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Thermal biology of the spotted snow skink, *Niveoscincus ocellatus*, in a warming world

By

Dra. Luh Putu Eswaryanti Kusuma Yuni, M.Sc.

School of Biological Sciences

A thesis submitted in fulfilment of the requirements

for the degree of Doctor of Philosophy

University of Tasmania

May 2016

Declaration of originality

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, and to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due acknowledgement is made in the text of this thesis, nor does the thesis contain any material that infringes copyright.

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Luh Putu Eswaryanti Kusuma Yuni Date: 03/05/2016

Statement of Co-Authorship

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Paper 1 (Chapter 2): Yuni, L.P.E.K., Jones, S.M., and Wapstra, E. Thermal biology of the spotted snow skink, *Niveoscincus ocellatus*: responses to different thermal conditions along an altitudinal gradient. *In final stage of preparation of submission for publication*.

Luh Putu Eswaryanti Kusuma Yuni (candidate and author 1) was the primary author and conceived of the idea, carried out its execution and was responsible for its presentation. Susan M Jones (author 2) and Erik Wapstra (author 3) contributed to the formalization, development, refinement and presentation.

Paper 2 (Chapter 3): Yuni, L.P.E.K., Jones, S.M., and Wapstra, E. Altitudinal and seasonal variation in the relationship between locomotor performance and temperature in a wide ranging spotted snow skink, *Niveoscincus ocellatus. In final stage of preparation of submission for publication*.

Luh Putu Eswaryanti Kusuma Yuni (candidate and author 1) was the primary author and conceived of the idea, carried out its execution and was responsible for its presentation. Susan M Jones (author 2) and Erik Wapstra (author 3) contributed to the formalization, development, refinement and presentation.

Paper 3 (Chapter 4): Yuni, L.P.E.K., Jones, S.M., and Wapstra, E. 2015. Energy expenditure of the spotted snow skink, *Niveoscincus ocellatus*, at two climatic extremes of its distribution ranges. *Journal of Thermal Biology* 52: 208-216. doi: 10.1016/j.therbio.2015.07.003

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Paper 4 (Chapter 5): Yuni, L.P.E.K., Jones, S.M., and Wapstra, E. Climate variation does not induce population-specific responses in date of birth in the climatically widespread spotted snow skink, *Niveoscincus ocellatus*. *In final stage of preparation of submission for publication*.

Luh Putu Eswaryanti Kusuma Yuni (candidate and author 1) was the primary author and conceived of the idea, carried out its execution and was responsible for its presentation. Susan M Jones (author 2) and Erik Wapstra (author 3) also carried out its execution and contributed to the formalization, development, refinement and presentation.

We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

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Abstract

Thermal environment vary geographically and over time and they can have direct and indirect effects on natural populations, especially in ectotherms. Ectotherms rely on their thermal environment for thermoregulation, and the body temperatures they are able to maintain affect their physiological processes such as locomotor performance, developmental and growth rates, and energy expenditure. This thesis focusses on the thermal flexibility of a widely distributed ectothermic species, the spotted snow skink (*Niveoscincus ocellatus*) along an altitudinal gradient and across seasons to understand population-specific responses to different environmental conditions which may be important in allowing the species to occupy divergent thermal habitats. Further, I use this as a base to predict some of the potential effects of future climate change on this species (and ectotherms more generally).

I began by investigating the thermal biology (field active body temperature and thermal preference in the laboratory) across seasons in three populations of the spotted snow skink, *Niveoscincus ocellatus*, living along an altitudinal gradient in Tasmania. I demonstrated that the field active body temperature of *N. ocellatus* is dependent on its thermal environment but that the thermoregulation strategy might vary among populations of this species to respond to the variation in thermal environments at their localities. After establishing their thermal preferences in the laboratory that did not vary with site or season, I showed that *N. ocellatus* met their thermal preference along the altitudinal gradient and in all seasons, with an exception for the high altitude population in autumn. Their ability to meet their preferred body temperatures despite geographic and seasonal variation in thermal opportunities is important in allowing them to inhabit a wide range distribution area.

Since the field active body temperature in *N. ocellatus* depended on their thermal environments, I then focussed on other key physiological traits, namely on locomotor performance, energy expenditure, and date of birth that might be affected by the variation in the field active body temperatures in this species. I focused on the altitudinal and seasonal variation in the relationship between locomotor performance (sprint speed and endurance) and temperature in this species with particular focus on thermal optimum (T_{opt}) and performance breadth (B_{80}). Geographic variation was found in locomotor performance of *N. ocellatus*. The high altitude population had lower thermal optimum (T_{opt}) and a wider performance breadth (B_{80}) due to a lower limit of B_{80} than populations at the lower

altitudes. The higher limit of B_{80}, however, remained consistent among populations. These shifts toward lower T_{opt} and lower limit of B_{80} reflect the conditions they experience at the high altitude compared to the lower altitude sites. This reflects divergence in temperatures at which they are likely to be active at the lower sites when they emerge and prior to reaching their optimal temperatures. In contrast, higher temperatures can easily be avoided through thermoregulation and thus selection acts less strongly on this trait. There was also evidence of a seasonal shift in the relationship between temperature and locomotor performance which suggests a capacity for plastic responses in these traits – however this was only evident for B_{80} of endurance. However, the strong site effect and the absence of widespread seasonal signals suggest local adaptation to local climatic conditions.

The third approach of this study was to examine the energy expenditure of the spotted snow skink (*Niveoscincus ocellatus)* living at two extremes of their distribution range (warm lowland versus cold alpine site) using the doubly labelled water method. *Niveoscincus ocellatus* expended more energy per gram per hour at the cold alpine site compared to their counterparts living at the warm lowland site. Lizards living at high altitude were active at lower temperatures compared with those at the low altitude site, which resulted in a longer activity time for the highland population. However, the differences in energy expenditure cannot be explained only by these differences in activity time. Lizards at the cold alpine site might compensate for the low temperatures by elevating their metabolism which subsequently increased their energy expenditure. An elevated metabolic rate combined with modified thermoregulatory behaviour is likely an important mechanism allowing *N. ocellatus* to cope with the cold environments at high altitude sites.

The final theme of my thesis focused on the relationship between date of birth and environmental temperature experienced by female *N. ocellatus* during the gestation period at the climatic extremes of the species distribution (warm lowland versus cold alpine populations) by using a long-term data set (14 years). In *N. ocellatus*, date of birth was strongly related to mean maximum air temperature during the gestation period in both populations. Geographic and annual variation in date of birth were observed in this species with earlier births between sites and among years within sites corresponding to warmer conditions experienced during gestation. Contrary to my predictions that there would be site-specific differences in the relationship between annual temperature variation and date of birth within populations, I found that the relationship was similar with each 1 °C

shifting birth dates by between five and six days at each of the sites. This shift in birth date is despite the fact that the inter-annual variation in gestation temperature within site could be buffered via thermoregulatory behaviour. However, there was a similarity in the length of gestation between sites suggesting a combination of effective thermoregulation at the cold site, potentially combined with an adjustment of their physiological optima (for developmental rates) by the lizards at the cold alpine site. The implications of these results on temperature dependent phenological shifts are discussed in relation to potential impacts of future climate change.

Overall, this thesis demonstrated the influence of temperature on the physiological characteristics of the spotted snow skink, *Niveoscincus ocellatus*, across sites and seasons. Populations of *N. ocellatus* responded differently to the variation in their environment which appears to be determined by the level of temporal and spatial temperature variation they typically encounter. The combination of thermoregulatory behaviour and physiological adjustments, especially a downward shift in field active body temperature and physiological optima for locomotor performance, wider tolerance toward low temperatures, and elevated metabolic rate, acted as important potential buffer to allow *N. ocellatus* to overcome the challenges of the cold alpine areas in which their ranges extend. This study also indicates that *N. ocellatus* will potentially benefit from, at least modest, future predicted climate change. Firstly, the risks of fatalities of the extreme hot events can be avoided by a well-developed capacity for behavioural thermoregulation by this species. Secondly, warming will also allow for greater activity time (at or near their optimal temperatures for key physiological traits such as performance) which could facilitate range expansion into cold areas currently unsuitable for the species. Finally, the thermally-phenological shift in birth dates may initially enhance offspring fitness and even lead to greater demographic vigour. Thus, I concur with recent work that suggests that the effects of climate change on temperature dependent species is not necessarily all bad news and is different to the dire predictions made for other reptiles in tropical and desert areas.

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Chapter 1

General Introduction

Introduction

It is now widely accepted that climate has warmed up globally (Walther et al. 2002; Hughes 2003). Climate warming will unquestionably have profound effects on many natural populations in both endotherms and ectotherms (e.g., Dunham and Overall 1994; Calosi et al. 2008; Moritz et al. 2008; Visser et al. 2006; Sinervo et al. 2010). They both can be affected by the warming climate through their physiology, phenology (the timing of life cycle events) or distribution (Hughes 2000; Parmesan 2006; Seebacher and Post 2015). There may be profound changes in species geographical distributions, in which species will move polewards in latitude or upwards in elevation (Parmesan 1996; Parmesan and Yohe 2003; Walther et al. 2002; Moritz et al. 2008). Each rise of 1 ºC is predicted to move ecological zones by about 16 km in latitude (Thuiller 2007). Hughes (2000) suggested that a 3ºC increase in mean annual temperature corresponds to a shift in 500 m in elevation in response to shifting climate zones. Accordingly, species living in high altitudes are particularly sensitive to changed conditions, in that upwards migration cannot occur (Hughes 2000; Chamaille-James et al. 2006; Thuiller 2007). The effect on phenology is predicted to enhance the timing of reproduction (i.e., hatching date) in many species (Dunn 2004; Visser and Both 2005; Both et al. 2009) while effects in physiology can be forecasted, for example, by an increase in energy for maintenance of animals, which subsequently puts pressure on their energy expenditure (Chamaille-James et al. 2006). At the broader ecological level, changes in physiology, phenology and distribution of individual species will inevitably alter interactions between species, with consequent feedbacks to local abundance and to geographic ranges (Hughes 2000).

