calculated, the number of days above thresholds can be counted. The definition of mild, moderate and severe THI thresholds was 68, 75 and 79, respectively (**Table 3-2**).

Gestation and lactation were divided into five 50-day increments (first 50 days of gestation, middle 50 days of gestation, last 50 days of gestation, first 50 days of lactation and second 50 days of lactation). For each of the 26 selected sites, we evaluated the number of days at mild, moderate and severe THI thresholds (**Table 3-5** to **Table 3-10**). Seasonal plots provided below show the number of days at each THI threshold for each climate scenario, demonstrating the current seasonal effects of heat stress on a national scale at well as the effect at +1 and +3°C warming (**Figure 30** to **Figure 37**).

Table 3-5 demonstrate that all sites are affected by mild heat stress throughout early gestation regardless of the joining month. For many sites which join ewes over the summer/early autumn period, the number of days at the mild THI threshold during early gestation can exceed 40 days (**Table 3-5**). For those sites joining ewes in autumn, the number of days at the mild THI threshold tends to increase during late gestation, then throughout early and late lactation. While the effect of moderate and severe heat stress events affect gestation, mild heat stress events are more like to affect lactation (**Section 2.10**).

In QLD, two of the selected sites have peak joining periods during early autumn; however, as joining occurs over a six to seven-month period, the effects of moderate and severe heat stress would affect some ewes at all stages of reproduction at these sites (Longreach 54.6k ewes; Cunnamulla 44.6k ewes). Longreach is the only site where mild, moderate and/ or severe heat stress events are present from joining through to weaning. Ewes in this region experience an average of 46 days at the mild THI, and nearly 10 days at the severe THI threshold (moderate: 27.8 days). While Cunnamulla has fewer total ewes, the effects are similar, in that 12% of ewes are unsuccessfully joined resulting in 16% decrease in lambs born.

The national plots (**Figure 22** to **Figure 25**) show that in other sites across central and arid QLD, currently > 10% ewes are unsuccessfully joined, which will increase up to 17% at +3°C warming if joined in late summer/early autumn. While sites along the east coast and inland, such as the Darling Downs, currently have more ewes successfully mated during the same joining period, by +3°C warming, up to 15% of ewes in these regions will be unsuccessfully joined.

In contrast, the peak joining period for St George occurs in late autumn (May), and while joining can happen over an eight-month period, the current low level of lambs lost (1%) indicates the effects of gestational heat stress is less likely to occur for ewes in this region. However, a high number of mild, moderate and severe heat stress events currently occur throughout lactation, with close to 29 days at

the severe THI threshold during late lactation. At +3°C warming, this number of days will increase to over 40 days at the severe THI threshold.

Five of the selected sites for NSW join ewes during the summer/autumn period (Wilcannia, Dubbo, Griffith, West Wyalong and Young). As a result, many ewes at these sites experience mild, moderate and severe heat stress events throughout gestation, whereas in the other three selected sites that join later in the year (Gunnedah, Armidale and Trangie) heat stress will effect lactation rather than gestation. Under current climate conditions, Wilcannia experiences the highest number of moderate and severe heat stress events, with an average of 50 days at or above the moderate THI threshold during early and mid-gestation. Dubbo and Young, have <20 days at these THI thresholds during early gestation. In contrast, Griffith and West Wyalong experience a longer duration of heat stress events, 100 and 106 days, respectively, at both moderate and severe THI thresholds from joining to late gestation. Of the 1.7 million ewes at these combined sites, between 3 and 13% of ewes are currently unsuccessfully joined, resulting in a 4 to 17% decrease in lambs born; approximately 92k lambs lost. At +1 and +3°C warming these will increase to 162k and 239k lost lambs due to heat stressed ewes at joining, respectively.

For Gunnedah, Armidale and Trangie, joining occurs during mid to late autumn resulting in a spring lamb drop, which indicates heat stress events occur during lactation to weaning. While the number of ewes mated and lambs born are higher for these three sites, growth and development of the lamb(s) is compromised as lactation, specifically milk production and yield, are affected when ewes reach mild THI thresholds. While Trangie reported 144k lambs marked, this site experiences over 65 days at the moderate and severe THI threshold during lactation. At +1 and +3°C warming, the number of days at these thresholds will increase to 80 and 110 days, respectively, indicating that ewes at this site will be under constant heat stress throughout lactation. For Gunnedah, the effects of heat stress are only prominent during late lactation, with over 20 days at moderate and/or severe THI threshold. This will increase to over 50 days at +3°C warming. The ewes at Armidale are largely unaffected by heat stress, with < 1% ewes unsuccessfully joined and lambs lost, and 3 day at a moderate THI threshold from joining to weaning.

Four of the five sites in VIC (Walpeup, Bendigo, Hamilton, Ballarat) have autumn joining periods, and subsequently, have less moderate and severe THI heat stress effects during early gestation; each site experiences < 5 days at moderate THI threshold (less than 1 day at severe THI threshold). Due to the later joining period, the effects of heat stress would be present during lactation. Bendigo has the greatest number of days at mild THI threshold (25 days), whereas Walpeup has the longest heat stress effects, with up to 13 days at the moderate and severe THI during early and late lactation.

Shepparton, on the other hand, experiences moderate and severe heat stress events throughout gestation (early: 27.4; mid: 34.4; late: 4.1 days, respectively). This equates to a 3% decrease in ewes joining and 4% lambs born due to heat stress events. At +1 and +3°C warming, the number of days at or above a moderate THI increases to 80 and 123 days, respectively, equating to a 7% decrease in ewes successfully joined and a 9% decrease in lambs born due to heat stress events.

For both SA and WA, the joining period is shorter (4 months) compared to the other states. While joining begins during late spring, peak joining predominantly occurs during summer (late summer in WA). The seasonal winter rains across the southern parts of the states means pasture availability for the winter/spring lamb drop. As a trade-off to feed availability in winter, ewes experience mild, moderate and severe heat stress events prior to, and during, joining, early and mid-gestation. For the SA region, ewes at Renmark reach moderate or severe THI thresholds for approximately 30% of gestation. The intensity of this heat stress event results in a 9% decrease in the number of ewes successfully mated, and a 12% decrease in lambs born due to heat stress events. Ewes in Struan are also affected by heat stress for 30% of gestation; however, comparable to Renmark, only 2% of ewes are unsuccessfully mated and 3% lambs lost due to heat stress events. In contrast, ewes in Minnipa and Rosedale experience between 50 and 60% of gestation at or above the moderate THI thresholds (Minnipa: moderate THI: 60 days; severe THI: 32 days; Rosedale: THI: 51 days; severe THI: 25 days). Further, Rosedale ewes experience a high number of days at the mild THI threshold during early lactation. This will impact lamb growth and survival to weaning at Rosedale, whereas the other three sites across SA have very minor (if any) heat stress event during lactation.

For the two climate change scenarios for SA, the impacts of heat stress will affect all stages of reproduction. For example, at Minnipa the number of days at moderate and severe THI will increase from 90 days currently to 109 and 149 days at +1 and +3°C warming, respectively. In the SA regions, the forecasted heat stress events will result in a 9% to 12% decrease in ewes successfully mated (up to 6k lambs) and over 40k lost lambs due to heat stressed ewes unsuccessfully mated during joining.

As joining occurs during late summer at the WA sites, the impacts of heat stress occur during early and mid-gestation. Two of the four WA sites (Geraldton and Northam) have > 50 days at moderate and severe THIs during early gestation, suggesting that ewes are heat stressed prior to the joining period as well as through early gestation. For these sites combined, nearly 14k ewes are unsuccessfully mated during the joining period, and close to 20k lambs lost due to these unsuccessful mating. At +1°C warming, ewes will experience moderate and severe THI thresholds for 60% gestation, and at +3°C warming, this will increase to 90% gestation under moderate and severe heat stress conditions.

Comparable to Geraldton and Northam, ewes at Katanning and Esperance currently experience moderate and severe heat stress events throughout approximately 30% gestation; <15 days at the severe THI threshold. As a result, only 5% ewes are unsuccessfully mated at Katanning, and <3% at Esperance. This equates to <10%, or 9k lambs, lost due to heat stress events during joining.

During gestation, heat stress restricts the growth and weight of the conceptus, as well as viability and therefore survivability. Under current climate conditions, there are three sites (Wilcannia, Geraldton and Northam) where ewes reach moderate heat stress for > 70% of early gestation, and an additional 5 sites where the moderate THI threshold makes up 50% early gestation. This equates to over 900k ewes experiencing moderate THI conditions from joining to d50 gestation. When evaluating the effects of heat stress through to mid-gestation (d0 to d100), there are over 400k ewes from 4 sites (Griffith, West Wyalong, Minnipa, Geraldton) where half of this gestational period is under moderate

heat stress conditions. At +1 and +3°C this number increases to 7 sites (>800k ewes) and 12 sites (1.1 million ewes), respectively.

While these specific site descriptions have detailed the effects on regions or producer sites across Australia during gestation and lactation, for many sites that join in late spring, summer and early autumn, the negative effects of heat stress begin prior to the joining period. For ewes, acute heat stress periods can have detrimental effects on the quality of the ovum, reduce fertilisation, and increase embryo wastage. Whereas in the working ram, the effects of high heat are delayed (between 6 and 90 days), with significant decreases in seminal quality such as motility, sperm number and fertilisation rate. This suggests that for the sites which join ewes in autumn to avoid heat stress reproduction failures, ram fertilisation may still be compromised from previous moderate and severe heat stress events during the summer. The national plots from **Figure 22** to **Figure 37** provide valuable information of when the effects of heat stress are limited and may be used to develop or adapt current management practices to optimise the number of ewes successfully joined, and increase the number of lambs born. While shifting joining times to a cooler period may reduce embryo wastage, the effect of heat stress during lactation will impact lamb growth, development and survival to weaning.

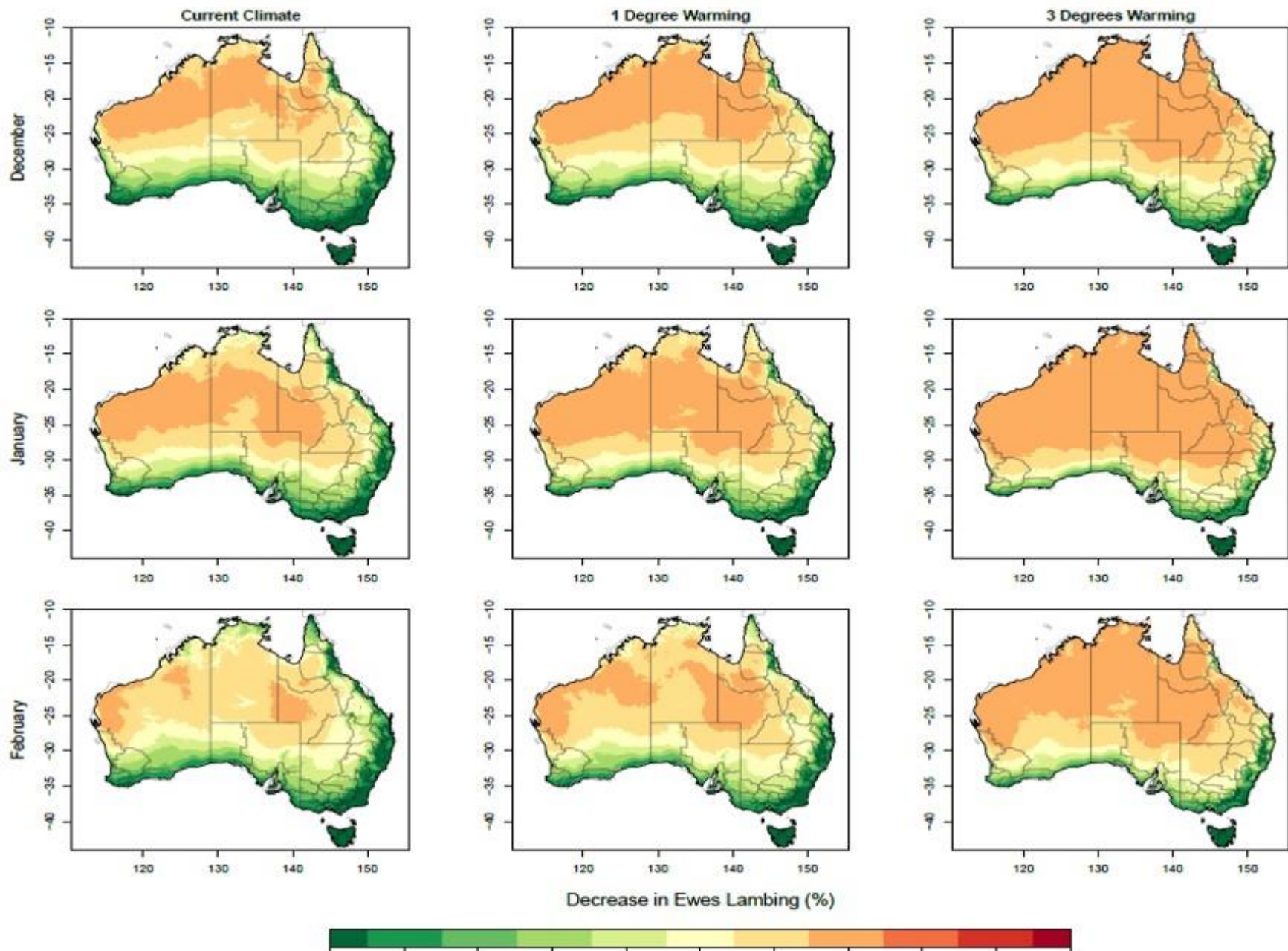


Figure 22. Decrease in ewes lambing (%) due to heat stress in summer

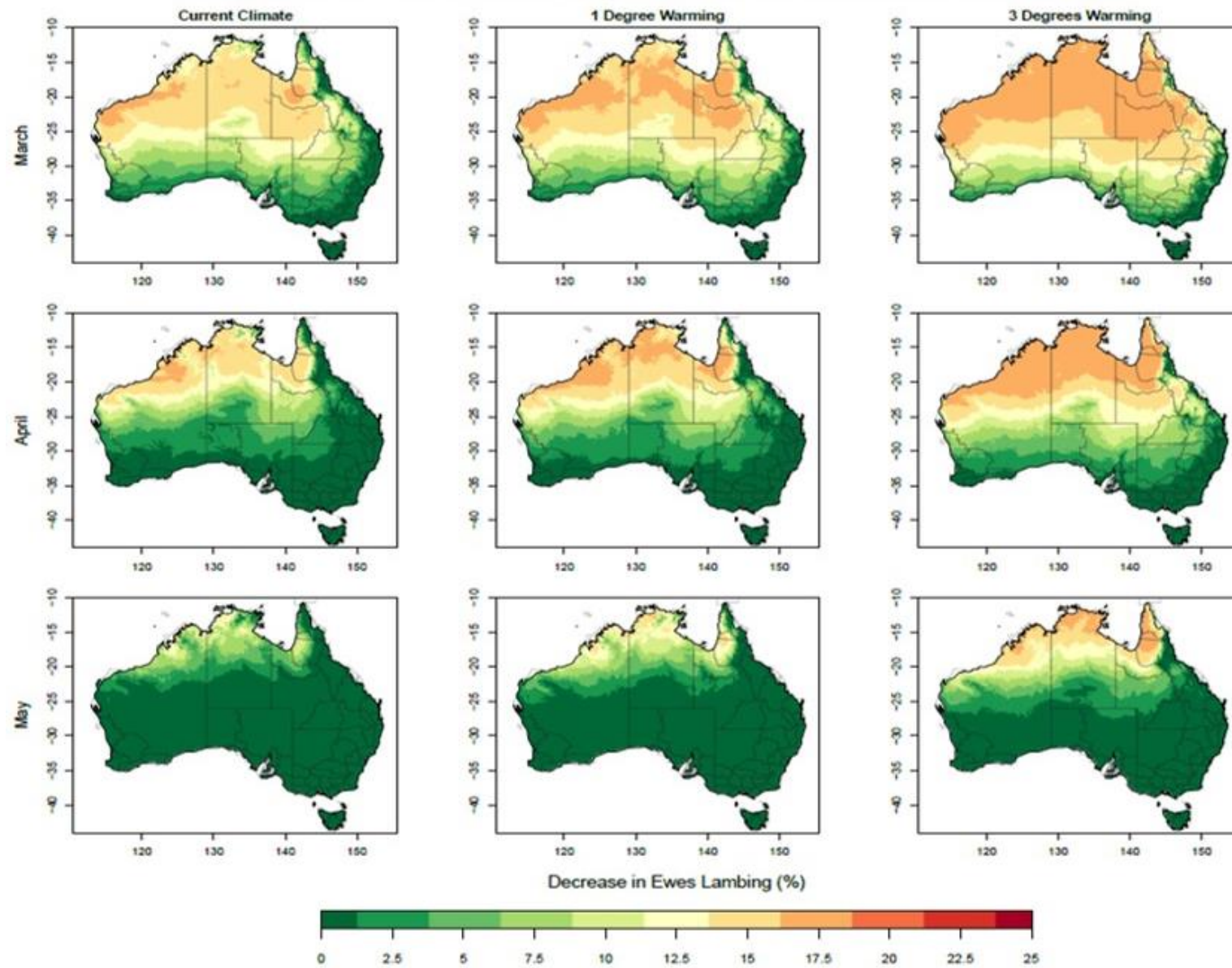


Figure 23. Decrease in ewes lambing (%) due to heat stress in autumn

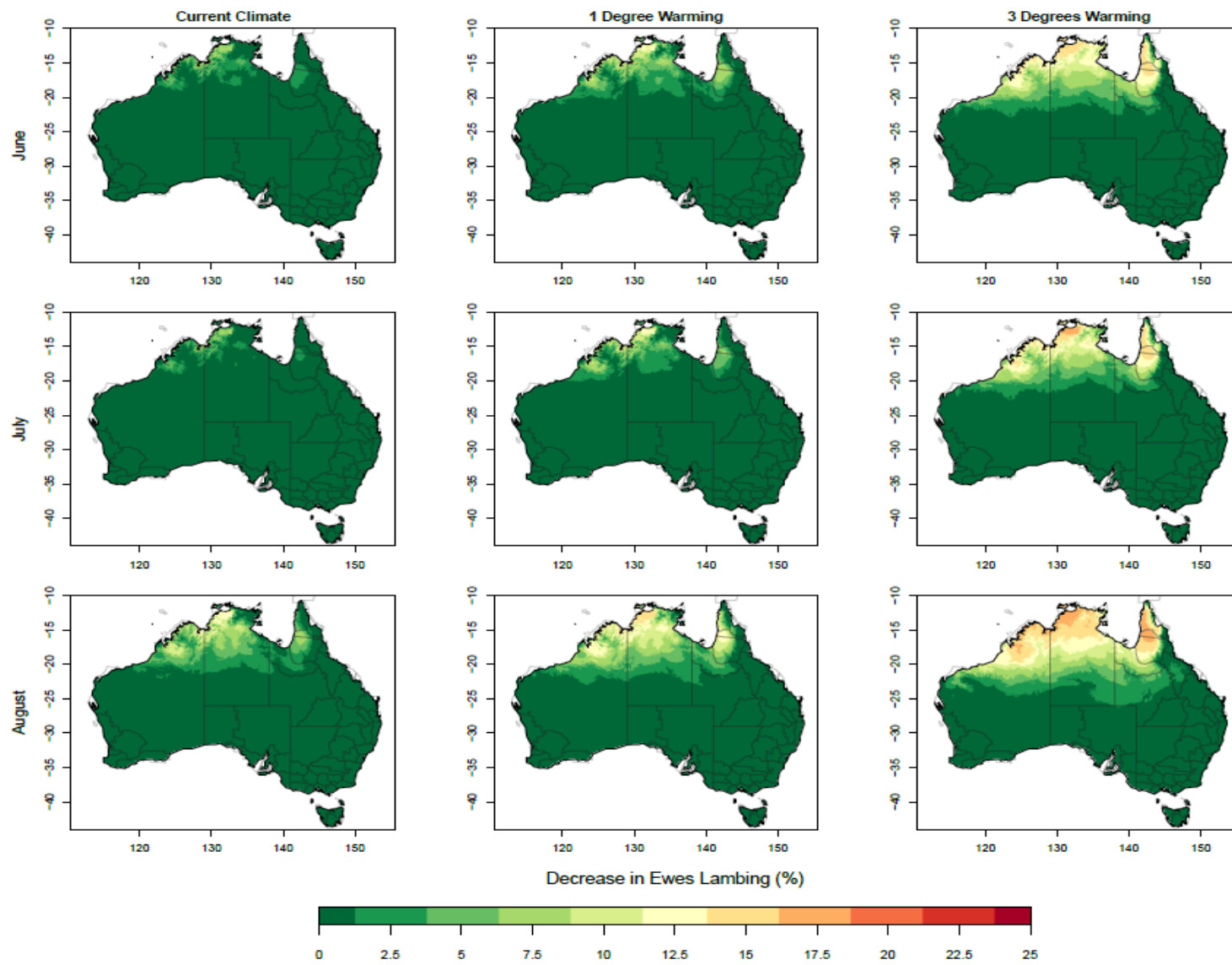


Figure 24. Decrease in ewes lambing due to heat stress in winter

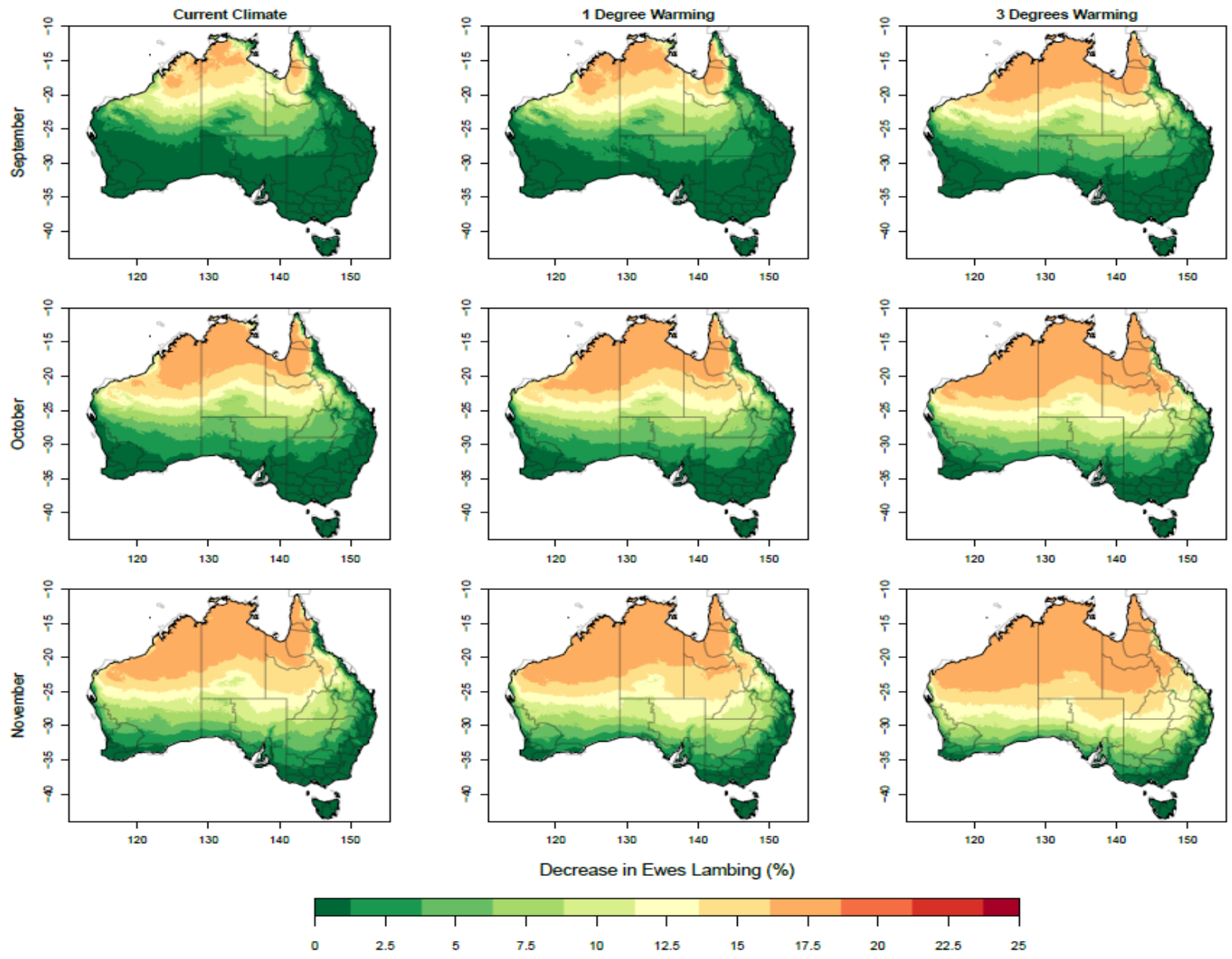


Figure 25. Decrease in ewes lambing due to heat stress in spring

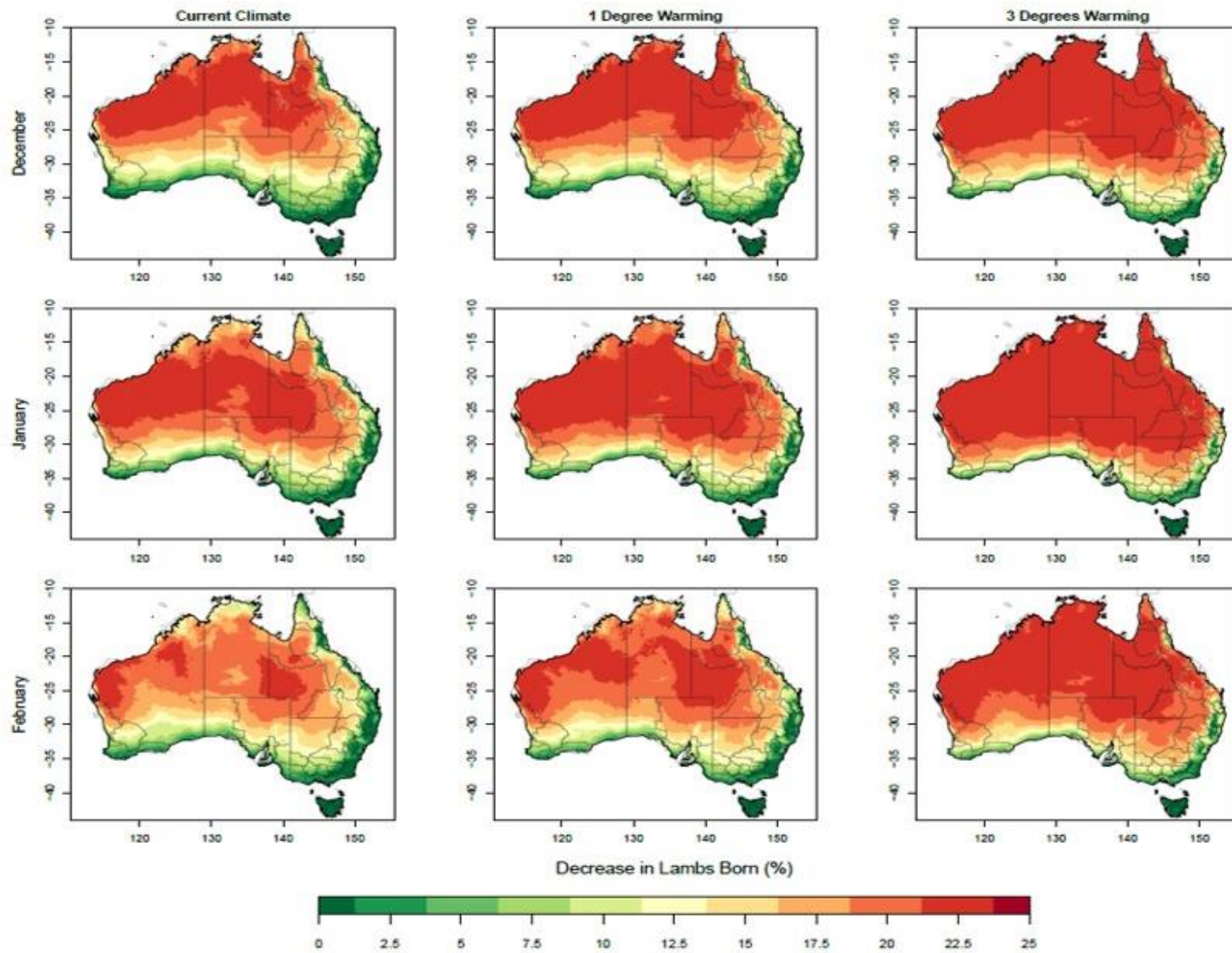


Figure 26. Decrease in lambs born (%) due to heat stress in summer.