Although the effects of climate change to natural populations have been widely observed, the mechanisms are often not well understood. Ectotherms may be particularly susceptible to perturbations in their thermal environments due to the strong effects of temperature on their body temperature (Pörtner et al. 2006; Amarasekare and Savage, 2012). Among ectothermic species, reptiles are particularly prone to perturbations in their thermal environments since they are not capable of long-distance dispersal (Araujo and Pearson 2005). Thus they will experience a major challenge from the future climate change (Araujo and Pearson 2005; Araujo et al. 2006; Pörtner et al. 2006; Amarasekare and Savage 2012). In reptiles, their body

temperatures affect many physiological processes, such as metabolic processes, developmental and growth rates and locomotor performance (Angilletta 2009; de Queiroz and Ashton 2004; Seebacher 2009; Bonino et al. 2011; Fernandez et al. 2011). In locomotor performance, for example, performance is constrained at low body temperatures, performance then increases with body temperature until a plateau is reached at or near the optimal body temperature range, before decreasing precipitously at higher body temperature (Huey and Stevenson 1979; Hertz et al. 1983). Accordingly, the animals always make efforts to regulate their body temperature to ensure performance and physiological processes function at an optimal level (Besson and Cree 2010). However, the thermal environment varies spatially which may constrain their effort to regulate body temperature at an optimal level. Thus body temperature may also vary spatially in ectothermic species living in a wide geographic distribution area (Huey and Slatkin 1976; Van Damme et al. 1989; Medina et al. 2009; Moritz et al. 2012).

Effect of environmental thermal gradients on body temperature has been linked to variation in life history in reptiles because of the influence of body temperature on the rate of metabolism and bioenergetics (Adolph and Porter 1993; Rohr 1997; Sears and Angilletta 2003; Sears 2005; Gutierrez et al. 2010). The early work by Grant and Dunham (1990) revealed that in the lizard *Sceloporus merriami,* changes to a number of life history characteristics were associated with variation in environmental variables. Body temperature is positively correlated with growth rate, which subsequently results in geographic variation in body size and age of first reproduction within species (Sinervo and Adolph 1989; Grant and Dunham 1990; Sinervo 1990; Niewiarowski 2001). For example, the high altitude population of sagebrush lizards, *Sceloporus graciousus,* grow faster compared with the lower altitude populations due to a greater proportion of energy allocated to growth rather than to maintenance by this population (Sears 2005). As another example, a study on the lizard *Liolaemus pictus argentinus* showed that lizards living at high altitudes limit their activity times, allocate more time and energy toward thermoregulation, and consequently experience life-history constraints such as reduced fecundity and supra-annual reproductive cycles compared with conspecifics living at lower altitudes (Gutierrez et al. 2010).

In species that live over wide geographic ranges, they are often composed of populations that spatially differentiated and locally adapted to different thermal regimes (Angert et al. 2011). Within those species, populations may respond not in the same way to climate change (Peck et al. 2009). For example, the thermal sensitivity to different climatic conditions may

vary substantially between populations (Seebacher et al. 2012). Janzen (1967) emphasized the importance of seasonal changes in temperature in shaping the thermal sensitivity of organisms to different climatic conditions. He pointed out that a species living within a temperature regime that is relatively uniform seasonally will probably be more acclimated and evolutionarily adapted to a narrower absolute range of temperatures than one which lives with more highly fluctuating seasonal temperatures ranges. Further, differences in limit of thermal sensitivity can lead to differences in fate of a species when the climate is changing, for example, for those with a narrow thermal tolerance limit will be prone to warming climate, and *vice versa* (Tomanek 2008; Calosi et al. 2008; Deutsch et al. 2008). Additionally, for those with a wide thermal tolerance limit but already live close to their maximum thermal tolerance limit will also be most affected by global warming climate (Tomanek, 2008; Hoffmann et al. 2013). A species which consists of connected populations with diverse physiological phenotypes then will be more resilient to climate change because of the increased likelihood that at least some populations will succeed under the changed conditions (Seebacher et al. 2012). Knowledge of responses to different thermal conditions at the population level within species, therefore, is essential as it enables us to make inferences about what may happen to a species during global climate change (Stillman 2002; Sorte et al. 2011; Seebacher et al. 2012).

One approach to understanding the potential impact of directional climate change at the population level within species is to conduct studies across populations of a species living along an altitudinal gradient. Such studies allow us to investigate the specific responses of different populations to different environmental conditions (Thuiller, 2007; Somero 2010). The altitudinal gradient mimics the expected gradient of environmental change; thus it is an effective surrogate to study the impacts of temperature change on natural populations across different climatic regions (Miles 1994; Williams et al. 2003).

To avoid fatalities when faced with pressures due to climate change, animals may disperse to suitable habitats elsewhere (Gienapp et al. 2008; Buckley et al. 2013). Dispersal will be possible if mechanism allow, for example, animals possess great endurance capacity to disperse (Araujo and Pearson 2005; Southwood and Avens 2010). Movement will also be facilitated when there are suitable habitat corridors (Miles 1994; Schloss et al. 2012). However, for those with limited dispersal ability such as reptiles (Araujo and Pearson 2005), other possible alternatives in responding the temperature change is by tolerating the new

conditions through either phenotypic plasticity or local adaptation (Salamin et al. 2010), thus allow animals to remain *in situ* and avoid local extinction (Merila 2012).

Phenotypic plasticity is defined as the phenomenon whereby a single genotype produces distinct phenotypes in distinct environments so that individuals are able to modify their behaviour, morphology, or physiology in response to environmental conditions (Price 2006; Salamin et al. 2010; Beldade et al. 2011). Phenotypic plasticity has been generally attributed to within generation changes such as seasonal events or variation in conditions between sites (Bradshaw and Holzapfel 2006). Plasticity can be context dependent and under selection itself so that the level of plasticity may diverge between populations of a species due to differences in environmental heterogeneity faced by those populations (Husby et al. 2010). When a species is exposed to the new environmental condition and its phenotypic response is a good match with the new environment, the species can adjust to the new condition without a change in genotype (Lynch and Gabriel 1987; Ghalambor et al. 2007; Hoffmann and Sgro 2011). Alternatively, to reduce the costs and constraints on plasticity, if environments are relatively more predictable within sites than between sites, a genotype that produces the locally optimal phenotype in each habitat can be favoured and local adaptation will be evident (Kawecki and Ebert 2004, Hoeksema and Forde 2008; Salamin et al. 2010; see also Cadby et al. 2014). However, local adaptation can then clearly constrain responses to environmental conditions, such as directional climate change, outside those typically experienced (Angert et al. 2011; Hareford 2009). If species cope only by phenotypic plasticity, then global temperature changes within the plastic tolerance range will not threaten the survival of local populations (Berg et al. 2010; Yampolsky et al. 2014). However, if species show a signature of adaptation to local temperatures, then global warming will put species under stress (Yampolsky et al. 2014).

In responding to variation in their thermal environment, most reptiles possess the capacity to behaviourally regulate their body temperature to attain their optimal level (Seebacher 2005). Thermoregulatory behaviour is of major importance for the survival and fitness of reptiles because physiological functions can be optimized through this way (Besson and Cree 2010). Within a species, animals may modify their thermoregulatory behaviour in response to different thermal environments, for example lizards living at a limited thermal opportunity environment (i.e., high altitude) increase basking at about 50% compared to those at the lower altitude (Gvozdik 2002). However, lizards do not always achieve their optimal body temperature through basking considering some potential costs may arise (Huey and Slatkin

1976; Sears 2005). Alternatively, they can modify their physiological and biochemical rates to shift their optimal temperatures for physiological and physical performance (Seebacher 2005; Angilletta 2009; Beldade et al. 2011). A study on reptiles revealed that there is potential for biochemical and physiological systems to be plastic or flexible, allowing them to maximise performance in different environments (Glanville and Seebacher 2006). Freshwater turtles, *Chelodina longicollis*, use a combination of muscle metabolic enzyme activity regulation and behavioural thermoregulation in response to variation of thermal conditions (Seebacher et al. 2004). Similarly, American alligators, *Alligator mississippiensis*, may not exclusively rely on thermoregulation, but they also acclimatise metabolic enzyme activities to cope with changing environmental conditions (Seebacher et al. 2003a, 2003b). Through that way, attainment of body temperature via thermoregulatory behaviour is not mandatory for the animals to optimize their physiological functions (e.g., Seebacher et al. 2003a, 2003b, 2004).

The study on thermal flexibility of a widely distributed ectothermic species along an altitudinal gradient and across seasons will allow us to understand population-specific responses of the species to different environmental conditions which may be important in allowing the species to occupy divergent thermal habitats. This is because the response of most species is still unknown, i.e., whether the populations of a species that occupy a wide geographic range respond in the same way to different environmental conditions (e.g., Peck et al. 2009; Seebacher et al. 2012). The knowledge on the thermal flexibility obtained, further can be used as a base to predict some of the potential effects of future climate change on this species and in ectotherms in more general.