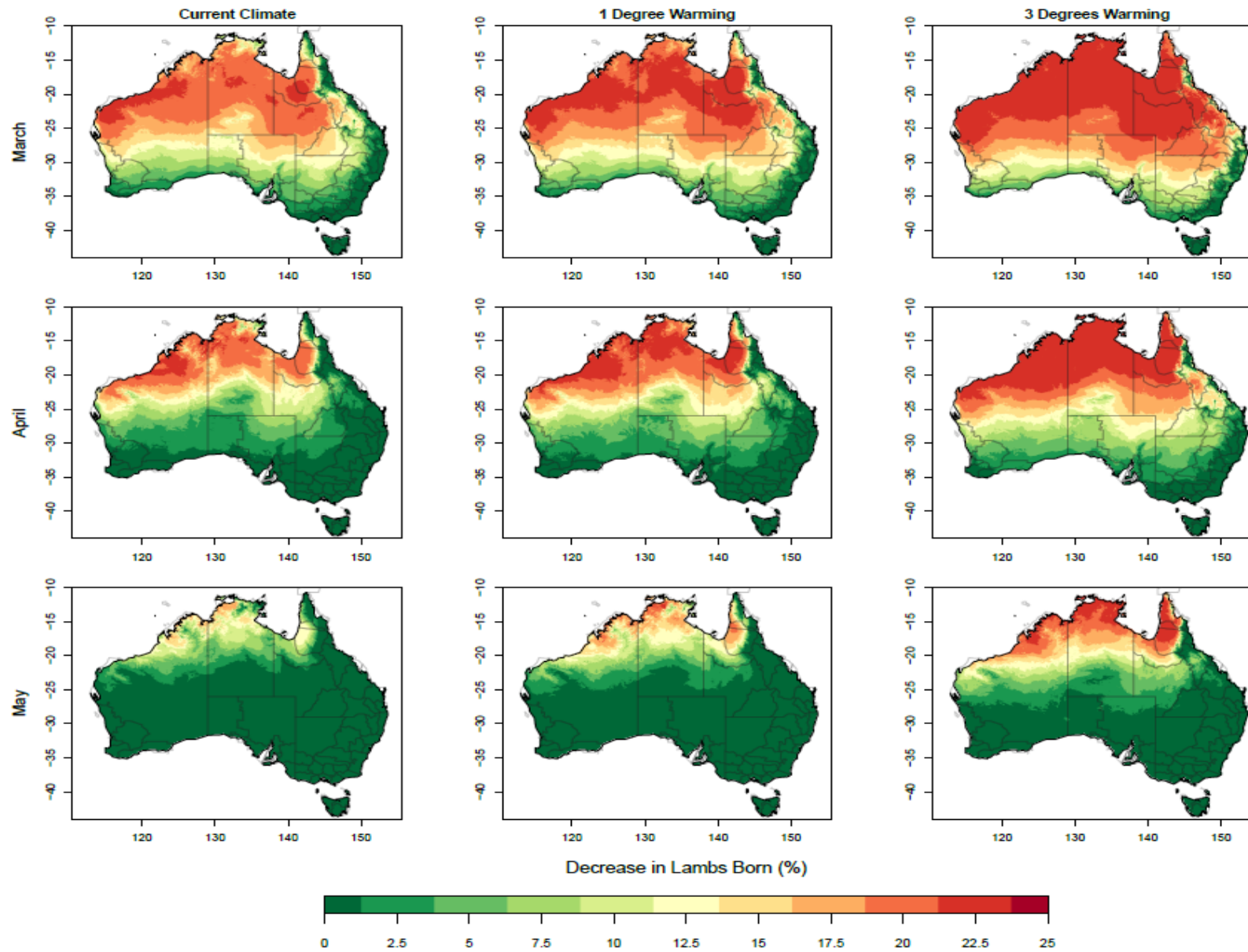


Figure 27. Decrease in lambs born due (%) to heat stress in autumn

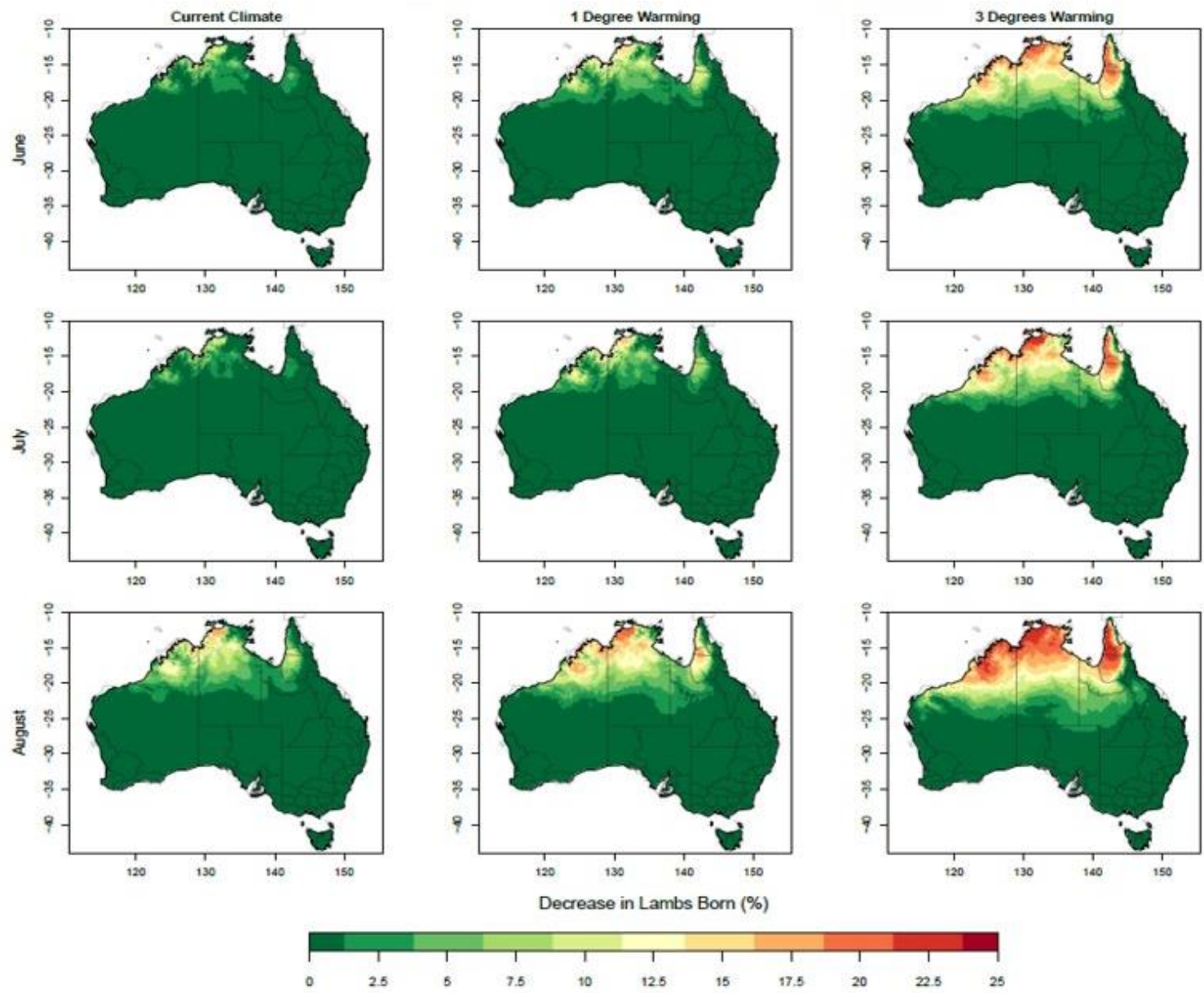


Figure 28. Decrease in lambs born (%) due to heat stress in winter

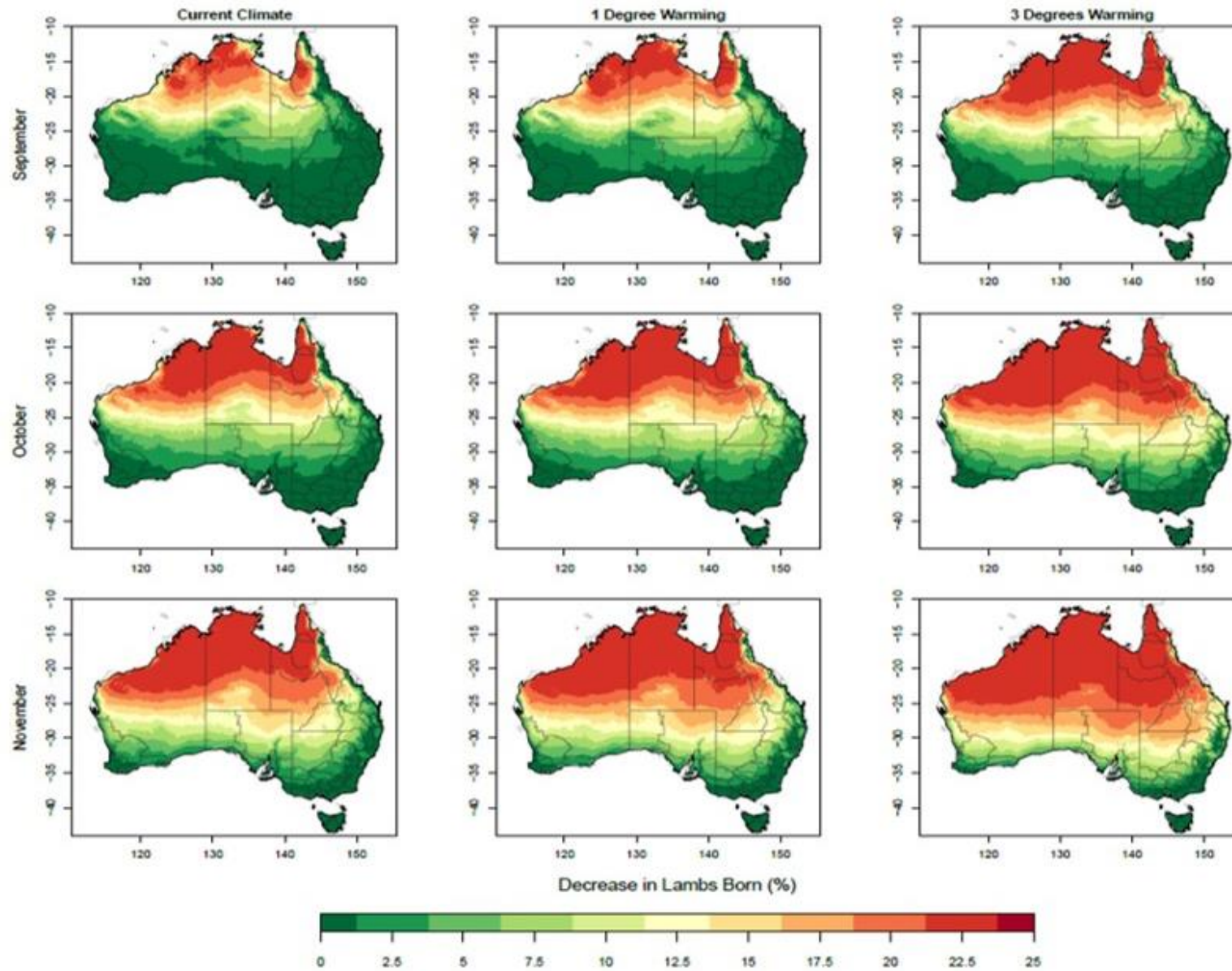


Figure 29. Decrease in lambs born (%) due to heat stress in spring

Table 3-5. Number (n) of mild heat stress (THI 68) occurrences throughout gestation calculated for 26 sheep producing sites across Australia

State	Site	Ewes (n)	Joining Month	Early gestation (d0-50)			Mid-gestation (d51-100)			Late gestation (d101-150)		
				Current	+1 °C	+3 °C	Current	+1 °C	+3 °C	Current	+1 °C	+3 °C
QLD	Longreach	54.6	Mar	45.8	47.8	49.8	24.0	27.7	37.5	23.7	28.4	36.8
	Cunnamulla	44.7	Mar	47.6	48.4	49.6	23.7	28.9	38.2	3.5	6.1	14.9
	St George	154.6	May	19.0	24.2	34.3	4.0	7.6	17.1	21.4	25.8	36.1
NSW	Wilcannia	288.2	Feb	48.6	49.2	50.0	33.9	39.0	45.0	4.2	7.7	16.4
	Gunnedah	31.9	Apr	28.7	33.9	43.5	1.1	2.7	11.0	1.5	3.0	9.3
	Armidale	298.7	Apr	3.3	6.8	19.3	0.0	0.1	0.1	0.2	0.3	0.9
	Trangie	149.1	May	5.4	9.0	20.7	0.1	0.3	3.1	9.6	12.8	21.6
	Dubbo	401.8	Mar	42.3	45.7	48.6	10.8	15.2	28.4	0.0	0.1	1.8
	Griffith	20.7	Dec	46.1	47.8	49.5	47.2	48.3	49.7	31.7	36.4	44.1
	West Wyalong	283.1	Dec	46.6	48.1	49.4	47.4	48.4	49.6	32.9	37.6	44.7
	Young	800.7	Mar	35.6	40.0	45.6	4.9	8.0	17.4	0.0	0.0	0.0
VIC	Walpeup	84.1	Apr	13.5	17.4	28.1	0.2	0.3	2.3	0.4	0.7	2.5
	Bendigo	108.5	Mar	23.6	29.0	37.1	1.8	2.9	7.4	0.0	0.0	0.0
	Shepparton	58.7	Dec	40.3	43.4	48.1	43.4	46.2	48.7	24.6	29.1	38.6
	Hamilton	1 068.5	Apr	17.1	21.4	31.4	1.7	3.0	5.9	0.0	0.0	0.0
	Ballarat	37.5	May	14.5	17.8	26.5	0.8	1.2	3.5	0.0	0.0	0.0
SA	Minnipa	70.9	Dec	42.8	45.8	49.0	46.1	48.3	49.8	32.6	38.0	46.3
	Rosedale	41.7	Dec	30.5	34.1	42.7	42.1	45.5	49.1	39.3	43.2	48.7
	Renmark	96.4	Feb	46.1	47.9	49.6	25.0	31.0	41.1	1.6	3.2	9.8
	Struan	502.7	Dec	30.9	36.1	43.3	37.2	40.3	47.8	17.3	22.1	34.0
WA	Geraldton	79.7	Feb	49.9	49.9	50.0	46.3	48.3	49.8	16.5	25.4	41.2
	Northam	75.4	Feb	49.5	49.8	50.0	38.7	43.3	48.1	4.6	8.6	22.2
	Katanning	103.4	Feb	45.2	46.8	49.2	37.3	41.7	46.3	10.6	14.7	26.7
	Esperance	302.7	Feb	34.7	40.5	48.1	23.7	30.5	42.4	3.1	6.1	17.2
TAS	Launceston	19.7	Apr	1.0	2.3	7.8	0.0	0.0	0.0	0.0	0.0	0.0
	Hobart	1.1	May	0.1	0.4	1.4	0.0	0.0	0.0	0.5	0.7	2.2

Table 3-8. Number of mild heat stress (THI 68) occurrences throughout lactation calculated for 26 sheep producing sites across Australia

State	Site	Ewes (n)	Lambing	Early lactation (d0-50)			Late lactation (d51-100)		
				Current	+1 °C	+3 °C	Current	+1 °C	+3 °C
QLD	Longreach	54.6	Mar	44.8	46.7	48.9	49.8	49.9	50.0
	Cunnamulla	44.7	Mar	13.8	18.2	29.1	41.8	44.5	48.3
	St George	154.6	May	45.1	47.2	49.4	49.6	49.8	50.0
NSW	Wilcannia	288.2	Feb	1.0	2.2	7.8	17.8	22.5	33.1
	Gunnedah	31.9	Apr	22.1	26.0	37.8	43.1	45.3	48.7
	Armidale	298.7	Apr	4.8	7.9	15.9	22.1	27.2	37.1
	Trangie	149.1	May	34.6	38.8	45.8	48.0	48.6	49.8
	Dubbo	401.8	Mar	3.1	5.1	12.1	27.6	33.3	41.2
	Griffith	20.7	Dec	3.7	6.5	15.1	0.0	0.1	0.8
	West Wyalong	283.1	Dec	2.8	5.3	12.9	0.0	0.0	0.4
	Young	800.7	Mar	0.7	2.0	5.1	16.8	21.8	31.8
VIC	Walpeup	84.1	Apr	8.5	11.6	19.8	29.0	33.2	43.0
	Bendigo	108.5	Mar	0.2	0.5	1.7	8.0	10.7	18.7
	Shepparton	58.7	Dec	0.9	2.4	7.6	0.0	0.0	0.0
	Hamilton	1 068.5	Apr	0.1	0.3	1.3	5.8	7.3	12.7
	Ballarat	37.5	Ma	0.0	0.0	0.5	3.4	5.6	9.5
SA	Minnipa	70.9	Dec	5.3	8.4	18.6	0.2	0.4	2.9
	Rosedale	41.7	Dec	15.3	19.5	31.6	0.4	1.0	3.7
	Renmark	96.4	Feb	0.5	0.9	3.1	9.8	13.3	23.1
	Struan	502.7	Dec	1.4	2.5	6.7	0.0	0.0	0.0
WA	Geraldton	79.7	Feb	3.6	7.8	26.6	11.9	19.6	38.1
	Northam	75.4	Feb	0.3	1.2	7.6	8.5	12.7	25.8
	Katanning	103.4	Feb	0.1	0.1	2.3	0.2	0.6	2.3
	Esperance	302.7	Feb	0.5	1.2	6.8	6.7	9.9	19.1
TAS	Launceston	19.7	Apr	0.1	0.4	2.1	5.4	8.5	17.9
	Hobart	1.1	May	4.1	5.7	11.1	10.9	14.4	25.1

Table 3-9. Number of moderate heat stress (THI 75) occurrences throughout lactation calculated for 26 sheep producing sites across Australia

State	Site	Ewes (<i>n</i>)	Lambing	Early lactation (d0-50)			Late lactation (d51-100)		
				Current	+1 °C	+3 °C	Current	+1 °C	+3 °C
QLD	Longreach	54.6	Aug	27.1	31.2	38.9	44.0	46.4	48.6
	Cunnamulla	44.7	Aug	1.3	2.5	6.7	19.0	23.6	32.3
	St George	154.6	Oct	23.1	27.7	37.1	41.9	45.4	48.0
NSW	Wilcannia	288.2	Jul	0.0	0.0	0.2	3.0	4.8	9.6
	Gunnedah	31.9	Sep	3.0	5.0	11.2	19.9	24.8	34.4
	Armidale	298.7	Sep	0.1	0.2	0.9	2.4	4.3	11.1
	Trangie	149.1	Oct	11.9	15.2	23.6	33.3	37.0	43.8
	Dubbo	401.8	Aug	0.0	0.1	0.6	5.2	8.3	15.7
	Griffith	20.7	May	0.0	0.1	0.6	0.0	0.0	0.0
	West Wyalong	283.1	May	0.0	0.0	0.5	0.0	0.0	0.0
	Young	800.7	Aug	0.0	0.0	0.1	2.1	3.6	8.2
VIC	Walpeup	84.1	Sep	1.2	1.7	3.8	9.7	13.2	20.2
	Bendigo	108.5	Aug	0.0	0.0	0.0	0.9	1.4	3.6
	Shepparton	58.7	May	0.0	0.0	0.2	0.0	0.0	0.0
	Hamilton	1 068.5	Sep	0.0	0.0	0.0	0.5	1.1	2.4
	Ballarat	37.5	Aug	0.0	0.0	0.0	0.3	0.4	1.7
SA	Minnipa	70.9	May	0.0	0.2	1.5	0.0	0.0	0.0
	Rosedale	41.7	May	1.6	2.6	7.3	0.0	0.0	0.0
	Renmark	96.4	Jul	0.0	0.1	0.1	1.1	2.0	4.5
	Struan	502.7	May	0.0	0.0	0.2	0.0	0.0	0.0
WA	Geraldton	79.7	Jul	0.0	0.0	0.4	0.7	1.4	3.9
	Northam	75.4	Jul	0.0	0.0	0.0	0.3	0.5	2.7
	Katanning	103.4	Jul	0.0	0.0	0.0	0.0	0.0	0.0
	Esperance	302.7	Jul	0.0	0.0	0.0	0.3	0.6	2.3
TAS	Launceston	19.7	Sep	0.0	0.0	0.0	0.2	0.6	1.8
	Hobart	1.1	Oct	0.4	0.5	1.1	1.7	2.4	4.8

Table 3-10. Number of severe heat stress (THI 79) occurrences throughout lactation calculated for 26 sheep producing sites across Australia

State	Site	Ewes	Lambing	Early lactation (d0-50)			Late lactation (d51-100)		
		(n)		Current	+1 °C	+3 °C	Current	+1 °C	+3 °C
QLD	Longreach	54.6	Aug	33.9	38.3	43.6	11.0	16.5	26.5
	Cunnamulla	44.7	Aug	5.7	9.7	18.7	0.1	0.2	1.3
	St George	154.6	Oct	28.6	33.6	41.4	9.5	13.2	22.7
NSW	Wilcannia	288.2	Jul	0.7	1.1	2.7	0.0	0.0	0.0
	Gunnedah	31.9	Sep	7.2	10.5	19.6	0.2	0.5	2.9
	Armidale	298.7	Sep	0.1	0.3	2.3	0.0	0.0	0.1
	Trangie	149.1	Oct	19.0	23.8	33.1	2.9	5.3	11.5
	Dubbo	401.8	Aug	0.8	1.8	5.1	0.0	0.0	0.0
	Griffith	20.7	May	0.0	0.0	0.0	0.0	0.0	0.0
	West Wyalong	283.1	May	0.0	0.0	0.0	0.0	0.0	0.0
	Young	800.7	Aug	0.2	0.4	2.0	0.0	0.0	0.0
VIC	Walpeup	84.1	Sep	3.6	5.2	9.4	0.2	0.4	1.2
	Bendigo	108.5	Aug	0.2	0.3	0.9	0.0	0.0	0.0
	Shepparton	58.7	May	0.0	0.0	0.0	0.0	0.0	0.0
	Hamilton	1 068.5	Sep	0.1	0.2	0.4	0.0	0.0	0.0
	Ballarat	37.5	Aug	0.0	0.0	0.3	0.0	0.0	0.0
SA	Minnipa	70.9	May	0.0	0.0	0.0	0.0	0.0	0.0
	Rosedale	41.7	May	0.0	0.0	0.0	0.3	0.6	1.6
	Renmark	96.4	Jul	0.1	0.2	1.1	0.0	0.0	0.0
	Struan	502.7	May	0.0	0.0	0.0	0.0	0.0	0.0
WA	Geraldton	79.7	Jul	0.0	0.1	0.7	0.0	0.0	0.0
	Northam	75.4	Jul	0.0	0.0	0.3	0.0	0.0	0.0
	Katanning	103.4	Jul	0.0	0.0	0.0	0.0	0.0	0.0
	Esperance	302.7	Jul	0.0	0.1	0.3	0.0	0.0	0.0
TAS	Launceston	19.7	Sep	0.0	0.0	0.2	0.0	0.0	0.0
	Hobart	1.1	Oct	0.4	0.7	1.5	0.0	0.0	0.4

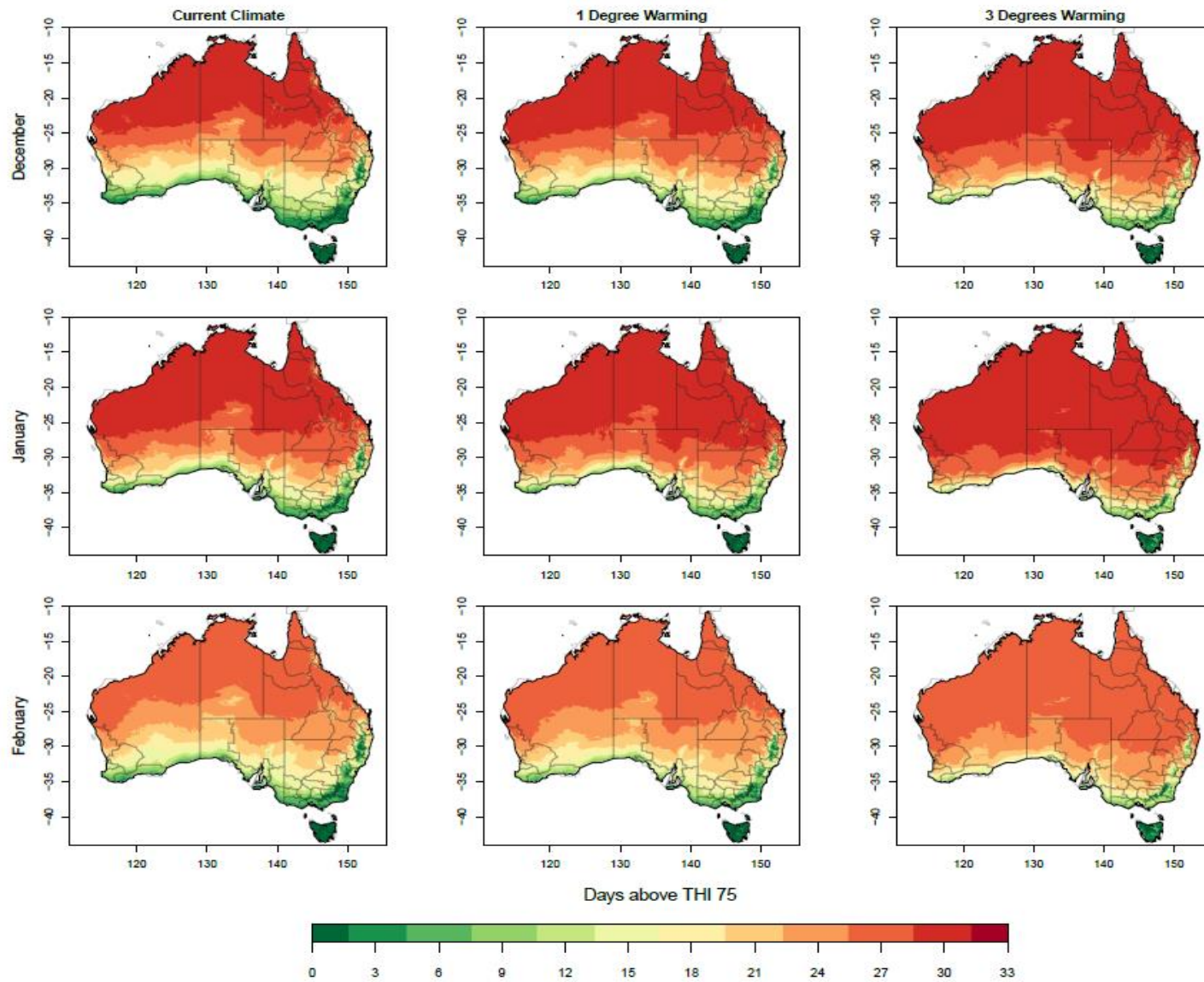


Figure 30. National plots of the count of day over the moderate THI (75) threshold in summer

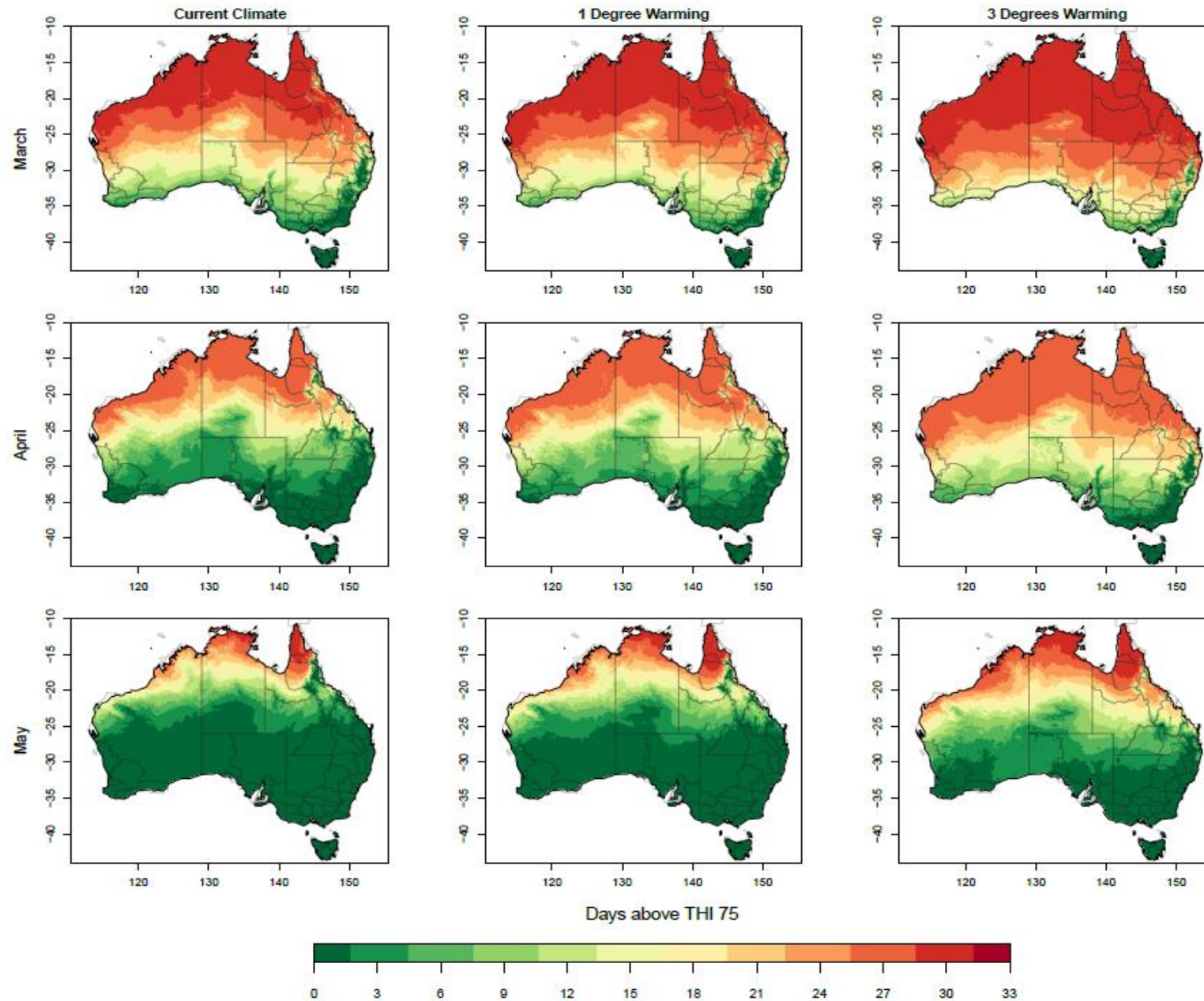


Figure 31. National plots of the count of day over the moderate THI (75) threshold in autumn

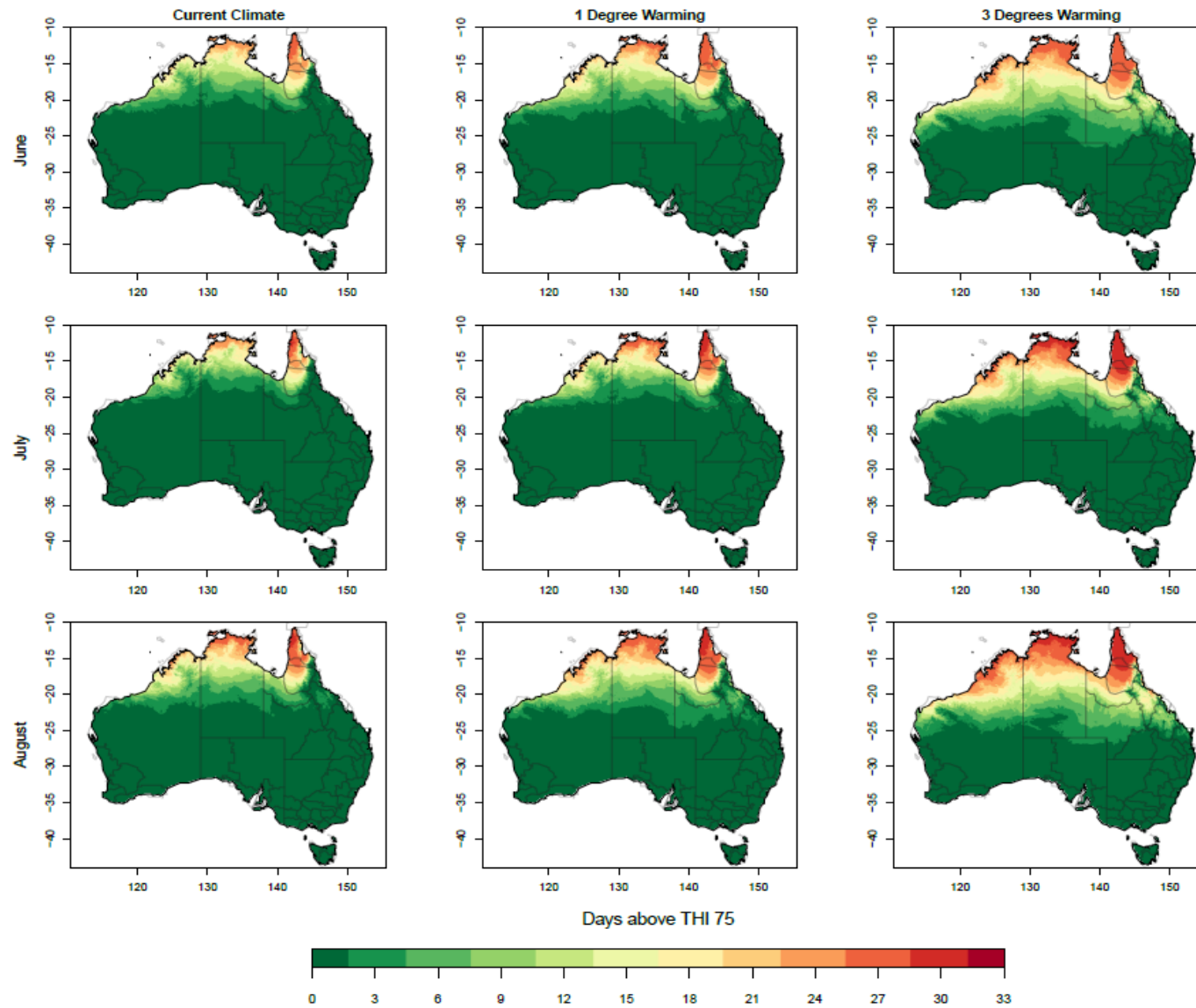


Figure 32. National plots of the count of day over the moderate THI (75) threshold in winter