In this thesis, I use a wide ranging ectothermic species, the spotted snow skink, *Niveoscincus ocellatus,* living along an altitudinal gradient in Tasmania to understand the responses of a species at the population level to different thermal conditions. This species is a small lizard (4-7 g, 55-75 mm snout vent length) that occurs over a wide geographic and climatic range in Tasmania, an island state of Australia (Wapstra et al. 1999; Uller et al. 2011) and it thus offers an excellent opportunity to study specific population level responses to environmental variation (e.g., Cadby et al. 2010; Pen et al. 2010; Uller et al. 2011). Across its range, *N. ocellatus* displays distinct life history variation (Wapstra and Swain 2001; Wapstra et al. 2001) which reflect a combination of local adaptation and plastic responses to climate variation which may be population specific (Melville and Swain 2000; Cadby et al. 2010, 2011, 2014; Uller et al. 2011; Cliff et al. 2015). *Niveoscincus ocellatus* at the warmer extremes of the species' range experience an earlier and longer active season (approximately

from September to May) than conspecifics living at the colder extremes (approximately October to April) of the species' range (Wapstra et al. 1999). Following that, key reproductive events such as mating and parturition occur approximately one month later at cold extreme sites compared with at lowland warmer sites (Wapstra et al. 1999; Pen et al. 2010; Uller et al. 2011). The lizards are considerably smaller in body size and in maturity at the warmer extremes than at the colder extremes of the species' range (Wapstra et al. 2001). They reach maturity earlier (~ 3 years old) at the warm lowland sites than those at the cold alpine sites (~ 4 – 5 years old) (Wapstra et al. 2001; Pen et al. 2010). Description of study sites is provided in Appendix 1.

This thesis focusses on the thermal flexibility of the spotted snow skink, *N. ocellatus*, along an altitudinal gradient and across seasons in Tasmania. I have two broad aims. Firstly, I aim to understand population-specific responses to different environmental conditions. I focus on key physiological traits (e.g., body temperature, locomotor performance, energy expenditure, and phenology which represented by date of birth) because physiological studies can help to determine the tolerance limit of a species to different thermal conditions (Tomanek 2008; Somero 2010; Hoffmann et al. 2013). Secondly, I aim to understand some of the potential effects of future climate change on this species (and in ectotherms in general) by using the knowledge obtained through the first major aim. For example, populations of a species with a thermal optimum close to the mean maximum daily air temperature at their current habitat are predicted to be more strongly affected by the predicted future warming (i.e., as it was studied in Chapter 3) because the increases in environmental temperatures would push their body temperature outside the optimal range (Huey et al. 2009).

The studies described in this thesis integrate (see Figure 1) to add to our knowledge of the thermal biology of a widely distributed ectothermic species. It seeks to understand how thermal biology allows the species to occupy divergent habitats, and how that knowledge can be used as a base to predict some of the potential effects on future climate change on the species. The dashed square represents the studies conducted to answer the first broad aim of this thesis. Thermal environments strongly affect ectotherms through physiology (chapters 2, 3, 4), phenology (chapter 5) or distribution. The animals may respond to variation in their thermal environment through adaptive processes. To avoid the risk of fatalities, ectotherms may respond to variation in the thermal environment either through thermoregulatory behaviour and/or through physiological adjustments to optimize their performance and/or physiological processes. The knowledge obtained through those studies (the dashed square) is

then used as a base to predict some of the potential effects of future climate change, namely on physiology (chapters 2, 3, 4) and phenology (chapter 5), of the study species, as well as on ectotherms in general (chapter 6).

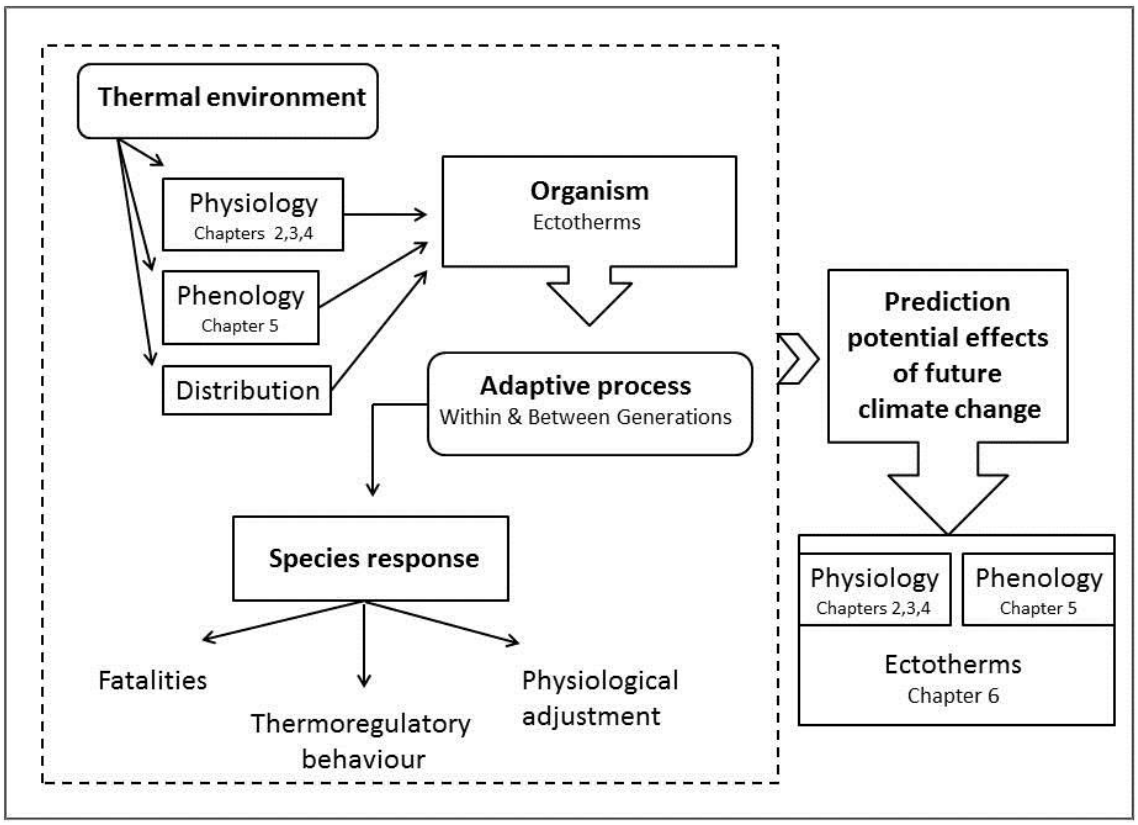

Figure 1. This diagram demonstrates how the works contained in this thesis (enclosed by the dashed square) adds to our knowledge of thermal flexibility of a widely distributed ectotherm allowing the species to occupy divergent habitats, and shows how that knowledge can be used as a base to predict some of the potential effects (e.g., on physiology, phenology) of future climate change on the study species, as well as on ectotherms in general.

Thesis presentation and thesis outlines

This thesis is composed of this General Introduction, four empirical chapters and a General Discussion. One chapter (Chapter 4) has been published in the *Journal of Thermal Biology*. The remaining three data chapters are in preparation for submission to relevant journals and

accordingly, all chapters have been written as stand-alone pieces of work. When fitted into the context of the thesis, these chapters provide the appropriate information to address the overall objectives of this study. Due to the nature of this thesis, each chapter may incur some repetitions, particularly in terms of animal collection and species description. Each chapter may vary with respect to formatting, as each has been formatted according to the specifications of the journal to which it was or will be submitted.

The thesis is presented as follows: **Chapter Two** examines thermal biology of the spotted snow skink, *Niveoscincus ocellatus*, in response to different thermal conditions along an altitudinal gradient. As ectotherms, these lizards have a particularly strong relationship to their thermal environment. They rely on it for thermoregulation, and the body temperatures ectotherms are able to maintain affect their physiological processes, e.g., their metabolic rate (Schaefer and Walters 2010; DeVries et al. 2013), energy expenditure (Christian et al. 2003, 2007), developmental and growth rates (Wapstra et al. 2001; Sears and Angilletta 2003; de Queiroz and Ashton 2004; Sears 2005) and locomotor performance (Angilletta et al. 2002; Fernandez et al. 2011). Given that many of these physiological functions are optimized within a narrow range of body temperatures (Besson and Cree 2010), there is strong selection on thermoregulatory behaviour to maintain body temperatures within narrow ranges when active. However, they do not always achieve their optimum body temperature due to several factors such as thermal quality or predator risk (Besson and Cree 2010; Gutierrez et al. 2010). This chapter seeks to reveal whether the field active body temperature of *N. ocellatus* is dependent on their thermal environment and whether this species could reach their preference body temperature at their localities.

I investigated the thermal biology (field active body temperature and thermal preference in the laboratory) in three populations of the spotted snow skink, *Niveoscincus ocellatus*, living along an altitudinal gradient in Tasmania. The field active body temperature of *N. ocellatus* was measured under natural living conditions at the low, mid, and high altitudes. The climate at the high altitude site is colder and is more variable than at the low and mid altitude sites (see Pen et al. 2010). To investigate whether the lizards' response to geographic variation across sites was consistent across seasons, the study was conducted in spring, summer, and autumn at each study site. Finally, I compared their field active body temperature to their thermal preference, which was a laboratory based study tested, to determine whether this species achieved their thermal preference in their distribution range along an altitudinal gradient. Since body temperature are related to many physiological

processes in ectotherms, the following studies were focused on other key physiological traits, namely locomotor performance (Chapter 3), energy expenditure (Chapter 4, Yuni et al. 2015), and phenology (Chapter 5), that could be affected by body temperature.