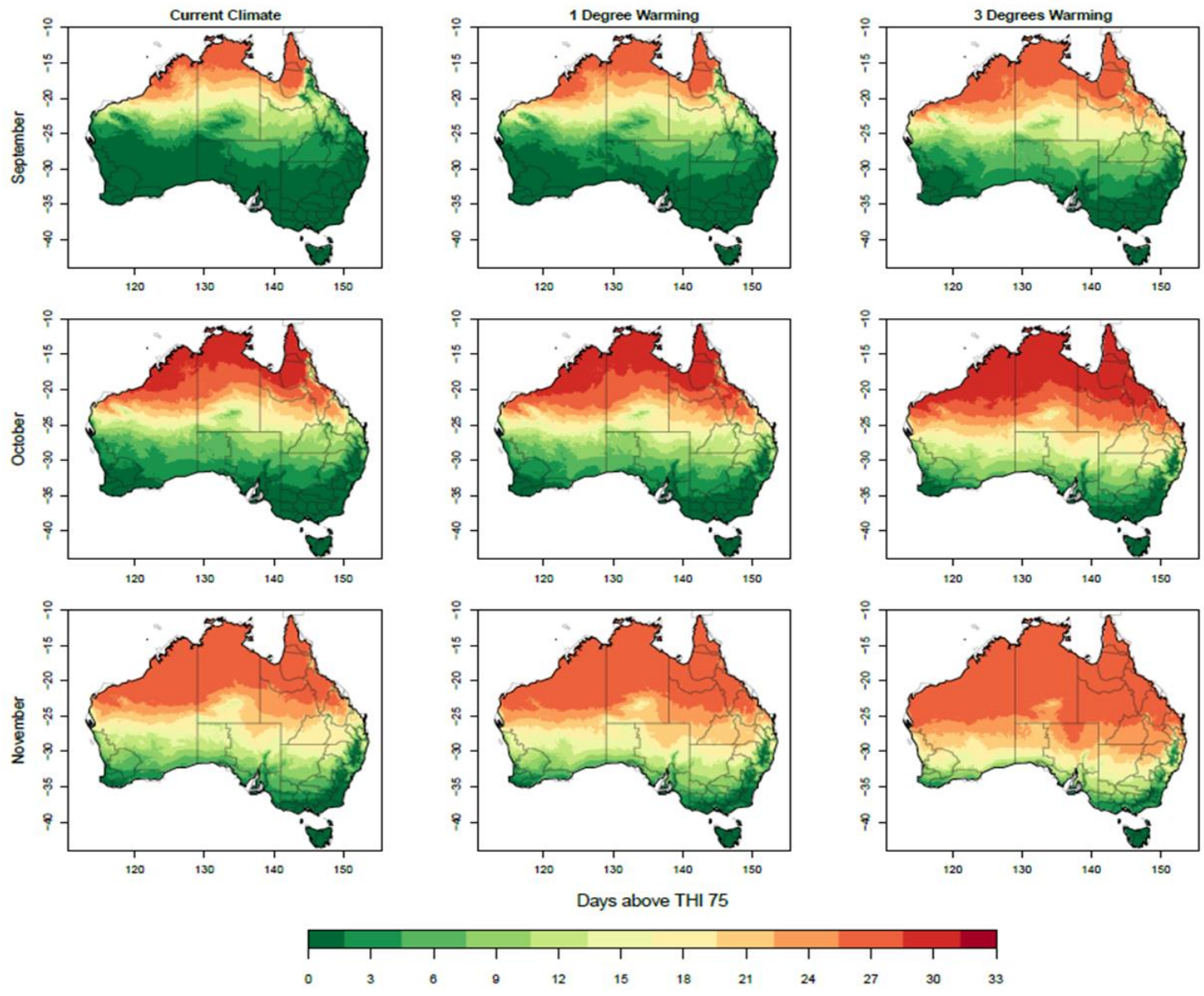


Figure 33. National plots of the count of day over the moderate THI (75) threshold in spring

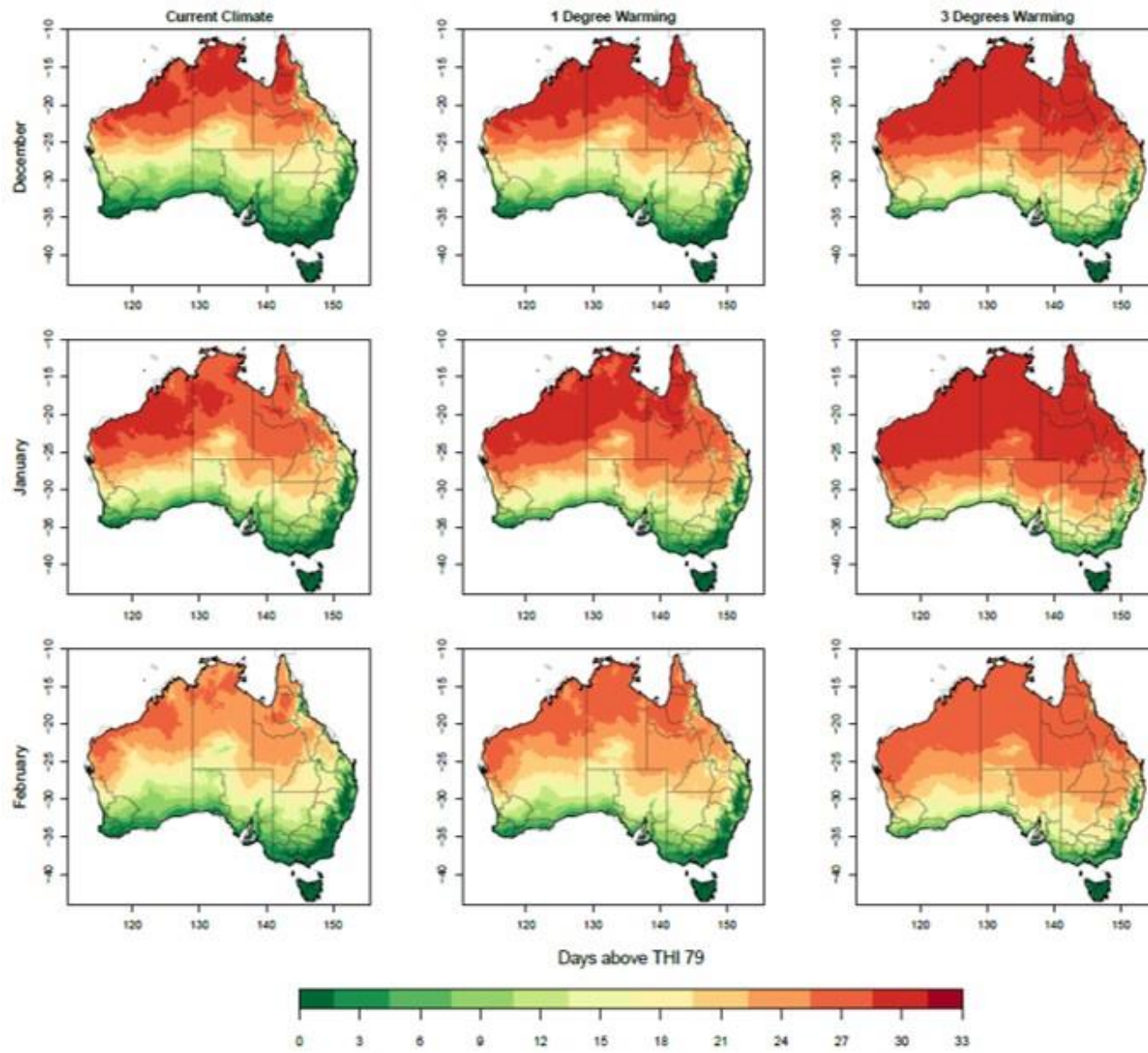


Figure 34. National plots of the count of day over the severe THI (79) threshold in summer

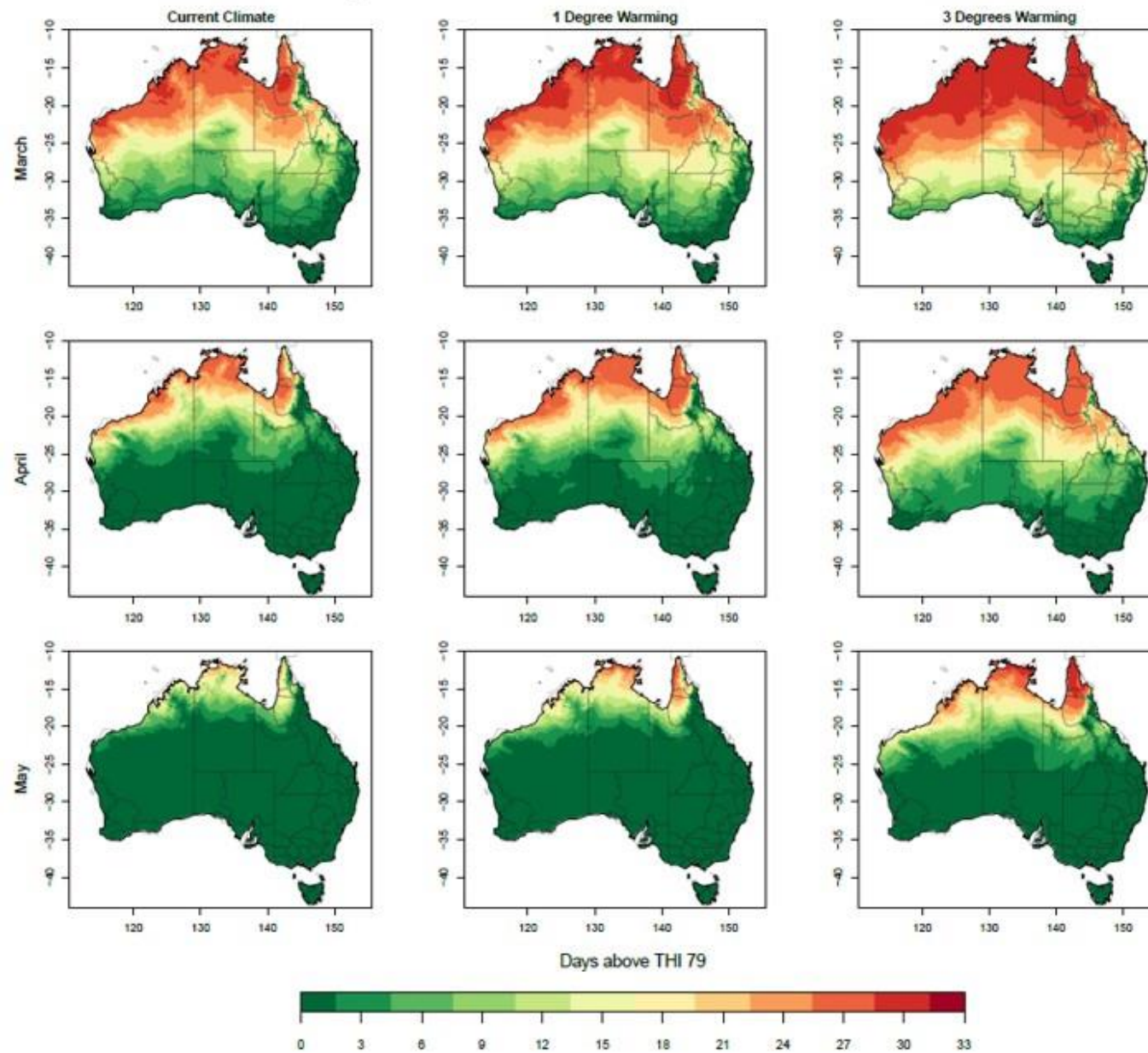


Figure 35. National plots of the count of day over the severe THI (79) threshold in autumn

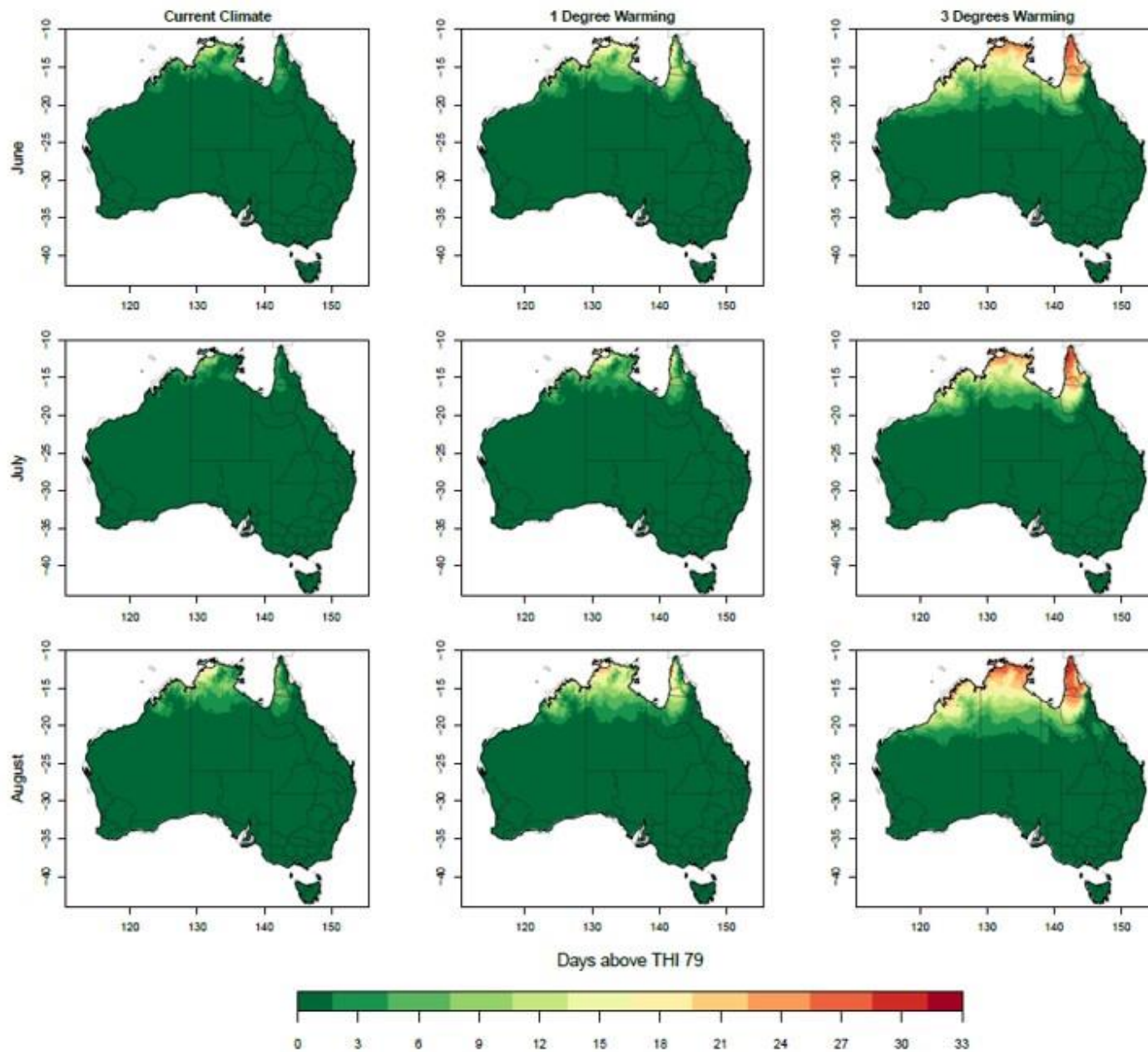


Figure 36. National plots of the count of day over the severe THI (79) threshold in winter

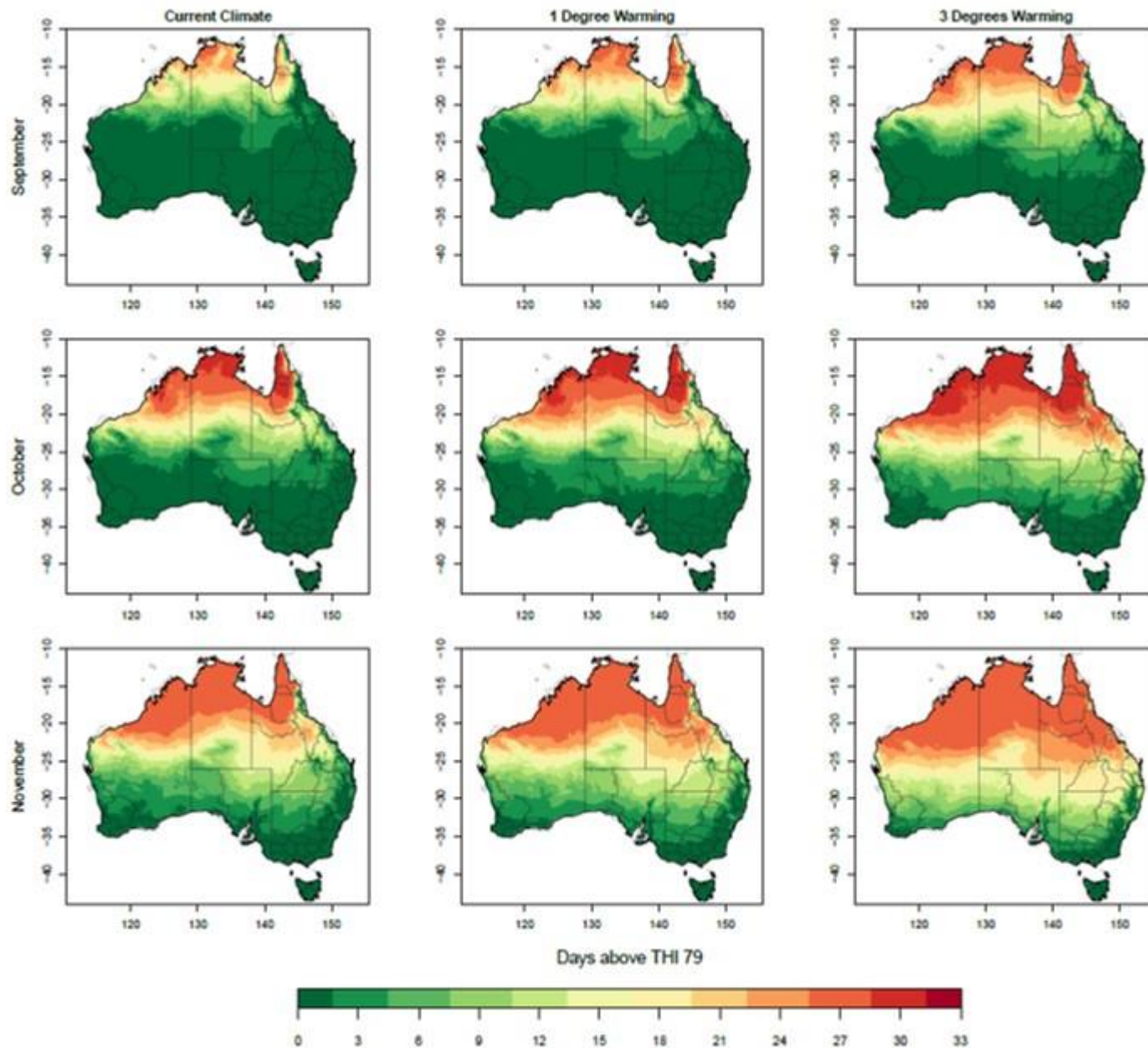


Figure 37. National plots of the count of day over the severe THI (79) threshold in spring

4. Summary of the effects of heat stress on reproduction of ewes and rams

It is clear that short- and long- term exposure to high ambient temperatures and humidity significantly impairs the fertility and fecundity of ewes, and decreases semen production and quality of rams, resulting in fewer lambs born per ewe mated. Furthermore, reductions in lamb birthweight and ewe mammary development resulting from heat stress during pregnancy, are likely to significantly decrease lamb survival and weight at weaning.

For the purposes of this review, the reproductive cycle of the ewe was separated into three fairly broad periods: the eight week period around oestrus/ mating, pregnancy and lactation. The impacts of heat stress during these periods on ewe and ram reproduction are summarised below. However, the majority of this work involved the use of hot-rooms, where the severity and duration of thermal stress experienced by the sheep was not reflective of temperature and humidity typical of the Australian climate. During summer, both chronic and acute heat stress are common and yet the relative impacts on reproductive function remains unclear. Equally important, the additive effects of other stressors which sheep typically experience during summer, including nutritional deprivation and walking long distances, were also rarely taken into account. However, there is general agreement that reductions in feed intake account for only a relatively minor portion of the negative effects of heat stress on reproduction and lactation.

4.1. Heat stress around mating decreases ewe reproduction

The results of field studies indicate that high ambient temperatures ($\geq 32^{\circ}\text{C}$) during the mating period adversely affect ewe fertility, resulting in fewer lambs born and fewer ewes lambing. This effect appears to be manifested around the time of fertilisation and early embryo development although the evidence to support this contention is limited. On the other hand, neither the incidence of oestrus nor ovulation rate is significantly affected by high ambient temperatures. Studies conducted to examine the effects of acute temperatures (using hot-room conditions) clearly identify the reproductive processes that are vulnerable to hyperthermia. These include the length of the oestrous cycle, duration of oestrus, quality of the ovum and its ability to fertilise as well as the survival of the embryo, particularly during the early stages of development. The period during which heat stress is most destructive extends from five days before oestrus until five days after oestrus. The parameters of oestrus are more affected the earlier heat stress is applied whilst ovum quality and fertilisation are most vulnerable when heat is applied around the time of oestrus and embryo wastage is greatest when heat stress is applied on the day of oestrus and during the ensuing five days. A number of these hot room studies used extremely high temperatures (approximately 40°C). However, reductions in ovum quality, fertilisation rate and embryo survival were also observed in ewes housed in hot-rooms at temperatures of 32.2°C , which supports evidence from field studies that ewe fertility is negatively correlated with the number of days $\geq 32^{\circ}\text{C}$ occurring during the mating period.

The identification of these critical period(s) provides a useful basis for the development of strategies for the future management of flocks as ambient temperatures increase. However, it is noteworthy that in the few hot-room studies where high temperatures were moderated by diurnal relief the negative impacts on specific sources of reproductive wastage were less severe. This indicates that

extreme weather events (e.g. prolonged heat-waves without some diurnal relief) during the mating period are likely to have the most significant impacts on flock reproductive performance.

4.2. Heat stress around mating impairs ram fertility

Experimentally exposing the testes of rams to an increased heat load reduces semen quality. However, in contrast to the immediate effects of hyperthermia on ova quality, reduced sperm motility and increased abnormalities are not observed until day 9 day post-heating. These negative effects can be sustained for 3-5 weeks, with the most significant effects observed at 14-21 days post-heating. Sperm numbers can also be reduced and may not return to normal levels until 50-60 days post heating. The reduction in semen quality affects fertility of the ram when mating occurs from approximately 14-50 days post-heat event, with reduced fertility due to both fertilisation failure and embryonic loss. Importantly, the effects of heat exposure on spermatogenesis, sperm quality, and hence ram fertility, are closely related to the increase in testicular temperature. There is variation between rams in the capacity to regulate scrotal temperature, with some rams able to maintain scrotal temperatures 2-3°C lower than others. Scrotal thermoregulatory capacity, its variation between rams and the level of heat exposure required to elevate testicular temperature above 39°C, has been little studied under Australian field conditions.

Experimentally induced hyperthermia and increased scrotal temperature clearly impair semen production and quality. However, the application of these results to field conditions and the ability to extrapolate the impacts on the productivity of the Australian flock is complicated for the following reasons. One, the extent and duration of heat stress differ from conditions typically experienced in the field. Two, variation in the response of individual rams to heat stress conditions and the use of teams of rams for natural joining may effectively reduce the impact on ewe fertility indices (eg pregnancy rates). Three, the delayed impact of heat stress on sperm quality compared with the immediate effect on ewe fertility may make it difficult to identify the effects and timing of heat stress under field conditions.

4.3. Pregnancy and lactation heat stress decreases birthweight, survival and milk output

The impacts of heat stress during pregnancy on fetal and lamb development are restricted primarily to studies using hot-rooms. In these studies, pregnant ewes were typically exposed to prolonged periods of hot days (ranges: 6-18 h, 35 – 44.4°C and THI of 83.2 to 97.0) with cooler nights (ranges: 6 – 18 h, 16.4 – 35.0°C, THI of 38.3 – 83.5), similar to those experienced in the field. However, in the majority of these studies heat stress conditions were maintained for 50 to 150 days, a continuity of heat stress which is unlikely under the current climate. Despite this, it is clear from these studies that even several hours of heat stress are sufficient to increase fetal temperature and alter blood supply to the fetus. Furthermore, prolonged periods of heat stress at any stage of pregnancy consistently reduced lamb weight and viability at birth, thus decreasing their chance of surviving to weaning. Similar to rams, ewes vary in their ability to thermoregulate when exposed to heat stress conditions, with lower core temperatures during periods of heat stress associated with heavier lamb birthweights and increased lamb survival. Phenotypic variation in lambs born to heat stressed ewes is also probable, and likely to be sufficient to alter their post-natal performance and productivity. Studies in cattle

indicate that ewe milk production may also be impaired by gestational heat stress, and based on evidence from dairy cattle and sheep, exposure to THI greater than 65 – 68 during lactation may also impair milk production of the Australian sheep flock.

4.4. Heat stress reduces reproductive performance of the Australian breeding flock

In this review, the impacts of heat stress on reproductive function have been discussed for distinct periods of the reproductive cycle (around mating, pregnancy and lactation). From a production perspective, heat stress two to nine weeks prior to mating retards semen production by rams, whilst heat stress around mating reduces ewe fertility and, thus, the number of ewes lambing and lambs born resulting in lower weaning rates per ewe mated. In addition, heat stress during pregnancy has the potential to decrease lamb birthweight and viability, thus also reducing the number of lambs weaned per mated ewe. Furthermore, heat stress during both pregnancy and lactation may also impair milk production and, therefore, lamb weight at weaning. Importantly, under Australian conditions the majority of breeding ewes and rams will experience repeated periods of heat stress during more than one of the periods described above, especially in those flocks mated during spring / summer. It is, therefore, expected that the impacts of heat stress will be additive. For example, in flocks which experience heat stress around mating and during pregnancy the number of ewes which lamb and the number of lambs which are born will be lower, and the birthweight and survival of these lambs will also be reduced, profoundly reducing weaning rates.

Using AGCD in conjunction with management data from 26 NRM sites across Australia and equations modified from Lindsay et al. (1975) the impacts of heat stress during the mating period on ewe fertility were calculated. This was conducted for the current climate, and a +1°C or +3°C increase in temperature. For Queensland, New South Wales, Victorian, South Australian and Western Australian flocks, which join ewes in spring and early autumn (October to March), the incidence of heat stress experienced under current climate conditions decreased the number of lambs born by 3 – 22% (mean: 9%), with this expected to rise to 4 – 23% (mean: 11%) and 5 – 24% (mean: 14%) should temperatures increase by 1°C or 3°C, respectively. In flocks which join during late autumn/winter (April to September), the impacts of heat stress during the joining period are less severe, causing a 0 – 1% (mean: 0.3%), 0 – 2% (mean: 0.5%) and 0 – 3% (mean: 1.6%) decrease in lambs born under current, +1°C and +3°C.

The impacts of heat stress during gestation on lamb birthweights and post-natal survival were difficult to quantify given the differences between experimental and field conditions. There is considerable variation in the frequency and duration of heat stress events experienced during the gestation of ewes joined between October and March varies. For flocks joined in October to March, days of moderate heat stress account for 9 – 75% (mean: 45%) of the first 50 days of pregnancy, with this increasing to 14 – 82% (mean: 52%) and 26 – 97% (mean: 67%) should temperature increase by 1°C or 3°C, respectively. Of the sites studied, days of moderate heat stress accounted for < 5% of the middle third of pregnancy at 5 sites, with this reducing to 4 sites should temperature increase by 3°C. However, for the remaining sites days of moderate heat stress accounted for 9 – 67% (mean: 36%), 13 – 74 (mean: 43%) and 25 – 87% (mean: 59%) of the middle third of pregnancy. Predictably, ewes mated during late

autumn (April – September) experienced very few days of moderate heat stress during gestation. However, days of mild heat stress (THI > 68) sufficient to impact milk production of dairy sheep and cattle breeds accounted for a reasonable proportion of lactation for spring lambing flocks. Specifically, for the current climate 33% and 58% of the first 50 and second 50 days of lactation had a THI > 68, with these values increasing to 38% and 62% should temperatures increase by 1°C and 49% and 72% if temperatures increase by 3°C. Whether this heat stress translates to a reduction in milk production by meat and wool breeds of sheep is not known, but is worthy of future investigation.

In conclusion, based on an extensive review of the scientific literature and detailed analysis of the impacts of current and future climate on ewe fertility it is clear that heat stress profoundly impairs the reproductive performance of the Australian Flock. It is clear that under current and future (+1°C or +3°C) temperatures, the fertility of the Australian sheep flock is, and will be, significantly impacted by heat stress during the mating period. This impact of heat stress on ewe fertility and fecundity is particularly profound for flocks which join during spring/summer and early autumn. However, based on the sites investigated, and regardless of season of joining, it is estimated that 1.3 million potential lambs are lost due to heat stress under the current climate. In addition to this, heat stress during gestation is likely to retard conceptus development, particularly for flocks mated in spring/summer, with the potential to reduce lamb birthweights by 0.6 – 1.4 kg and decrease survival to weaning by 20 – 30%. Should a 1°C or 3°C increase in temperatures occur, as is likely based on projections from climate models, the number of potential lambs lost will rise to 1.5 and 2.0 million, respectively. In conjunction with this the severity of fetal growth retardation due to heat stress during gestation will increase, further decreasing the weaning rates of the Australian Flock. Based on the outcomes of this review, it is clear that the profitability of the Australian sheep flock is extremely vulnerable to heat stress. In light of this, the next steps for this review process will be to identify potential strategies to ameliorate and mitigate the risk associated with heat stress, and establish the economic costs of current and future heat stress for the Australian flock.

5. Supplementary Material 1: The effects of heat stress on ewe reproduction

Table 5-1. Summary of the effects of heat stress on the parameters of oestrus

Parameter	Thermal conditions	Heat treatment (d0=day of oestrus)	Outcome		Difference %	Reference (breed)
			Control	Heat stress		
Incidence of oestrus (%)	90°F 60%rh	-d5 to d0		100.0 (shorn) 100.0 (unshorn)	0.0 0.0	Dutt et al. (1959) (Northwestern)
	41.9 ± 0.42°C 2.07-3.00 kPa	-d5 to d0 d1 to d9 Combination	85.0	65.0 95.0 70.0	-20.0 (P<0.05) +10.0 ns +15.0 ns	
	42.0±0.34 2.58-3.08 kPa	-d5 to d0 + d1-4 -d5 to d0 + d5-8	100.0	75.0 72.0	25.0 (P<0.05) 28.0 (P<0.05)	Sawyer et al. (1979) (Merino)
	40.0±0.21-41.5±0.31°C 2.65- 3.60 kPa Multiple expts	-d5 to d0 -d5 to d0 -d7 to -d4 -d3 to d0	87.0 95.6 85.0 85.0	65.2 66.6 95.0 100.0	-21.8 ns -29.0 (P<0.05) +10.0 ns +15.0 ns	Sawyer (1979b) (Merino)
	21.1-32.2°C 65%rh Max. temp. between 1000-1600h Diurnal	-d5 to d5	73.1	74.1	1.0 ns	Rich and Alliston (1970) (unknown)
	38.0-44.0°C Applied 1000-1600h Diurnal	-d35 to d0 1st cycle 2nd cycle	100.0 100.0	100.0 87.5	0.0 -12.5 ns	Indu et al. (2015) (Malpura)

Table 5-1. Cont

Parameter	Thermal conditions	Heat treatment (d0=day of oestrus)	Outcome		Difference %	Reference (breed)
Length of cycle (days)	90°F 60%rh	-d5 to d0	16.2±0.13	16.8±0.20 (shorn) 17.1±0.28 (unshorn)	0.6 (P<0.05) 0.9 (P<0.01)	Dutt et al. (1959) (Northwestern)
	42.0±0.34°C 2.58-3.08 kPa	-d5 to d0 + d1 to d4 -d5 to d0 + d5 to d8	16.9±0.20	18.2±0.30 17.6±0.20	1.3 (P<0.01) 0.7 (P<0.05)	Sawyer et al. (1979) (Merino)
	40.0±0.21-41.5±0.31°C 2.65- 3.60 kPa Multiple expts	-d5 to d0 -d5 to d0 -d7 to -d4 -d3 to d0	17.3±0.37 16.4±0.16 17.1±0.30 17.1±0.30	19.0±0.55 17.0±0.19 16.6±0.14 17.1±0.22	1.7 (P<0.05) 0.9 (P<0.05) -0.5 ns 0.0	Sawyer (1979b) (Merino)
	38.0-44°C Applied 1000-1600h Diurnal	-d35 to d0 1st cycle 2nd cycle	13.4±0.18 16.1±0.27	13.3±0.25 15.7±0.44	-0.1ns -0.6 ns	Indu et al. (2015) (Malpura)
	40.0±0.21-41.5±0.31°C 2.65- 3.60 kPa Multiple expts	-d5 to d0 -d7 to -d4 -d3 to d0	24.5±1.05h 20.9±1.14h 20.9±1.14h	16.1±1.44h 21.2±1.54h 15.4±1.08h	8.4h (P<0.05) 0.3h ns 5.5h (P<0.05)	Sawyer et al. (1979) (Merino)
Oestrous Duration	21.1-32.2°C 65%rh Max. temp. between 1000-1600h Diurnal	-d5 to d5	28.8h	35.3h	6.5h ns	Rich and Alliston (1970) (Columbia, Rambouillet)
	40.0°C Applied 1000-1600h daily Diurnal	-d28 to d0	37.7±1.6h	31.7±3.6h	6.0h (P<0.05)	Naqvi et al. (2004) Maurya et al. (2005)
	38.0-44.0°C Applied 1000-1600h Diurnal	-d35 to d0 1st cycle 2nd cycle	48.0±1.66h 45.0±1.43h	43.5±2.71h 36.9±3.34h	4.5h ns 8.1h (P<0.05)	Indu et al. (2015) (Bharat)

Table 5-2. Summary of the effects of heat stress on rates of fertilisation

Thermal conditions	Heat treatment (d0=day of oestrus)	Fertilisation %		Difference	Reference (breed)
		Control	Heat stress		
90°F 60%rh	-d5 to d0	92.6	64.0 (shorn) 40.7(unshorn)	-28.6 (P<0.05) -51.9 (P<0.01)	Dutt et al. (1959) (Northwestern)
70°C or 90°C 65%rh Applied up to d30	70°F 90°F Embryo transfer d3: 70°F to 70°F 70°F to 90°F 90°F to 70°F	88.4 56.5	59.0 24.0 9.5	-29.4 (P<0.01) -32.5 ns -46.0 (P<0.05)	Alliston and Ulberg (1961) (unknown)
92°F	-d7 to d2	88.2	71.4	-16.8 ns	Alliston et al. (1961) (unknown)
90°F 60-65%rh	d0 to d3 d1 to d3	96.3	69.2 100.0	-27.1 (P<0.01) + 3.7 ns	Dutt (1963) (cross-bred)
32°C 60-65%rh Applied up to d24	-d5 d0 d1	94.2	40.7 69.2 100.0	-53.5 (P<0.01) -25.0 (P<0.01) +5.8 ns	Dutt (1964) (Rambouillet cross-bred)
21.1-32.2°C 65%rh Max. temp. between 1000-1600h Diurnal	-d5 to d5	77.8	100.0	+22.2 ns	Rich and Alliston (1970) (Columbia, Rambouillet)
40.0°C Applied 1000-1600h daily Diurnal	-d28 to d0	71.4	71.4	0.0	Naqvi et al. (2004)

Table 5-3. Summary of the effects of heat stress on rates of abnormal ova

Thermal conditions	Heat treatment (do=day of oestrus)	Abnormal ova %		Difference	Reference (breed)
		Control	Heat stress		
90°F 60%rh	-d5 to d0	3.7	32.0 (shorn) 55.6 (unshorn)	28.3 (P<0.01) 51.9 (P<0.001)	Dutt et al. (1959) (Northwestern)
92°F	-d7 to d2/14.5	29.4	85.7	-56.3 (P = ?)	Alliston et al. (1961) (unknown)
90°F 60-65%rh Applied until d24 (max)	d0 to d3 d1 to d3	3.7	46.2 30.8	42.5 (P<0.01) 27.1 (P<0.05)	Dutt (1963) (cross-bred)
32°C 60-65%rh	-d5 to d3 d0 to d3 d1 to d3	3.8	55.6 46.2 30.8	51.8 (P<0.01) 42.4 (P<0.01) 27.0 (P<0.01)	Dutt (1964) (Rambouillet cross-bred)
90°C 65%rh	-d5 to d3	18.4	7.7	-10.8 ns	Alliston and Ulberg (1961) (unknown)
21.1-32.2°C 65%rh Max. temp. between 1000-1600h Diurnal	-d5 to d5	11.1	28.6	17.5 ns	Rich and Alliston (1970) (Columbia and Rambouillet)

Table 5-4. Summary of the effects of heat stress on rates of embryo mortality

Thermal conditions	Heat treatment (do=day of oestrus)	Embryo mortality %		Difference	Reference (breed)
		Control	Heat stress		
90°F 60%rh	-d5 to d0	16.7	85.7 (shorn) 100.0 (unshorn)	69.0 (P<0.01) 83.3 (P<0.01)	Dutt et al. (1959) (Northwestern)
	d8 to d24		0.0	0.0 ns 38.5 ns	
70°F or 90°F 65%rh Applied from -d5 to d3	Embryo transfer d3: 70°F to 70°F 70°F to 90°F 90°F to 70°F	43.5	76.0 90.5	32.5 ns 47.0 (P<0.01)	Alliston and Ulberg (1961) (unknown)
90°F 60-65%rh Applied up to d24	d0 d1 d3 d5		19.2	100.0 77.8 61.5 65.4	80.8 (P<0.05) 58.6 (P<0.05) 42.3 (P<0.05) 46.2 (P<0.05)
32°C 60-65%rh Applied tol d24 (max)	-d5 d0 d1 d3 d5 d8	12.2		100.0 100.0 77.8 61.5 55.4 38.5	87.8 (P<0.05) 87.8 (P<0.05) 75.6 (P<0.05) 49.3 (P<0.05) 43.2 (P<0.05) 26.3 ns
100.0±1°F 55-60%rh	d0 to d20 Merino Southdown		28.6 36.4	(inc. ova wastage) 100.0 100.0	71.4 (P<0.001) 63.6 (P<0.001)
36.1-38.6°C 42-54%rh	-d2 to d0 Maiden ewes Mature ewes	37.5 50.0	(inc. ova wastage) 100.0 100.0	(P<0.001 ages combined) 62.5 50.0	(Thwaites, 1969) (Merino)

Table 5.4. Cont.

Thermal conditions	Heat treatment (do=day of oestrus)	Embryo mortality %		Difference	Reference (breed)
		Control	Heat stress		
106.0°F 40%rh Applied for 8h followed by 95°F/30%rh overnight Diurnal	d0 to d15	22.2	35.0	12.8 ns	(Thwaites, 1970) (Merino)
	d0 to d15 Shorn Unshorn	0.0 33.3	66.6 100.0 (inc. ova wastage)	66.6 (P<0.001) 66.7 (P<0.001)	
40.6°C 30%rh Applied during first week after mating	d1 to d4	9.1	54.5	45.4 (P<0.05)	Thwaites (1971) (Merino)
	d2 to d5		20.0	10.9 ns	
	d3 to d6		36.4	27.3 ns	
	d4 to d7		36.4	27.3 ns	
	d1 to d7	18.2 (inc. ova wastage)	83.3 (inc. ova wastage)	65.1 (P<0.01)	
41.1°C 60%rh Increased over 9d to 8h heat/day Diurnal	-d17 to d6	18.0	39.0	21.0 ns	Ryle (1961) (Merino)
21.1-32.2°C 65%rh Max. temp. 1000-1600h Diurnal	-d5 to d5	14.3	57.2	42.9 ns	Rich and Alliston (1970) (Columbia and Rambouillet)
		34.6	41.2	6.6 ns	
40.0°C Applied 1000-1600h daily Diurnal	-d28 to d0	1.0	54.8	53.8 (P<0.05) (data for poor quality embryos)	Naqvi et al. (2004) (Bharat)

Table 5-5. Summary of the effects of heat stress on pregnancy rates

Thermal conditions	Heat treatment (do=day of oestrus)	Pregnancy %		Difference	Reference (breed)
		Control	Heat stress		
90°F 60%rh	d8 to d24 *shorn *unshorn	100.0	100.0 90.0	0.0 10.0 ns	Dutt et al. (1959) (Northwestern)
92°F	-d7 to d2/14.5	30.0	13.0	-17.0 (sig. difference but level not given)	Alliston et al. (1961) (unknown)
41.1°C 60%rh Increased over 9d to 8h heat/day Diurnal	-d17 to d6	83.0	62.0	-21.0 (P=?)	Ryle (1961) (Merino)
90°F 60-65%rh Applied until d24 (max)	Control Hot-room d0 + d1 d3 d5	85.0	10.0 35.0 40.0b	-75.0 (P<0.01) -50.0 (P<0.01) -45.0 (P<0.01)	Dutt (1963) (cross-bred)
32°C 60-65%rh Applied until d24 (max)	-d5 d0 d1 d3 d5 d8	87.5	0.0 0.0 20.0 35.0 40.0 70.0	-87.5 (P<0.01) -87.5 (P<0.01) -67.5 (P<0.01) -52.5 (P<0.01) -47.5 (P<0.01) -17.5 ns	Dutt (1964) (Rambouillet cross-bred)
89.9±4.8°F 57.3±6.3rh -d5 to -d3	Embryo transfer d3 Control 70°F 90°F to 90°F 70°F to 90°F 90°F to 70°F		71.4 35.0 66.7 16.7	(P<0.05) (P<0.05)	Woody and Ulberg (1964) (Rambouillet cross-bred)

Table 5-6.Cont

Thermal conditions	Heat treatment (do=day of oestrus)	Pregnancy %		Difference	Reference (breed)
		Control	Heat stress		
40.5°C	d1 to d7	62.5	9.1	-53.4 (P<0.001)	Smith et al. (1966) (Merino)
21.1-32.2°C 65%rh Max. temp. 1000-1600h Diurnal	-d5 to d5	50.0	33.3	-16.7 (P=?)	Rich and Alliston (1970) (Columbia and Rambouillet)
40-43°C 1.36-3.06 kPa	d1 to d3	70.6	31.2	39.4 ns	Sawyer (1979a) (Merino)
	d4 to d6		59.1	11.5 ns	
	d7 to d9		63.6	7.0 ns	
	d1 to d3	78.9	50.0	28.9 ns	
	d1 to d6		52.5	26.4 ns	
	d1 to d9		58.6	20.3 ns	
42.0±0.34°C 2.58-3.08 kPa	-d5 to d0	70.6	84.6	14.1 ns	Sawyer et al. (1979) (Merino)
	d1 to d9		47.4	-23.1 ns	
	-d5 to d0 + d1 to d9		42.9	-27.6 (P<0.05)	
	-d5 to d0 + d1 to d4	67.9	55.6	-12.3 ns	
	-d5 to d0 + d5 to d8		66.7	-1.2 ns	
40.8±0.26 3.6kPa 40.0±0.21 2.79 kPa 41.5±0.31 2.65 kPa	-d5 to d0	70.0	66.0	-4.0 ns	Sawyer (1979b) (Merino)
	-d5 to d0	63.6	56.2	-7.4 ns	
	-d7 to -d4	64.7	57.9	-6.8 ns	
	-d3 to -d0		52.4	-12.3 ns	
42°C Applied for two cycles	Thermal stress	83.3			Sejian et al. (2012) (Malpura)
	Multiple stress combined Thermal Nutritional Exercise		50.0	-33.3 (P<0.05)	

6. Supplementary Material 2: The effects of heat stress on ram reproduction

Table 6-1. Summary of studies on increased testicular temperature induced by hot-room exposure or scrotal insulation on semen quality

Thermal conditions	Breed		Subcutaneous scrotal temp	Rectal temp	Semen quality measures	Timing of effects on semen quality	Reference
6h, 40.5°C, 8.5 mm Hg and 6h, 40.5°C, 31.5 mm Hg 42h after first trt	Merino	Variable response between rams, divided into 3 groups based on response	@ 40.5°C 8.5 mm Hg A: 39.3°C (n=3) B: 37.9°C (n=4) C: 36.9°C (n=5)	A: 39.4°C B: 39.8°C C: 39.3°C	D1 = day of first trt Ejaculates with >50% dead (from 17 ejaculates in 60d post-trt): A:7-9, B:2-5, C:0-2 Ejaculates with >15% tailless (from 17 ejaculates in 60d post-trt): A:6-9, B:2-5, C:0-2 Group A reduced sperm conc ⁿ	Ejaculates <75% pre-trt composite score (based on viability, morphology, motility) A: 11/17 from d13, for 35-39d B: 5/17 from d14-21, for 14-28d C: 2/17 from d17-20, for 4-8d Group A: ↓ conc ⁿ from d14, remained low to d60, 1-4 ejaculates/ram sperm almost absent between d21-32	Moule & Waites (1963)
4d, 32°C 65%rh (n=8)	Not stated	Control, ambient, avge max 13°C (n=8)		Con: 39.4 Heat: 39.9 ± 0.2°C	% Motile <10% at d14 post-trt % Abnormal 50-60% from d7-21 post-trt Conc ⁿ ~50% lower than control d7-21	↓ Conc ⁿ ↓%motile d7-21, peak effect d14 ↑ Abnormal sperm d7-21, peak effect d21 Assessment ended at d21 post-trt	Howarth (1969)
4h, 6h, 9h and 13.5h, 41°C, 50d between trt (n=9)	Not stated		4h: 37.3°C 6h: 37.5°C 9h: 38.9°C 13.5h: 39.6°C		Sperm measures pre-trt and across 42d after 4h, 6h, 9h, 13.5h trt % Abnormal: 9.0 (pre), 9.2, 8.8, 16.0, 20.9 Motility score (0-5): 4.6 (pre), 4.5, 4.5, 4.0, 3.9 Conc ⁿ (x10 ⁹ /ml): 2.6 (pre), 2.0, 2.4, 2.8, 2.8 Minimal effects after 4h trt; ↑ abnormal sperm, ↓ motility after 9h and 13.5 h trt	↑ Abnormal sperm, ↓ motility d14-28 Peak effects at d21	Smith (1971)
2d x 40.5°C or 4d x 40.5°C 8h/d, 45%rh (n=4)	Merino	Control, ambient (n=2)			% Pyriform sperm ↑ following heat: 2d trt: 17.5% pyriform at d15-18 4d trt: 31.5% pyriform at d18 % Abnormal acrosome increased following heat: 4d trt: 18.5% abnormal acrosome at d15-18 Conc ⁿ across 45d post-trt not different vs control	Pyriform sperm from d9-30 post-trt. Peak number pyriform sperm d15-18 Abnormal acrosome (4d trt) from d9 Peak number abnormal acrosome d15-18	Rathore (1970a,b)

Thermal conditions	Breed		Subcutaneous scrotal temp	Rectal temp	Semen quality measures	Timing of effects on semen quality	Reference
1.5d x 40.5°C or 2.5d x 40.5°C or 4.0d x 40.5°C 8h/d, 45%rh (n=6)	Merino	Control ambient, (n=2)			% Pyriform sperm ↑ following heat: 1.5d trt: 11% pyriform at d17 2.5d trt: 17% pyriform at d17 5d trt: 31.5% pyriform at d17 % Abnormal acrosome increased following heat: 5d trt: 25, 24, 23% abnormal at d14, d17, d20 Conc ⁿ across 48d post 5d-trt ↓ vs con (-20%)	Pyriform sperm from d10-34 post-trt Peak number pyriform sperm from d17 Abnormal acrosome (5d trt) from d10 Peak number abnormal acrosome d14-20	Rathore (1970a,b)
40.5°C x 2d or 40.5°C x 4d 8h/d, 45%rh (n=4)	Merino	Control ambient, (n=2)		Con: 38.4 ± 0.4°C Heat: 40.9 ± 0.2°C	% Abnormal midpiece ↑ following heat: 2d trt: 15% abnormal at d24 4d trt: 22% abnormal at d24 Conc ⁿ across 42d post 4d-trt ↓ vs control (-24%)	Abnormal midpiece from d15 post-trt Peak number abnormal midpiece at d24	Rathore (1969)
7d x 21, 26.7, 32.2, 37.8, 43.3, 37.8, 32.2 26.7°C; 7d at each temp	Merino, Border-Leicester, Dorset (n=4 per breed)	Temp ↑ d0-21, then ↓ d21-42		Dorset ~0.4°C < BL & Merino	Motility score: ↓ at 37.8°C in Merino and Border Leicester (BL); ↓ after 43.3°C in Dorset Horn ↓ of ~4 points (score of 0-5) in Merino and BL ↓ of ~3 points in Dorset Horn	Merino & BL, ↓ motile sperm 14-42d Dorset, ↓ motile sperm 28-42d Lowest at d35 all breeds (temp ↓ 32.2°C)	Lindsay (1969)
21d, 30-35°C High skin fold (n=4) Low skin fold (n=4)	Merino High skin folds (+) Low skin folds (-)	Control, ambient avge max ~16°C (n=4)			Motility score (score of 0-10) ↓: Folds (-); pre-trt ~7-8, d17 ~4 Folds (+); pre-trt ~6-7, d25 ~2, > ↓ in Folds (+) % Live sperm ↓: pre-trt ~80%, d17 Folds (+) ~40%; Folds (-) ~30% Conc ⁿ ↓ in Folds (+) only	Motility score and % live ↓ from d17 Folds (-): restored by d30 (10d post-trt) Folds(+): remained low to d50 (30d post-trt); peak effect at d25 Conc ⁿ ↓ from d20 – d50 in Folds (+)	Fowler & Dun (1966)
45d, 42°C, 6h/d, 55%rh, (n=7)	Malpura	Control ambient, (n=7)			Sperm measures mean d0, 15, 30 and 45 Motility score (score of 0-5) ↓: Control: 4.4 ± 0.2; Heat: 1.8 ± 0.2 Conc ⁿ (x10 ⁹ /ml) ↓: Control: 4.1 ± 0.4; Heat: 1.2 ± 0.1	Values reported as mean across 45 d trt	Maurya et al (2016)

Thermal conditions	Breed		Subcutaneous scrotal temp	Rectal temp	Semen quality measures	Timing of effects on semen quality	Reference
Scrotal insulation (Ins) 8h/d for 162d 16h/d for 144d 24h/d for 30d (n=4 / trt)	Merino	Control not insulated (n=4 / trt)	220 min insulation: Pre: 33.7 ± 0.1°C Ins: 35.8 ± 0.1°C 24h insulation: Con: 34.5 ± 0.1°C Ins: 36.4 ± 0.06°C	24h Con: 38.8 ± 0.03°C Ins: 38.1 ± 0.03°C	Sperm measures mean across trt period 8h/d: % motile ↓ 58 ± 2% vs 73 ± 1% (control) Conc ⁿ not different vs control; 16h/d: % motile ↓ 41 ± 3% vs 75 ± 3% (control) Conc ⁿ ↓ by ~28% vs control; 24h/d: % motile ↓ 22 ± 9% vs 78 ± 3% (control) Conc ⁿ ↓ by ~53% vs control	16h/d: %motile ↓ d20, restored ~d180 (40d post-trt) 24h/d: % motile ↓ d9, restored by d70 (40d post-trt); < 5% motility d20 to d55; 24h/d: conc ⁿ ↓ d12, restored by d110 (80d post-trt); < 0.05x10 ⁹ sperm per ejaculate from d19 to d58	Mieusset et al (1991)
Scrotal insulation 16h/d for 21d (n=4)	Merino	Control not insulated (n=4)			Sperm measures pre, d4, d15, d21 of insulation % Dead ↑: 17 ± 3 (pre), 29 ± 10, 38 ± 2, 57 ± 7 % Motile ↓: 79 ± 11 (pre), 48 ± 12, 55 ± 8, 17 ± 6 Conc ⁿ (10 ⁹ /ejaculate) ↓: 4.5 ± 0.3 (pre), 2.7 ± 0.4, 2.9 ± 0.8, 2.6 ± 0.5	↑ dead sperm at d15 ↑ dead sperm, ↓% motile, ↓ conc ⁿ at d21	Mieusset et al (1992)
Scrotal chamber, 41-44°C water 1.5h (n=2), 2h (n=1), or 4h (n=2)	Merino	Control not insulated (n=2)	2h: 40.5 – 41.5°C 1.5h: 40.4 - 40.6°C 4h: 39.4-39.6°C		2h at 41°C, Conc ⁿ ↓; % dead ↑ from ~d25; 10% dead pre-trt, 85% dead d47 post-trt 1.5h at 40.5°C, Conc ⁿ ↓; % dead ↑ from ~d20, 15% dead pre-trt, >90% dead ~d47 post-trt 4h at 39.5°C, % dead ↑ from ~d22, 10-35% dead d0-22, 40-55% dead d22-d47	% dead ↑ from ~d15 post-trt, peak effect at d47, restored by ~d50-60 Conc ⁿ ↓ from ~d25, very low d34-41 (~0.1 x 10 ⁹ / ejaculate), restored by d50-60 (rams used for mating expt, therefore results affected by ejaculation frequency)	Braden & Mattner (1970)
Scrotal insulation 30h, (n=9)	Canadian Arcott	Control not insulated (n=9)			% Motile ↓ at d24 post-trt % Abnormal ↑ at d21 post-trt	% Motile ↓ at d24 post-trt % Abnormal ↑ at d21 post-trt, restored by d35 post-trt	Kastelic et al. (2017)
Scrotal insulation 24 or 48h, (n=6)	Dorset x Romney				↑ DNA fragmentation index	DNA fragmentation index ↑ d21 post-trt. Recovered by d38-43 post 24h trt Remained high at d49 post 48h trt	McDonald et al. (2007)

7. Supplementary Material 3: The effects of heat stress during pregnancy

Table 7-1. Summary of studies on the effects of heat stress on ovine pregnancy

Ref	Breed and study size	Heat stress (temperature, humidity) and control conditions	Timing of HS	Main outcomes
Alexander and Williams, 1971	Merino See results summary for numbers	CON: un heated animal house, monthly average temperatures 18-32 (max) and 8-18 (min) HS: 44.4C +rh 47% for 9 h then 32.2C andrh ~50% Expt C also included a "modified" HS group returned to animal house (cool overnight) with 9 h heating as above.	Various. Expt A, last 2 thirds of pregnancy plus pair-fed control. Expt B, second, third, both or neither third of pregnancy	Expt A: HS throughout the last two thirds of pregnancy significantly reduced lamb BW (CON (n=7): 3.62±0.07 kg; HS (n=9):1.79±0.19 kg), effects of maternal food restriction were intermediate (UN (n=11):2.44±0.08 kg). Similarly, maternal food restriction resulted in intermediate placental weight (CON: 377±28 g; HS:120±17 g; UN: 293±19 g). Number of placental cotyledons was unaffected by treatment. Expt B: HS through last two-thirds of pregnancy reduces BW more than HS in only thirds 2 or 3 (CON (n=5): 3.23±0.11 kg; HS2+3 (n=5): 1.63±0.26 kg; HS2 (n=5): 2.45±0.36 kg; HS3 (n=4): 2.29±0.21 kg). Similar effects for placental weight (CON: 423±28 g; HS2+3: 139±26 g; HS2: 288±67 g; HS3: 251±28 g). Ambiguous results and some issues with variation in Expts C and D
Andrianikis and Walker, 1994	Border Leicester TN: 5 ewes HS: 7 ewes	TN: 24C HS: 43C for 8 h	Late pregnancy: 24 h experiment at between gd122-130	<ul style="list-style-type: none"> • Maternal and fetal temperatures increased over 6 h then stabilised until 8 h and fell rapidly once heating stopped. At 8 h, maternal temp +2.0C, fetal temperature +1.9C. • Respiratory alkalosis (↑pH, ↓pCO₂) due to maternal panting • Plasma PGE₂ = ↑ in maternal & fetal circulation during heat. • Plasma PGF_{2α}= in maternal circulation, ↑ in fetal during heat. • Uterine activity measured by EMG ↑ incidence (from 24% to 42% of time) and 45%↑ amplitude during heat
Barbera et al 1995	Columbia– Rambouillet TN: 43 ewes HS: 4 ewes (all singletons, all fed ad lib)	TN: 20C HS: 40C for 18 h/d and 35C for 6 h/d, 35-40% rel humidity.	Early-mid to mid-late pregnancy Starting gd35, duration 80 d (to ~gd115)	Essentially a pilot study for the heat effect with only n=4 heat-stressed ewes but 43 controls. Does show fetal growth reduction is apparent by about mid-pregnancy – evident from gd70 Fetal growth measured by ultrasound weekly from gd50: <ul style="list-style-type: none"> • Abdominal circumference HS<TN from gd70 • Femur & tibia lengths HS<TN from gd80 • BPD (skull width) HS<TN from gd90 • By gd125 fetal size in HS group is around or below the 5th centile for TN group

Bell et al 1987	Cambridge – Rambouillet N=5 control and n=5 HS ewes	38-40C for 9h/d and 30-32 for 15 h/d at 40-50% rh in HS versus "not heat exposed" controls	gd 45 - 120	<p>Outcomes were studied at gd 132 – 137. This is basically a pilot study with n=5 control and n=5 HS ewes. Much more detailed & larger studies including these outcomes were done subsequently by Tim Regnault and B de Vrijers.</p> <p>Essentially similar <u>growth results</u> to other studies in the model:</p> <ul style="list-style-type: none"> • Fetal weight 2.225±0.309 cf 3.067±0.182 kg (means ± SEM), ↓27%, P<0.05 • Placental weight 160±32 cf 329±20 g, ↓51%, P<0.01, reflecting smaller rather than fewer cotyledons • Higher fetal:placental weight indicating some placental adaptation to maintain fetal growth <p>Several <u>placental functional measures</u> reported:</p> <ul style="list-style-type: none"> • Uterine blood flow 866±166 cf 1431±51 ml/min, ↓39%, P<0.05 • Umbilical blood flow 316±46 cf 607±40 ml/min, ↓48%, P<0.01 • Relative umbilical blood flow 144±16 cf 198±10 ml.kg/min, ↓37%, P<0.01 (i.e. reduced blood flows through placenta, and reduced even after correcting for the fetus being smaller) • Placental clearance of ethanol (passively diffuses from maternal to fetal circulations so used as a marker of placental flow) 178±27 cf 336±23, ↓47%, P<0.001 • ↓total fetal oxygen uptake, but proportional to the reduced fetal weight. Nevertheless, poorer oxygen transfer from maternal to fetal blood (greater uterine-umbilical oxygen difference), resulting in fetal hypoxia (pO₂ 16.2±0.8 cf 19.8±0.6 torr and 42.9±3.2 cf 58.0±3.0% O₂ saturation) • ↓placental glucose transfer capacity 20.9±4.2 cf 33.3±3.0 ml/min, P<0.05, resulting in ↓fetal circulating glucose 10.6±2.0 cf 16.4±1.0 mg/dL, P<0.05
Bell et al 1989	Suffolk TN: N=7 HS: N=6	TN: 18-20C, 30%rh HS: 9 h at 40C, 15 h at 30C, 40%rh	Mid-late pregnancy; gd 64 until gd 134-141	<ul style="list-style-type: none"> • HS increased ewe core temperature ~1C at the end of 40C period, normalised by end of 30C period • Loss of one lamb in two of five HS pregnancies initially carrying twins <p>At late gestation (gd 134-141)</p> <ul style="list-style-type: none"> • = BW (TN: 4.07±0.43 kg; HS: 3.39±0.43 kg) • Fetuses thinner • 54% ↓ placental weight (TN: 398±37 g; HS: 185±37 g)

Brown et al., 1977	Targhee x Suffolk CON: N=16 (range-housed) Restrict: N=16 pair-fed to HS group HS: N=16	CON: Range housed HS: fluctuating from 28 or 32C minimums to 38C max daytime temp.	Late pregnancy, variable starting from ~3-4 months after mating	HS but not restricted feeding decreased: <ul style="list-style-type: none"> • Live lambing rate per ewe (CON: 167±19%; Restrict: 173±15%; HS: 100±26%) • Average BW of all lambs born (CON: 4.57±0.26 kg; Restrict: 4.16±0.16 kg; HS: 3.18±0.22 kg) • Average BW of live-born lambs (CON: 4.69±0.26 kg; Restrict: 4.16±0.16 kg; HS: 3.26±0.31 kg)
Brown & Harrison 1981	Targhee x Texel N=11 ewes; 5 infused with saline and 6 infused with hexamethonium (blocker for sympathetic nervous system) during HS	Acute HS reaching 40C, controls at 20-22C. Humidity not stated, must be high (animals in enclosed chambers, airflow turned off during HS)	Repeated acute studies of ~95-120 min, at least 48 h apart, day 120-140	During acute HS: <ul style="list-style-type: none"> • Ewe rectal temperature ↑ 1.4-1.5C • Uterine blood flow (UBF) ↓ 40-50% (from ~300-400 ml/min in TN to ~140-260 after ~80-100 min when UBF stabilised), effect of HS not affected by central sympathetic blocker • Uterine vascular resistance unchanged (indirectly calculated as UBF/BP) • Implies local vasoconstriction within uterine bed (placenta)
Cartwright and Thwaites (1976)	Merino <u>Experiment 1:</u> N=20 HS ewes, fed to maintain maternal body weight N=45 CON ewes, split into 3 groups of 15, fed differently from gd82-89 until term, to lose 5 kg, maintain weight, or gain 5 kg <u>Experiment 2:</u> N=10 HS ewes, fed <i>ad libitum</i> N=10 TN ewes, fed <i>ad libitum</i> N=9 TN ewes, pair-fed to HS	CON: outdoor yard housed at -2 to 16.4C HS: 8h 42.2C and 16 h 32.2	gd 44-51 until term	Experiment 1: <ul style="list-style-type: none"> • BW lower in HS group (2.30±0.15 kg) than any TN group (wt loss TN: 2.99±0.16 kg; wt maintenance TN: 3.60±0.15 kg; wt gain TN: 4.22±0.15 kg). Experiment 2: <ul style="list-style-type: none"> • HS ↓ maternal food intake 40% • BW reduced by HS but not restricted-feeding (TN <i>ad libitum</i>: 3.40±0.11 kg ; HS: 1.27±0.16 kg ; TN pair-fed: 3.75±0.12 kg)
De Vrijer et al. 2004	Columbia-Rambouillet ewes, 2-3 y old, all singletons	TN: 20±2C continuous, 30% rel humidity HS: [conditions and feeding as per Regnault j	Early-late pregnancy gd37-120	At gd134: <ul style="list-style-type: none"> • Variable reduced fetal growth in HS – 6/10 classified as moderate FGR (wt ↓23%), 4/10 classified as severe FGR (wt ↓53%)

	N=7 TN and N=10 HS ewes	endocrinol 1999, i.e. pair-fed] 40C for 12 h/d and 35C for 12 h/d, 30% rel humidity. <u>TN ewes were pair-fed to the intakes of HS ewes</u>		<ul style="list-style-type: none"> Placental weight ↓26% in moderate FGR and ↓60% in severe FGR Brain sparing in severe group Within the severe FGR group, impaired placental uptake and impaired transfer to fetus of a BCAA analog (system L amino acid transport reduced)
De Vrijer et al. (2006)	Columbia-Rambouillet ewes, 2-3 y old, all singletons N=10 TN and n=11 HS ewes (? Overlap in animals as Regnault et al 2002)	TN: 20±2C continuous, 30% rel humidity HS: [conditions and feeding as per Regnault 1999, i.e. pair-fed] 40C for 12 h/d and 35C for 12 h/d, 30% rh <u>TN ewes were pair-fed to the intakes of HS ewes</u>	Early-mid pregnancy gd35-end	<p>Outcomes measured at gd55 and gd90.</p> <p>At gd55 (after 20 d HS):</p> <ul style="list-style-type: none"> CBT 39.9 in HS ewes cf 39.2 in TN ewes No difference in fetal or placental weights Fetal:placental wt ratio HS>TN Higher brain:liver wt ratio (indicating brain sparing) <p>At gd90 (after 55 d HS):</p> <ul style="list-style-type: none"> NSD placental weight (33% lower in HS) Fetal wt ↓24% in HS (P<0.05) <p>Some changes in placental IGF system gene expression – suggested as mechanism leading to reduced placental fn but no functional measures to confirm this. Appears to be some compensatory changes in IGFs in placenta – increased IGF1 in caruncles at gd90 (maternal placenta), increased IGF2 expression in cotyledons at gd55 (fetal placenta)</p>
Early et al. (1991)	Suffolk TN: 7 ewes HS: 6 ewes started, 5 completed	TN: 18-20C, 30% rel humidity HS: 40C for 9h/d and 30C for 15 h/d 40% rel humidity	Mid and late pregnancy, gd64 to end at gd136-141	<p>At gd136-141:</p> <ul style="list-style-type: none"> Fetal weight ↓27%, P<0.01, 3.46 cf 4.74 kg, SE 0.18 kg* Placental weight ↓58%, P<0.001, 189 cf 448 g, SE 21 g* * these are the same animals and data as Bell 1989 Normal placental concentrations of protein, RNA, DNA; implying ↓cell number and = cell size; HS inhibits placental cell division Decreased fetal placental oxygen consumption in vitro – likely adaptation to lower placental oxygen transfer (may inhibit mitosis)
Galan et al (1998)	Columbia– Rambouillet TN: 5 ewes in current study + 37 from prior studies	TN: 20C HS: 40C for 12 h/d and 35C for 12 h/d, 35-40% rel humidity.	Early-mid pregnancy gd35-90 or early-late	<p>At gd135:</p> <ul style="list-style-type: none"> Fetal weight ↓45% (HS, 55 d) or ↓74% (HS, 80 d)