Chapter Three focuses on altitudinal and seasonal variation in the relationships between locomotor performance (sprint speed and endurance) and temperature in the spotted snow skink, *Niveoscincus ocellatus*, with particular focus on thermal optimum (T_{opt}) and performance breadth (B_{80}). The study was conducted in three populations of the lizards, living along an altitudinal (and thus climatic) gradient in spring and summer. Locomotor performance attributes such as sprint speed and endurance integrate a variety of organismal traits at physiological, morphological, and ecological levels which influence the fitness (including survival) of an organism (Miles 1994, 2004; Ghalambor et al. 2003; Le Gallard et al. 2004). Sprint speed and endurance are important to capture prey and escape from predators (Van Damme et al. 1989; Husak 2006). The relationship between body temperature (T_{body}) and locomotor performance is reflected by a thermal performance curve (Fernandez et al. 2011; Huey et al. 2012). Within a thermal performance curve, thermal optimum (T_{opt}) and performance breadth (B_{80}) are the physiological parameters which are ecologically related to the animal's thermal niche (Hertz et al. 1983). The T_{opt} is the T_{body} at which performance is maximal (Hertz et al. 1983; Amarasekare and Savage 2012), and the B_{80} is the range of T_{body} at which performance is greater than or equal to 80% of the maximal performance (Hertz et al. 1983; Schulte et al. 2011). Since the climate at the high altitude site is colder and is more variable than that at the low and mid altitude sites (see Pen et al. 2010), I predicted that lizards at the high altitude would have greater tolerance of performance toward low temperatures (i.e., maintain higher performance measures at lower temperatures) than their counterparts at the lower altitudes. Further, I also predicted that the more variable climate at the high altitude might induce variation in locomotor performance between seasons in this population.

Chapter Four examines the energy expenditure of the spotted snow skink (*Niveoscincus ocellatus)* living at two extremes of their distribution range (warm lowland versus cold alpine site) using the doubly labelled water method. Studying energy expenditure and energy allocation by an animal in nature helps us to understand how organisms have adapted to the environmental conditions. Such studies can also provide insights into how species respond to temperature differences between different latitudes or altitudes (Porter 1989; Angilletta 2001). In ectotherms, energy expenditure is typically related to activity patterns (e.g., Sears 2005; Smith et al. 2008) because higher level of activity is typically associated with higher body

temperatures: thus, within species, energy expenditure varies with season or geographic variation in altitudes or latitudes (Karasov and Anderson 1984; Patterson and Davies 1989; Grant and Dunham 1990; Clarke 1991, 2003; Hare et al. 2010; Powers and Anderson 2010; Schaefer and Walters 2010). In general, ectotherms living in warmer environments have higher resting metabolic rates than those at the colder environments because the speed of chemical reactions, including aerobic respiration, increases exponentially with temperature (Clarke and Fraser 2004; Martinez del Rio and Karasov 2010). However, the relationship is not as straightforward as that because lizards at the cold alpine site might have physiological adaptations that allow them to cope with cold environments. Accordingly, I suggested that this can be resulted in a higher energy expenditure in this population than that at the warm lowland site.

Chapter Five focusses on the relationship between date of birth and environmental temperature experienced by female during gestation period at the climatic extremes of the species distribution (warm lowland versus cold alpine populations). In viviparous lizards, parturition date is largely driven by thermal conditions experienced by females because they influence the rate at which her embryos develop (Wapstra 2000). Accordingly, pregnant females experiencing higher temperature such as at warm lowland site would give birth earlier than those experiencing lower temperature, and *vice versa* (e.g., Wapstra et al. 2001; Pen et al. 2010). However, there are number processes that also affect the relationship between thermal environments and the date of birth within populations (i.e., among years) and between populations, including selection on basking behaviour to buffer the poor thermal conditions (Gvozdik and Castilla 2001; Gvozdik 2002; Cadby et al. 2010, 2014; Uller et al. 2011), body temperatures the lizards are able to maintain (Le Galliard et al. 2003; Uller et al. 2011; Wang et al. 2014), and potentially shift in optimum temperature to facilitate physiological processes (Seebacher 2005; Beldade et al. 2011).

A long-term data set (14 years) on date of birth of the viviparous spotted snow skink, *Niveoscincus ocellatus*, was used to investigate the relationship between date of birth and environmental temperature experienced by females during their gestation period at the climatic extremes of the species distribution (warm lowland versus cold alpine populations). In this species, there is strong selection on basking behaviour in females from high altitude populations which means they thermoregulate more actively (i.e., bask more) than low altitude lizards especially under reduced thermal opportunities (Uller et al. 2011; Cadby et al. 2014), even to the extent that counterintuitively they target higher body temperature than their

lowland counterparts. As a result, the effect of temperature on date of birth might be predicted to be less at the cold alpine site than at the warm lowland site because they behaviourally compensate for poorer weather by increasing basking frequency and raising their preferred body temperatures more than those from warm lowland site.

The **General Discussion** integrates the main outcomes from each study (4 chapters) to provide an understanding of how an ectothermic species, *Niveoscincus ocellatus*, responds to temporal and spatial temperature variation in their environment. I also discuss the potential effect of climate change to this species

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Chapter Two

Thermal biology of the spotted snow skink, *Niveoscincus ocellatus*: responses to different thermal conditions along an altitudinal gradient

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Abstract

Body temperatures in ectotherms are strongly affected by their thermal environment. Ectotherms respond to variation in the thermal environment either by modification of behavioural thermoregulation to maintain their optimal body temperature or by shifting their optimal body temperature. In this study, the body temperatures of three populations of male spotted snow skink, *Niveoscincus ocellatus*, living along an altitudinal gradient (low, mid, and high altitude) were studied in the field and laboratory in spring, summer, and autumn representing the full activity period of this species. The environmental variation across sites and seasons affected the field active body temperatures of *N. ocellatus.* At the low and mid altitude, lizards had a higher body temperature than those at the high altitude. The lizards achieved their thermal preference at the low and mid altitude sites in all seasons. At the high altitude, however, the lizards struggled to reach their preferred body temperatures, especially in autumn. The lower body temperature at the high altitude is likely due to limited thermal opportunity and/or an effect of avoiding the cost associated with increased intensity of basking.

Keywords: Body temperature, thermal preference, thermoregulation, lizards, altitudinal gradient

Introduction

Ectotherms have a particularly strong relationship to their thermal environment. They rely on it for thermoregulation, and the body temperatures ectotherms are able to maintain affect their physiological processes, e.g., locomotory performance (Angilletta et al. 2002a), metabolic rate (Schaefer and Walters 2010), energy expenditure (Christian et al. 1999, 2003, 2007) and developmental and growth rates (e.g., Wapstra et al. 2001; Sears and Angilletta 2003; Sears 2005). Through these effects, variation in body temperatures (within or between populations) influences life history traits in ectotherms (e.g., Adolph and Porter 1993; Grant and Dunham 1990; Rohr 1997; Wapstra and Swain 2001; Sears and Angilletta 2003; Uller et al. 2011; Cadby et al. 2014). Additionally, since body temperatures affect many physiological processes in ectothermic species, some aspects of thermal biology in ectothermic species may co-evolve in order to maximize their physiological functions at the temperature range typically experienced in the field (Angilletta et al. 2002a, 2002b; Kohlsdorf and Navas 2006). Further, given that many of these physiological functions are then optimized within a narrow range of body temperatures (Besson and Cree 2010), there is strong selection on thermoregulatory behaviour to maintain body temperatures within narrow ranges when active.

Body temperatures can vary between populations of ectothermic species living in different thermal environments (Hertz and Huey 1981; Hertz et al. 1983; van Berkum 1986; Gabirot et al. 2013; Martins et al. 2014). It also can vary among seasons within a population (Diaz and Cabezas-Diaz 2004; Sepulveda et al. 2008; Hadamova and Gvozdik 2011; Sanabria et al. 2011; Ortega et al. 2014). Ectotherms respond to the variation in their thermal environment by behaviourally regulating their body temperatures (Seebacher et al. 2004; Blouin-Demers and Nadeau 2005; Kearney et al. 2009; Beldade et al. 2011; Zamora-Camacho et al. 2013; Sunday et al. 2014) and by modifying physiological and biochemical rates to shift their optimal temperatures for physiological and physical performance (Seebacher 2005; Angilletta 2009; Beldade et al. 2011). Behavioural thermoregulation contribution to body temperature can be up to four to five times greater than those provided by physiological mechanisms (Stevenson 1985; Hertz and Huey 1981; Crowley 1985; Van Damme et al. 1989). If body temperatures do not vary despite geographic or seasonal shifts in environmental temperature, then this reflects a change in thermoregulatory behaviour (Hertz and Huey 1981; Huang and Tu 2009; Sunday et al. 2014). Behavioural thermoregulation, however, may be limited by several factors, e.g., thermal quality of the environment (Scheers and Van Damme 2002; Besson and Cree 2010), predation risk (Gutierrez et al. 2010), and

time allocation for activities other than thermoregulatory behaviour (Huey and Slatkin 1976; Sinervo et al. 2010).

If variation in thermal environments cannot be compensated via behavioural thermoregulation, body temperatures will vary along an altitudinal gradient or across seasons (Hertz and Huey 1981; Rodriguez-Serrano et al. 2009; Seebacher 2009; Gutierrez et al. 2010; Hadamova and Gvozdik 2011). For example, individuals living in a colder environment such as in high altitudes or latitudes may have a lower body temperature than those living in warmer environment of low altitudes or latitudes (van Damme et al. 1989; Gutierrez et al. 2010; Valdecantos et al. 2013). Thus, how body temperatures relate to environmental temperatures is not straightforward because thermoregulatory decisions reflect a trade-off between multiple costs and benefits (Huey and Stevenson 1979; Seebacher 2009).