	HS, 55 d: 5 ewes in current study HS, 80d: 4 ewes from prior study	<u>Both groups ad libitum fed</u>	pregnancy gd35-115	<ul style="list-style-type: none"> • From serial ultrasounds, in HS 55 d group, relative to controls, smaller abdominal circumference from 77 gD, femur length from GD101, tibia length from gD80. • Evidence of IUGR including brain sparing • Placental weight ↓41% (HS, 55 d) or ↓70% (HS, 80 d) • = numbers of placental cotyledons
Regnault et al (1999)	Columbia– Rambouillet TN: 8 ewes HS: 6 ewes	TN: 20C, 30% rel humidity HS: 40C for 12 h/d and 35C for 12 h/d, 30% rh <u>TN ewes were pair-fed to the intakes of HS ewes</u>	Early-mid pregnancy gd37-93	<p>During treatment:</p> <ul style="list-style-type: none"> • Maternal core temp ↑ 0.8C • ↓ maternal circulating progesterone (from gd72, when placental prod'n takes over from ovarian) • ↓ maternal circulating placental lactogen (oPL, from gd62), • Immediate and sustained 4-fold ↑ maternal circulating prolactin, thought to be response to physiological stress. ?effect on pregnancy • =maternal feed intake <p>At gd93:</p> <ul style="list-style-type: none"> • Fetal weight ↓27%, P<0.05, 515±49 cf 703±45 g. Thinner with ↑brain:liver indicating “sparing”/chronic IUGR • Placental weight ↓36% but NS, 364±63 cf 571±96 g, NS ↓ in both placentome number and weight • =fetal:placental weight ratio (placental function not compensating) • =placental mRNA & protein oPL content at gd93 implying impaired development (less) of fetal bi-nucleated trophoblast cells (BNC, only cell type that produces oPL), or decreased trophoblast migration into maternal compartment • Major disruption of early placental function and development – likely particularly trophoblast BNC
Regnault et al (2002)	Columbia-Rambouillet ewes, 2-3 y old, all singletons N=10 TN and n=11 HS ewes (same animals as Regnault et al 1999)	TN: 20±2C continuous, 30% rh HS: [conditions and feeding as per Regnault 1999, i.e. pair-fed] 40C for 12 h/d and 35C for 12 h/d, 30% rh <u>TN ewes were pair-fed to the intakes of HS ewes</u>	Early-mid pregnancy gd37?-end	<p>Outcomes measured at gd55 and gd90.</p> <p>At gd55 (after 15 d HS, n=5 TN, 6 HS):</p> <ul style="list-style-type: none"> • CBT 39.9 in HS ewes cf 39.2 in TN ewes • No difference in fetal or placental weights • Fetal:placental wt ratio HS>TN • Higher brain:liver wt ratio (indicating brain sparing)

				<p>At gd90 (after 55 d HS, n=5 TN, 5 HS):</p> <ul style="list-style-type: none"> • NSD placental weight (33% lower in HS) • Fetal wt ↓24% in HS (P<0.05) <p>Some changes in placental VEGF system gene expression:</p> <p>At gd55:</p> <ul style="list-style-type: none"> • inc VEGF mRNA and protein in cotyledon in HS (hypothesised due to hypoxia which induces VEGF), • ↓VEGF protein in caruncle and = VEGFR mRNA in both <p>At gd90:</p> <ul style="list-style-type: none"> • ↓50% VEGFR mRNA in cotyledon. Likely to reduce vascularisation and function of the placenta (gd90 is period of maximal placental growth)
Regnault et al (2003)	<p>Columbia-Rambouillet ewes, 2-3 y old, all singletons</p> <p>N=6 TN and n=10 HS ewes</p>	<p>TN: 20±2C continuous, 30% <i>rh</i></p> <p>HS: [conditions and feeding as per Regnault 1999, i.e. pair-fed] 40C for 12 h/d and 35C for 12 h/d, 30% <i>rh</i></p> <p><u>TN ewes were pair-fed to the intakes of HS ewes</u></p>	<p>Early-late pregnancy gd39-120</p>	<p>Outcomes measured at gd123 (2-3 d after end of HS):</p> <ul style="list-style-type: none"> • Ultrasound/blood flow resistance in HS <p>Outcomes measured at gd135 (15 d after end of HS):</p> <ul style="list-style-type: none"> • Fetal hypoxia (arterial PO₂ ↓31%) despite increased uterine blood flow ↑37%, associated with blood flow resistance through placenta • Altered placental expression of VEGF receptor and placental growth factors
Shelton and Huston (1968)	<p>Breed not stated.</p> <p>Different N and groups in multiple experiments</p>	<p>TN: 24C</p> <p>HS: either 32C or 40.5C (different experiments).</p> <p>For 12 h HS experiments, 12 h @ 32C and 12 h @ 20-27C</p>	<p>Last half to two-thirds of pregnancy</p>	<ul style="list-style-type: none"> • Across all experiments, lamb survival 80.8% in TN and 56.8% in HS, BW TN: 3.95 kg singles & 2.89 kg in twins, HS: 2.79 kg singles & 1.90 kg twins • More severe ↓ in BW and survival in continuous HS compared to 12 h HS (Birthweight: TN: 4.36 kg, 12h HS 3.63 kg, 24h HS 2.60 kg; Lamb survival: TN: 100%, 12h HS 80%, 24h HS 55%) – HS pair-fed to TN • HS but not feed restriction ↓BW (TN ad lib 4.18 kg, TN restricted 3.77 kg, HS ad libitum 2.23 kg, HS restricted 2.14 kg) • High protein diets did not improve lamb outcomes, and under HS appeared to have an adverse effect
Thureen et al. (1992)	<p>Columbia– Rambouillet</p> <p>TN: 7 ewes</p> <p>HS: 6 ewes</p>	<p>TN: 20C, 40-45% <i>rh</i></p> <p>HS: 40C for 18 h/d and 35C for 6 h/d, 40-45% <i>rh</i></p>	<p>Early-mid to mid-late pregnancy</p> <p>Starting gd35-43,</p>	<p>During treatment:</p> <ul style="list-style-type: none"> • Maternal core temp ↑ 1.0C

	(all singletons, all fed ad lib)		duration 84-7 d (to ~gd125)	<p>Studied in TN conditions at gd ~135±1 in HS, 130±1 in TN, after 8-10 d in TN:</p> <ul style="list-style-type: none"> • Fetal weight ↓47%, P<0.01, 1645±264 cf 3112±224 g. Brain weight = but liver weight ↓56% • Placental weight ↓58%, P<0.001, 149±27 cf 356±30 g. = placentome number, ↓weight. • =fetal:placental weight ratio – indicating no compensation of function (essentially reflects lack of compensation of function for a smaller placenta during HS since the few days post-HS wouldn't be enough to restore fetal growth especially given surgery) • Uterine blood flow ↓33%, P<0.05, 853±127 cf 1270±120 ml/min. • Umbilical blood flow ↓52%, P<0.001, 326±63 cf 678±38 ml/min, but normal per kg fetus or placenta • Placental ethanol clearance (diffusion) ↓52%, P<0.001, 181±29 cf 379±14 ml/min, but normal per kg fetus or placenta • Fetal hypoxia in HS: O₂ satn ↓34%, P<0.02, 37.3±6.1 cf 56.8±3.8 % • Oxygen conc in umbilical vein =, greater extraction in HS fetuses (lower O₂ in umbilical artery – returning to placenta), likely compensation for lower oxygen delivery to fetus in HS – calc as umbilical blood flow x oxygen concentration: ↓21%, P<0.05, 1.28±0.12 cf 1.63±0.10 mM.min⁻¹.kg fetus⁻¹ • At the same maternal [glucose], lower fetal plasma [glucose] in HS: ↓37%, P<0.001, 13.3±1.2 cf 21.1±0.8 mg/dL – normal umbilical glucose uptake per kg fetus achieved by increased umbilical glucose uptake per kg placenta. i.e. impaired placental capacity for glucose delivery – lower transport for a given transplacental concentration difference. Not a blood flow effect given normal ethanol clearance (diffusion) and also not due to placental glucose metabolism, therefore impaired transport capacity, probably due to smaller exchange surface area.
Vatnick et al 1991	Dorset TN: n=6 HS: n=5	TN: 21C HS: 40C for 9 h/d and 30C for 15 h/d	Mid-pregnancy, gd50-75	<p>Maternal core temperature</p> <ul style="list-style-type: none"> • ↑0.6C during 40C period, ↑0.3C during 30C period <p>At gd75:</p>

	All twin-bearing	<i>rh</i> not controlled and always <50%		<ul style="list-style-type: none">• = Fetal weight, TN:232±7, HS:222±7• Placental weight ↓19%, P=0.02, TN:635±39, HS:514±24• N placentomes ↑30%, P<0.05, TN: 46±2, HS:60±5• Weight/placentome ↓36%, P<0.001, TN:14.0±1.0, HS:9.0±0.7• = placental DNA and protein concentrations (implying normal cell size and fewer cells)• Placental DNA synthesis ↑55%, P=0.06, TN:320±73, HS:495±43 – possibly greater cell turnover
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8. Supplementary Material 4: Site Plots

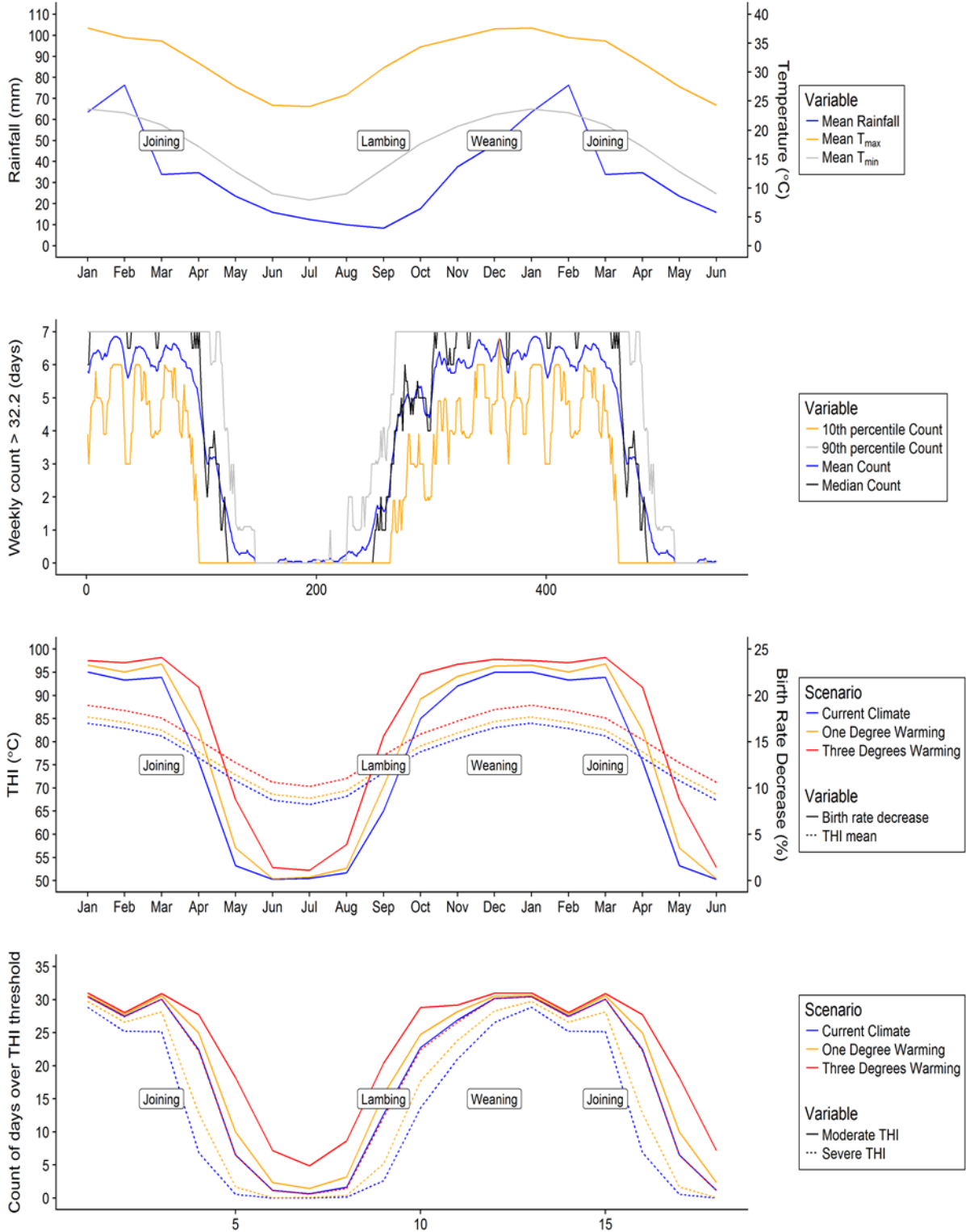
8.1. BOM weather stations for selected site

Table 8-1. List and location of BOM weather stations for selected site

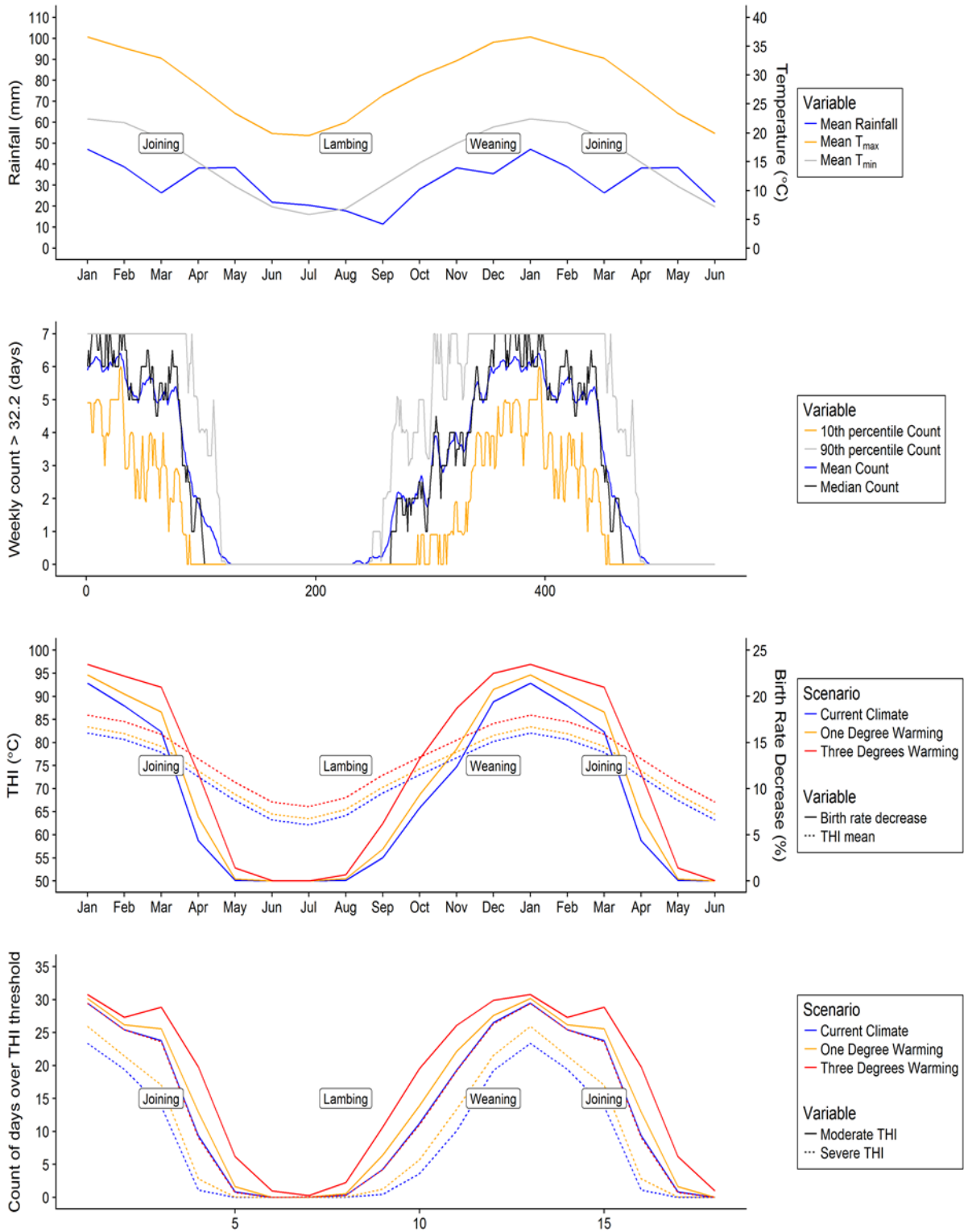
Map ID	BoM Weather Station	Longitude	Latitude
1	Longreach Aero	144.28	-23.44
2	Cunnamulla (Post Office)	145.68	-28.07
3	St George Airport	148.59	-28.05
4	Wilcannia (Reid St)	143.37	-31.56
5	Gunnedah Research Centre	150.27	-31.03
6	Armidale Radio Station	151.67	-30.52
7	Trangie Post Office	147.98	-32.03
8	Dubbo Airport	148.58	-32.22
9	Griffith (CSIRO)	146.07	-34.32
10	West Wyalong Airport AWS	147.2	-33.94
11	Young Airport	148.25	-34.25
12	Walpeup Research	142.36	-35.72
13	Bendigo Airport	144.33	-36.74
14	Shepparton Airport	145.39	-36.43
15	Hamilton Airport	142.06	-37.65
16	Ballarat (Aerodome)	143.79	-37.51
17	Minnipa Agricultural Centre	135.15	-32.84
18	Rosedale (Turretfield Research Centre)	138.83	-34.55
19	Renmark	140.75	-34.17
20	Struan	140.79	-37.1
21	Geraldton Town	114.61	-28.78
22	Northam	116.66	-31.65
23	Katanning	117.56	-33.69
24	Esperance	121.89	-33.83
25	Launceston Airport	147.2	-41.54
26	Hobart Airport	147.5	-42.83

8.2. Site plots and calendars for selected sites

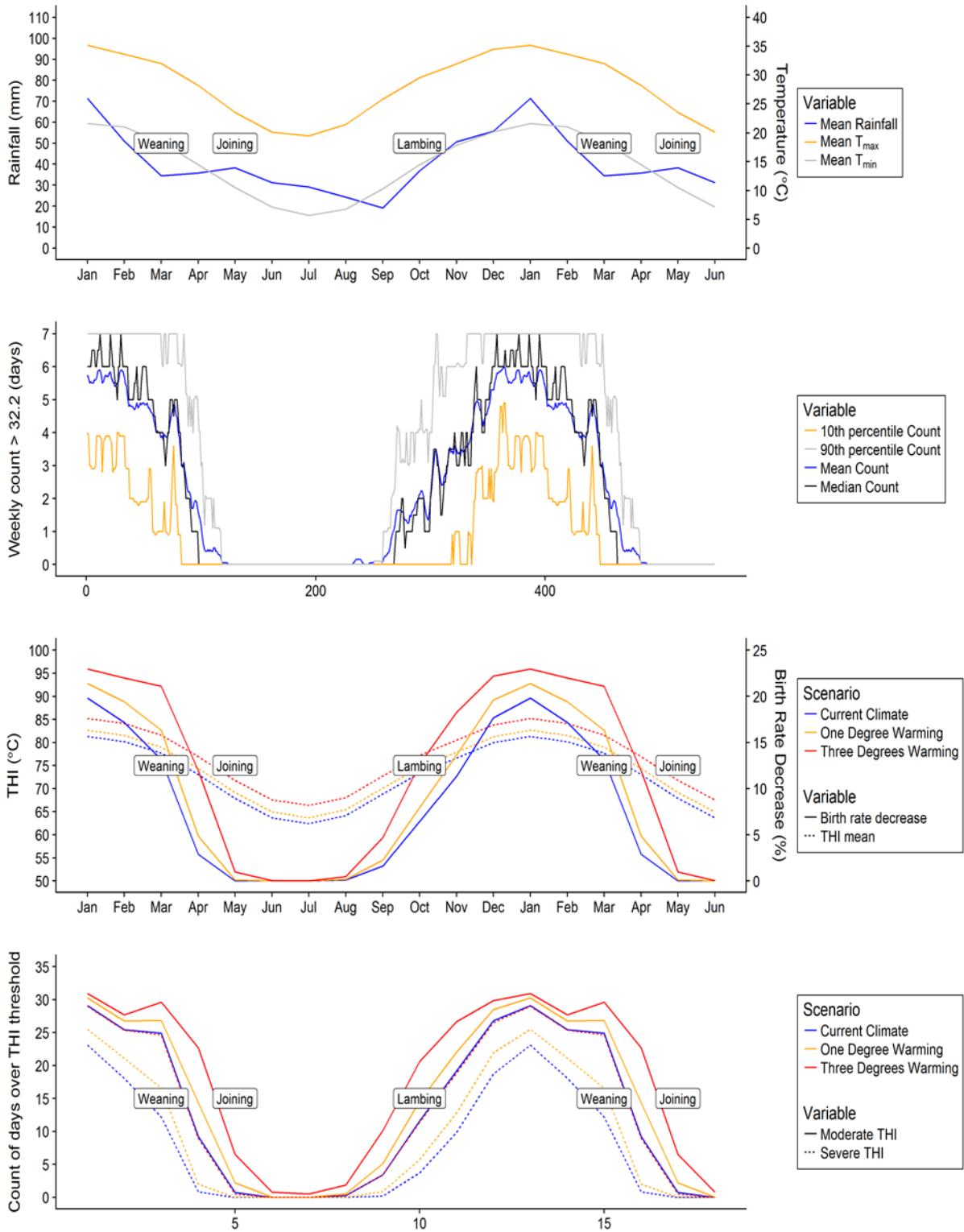
1. Longreach Aero - QLD



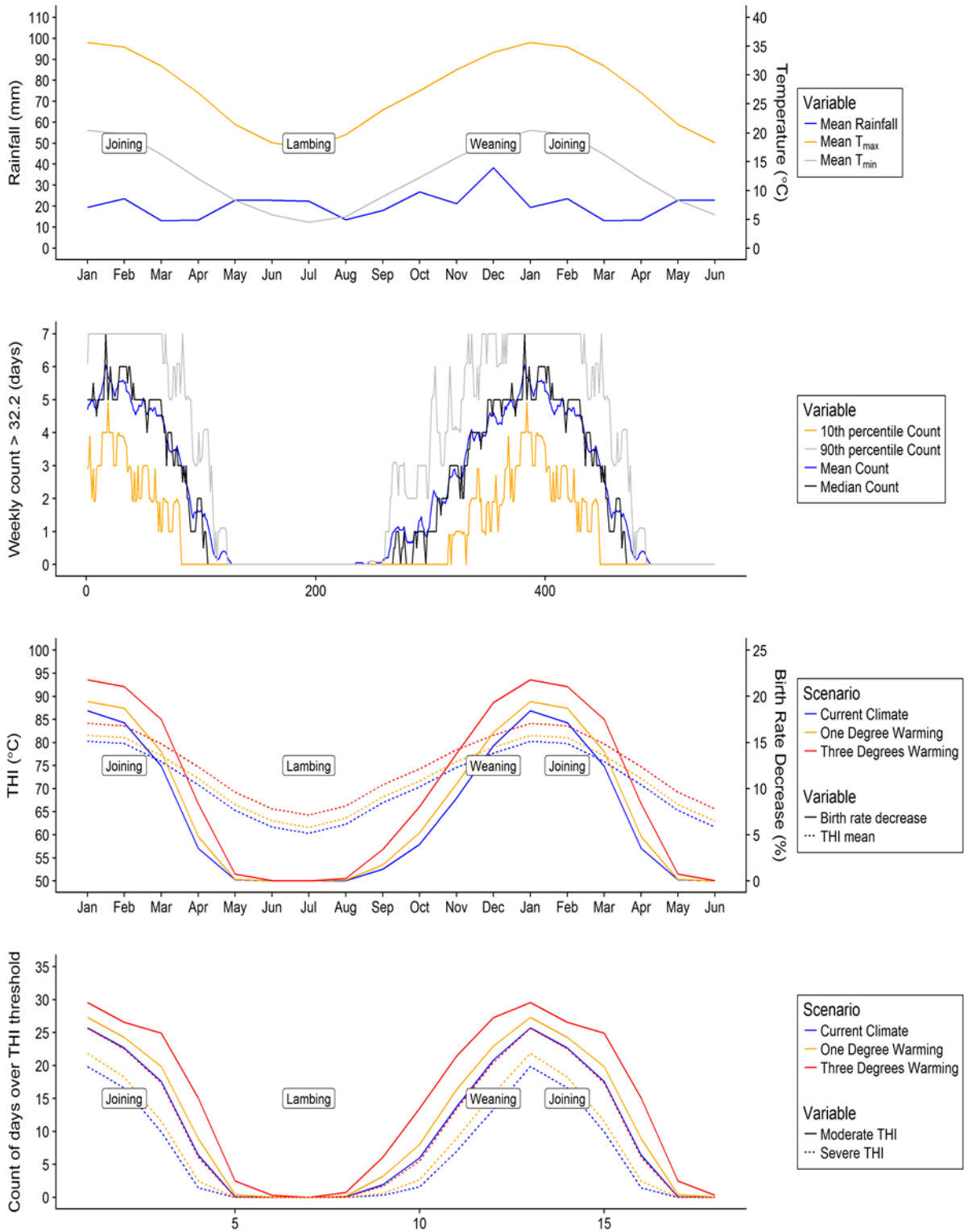
2. Cunnamulla (Post Office) - QLD



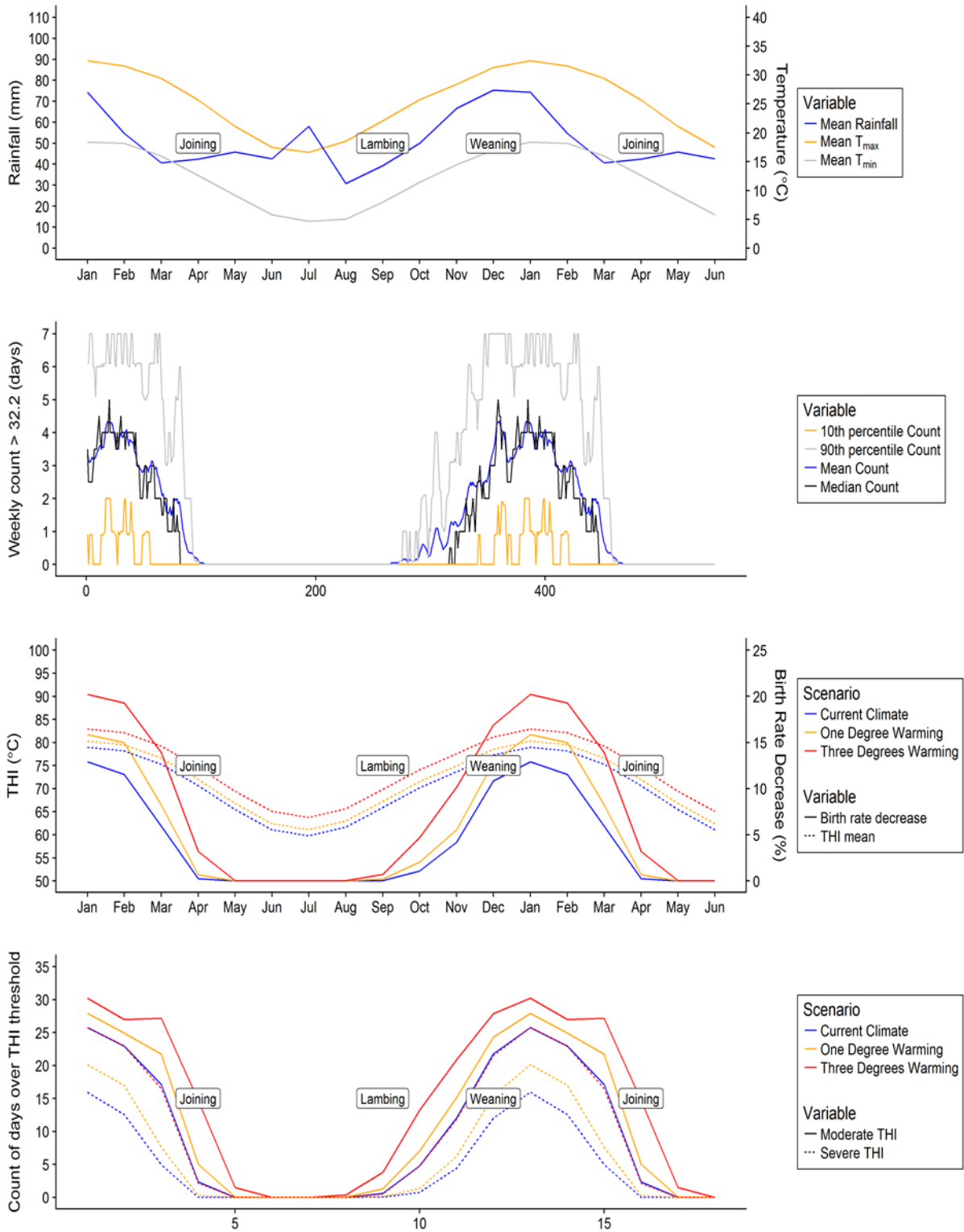
3. St George Airport - QLD



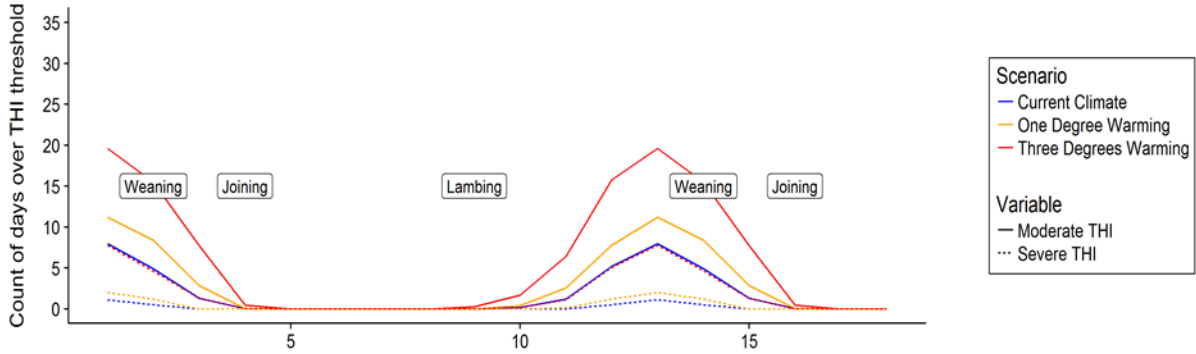
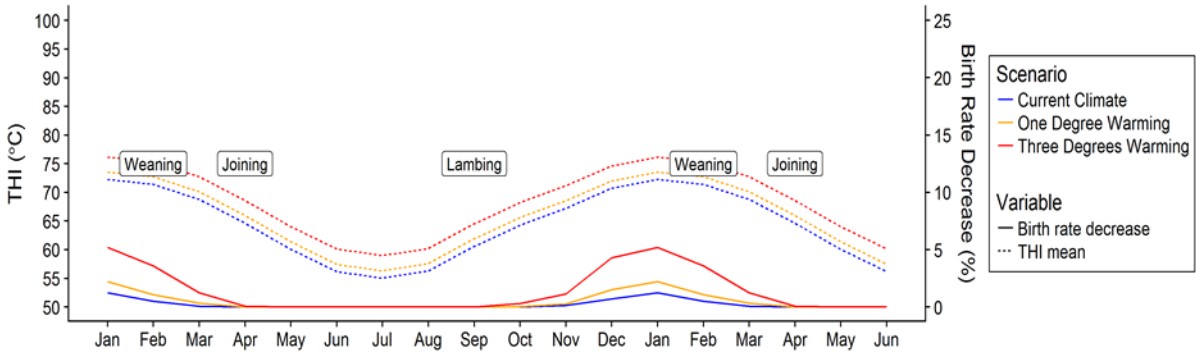
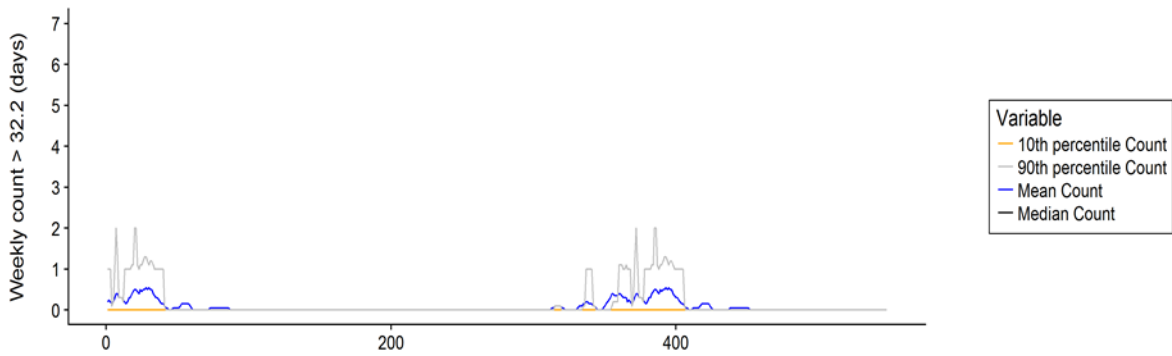
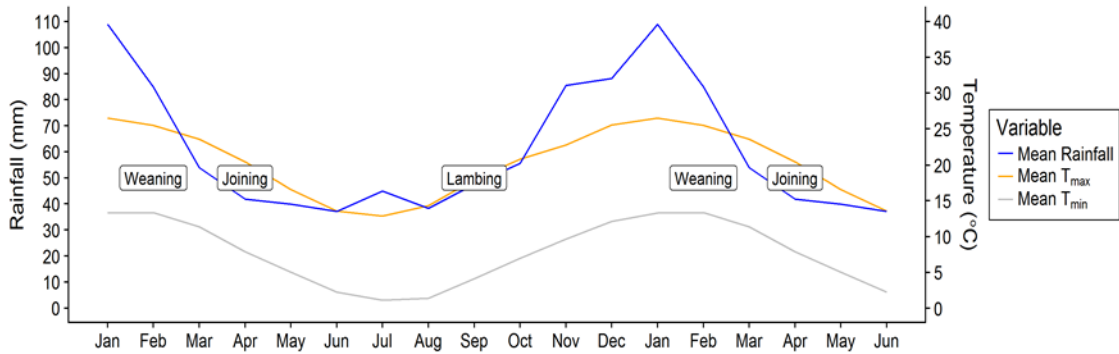
4. Wilcannia (Reid St) - NSW



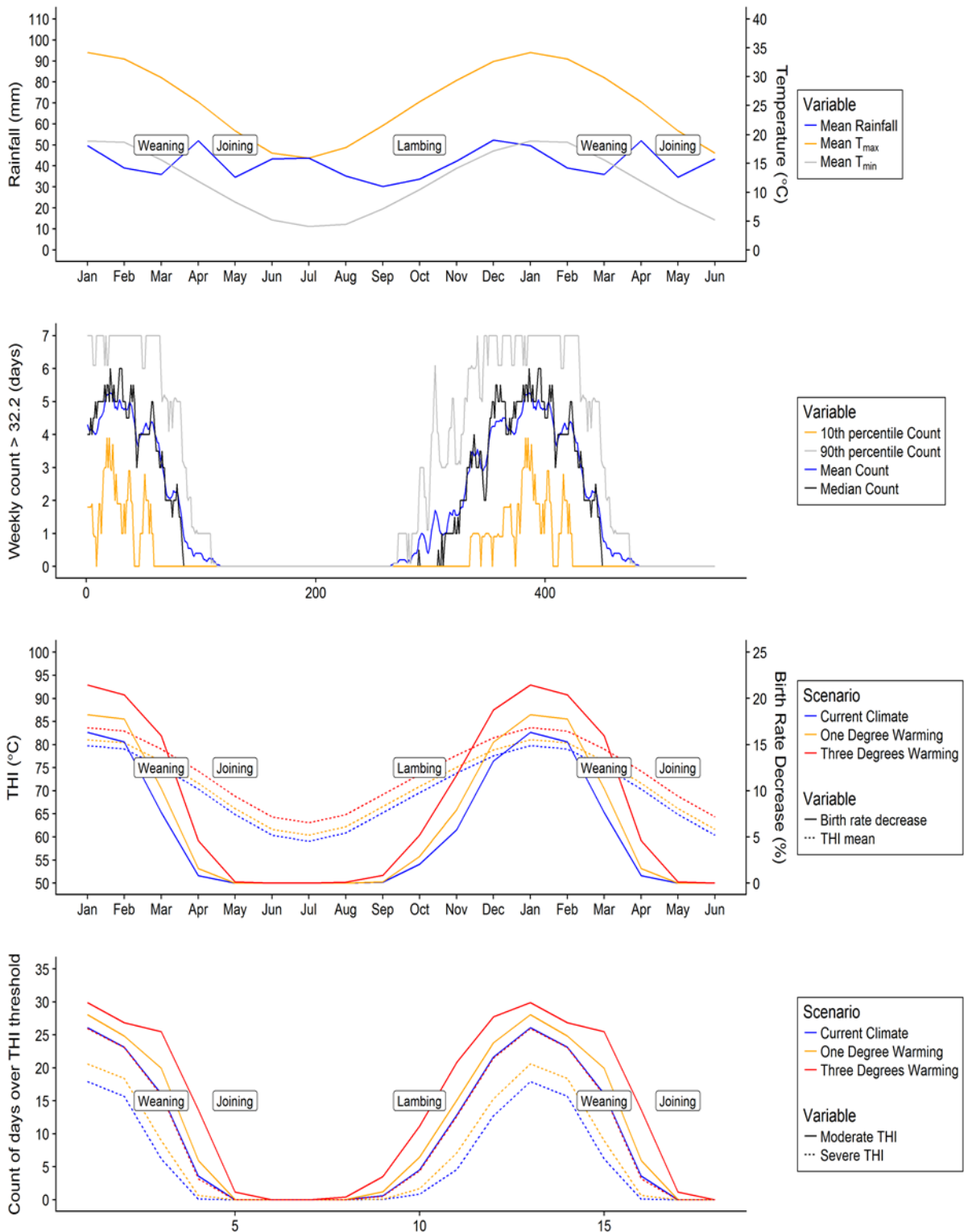
5. Gunnedah Research Centre - NSW



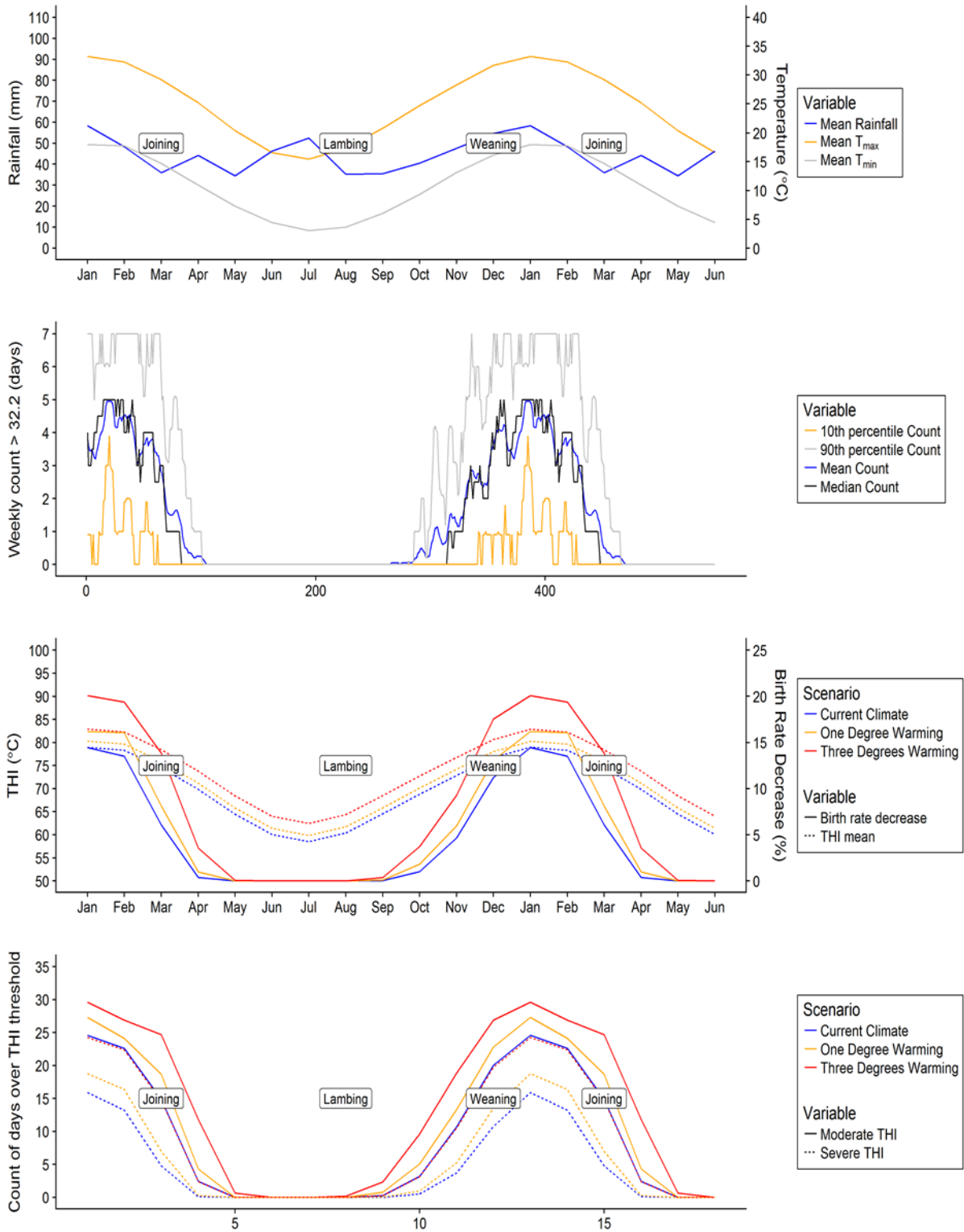
6. Armidale Radio Station - NSW



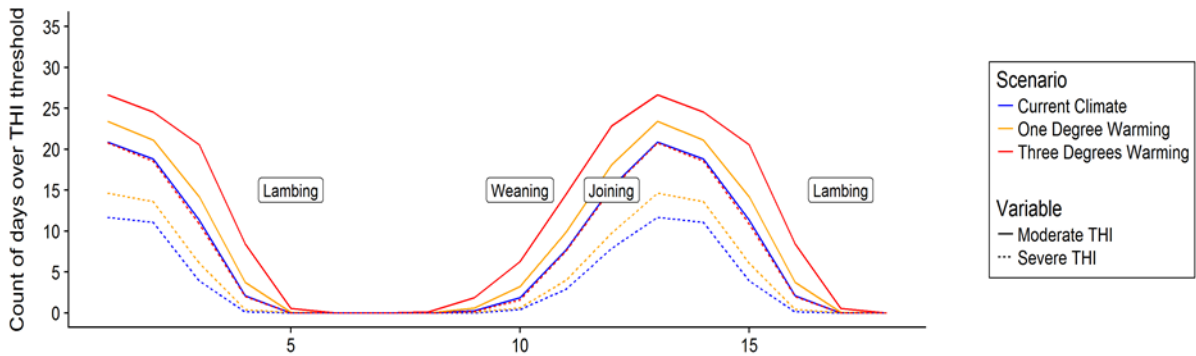
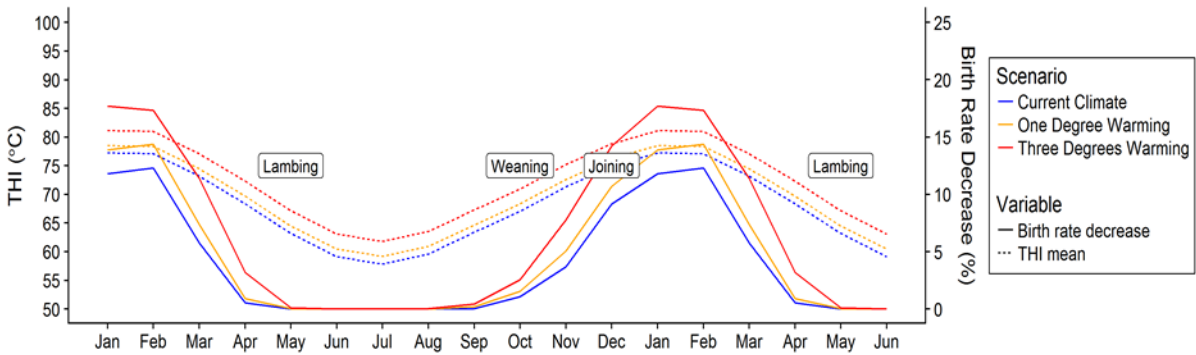
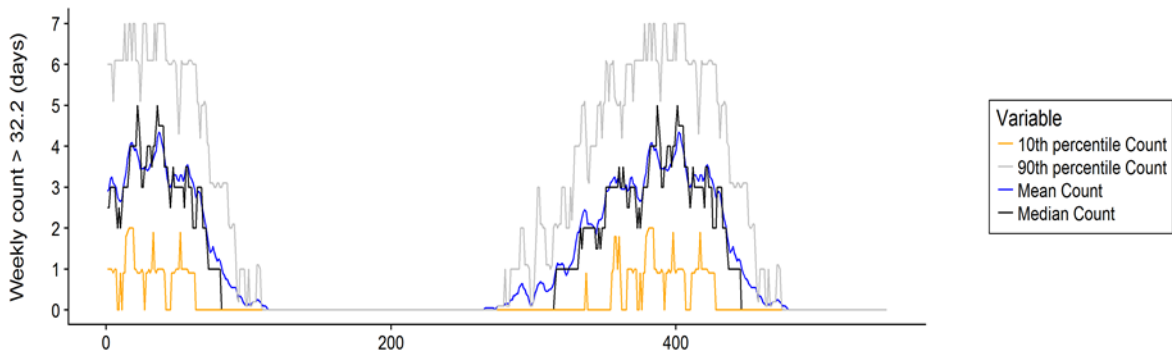
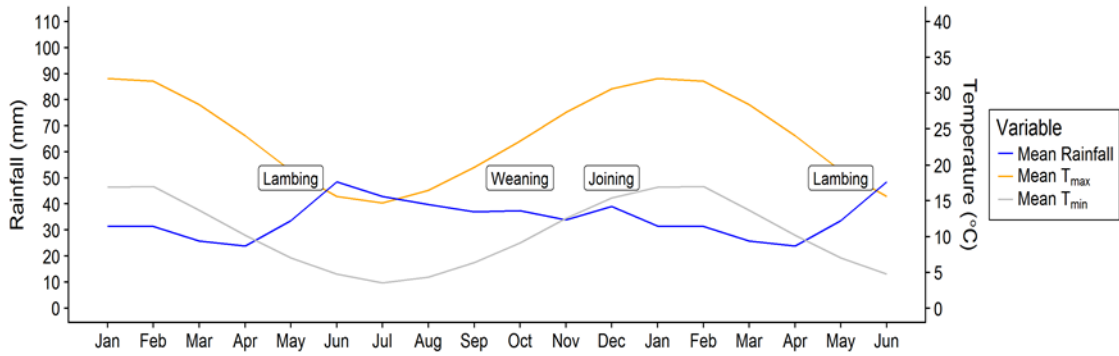
7. Trangie Post Office - NSW



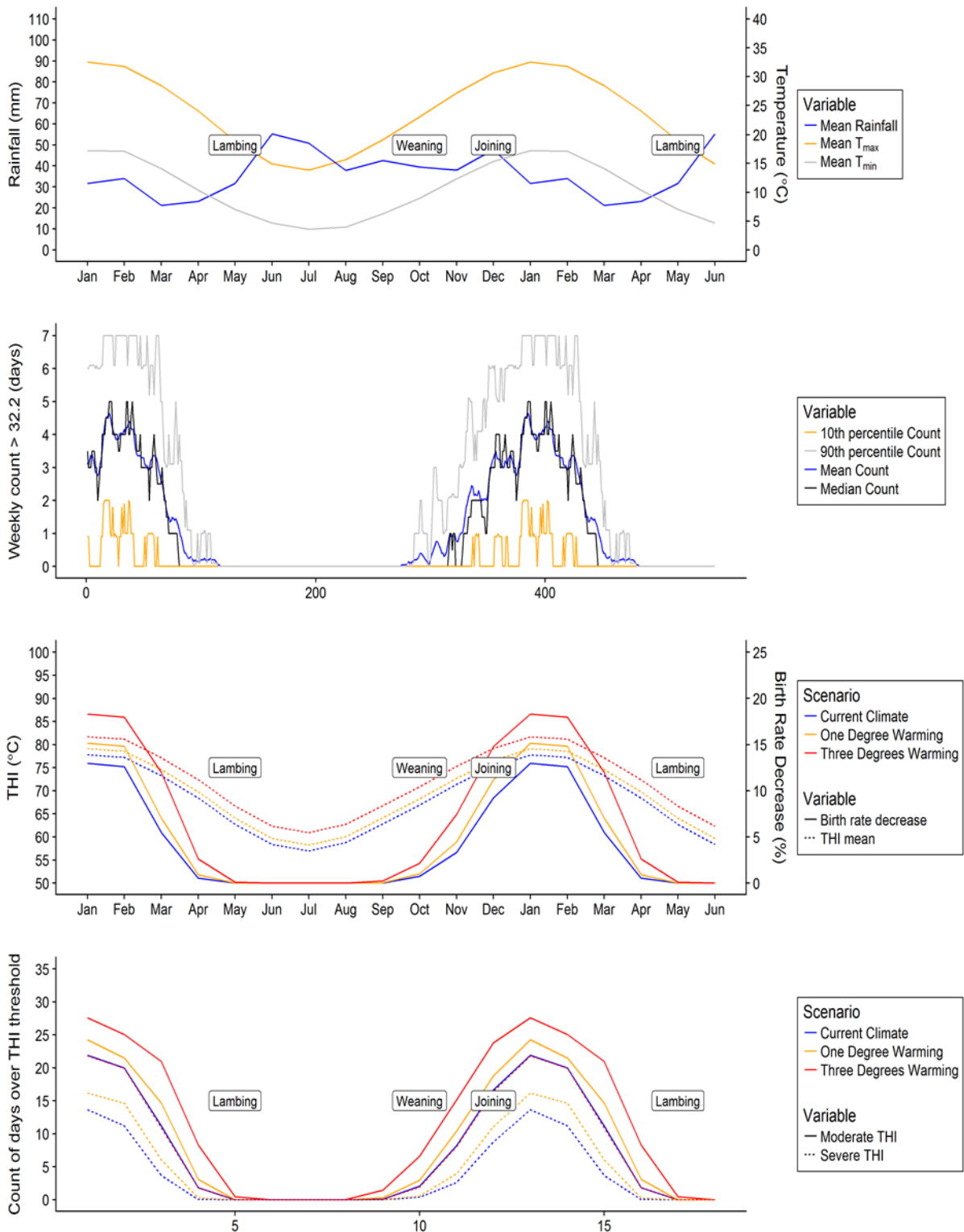
8. Dubbo Airport - NSW



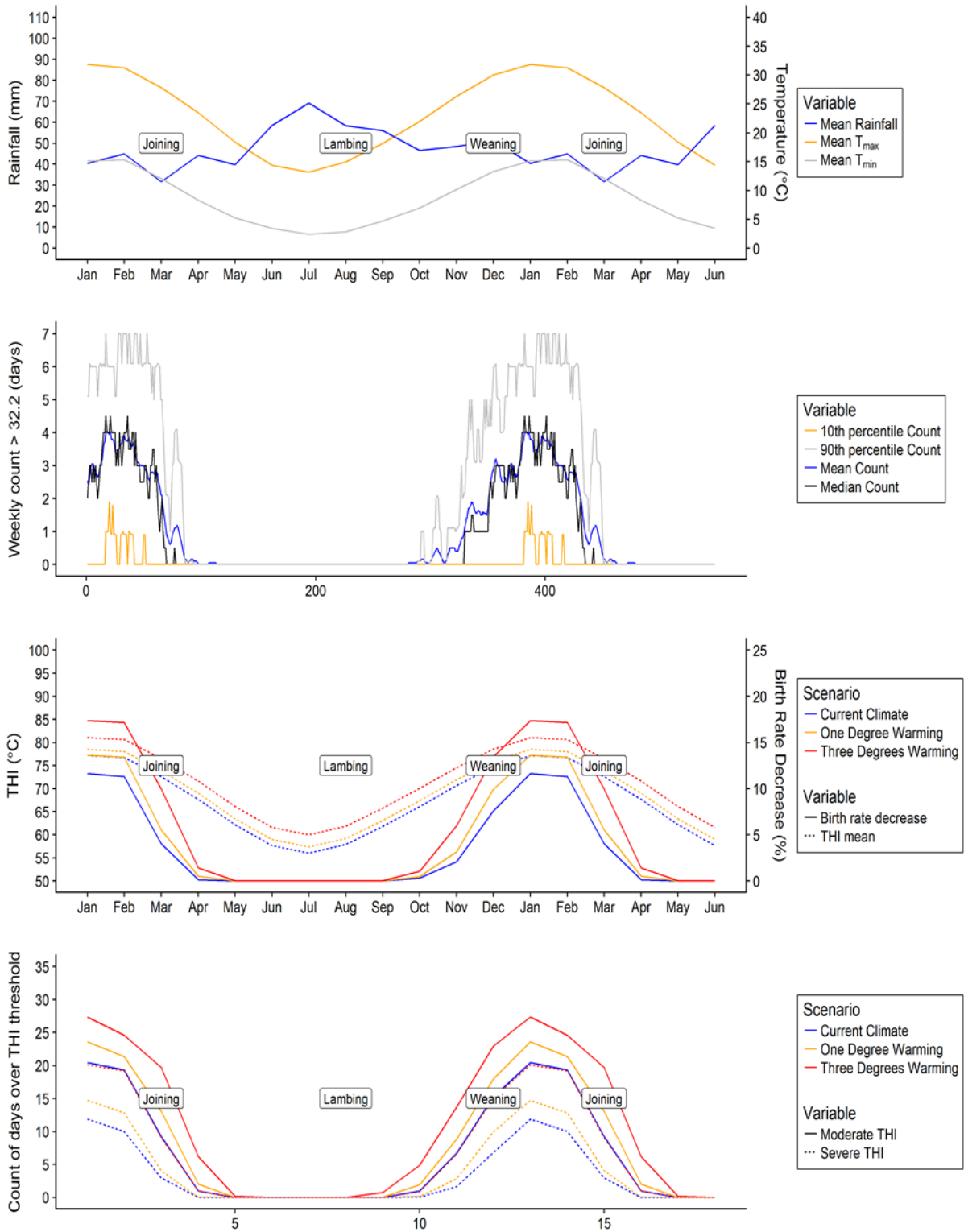
9. Griffith (CSIRO) - NSW



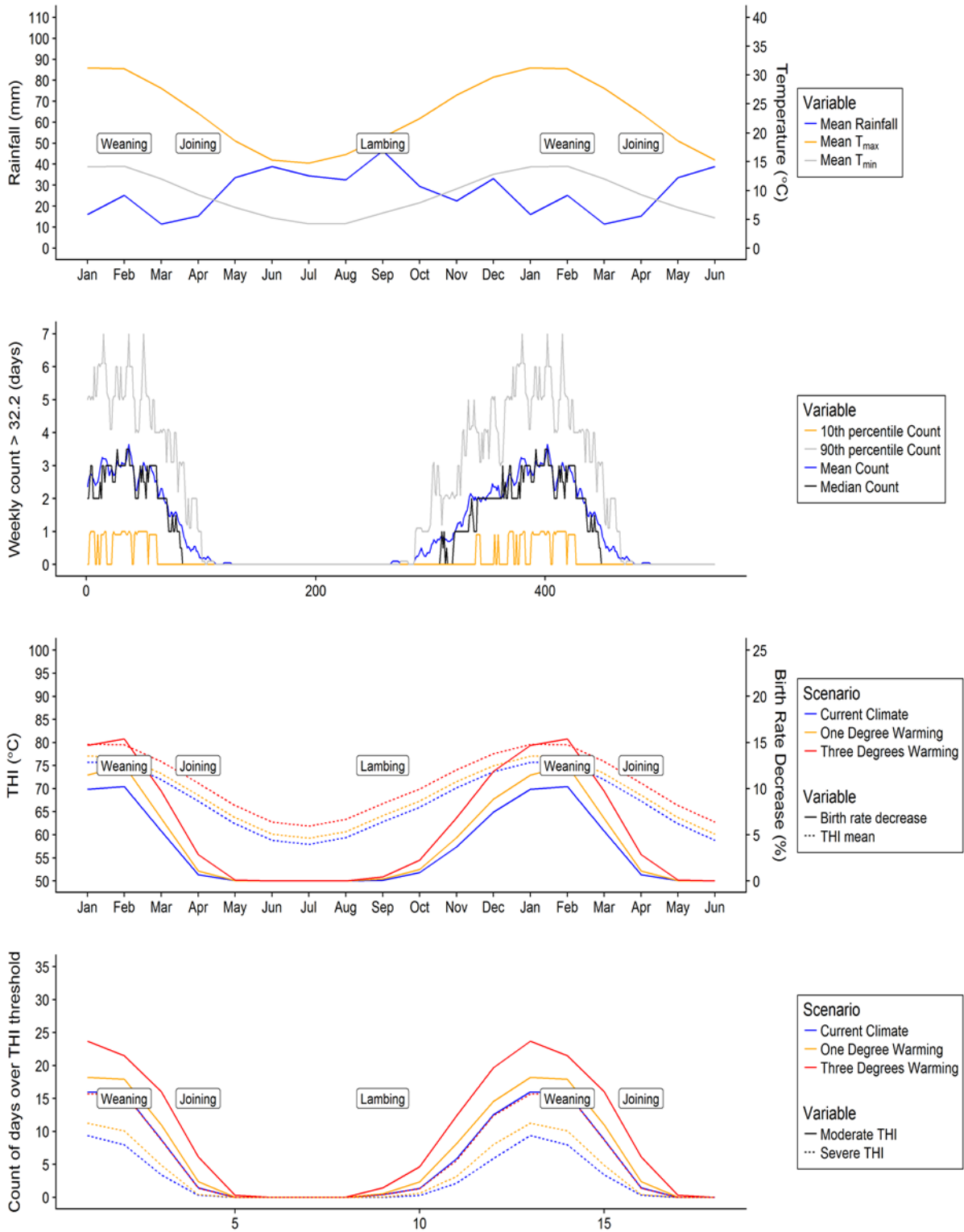
10. West Wyalong Airport AWS - NSW



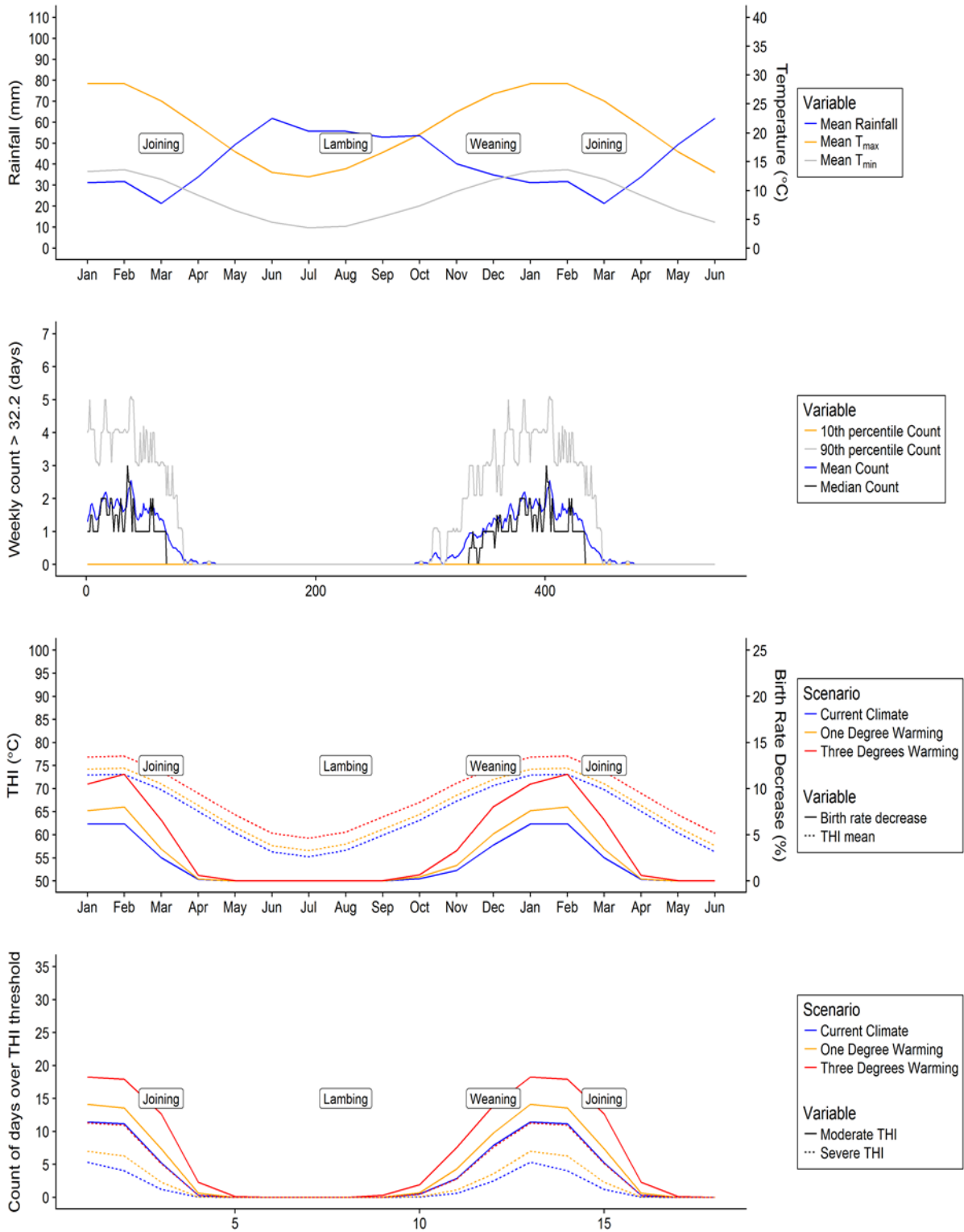
11. Young Airport - NSW



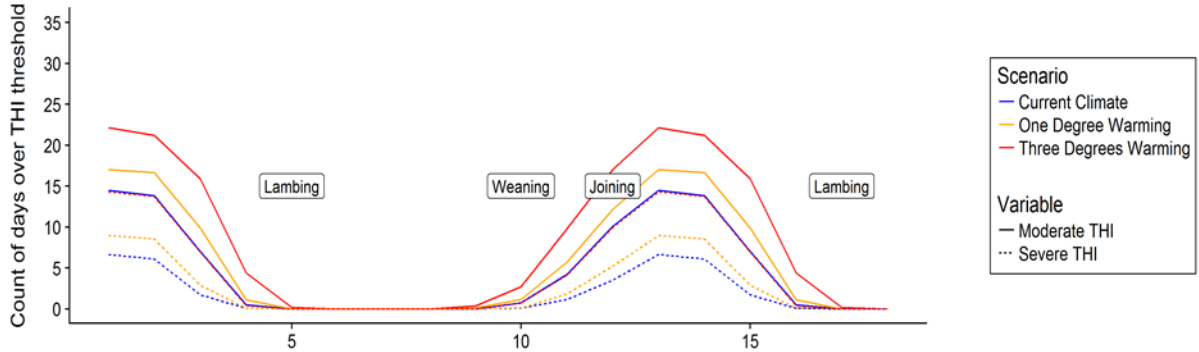
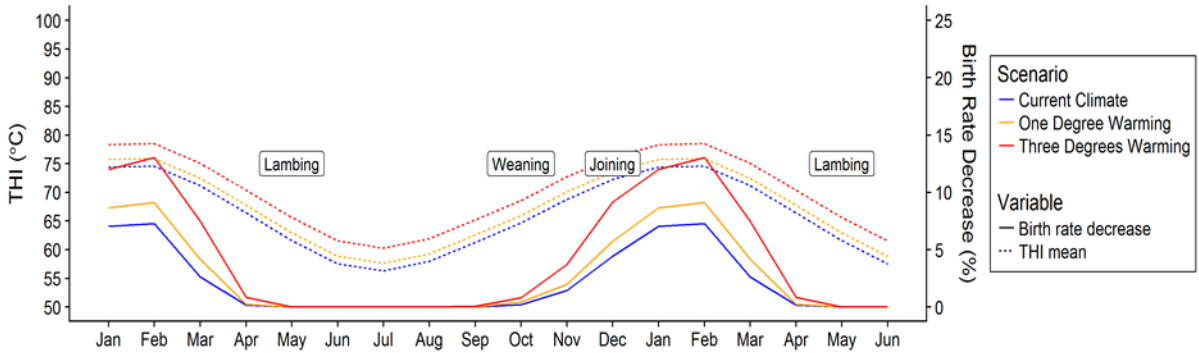
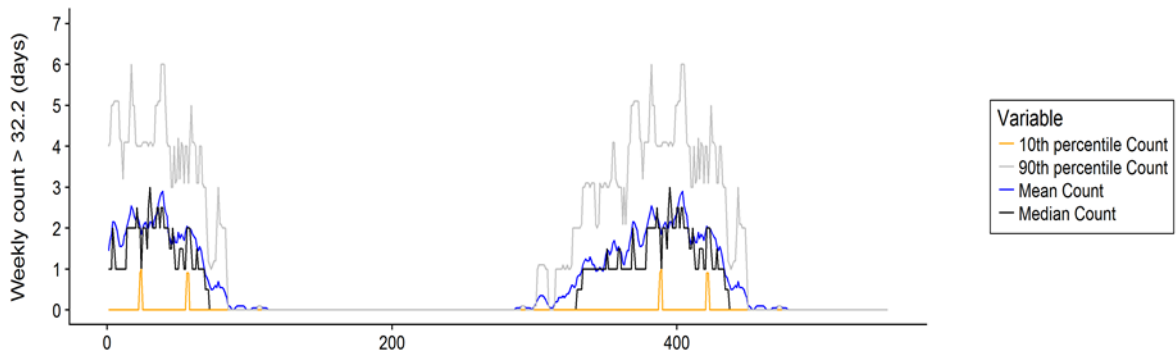
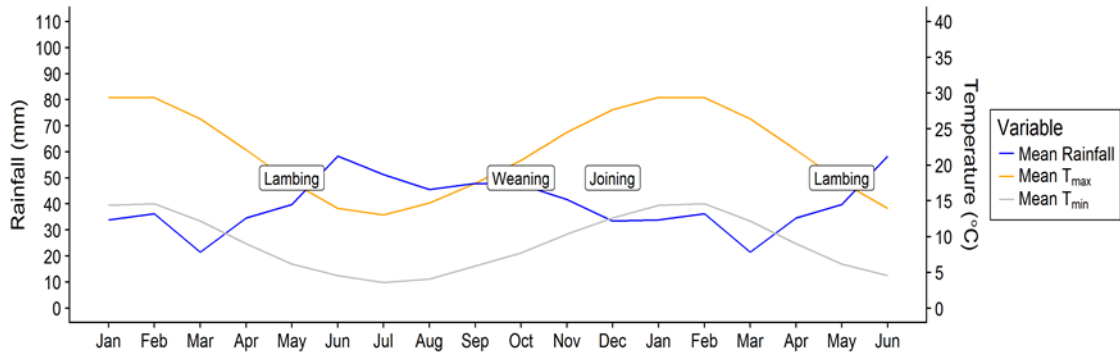
12. Walpeup Research - VIC