Since body temperature determines the optimal physiological processes in ectotherms (e.g., Angilletta et al. 2002a; Sears 2005; Christian et al. 2007; Schaefer and Walters 2010), it is essential to understand how well populations of an ectothermic species living in different thermal conditions achieve their thermal optimum in the area they live, given spatial (e.g., altitudinal gradient) and temporal (e.g., seasonal) variation in environmental opportunities. Data on thermal preferences in the laboratory, it allows us to determine the body temperature that is maintained under ideal conditions (largely via behavioural adjustment), without any limiting factors such as competition/predator risk or limitations in thermal opportunity (Scheers and Van Damme 2002; Diaz et al. 2006). Thermal preference is generally correlated with optimal temperature of other physiological traits such as locomotor performance (Angilletta et al. 2002b; Kubisch et al. 2011) and is widely used to determine the efficiency of thermoregulation (Clusella-Trullas and Chown 2014). Thermal preference is often conservative within species (or even genera), indicating that this trait evolves slowly in response to spatial and temporal heterogeneity of the thermal environment (Gvozdik and Castilla 2001; Gvozdik 2002; Clusella-Trullas et al. 2007; Valdecantos et al. 2013). If there is a mismatch between temperatures maintained in the field and the laboratory it suggests there are underlying constraints.

A study of an ectothermic species living along an altitudinal gradient allows to investigate the response of a species to different thermal conditions (Miles 1994; Williams et al. 2003; Thuiller 2007). Further, an understanding of how animals respond to temperature variation has been used for forecasting what may happen to a species during future predicted climate change (Stillman 2002; Tomanek 2010; Seebacher et al. 2012). For example, field

active body temperatures and thermal preferences are being used in modelling studies to predict the effect of global climate change on distributional shifts and species extinction (e.g., Sinervo et al. 2010; Kearney et al. 2013; Ceia-Hasse et al. 2014). It has been predicted that animals with body temperatures below their optimal might obtain benefit from the modest climate warming, because extensive basking may not be required to reach optimal body temperatures (e.g., Kearney et al. 2009; Huey et al. 2012; Sunday et al. 2014; Caldwell et al. 2015). Conversely, the increasing environmental temperature in the future may add the risk of overheating for lizards living in warmer (lower altitudes or latitudes) environments, although the risk of overheating can be avoided if a species has a well-developed capacity for behavioural thermoregulation and lives in a thermally heterogeneous environments (Chamaille-James et al. 2006; Kearney et al. 2009; Sinervo et al 2010; Huey et al. 2012; reviewed in Clusella-Trullas and Chown 2014).

In this study, we investigated the thermal biology (field active body temperature and thermal preference in the laboratory) in three populations of the spotted snow skink, *Niveoscincus ocellatus*, living along an altitudinal gradient in Tasmania. The field active body temperature of *N. ocellatus* was measured under natural living conditions at the low, mid, and high altitudes. The climate at the high altitude site is colder and is more variable than at the low and mid altitude sites (see Pen et al. 2010 and Figure 2). Accordingly, we predicted that lizards at the high altitude might be active at lower body temperatures than their counterparts at the lower altitudes, and lizards at the high altitude might have difficulty to reach their thermal preference in the field. In order to investigate whether the lizards' response to geographic variation across sites was consistent across seasons, the study was conducted in spring, summer, and autumn at each study site. We predicted that field active body temperature at the high altitude would be more variable than those from lower altitudes due to more variable climate at this site. For the thermal preference, because this trait is relatively slow to evolve in responding to the heterogeneity in thermal environment, we predicted that the thermal preference would be consistent across sites in *N. ocellatus*.

Methods

Study species

Niveoscincus ocellatus is a small viviparous lizard occurring over a wide geographical and climatic range in Tasmania (Atkins et al. 2007; Cadby et al. 2010; Uller et al. 2011; Yuni et al. 2015). It is a saxicolous (rock-dwelling) species; their occurrence is typically associated with rocky outcrops which are used as basking sites (Melville 2007). Across its range, *N. ocellatus* displays distinct life history variation (e.g., Wapstra and Swain 2001; Wapstra et al. 2001; Pen et al. 2010; Uller et al. 2011; Cadby et al. 2011, 2014). Individuals are considerably larger in body size and significantly larger at the first age of maturity at the colder extremes than those at the warmer extremes of the species' range (Wapstra et al. 2001) where the active season is reduced to approximately October to April, compared to their counterparts that are active approximately from September to May at the warmer extreme of the species' range (Wapstra et al. 1999). Key reproductive events are delayed at cold highland sites compared with lowland warmer sites (Wapstra et al. 1999; Pen et al. 2010; Uller et al. 2011). The effect of climate on life history traits, especially embryonic development and concomitant effects on offspring traits including offspring birth date, size and sex, has created divergent selection pressure on basking behaviour (Wapstra et al. 2004, 2009, 2010; Atkins et al. 2007; Pen et al. 2010; Cadby et al. 2010, 2014; Uller et al. 2011). This species thus presents ideal opportunity to understand how a wide ranging ectothermic species has adapted to the environmental conditions they live in.

Description of study sites

The study was conducted using three populations that occurred along the full climatic (and altitudinal) gradient occupied by *N. ocellatus*. These were a low altitude site at Orford (147º44'E; 42º33'S; 50 m asl), a mid-altitude site at Tooms lake near Oatlands (147º45'E; 42º12'S; 550 m asl) and a high altitude site at Miena on the Central Plateau (146º45'E; 41º55'S; 1050 m asl) in Tasmania, Australia (Figure 1). The three sites differ in temperature in line with their altitude. The low altitude site experiences a warm coastal temperature with a mild winter, while the high altitude site is much cooler where snow or hail can fall in any month (www.bom.gov.au). Monthly mean maximum and minimum air temperature (2001 – 2010) at the nearest weather station to each study site from the Bureau of Meteorology

Australia is displayed in Figure 2. The mid altitude site is intermediate in temperature between these two extremes. The study sites were each separated by about 100 km.

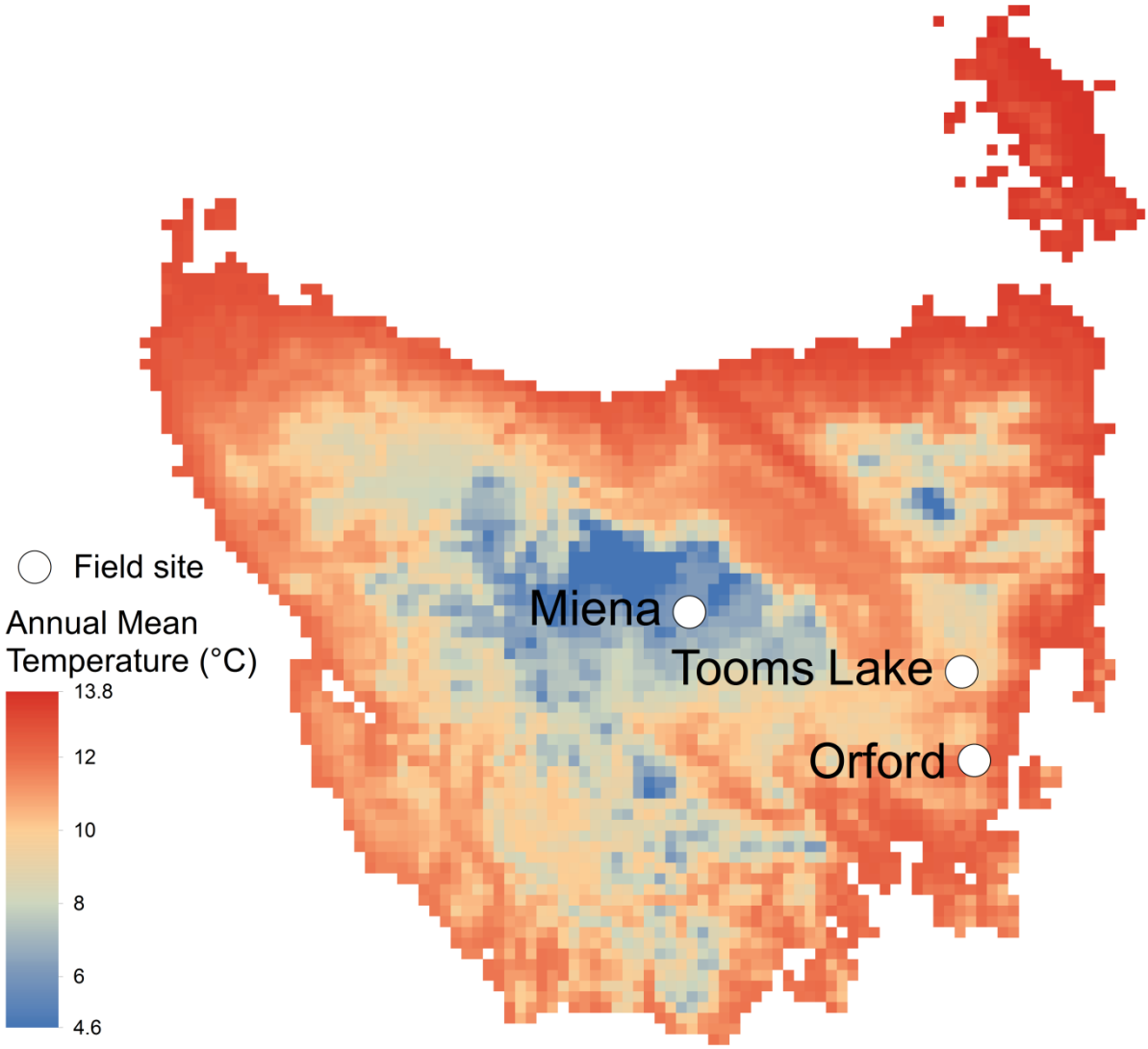

Figure 1. Temperature profile of Tasmania showing the three study sites from different climatic regions. The low altitude site is located at Orford (50 m asl); Mid altitude site is located in Tooms lake near Oatlands (550 m asl); High altitude site is located in Miena on the Central Plateau (1050 m asl). Data presented as the annual maximum air temperature which were derived from climate records from 1950 to 2000 (source: http://worldclim.org/current).