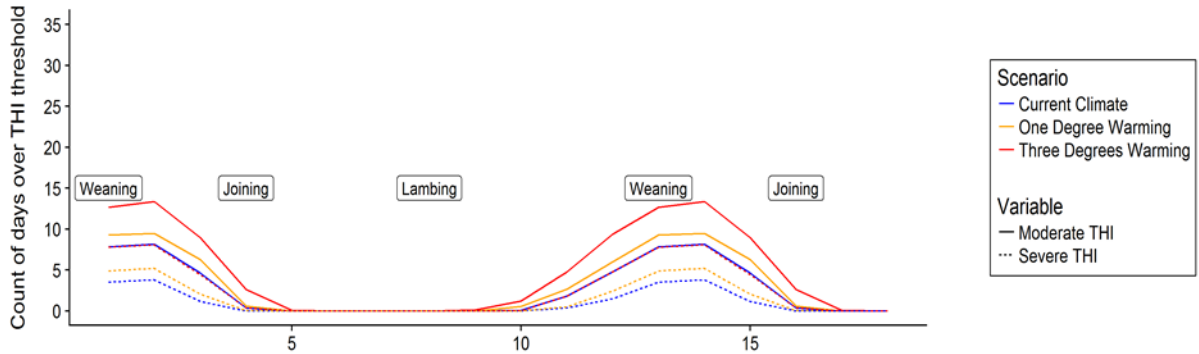
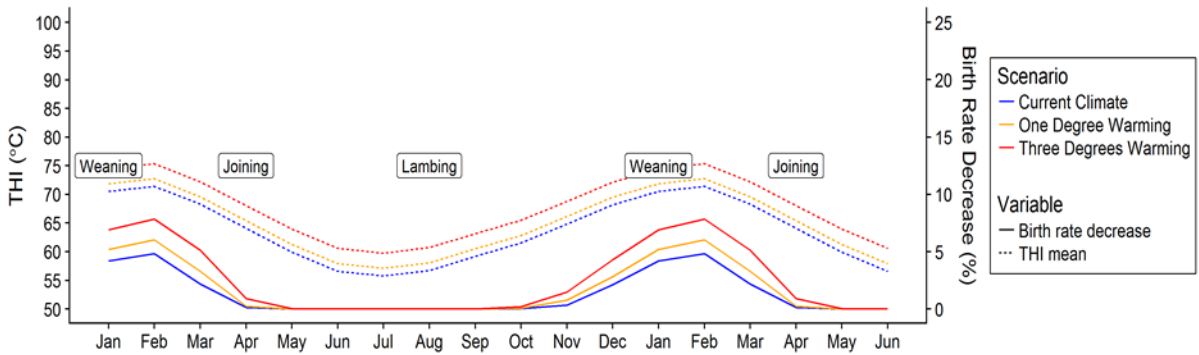
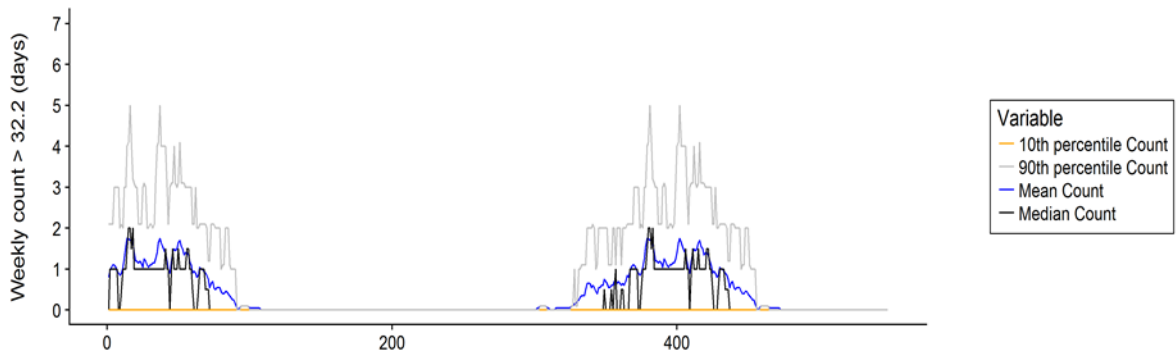
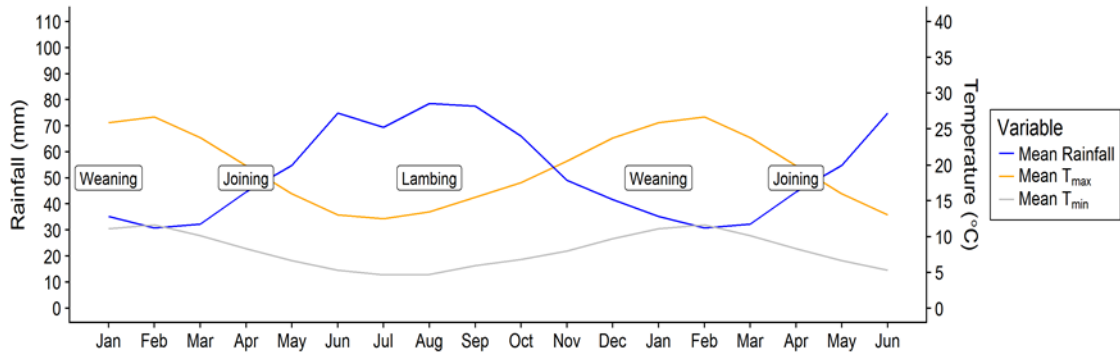
13. Bendigo Airport - VIC



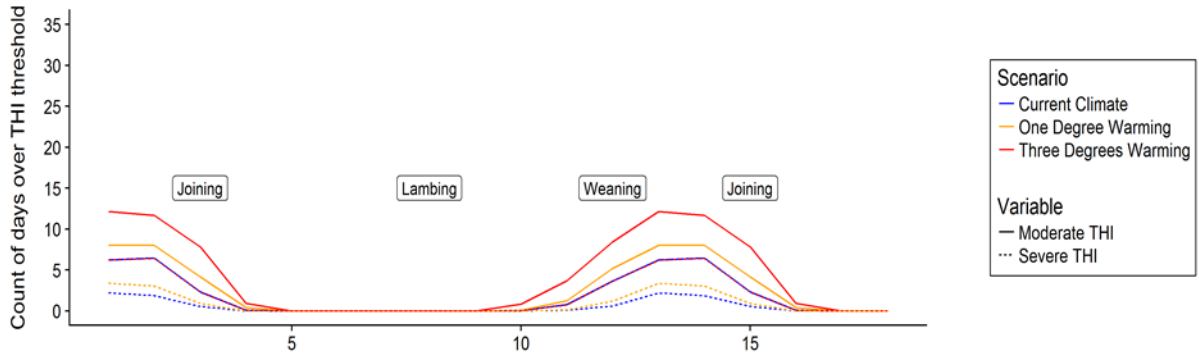
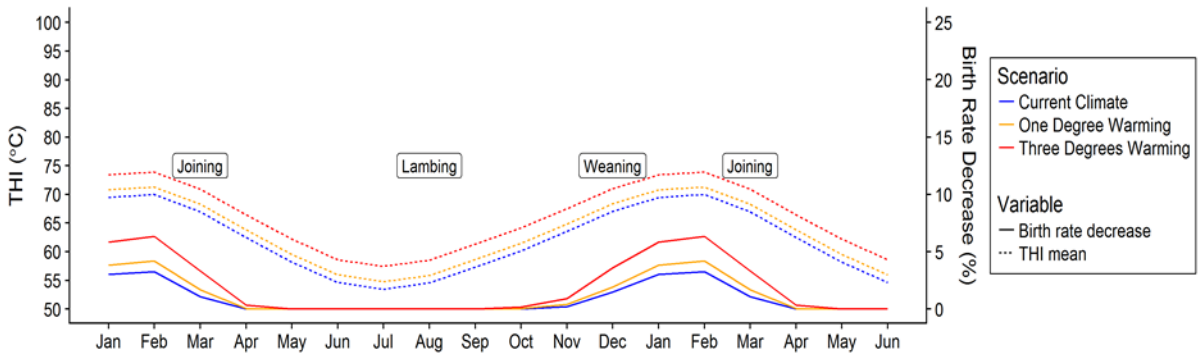
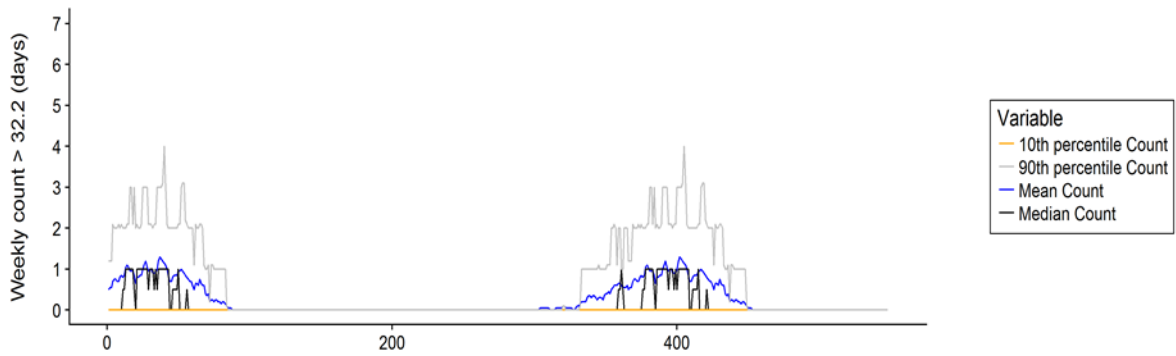
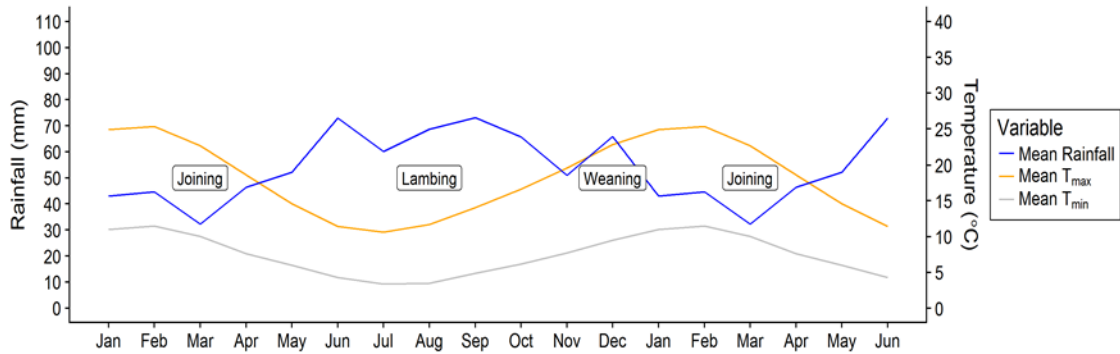
14. Shepparton Airport - VIC



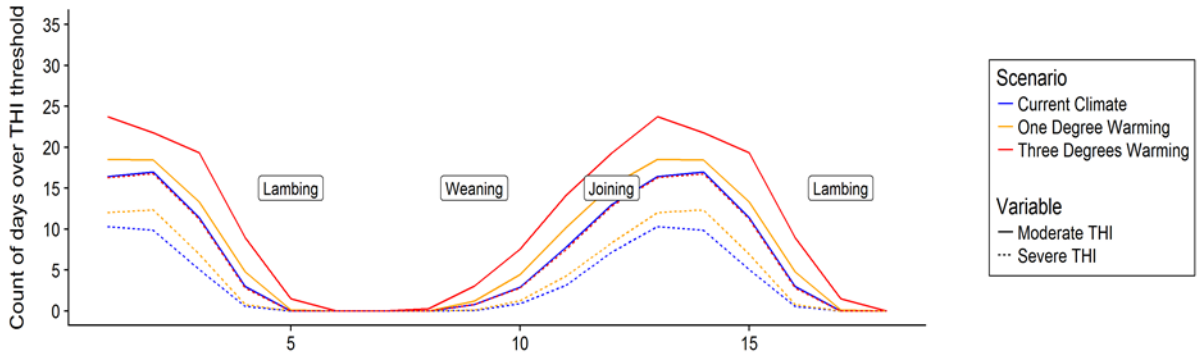
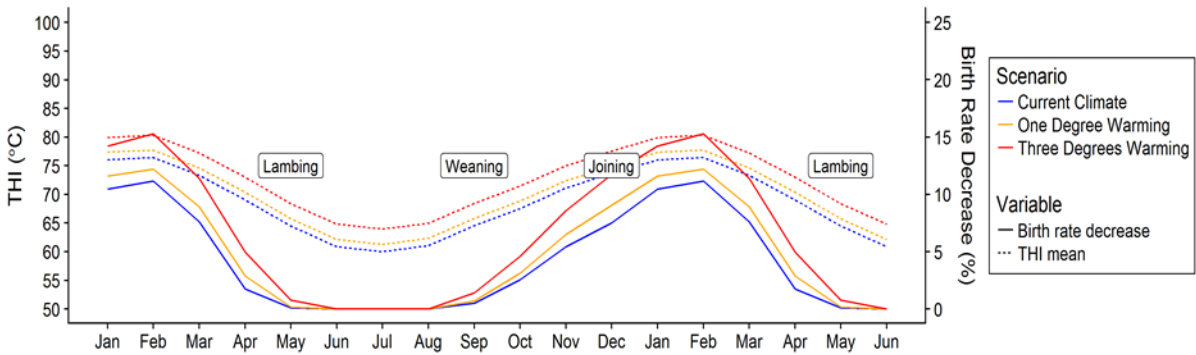
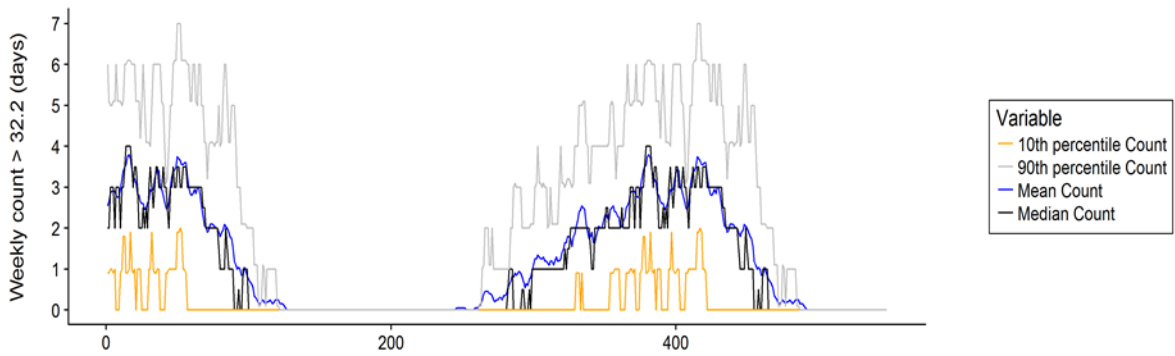
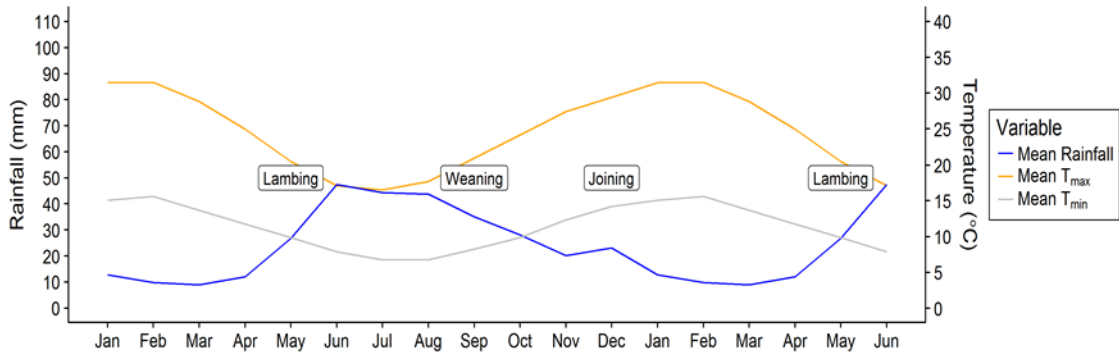
15. Hamilton Airport - VIC



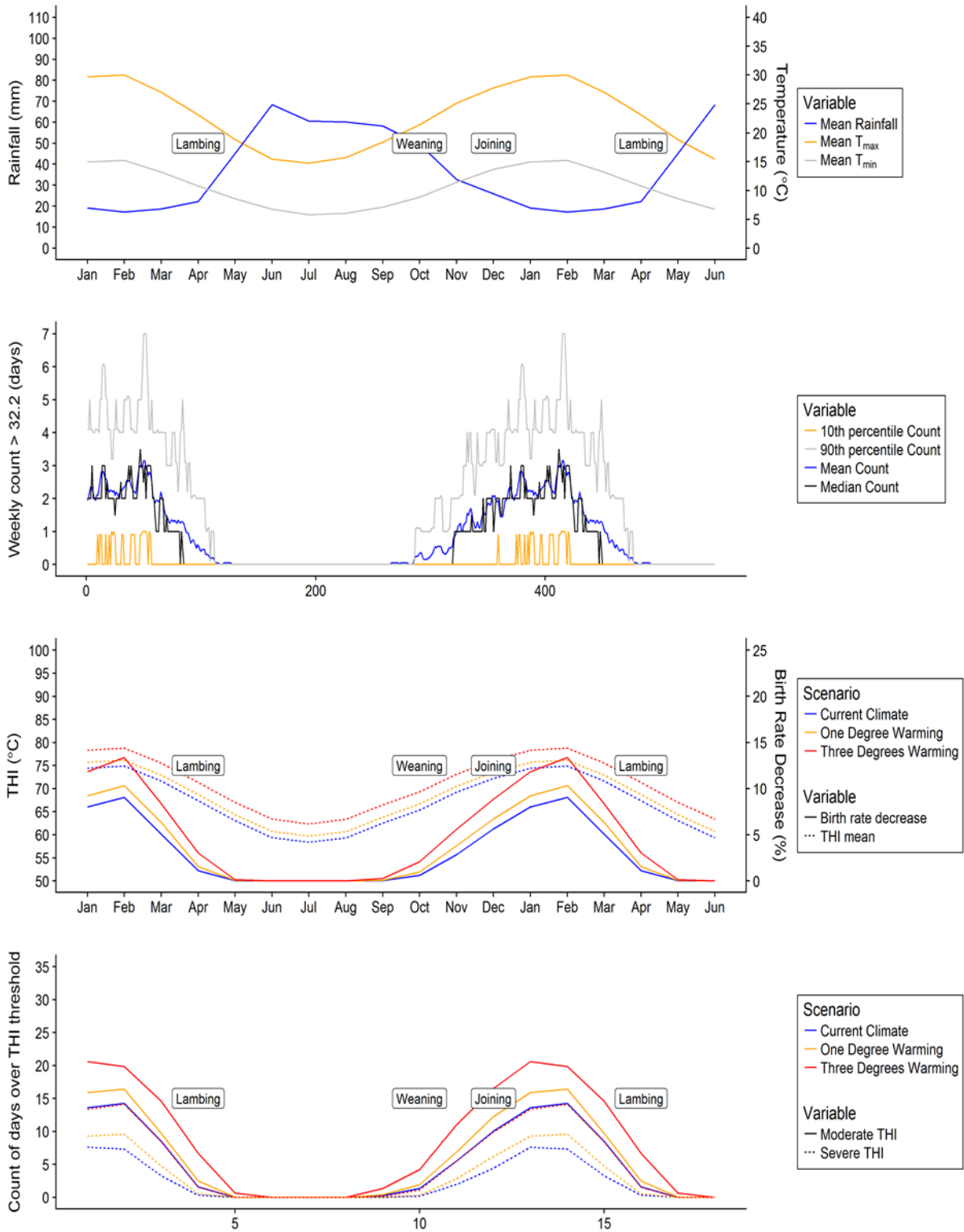
16. Ballarat (Aerodome) - VIC



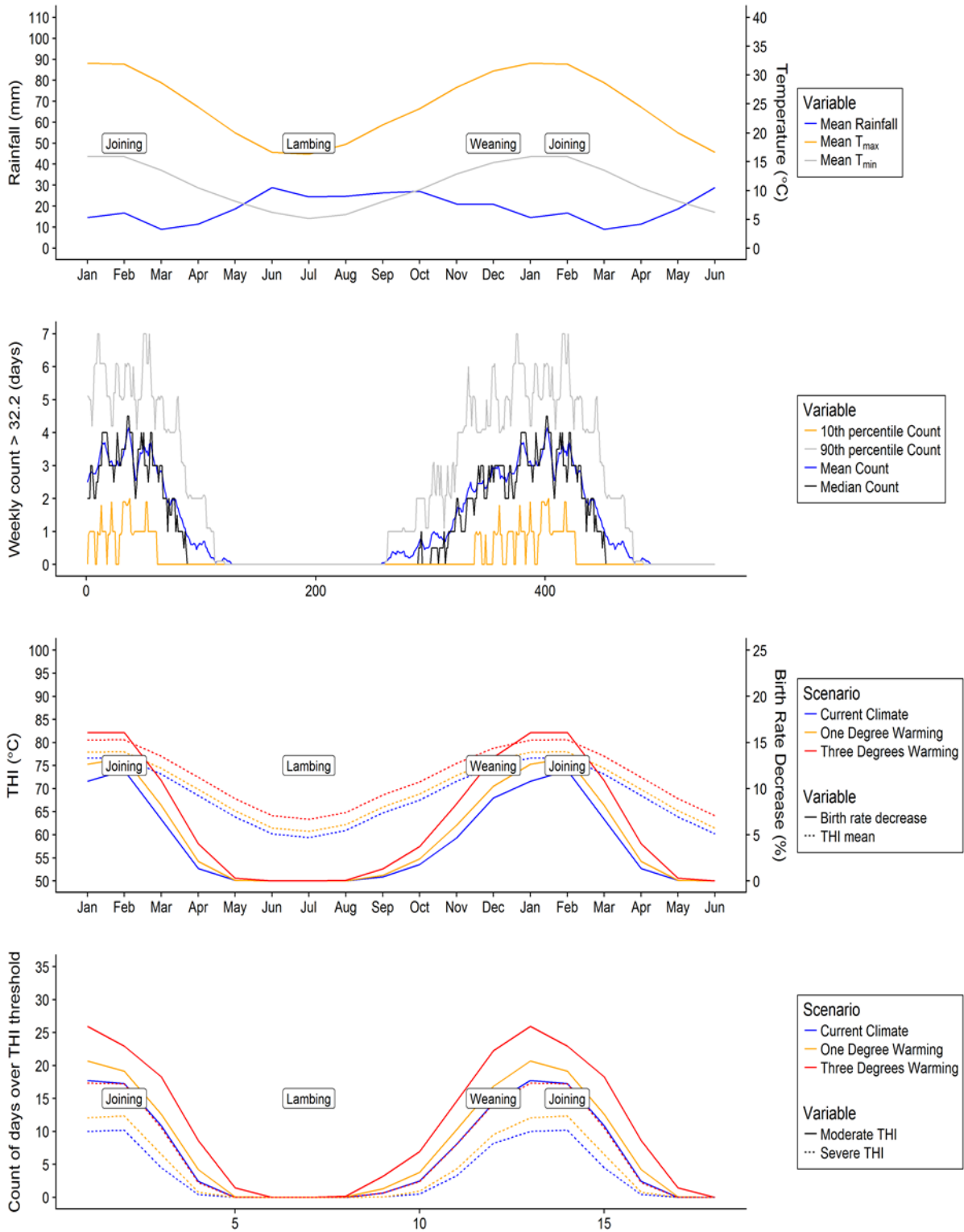
17. Minnipa Agricultural Centre - SA



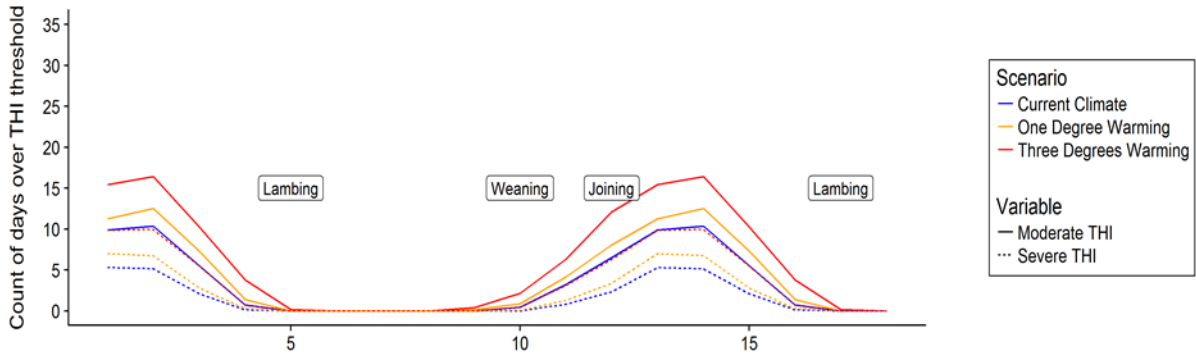
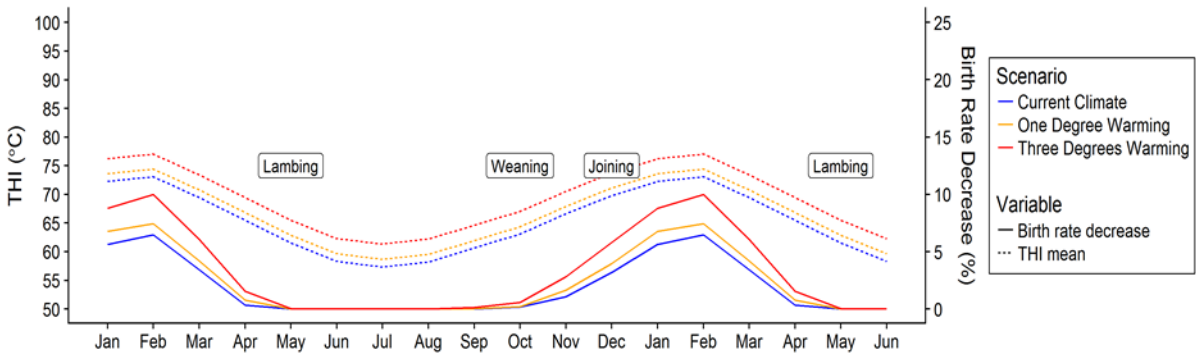
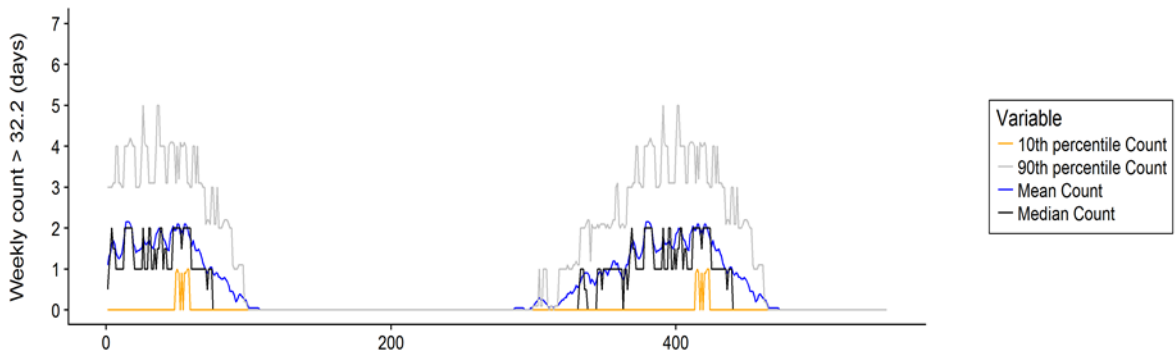
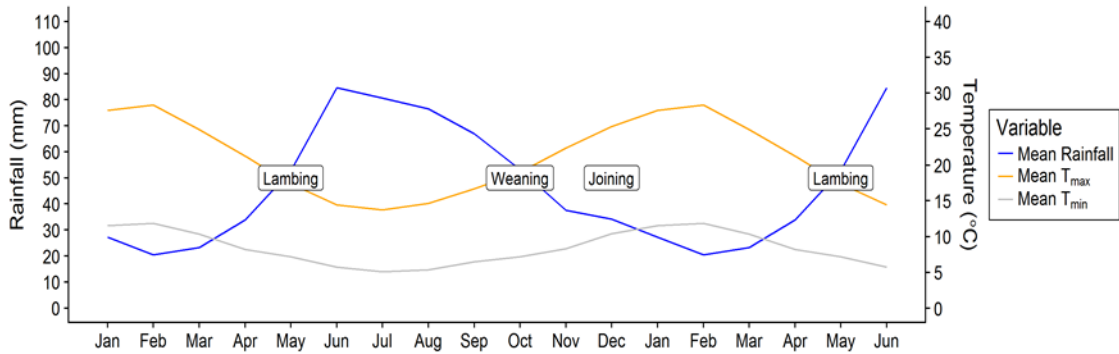
18. Rosedale (Turretfield Research Centre) - SA