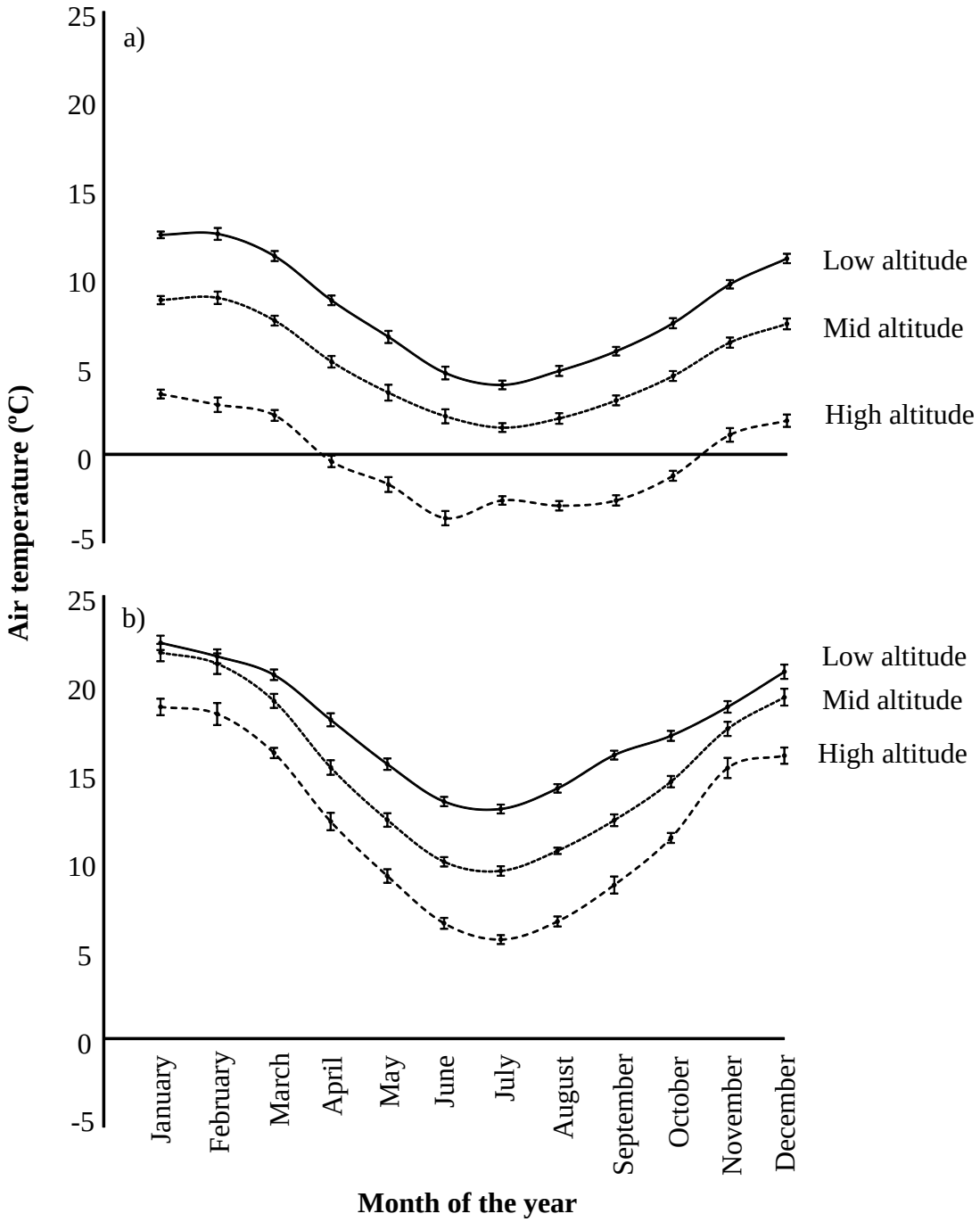

Figure 2. Mean minimum (a) and mean maximum (b) monthly air temperature ± S.E. recorded from the nearest Bureau of Meteorology weather station from each study site from 2001 to 2010.

Field active body temperature measurement

Male *N. ocellatus* were caught in the field by fishing with a mealworm tied onto a piece of cotton in over two days each in Spring 2010, Summer 2010/11 and Autumn 2011 at the low altitude (N = 34, 46, 37 respectively), mid altitude (N = 35, 29, 31 respectively), and high altitude (N = 46, 42, 44 respectively) sites (Table 1). Only males were used in this study because stage of reproduction affects thermal preferences in females of many lizard species (Mathies and Andrews 1997; Gvozdik and Castilla 2001; Le Galliard et al. 2003; Lin et al. 2008).

Table 1. Date of collection and sample size of male *Niveoscincus ocellatus* used for field active body temperature measurement.

Site	**Season**	**Dates**	**Number**
Low altitude	Spring 2010	14 October 2010	17
		9 November 2010	17
	Summer 2010/11	6 January 2011	24
		6 February 2011	22
	Autumn 2011	6 March 2011	21
		8 April 2011	16
Mid altitude	Spring 2010	20 October 2010	19
		29 November 2011	16
	Summer 2010/11	7 January 2011	15
		23 February 2011	14
	Autumn 2011	26 March 2011	13
		26 April 2011	18
High altitude	Spring 2010	27 October 2010	21
		20 November 2010	25
	Summer 2010/11	22 January 2011	20

	22 February 2011	22
Autumn 2011	15 March 2011	27
	17 April 2011	17

Immediately at capture, body temperature of each lizard was measured to ± 0.1ºC temperature using a thermocouple probe (K-type, 0.5 mm diameter) connected to a digital micro-processor thermometer (HH 21, OMEGA Engineering, Stamford, CT, USA), which was inserted 5 mm into the lizard's cloaca. To establish the relationship between body temperature and the environment, the substrate and the air temperatures in where the lizard was sighted were also measured. The temperature of the substrate was measured to ± 0.1 ºC using an infrared handheld thermometer (Testo 805) and the air temperature was measured 1 m above the ground in the shade to ± 0.1 ºC using an air thermometer (Humidity/temperature pen, ProSciTech E800012). Captured lizards were marked on their backs with non-toxic silver pen for temporary identification as a precaution against repeated measurements on the same individuals within a season. Each lizard was released at its point of capture as soon as the measurement was completed.

Measurement of preferred body temperature in the laboratory

Male *N. ocellatus* were caught by fishing with a mealworm tied onto a piece of cotton in Spring 2010, Summer 2010/11 and Autumn 2011 at low altitude (14 October 2010, 6 January 2011, and 6 March 2011 respectively), mid altitude (20 October 2010, 7 January 2011, and 26 March 2011 respectively), and high altitude (27 October 2010, 22 January 2011, and 15 March 2011 respectively). After capture, lizards were placed in dark cloth bags and transported to the Herpetology Laboratory at the School of Biological Sciences at the University of Tasmania on the afternoon of their capture. On arrival at the laboratory, the lizards were treated to kill any mites by having their tails dipped in oil for 30 sec. Lizards were weighed to the nearest 0.001 g using an electronic balance (Sartorius TE 153S) and the snout vent length (SVL) was measured to the nearest 1 mm using a plastic ruler. The lizards were housed individually in plastic cages measuring 30 x 20 x 10 cm. Each cage contained paper towel as substrate, a piece of wood (10 x 8 x 2 cm) and two terracotta saucers as basking surface and/or as a hiding place. Water was available *ad libitum*. Each lizard was fed

two mealworms (tenebrionid larvae) dusted with multi-vitamins (Aristopet repti-vite) and baby food (Heinz, fruity pears or apples) mixed with calcium (Aristopet repti-cal), three times a week.

Lizards were kept under a light and temperature regime that resembled their natural environment. A photo period was established by overhead lights (fluorescent tubes) which were set for 10 Light: 14 Dark during spring and autumn, and 14 Light: 10 Dark during summer. A 25-watt spotlight situated above each cage provided basking opportunity that approximately matched the thermal opportunities available at each of the sites (e.g., Uller et al. 2011; Cadby et al. 2014). The spotlights were set for 8 hours, 5 hours, and 2 hours for the low, mid, and high altitude population respectively in spring and in autumn, and 10 hours, 7 hours, and 4 hours in summer. Lizards were housed for two weeks to allow acclimation to captive conditions (Beck and Lowe 1994; McConnachie et al. 2009) before their thermal preference in the laboratory was assessed. Therefore, all lizards had similar conditions prior to the test (e.g., McConnachie et al. 2009; Valdecantos et al. 2013).

Each lizard was fed on the day prior to the measurement of preferred body temperature. Each lizard was placed in a chamber (1500 mm, 100 mm, 60 mm) with a base and walls constructed from 10-mm-thick aluminium fitted with a plastic floor. A plexiglas lid (5-mm thick) fitted with an inlet and outlet port for air sealed the chamber. The chamber was preheated at one end by 49°C water and cooled at the other end by 7°C water; this established an approximately linear thermal gradient from 8°C to 40°C. The sides of the chamber were fitted with a series of infrared phototransistors spaced at 40 mm intervals; the photocell closest to the warm end of the gradient that was obscured by the lizard recorded the position (i.e., preferred temperature) of the lizard within the thermal gradient. Movement of the lizard was also recorded by the movement between photocells.

During the experiment, a light-dark photoperiod matching the housing photoperiod of the season and locality was used. The experiment was conducted for 36 hours, and the recording was taken every 10 minutes for each lizard. The recording was started immediately when the lizard was placed in the gradient at about 18.00 hours. However, the data used was from 12.00 on day 2 to 12.00 on day 3 (i.e., a 24 hour period) in order to allow the lizard to explore and to become familiar with the chamber during their active phase. The daytime period data was used for further analysis to avoid confounding effects of diel variation and differences in day length between seasons (Christian and Bedford 1995; Angilletta et al. 1999; reviewed in Clusella-Trullas and Chown 2014; see also Seebacher 2005). The daytime period was

determined according to the season in which the thermal preference experiment was conducted, which was 10L: 14D for spring and autumn (light on from 07.00 to 17.00), and 14L: 10D for summer (light on from 06.00 to 20.00). At the end of the experiment, lizards were returned to their holding cage. In total, the preferred body temperature measurement was conducted on 5 – 6 individuals male *N. ocellatus* per season per site. The lizards were then released at their site of capture.

Data analysis

All statistical analyses were conducted in SAS 9.2. with significance, α, set at 0.05. Data for the field active body temperature and the preferred temperature in the laboratory were normally distributed (confirmed using the normal quantile plot prior to analysis). Field active body temperatures were compared among sites and seasons using a 2 way ANCOVA, with snout vent length as covariate because body size can affect the heating and cooling rates in lizards (e.g., Porter et al. 1973; Rice et al. 2006). When the full models were significant, planned comparison tests were used to isolate significant effects (including interactions) between sites and seasons. In order to determine whether field active body temperatures were related to their thermal environment (air or substrate temperatures), linear regressions analyses were conducted. One might expect that since we had 3 populations and 3 altitudes, the differences we found in this study could be because of population effects rather than altitude. One way to address this was the possibility to replicate the three sites, which is beyond the scope of this study. Given we know that temperature drives many processes in ectotherms (rather than altitude per se), it is more parsimonious to accept that the population differences (see later) are in fact likely driven by temperature.

The preferred thermal range in the laboratory was determined as the central 50% of all temperature recordings around the median value (Hertz et al. 1993; Scheers and Van Damme 2002). Accordingly, the second and third quartile of the temperature preferred range distribution were defined as the lower and upper set of the thermal preferred range respectively (Hertz et al. 1993; Scheers and Van Damme 2002). Temperatures measured in the laboratory that fell within the lower set and upper set were considered as the thermal preference range because earlier studies shows that ectotherms regulate between upper and lower set temperatures rather than around a single body temperature (Barber and Crawford 1977; Guiterrez et al. 2010; Valdecantos et al. 2013). Similar to the analysis for the field active body temperature, the lower and upper set of thermal preference were compared among

sites and seasons using a 2 way ANCOVA, with SVL as the covariate. If there was a significant interaction or main effect, a post hoc test (Tukey-Kramer) was used to determine where the differences lay. In order to determine if the lizards were able to reach their thermal preferences in their natural environment, the field active body temperature data was compared with the lower set of thermal preference using a two sample T test for each site and season (Valdecantos et al. 2013).

Results

A. Field active body temperature measurements

Niveoscincus ocellatus maintained mean field active body temperature higher than 28 °C at all sites and seasons (Figure 3a-c). The range of body temperatures in which the lizards were active was wider at the high altitude site (22.3 - 33.4 °C) than those at the low altitude (29.1 - 34.2 ºC) and at the mid altitude sites (29.1 - 34.4 ºC), with the principle difference being in the lower temperatures at which they were active at the high altitude site. The field active body temperature in *N. ocellatus* showed significant interaction among sites and seasons ($F_{4, 343} = 10.12$, $P < 0.0001$). The field active body temperature of the high altitude population was significantly lower than that of both at the low and mid altitudes in spring (low altitude $F_{2,248} = 43.99$, $P < 0.0001$; mid altitude $F_{2,226} = 30.61$, $P < 0.0001$), summer (low altitude $F_{2,248} = 57.92$, $P < 0.0001$; mid altitude $F_{2,226} = 30.16$, $P < 0.0001$), and autumn (low altitude $F_{2,248} = 146.97$, $P < 0.0001$; mid altitude $F_{2,226} = 168.55$, $P < 0.0001$). The field active body temperature of *N. ocellatus* at the low altitude was similar to those at the mid altitude in all seasons (spring: $F_{2,211} = 1.16$, $P = 0.2827$; summer: $F_{2,211} = 1.58$, $P = 0.2091$; autumn: $F_{2,211} = 1.95$, $P = 0.1634$) (Figure 3a,b).

Within a site, there was a seasonal variation in field active body temperatures at the low and high altitude populations (Figure 3a,c). In both of these populations, the field active body temperature was significantly lower in autumn than in spring (low altitude $F_{2,116} = 5.11$, $P = 0.0245$; high altitude $F_{2,131} = 68.16$, $P < 0.0001$) and summer (low altitude $F_{2,116} = 6.60$, $P = 0.0106$; high altitude $F_{2,131} = 58.31$, $P < 0.0001$). However, there was no difference in field active body temperature between spring and summer at the high altitude ($F_{2,131} = 0.19$, $P = 0.6612$) and low altitude ($F_{2,116} = 0.02$, $P = 0.8923$). In the mid altitude, there was no seasonal variation in the field active body temperature.

There was a significant but weak relationship between field active body temperature and air temperature at the low altitude site in all seasons (Figure 4a-c; spring $F_{1,33} = 11.21$, $P = 0.0021$, $r^2 = 0.26$; summer $F_{1,45} = 24.23$, $P < 0.0001$, $r^2 = 0.34$; autumn $F_{1,36} = 8.81$, $P = 0.0054$, $r^2 = 0.20$). A significant and weak relationship was also observed at the mid altitude in spring ($F_{1,34} = 6.03$, $P = 0.0195$, $r^2 = 0.15$) and summer ($F_{1,28} = 14.95$, $P = 0.0006$, $r^2 = 0.36$), but not in autumn ($F_{1,30} = 0.10$, $P = 0.7544$, $r^2 = 0.003$) (Figure 4d-f). At the high altitude, there was no significant relationship between the field active body temperature and the air temperature in spring ($F_{1,45} = 0.33$, $P = 0.5695$, $r^2 = 0.007$). However, there was a significant (and strong) relationship between field active body temperature and air temperature in summer ($F_{1,41} = 91.91$, $P < 0.0001$, $r^2 = 0.70$) and autumn ($F_{1,43} = 26.18$, $P < 0.0001$, $r^2 = 0.38$) (Figure 4g-i). The range of air temperatures at which lizards were active at the low altitude was 20.7-29.4 ºC (mean 24.1 ± 0.4 ºC), 19.7-29.4 ºC (mean 26.1 ± 0.3 ºC), and 16.5-28.7 ºC (mean 23.5 ± 0.5 ºC) in spring, summer, and autumn, respectively (Figure 4a-c). At the mid altitude, the lizards were active at the range of air temperatures of 13.4-22.2 ºC (mean 17.8 ± 0.4 ºC), 20.7-28.4 ºC (mean 24.6 ± 0.3 ºC), and 17.7-24.6 ºC (mean 22.1 ± 0.3 ºC) in spring, summer, and autumn, respectively (Figure 4d-f). The range of air temperatures at which the lizards were active at the high altitude were 12.9-24.1 ºC (mean 18.2 ± 0.4 ºC), 14.9-26.6 ºC (mean 20.2 ± 0.5 ºC), 12.2-23.6 ºC (mean 17.7 ± 0.4 ºC) in spring summer, and autumn, respectively (Figure 4g-i).

There was a significant relationship between the field active body temperature and the substrate temperature in summer and autumn at the low altitude (Figure 5b,c) (summer $F_{1,45} = 6.05$, $P = 0.0182$, $r^2 = 0.12$; autumn $F_{1,36} = 18.84$, $P = 0.0001$, $r^2 = 0.35$) and high altitude sites (Figure 5h,i) (summer $F_{1,41} = 77.78$, $P < 0.0001$, $r^2 = 0.66$; autumn $F_{1,43} = 33.05$, $P < 0.0001$, $r^2 = 0.44$), but no significant relationship was found in spring at either the low or high altitude site (Figure 5a,g) (low altitude $F_{1,33} = 0.26$, $P = 0.6166$, $r^2 = 0.008$; high altitude $F_{1,45} = 0.04$, $P = 08399$, $r^2 = 0.0009$). At the mid altitude, the field active body temperature was significantly related to the substrate temperature in spring ($F_{1,34} = 6.21$, $P = 0.0179$, $r^2 = 0.16$) and summer ($F_{1,28} = 27.00$, $P < 0.0001$, $r^2 = 0.50$) (Figure 5d,e), but not in autumn ($F_{1,30} = 0.18$, $P = 0.6725$, $r^2 = 0.006$) (Figure 5f). The range of substrate temperatures at which lizards were active at the low altitude was 19.2-34.3 ºC (mean 28.6 ± 0.7 ºC), 22.2-35.3 ºC (mean 30.4 ± 0.5 ºC), and 15.9-7.8 ºC (mean 24.8 ± 0.7 ºC) in spring, summer, and autumn, respectively (Figure 5a-c). At the mid altitude, the lizards were active at substrate temperatures of 17.0-37.5 ºC (mean 27.7 ± 0.9 ºC), 21.8-35.4 ºC (mean 27.0 ± 0.7 °C), and 20.6-30.6 ºC (mean 26.2 ± 0.6 ºC) in

spring, summer, and autumn respectively (Figure 5d-f). The range of substrate temperatures at the high altitude at which the lizards were active were 23.5-34.3 ºC (mean 29.8 ± 0.4 ºC), 18.9-38.8 ºC (mean 28.4 ± 0.9 ºC), and 16.6-31.8 ºC (mean 24.9 ± 0.7 ºC) in spring summer, and autumn, respectively (Figure 5g-i).