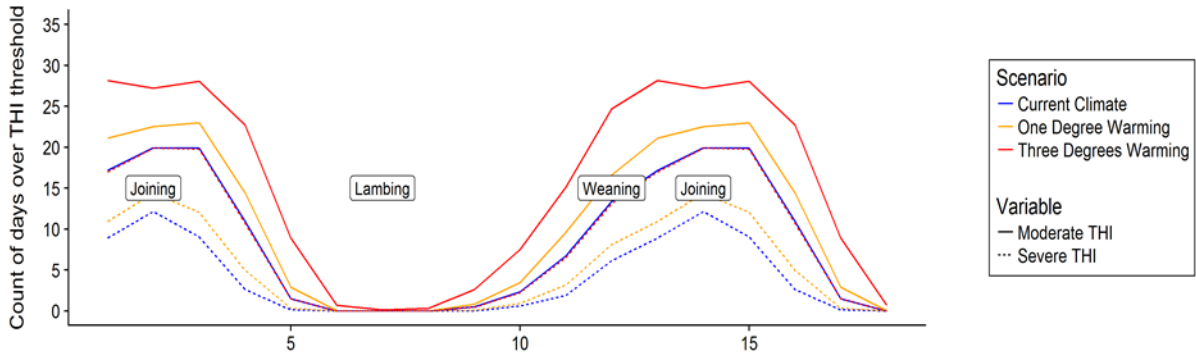
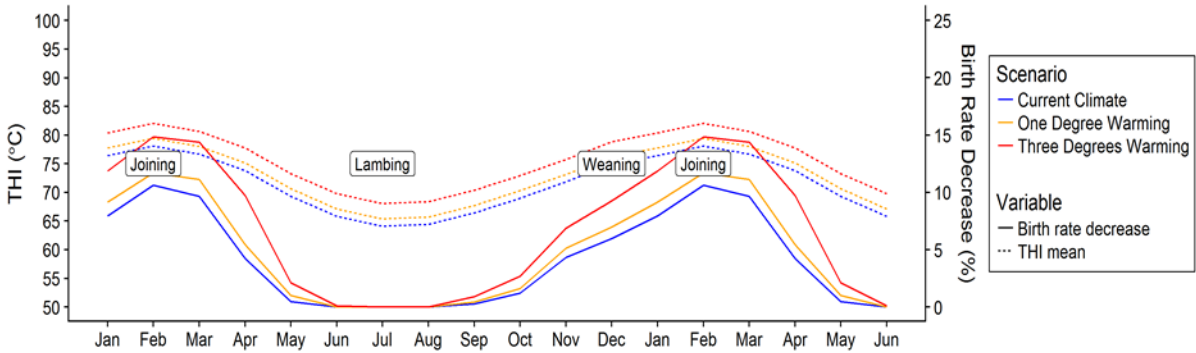
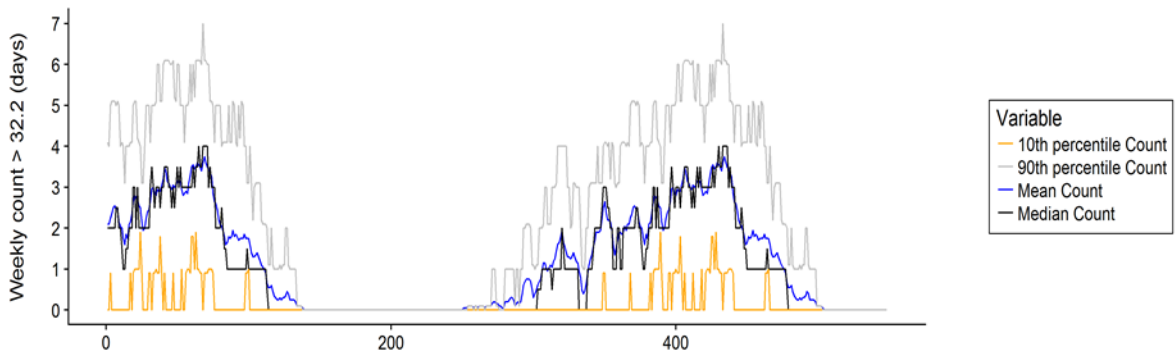
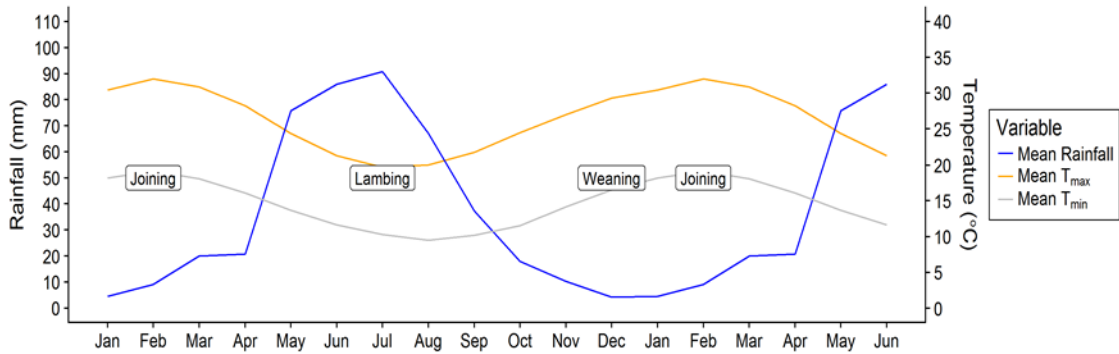
19. Renmark - SA



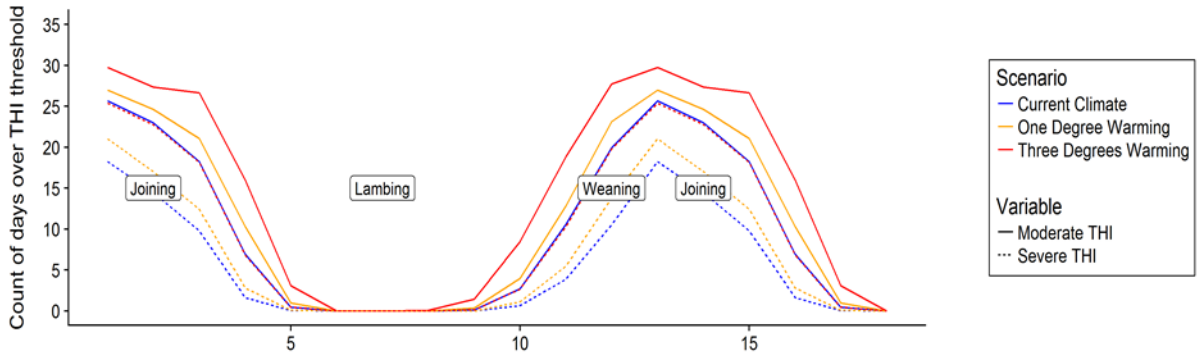
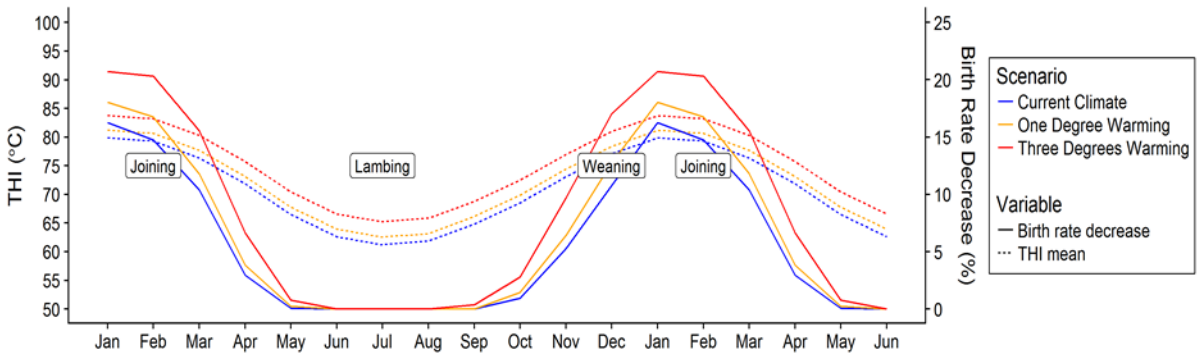
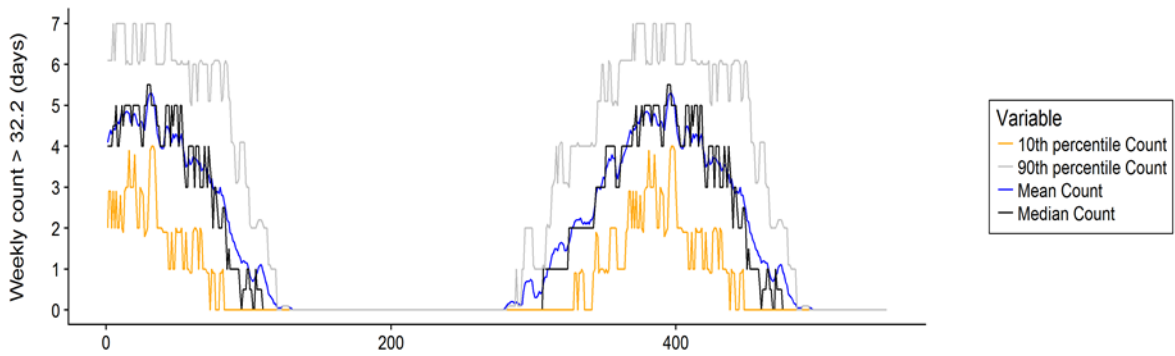
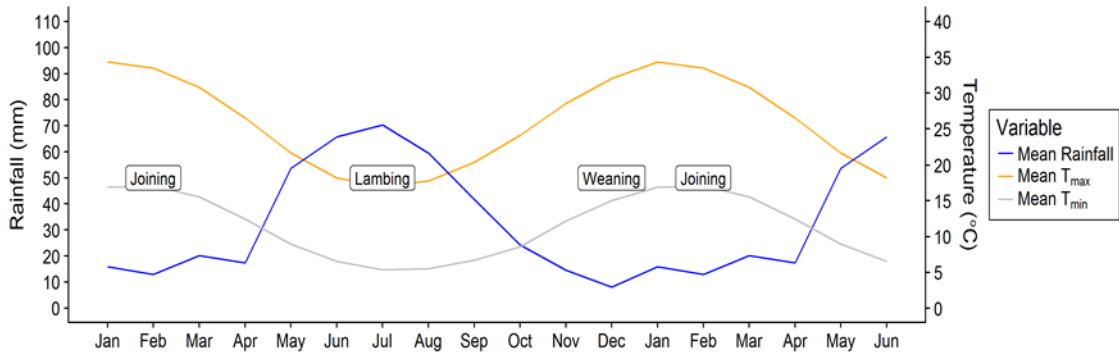
20. Struan - SA



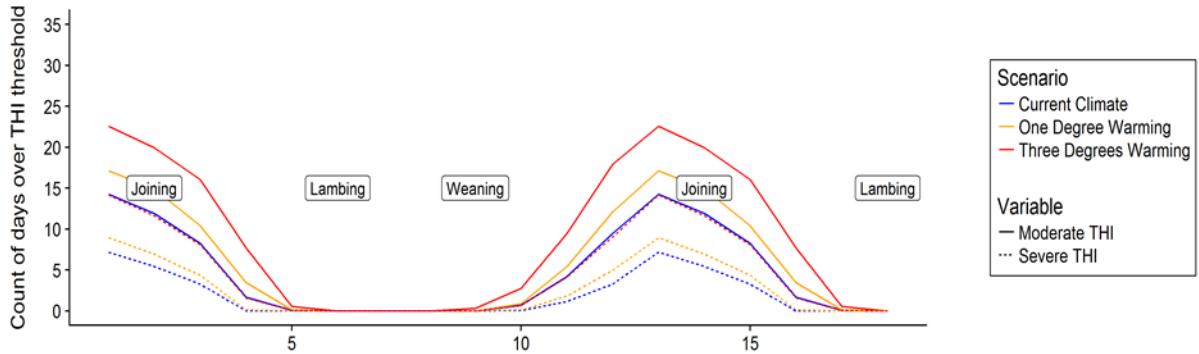
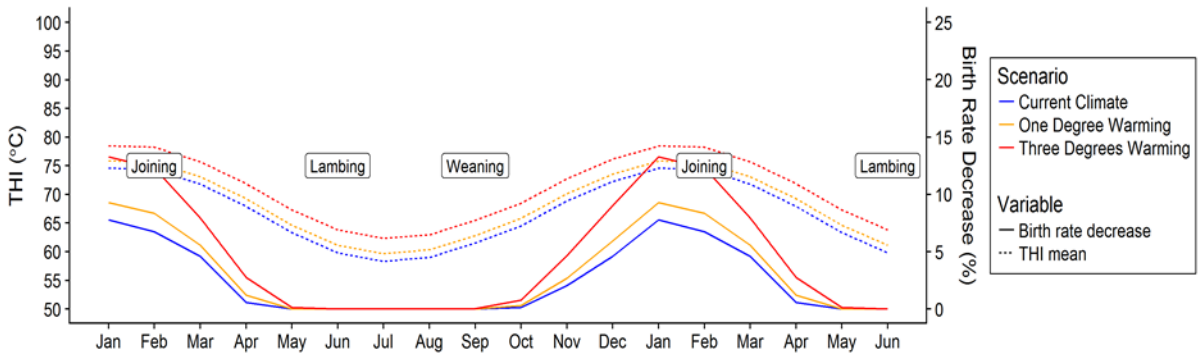
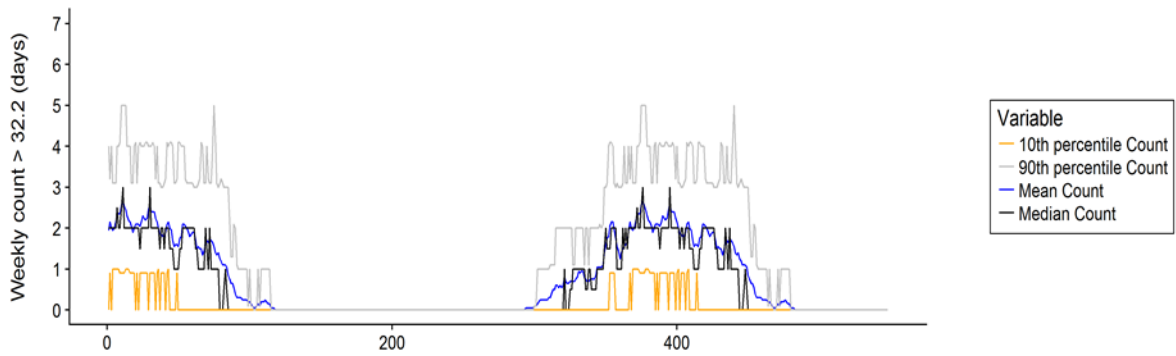
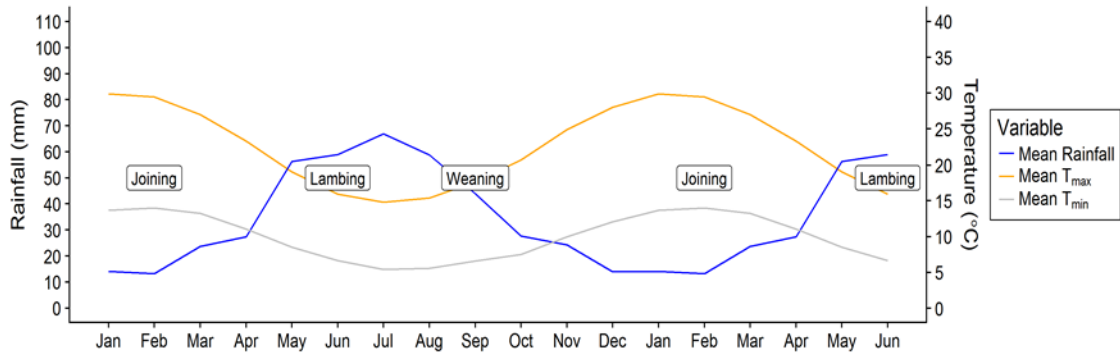
21. Geraldton Town - WA



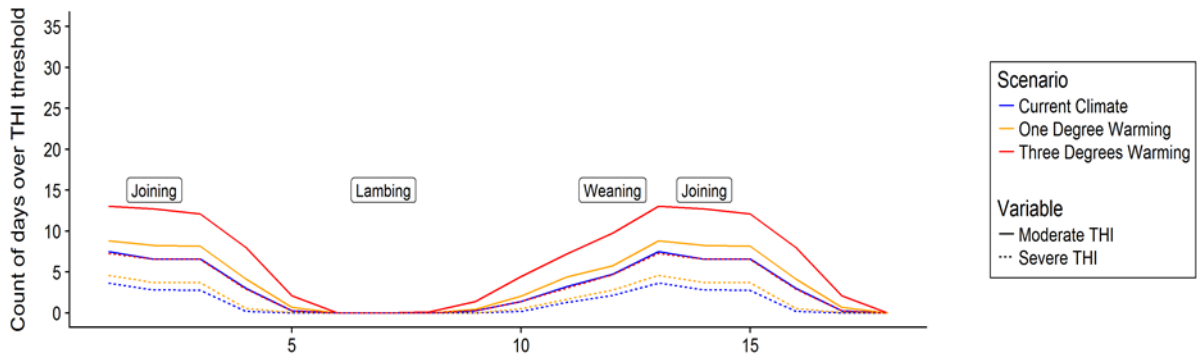
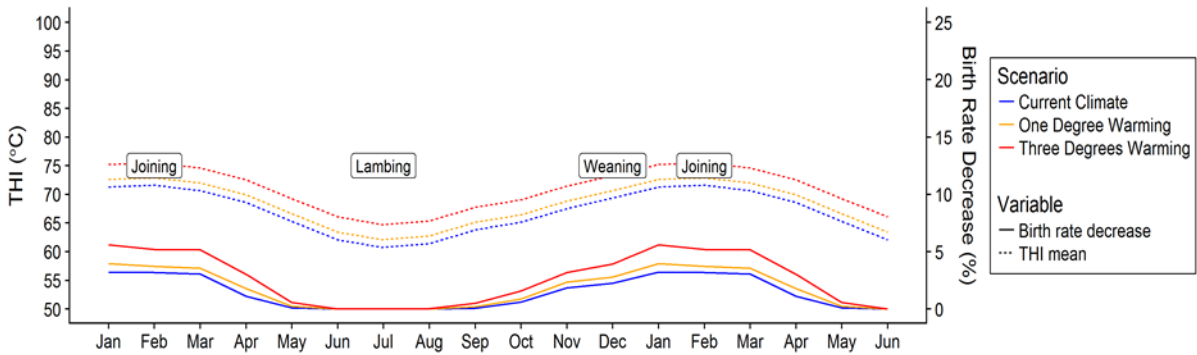
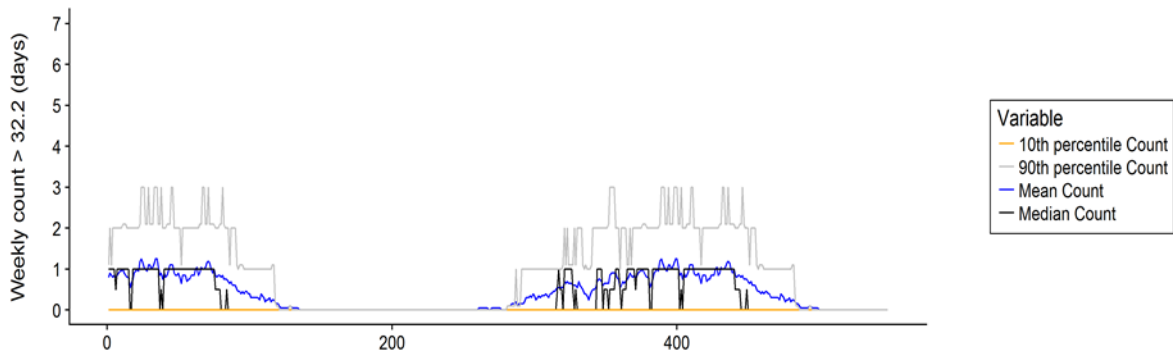
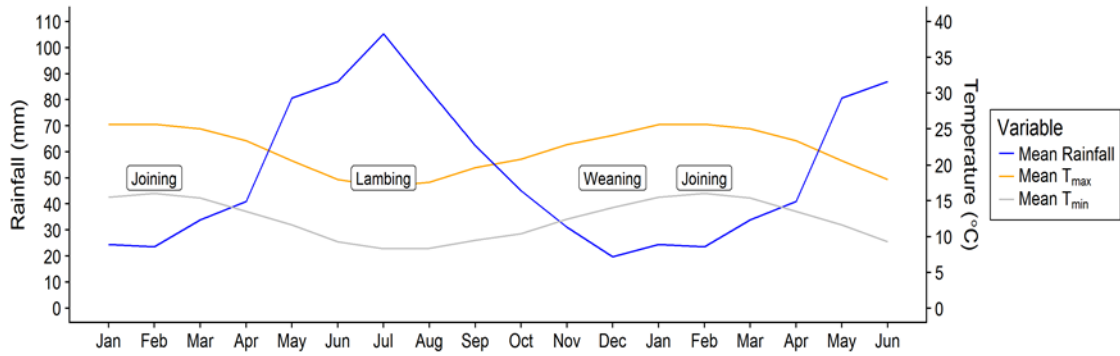
22. Northam - WA



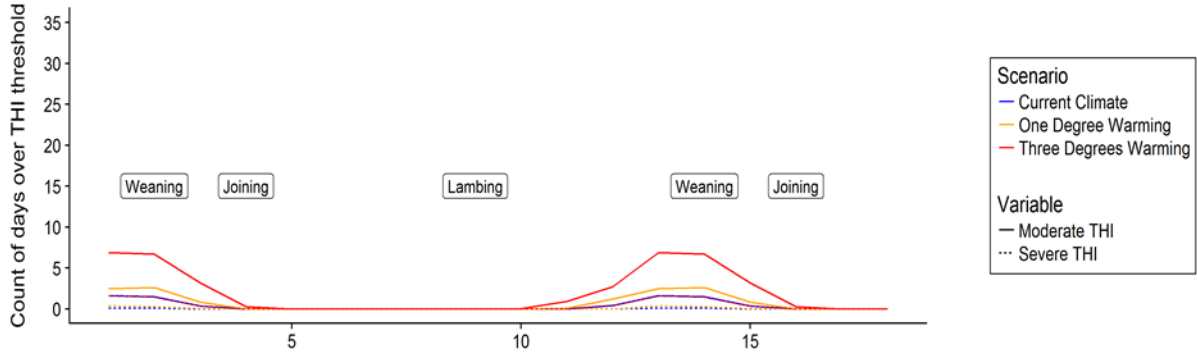
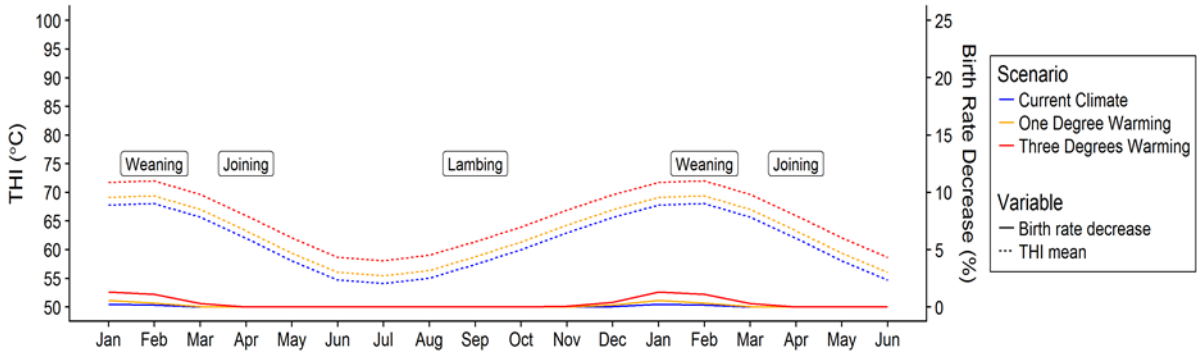
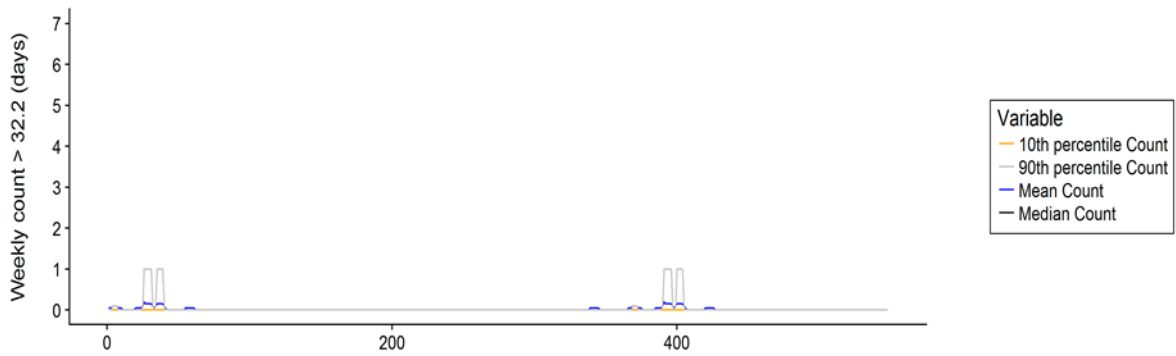
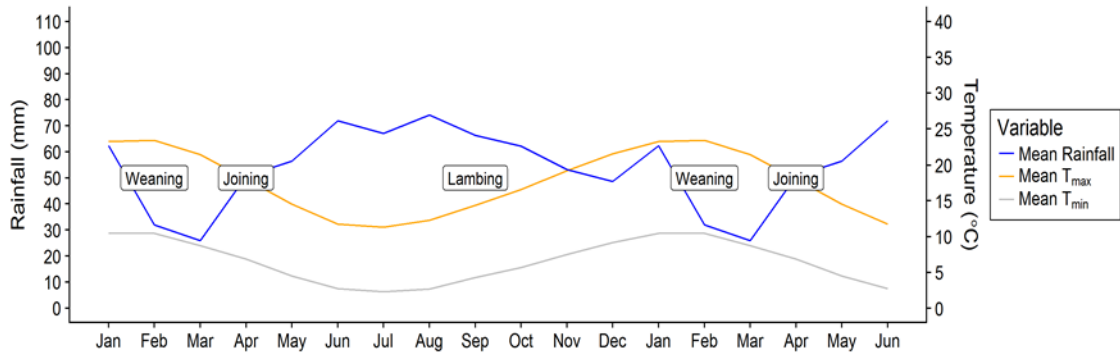
23. Katanning - WA



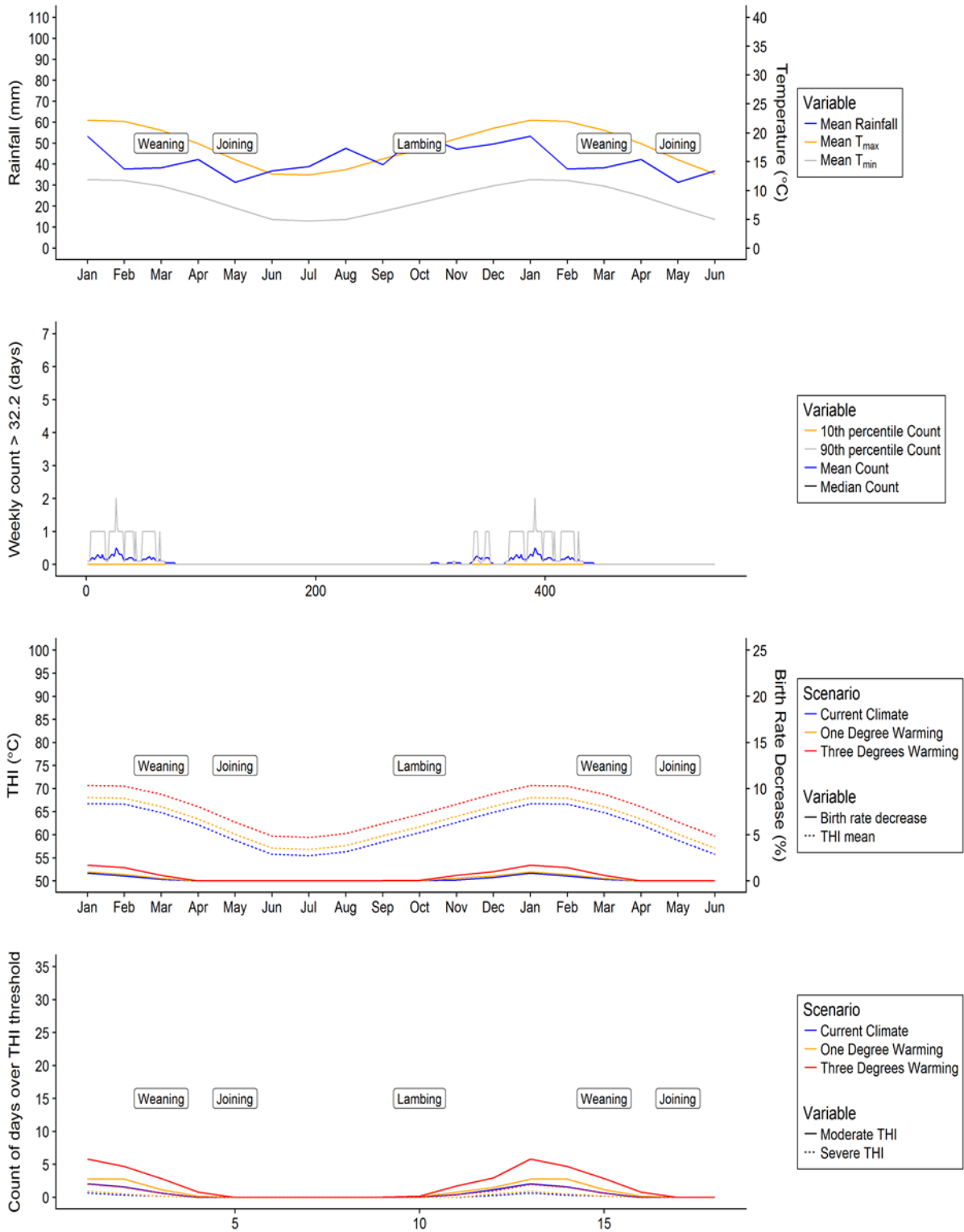
24. Esperance - WA



25. Launceston Airport - TAS



26. Hobart Airport - TAS



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