B. Preferred body temperature measurement in the laboratory

The mean of lower set and upper set of thermal preference at low, mid, and high altitudes in spring, summer, and autumn is presented in Table 2. The snout vent length did not affect the lower or upper set of thermal preference in the laboratory ($F_{4,46} = 0.90$, P = 0.4760 and $F_{4,46} = 0.06$, P = 0.9938, respectively). The lower set of the thermal preference was not influenced by either of the main effects of site ($F_{2,46} = 0.16$, P = 0.8540), season ($F_{2,46} = 2.05$, P = 0.1420) or their interaction ($F_{4,46} = 1.61$, P = 0.1911) (Figure 3). Similarly, the upper set point was not influenced by site ($F_{2,46} = 0.04$, P = 0.9603) or season ($F_{2,46} = 2.82$, P = 0.0716) or their interaction ($F_{4,46} = 2.33$, P = 0.0728) (Figure 3).

The lizards reached their preferred body temperature in the field at the low and mid altitudes in all seasons observed (Figure 3) but not always at the high altitude site.

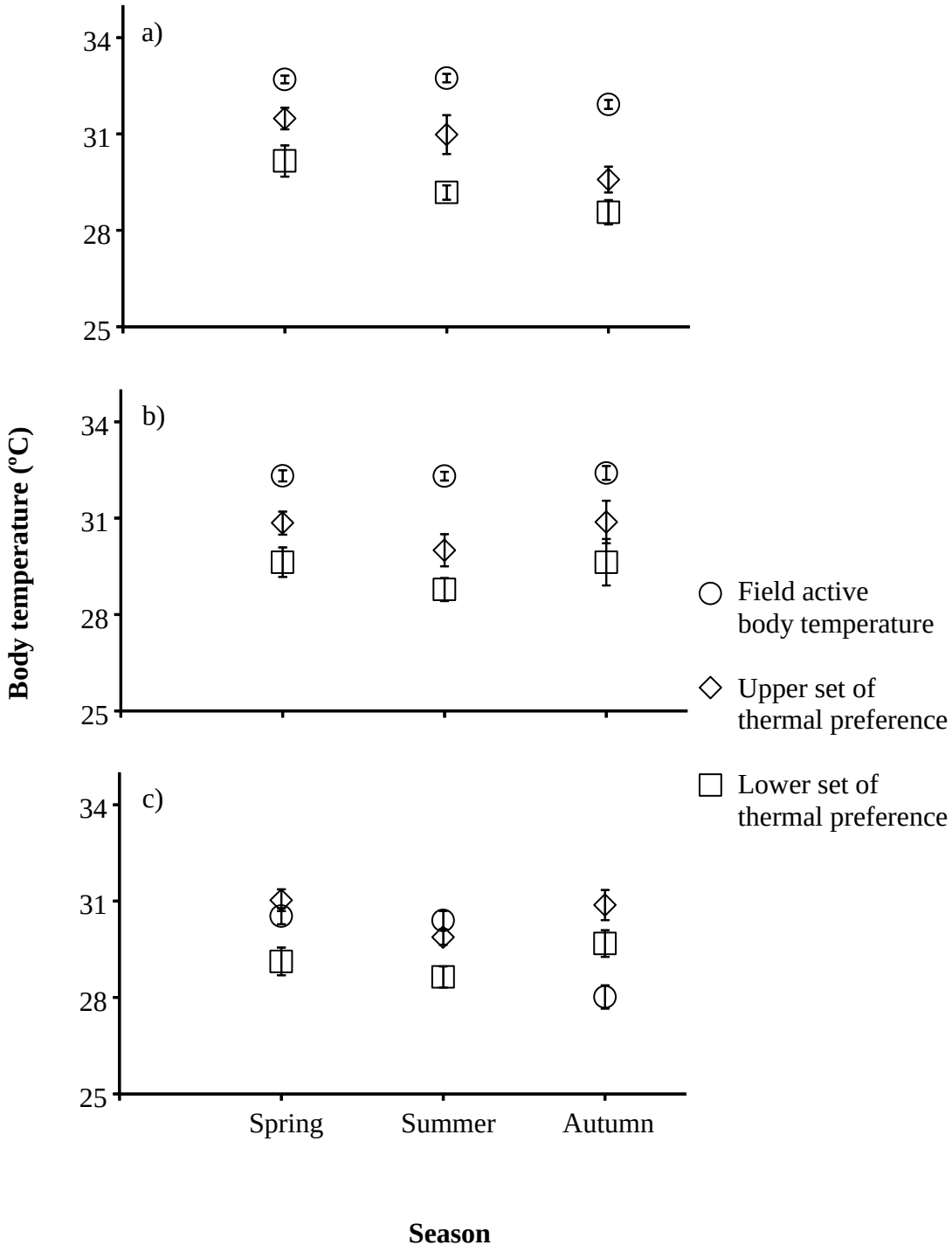

Figure 3. Seasonal and geographic variation in mean ± S.E body temperature of the spotted snow skink, *Niveoscincus ocellatus*. Figures 3a-c are the low, mid, and high altitude population respectively.

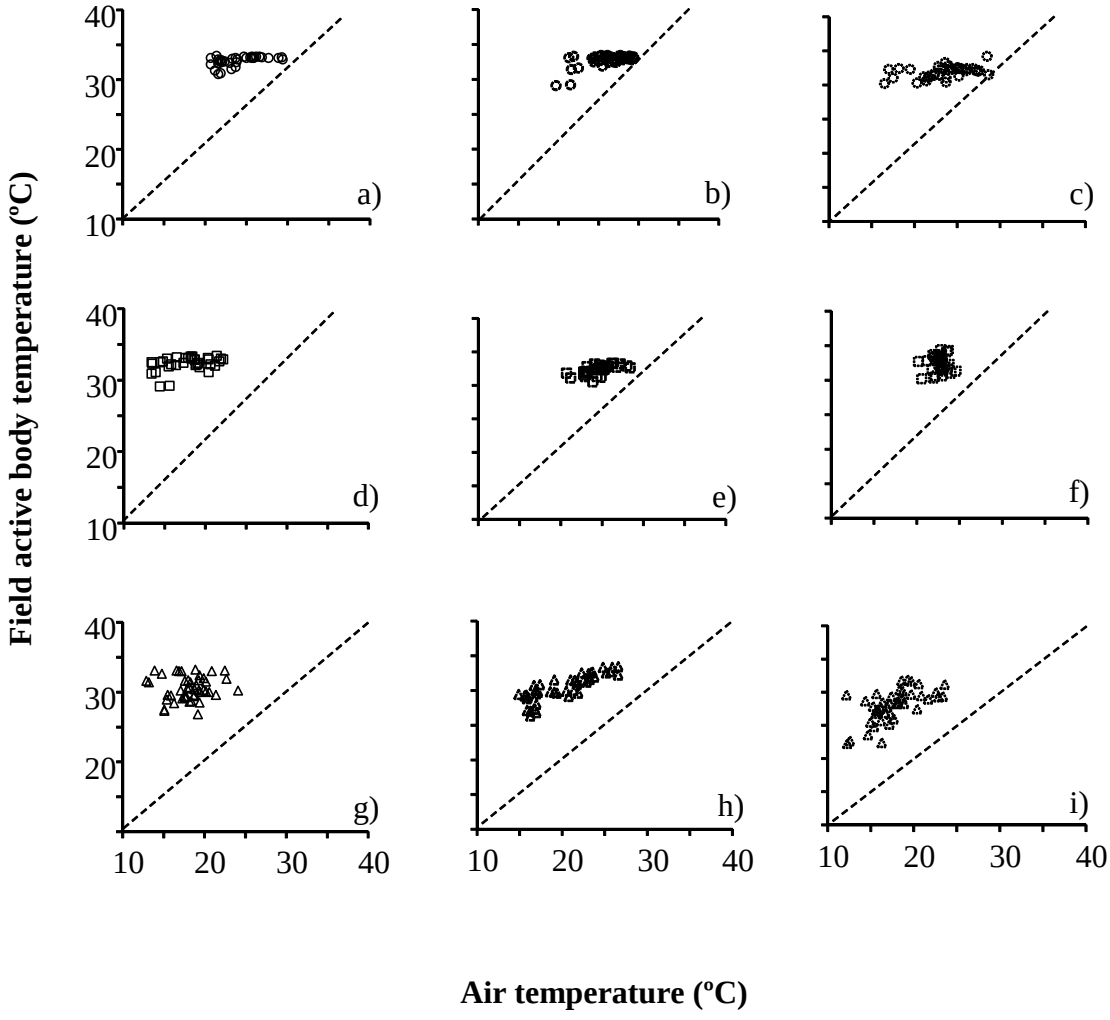

Figure 4. The relationship between the field active body temperatures and the air temperatures of the spotted snow skink, *Niveoscincus ocellatus,* from the three sites studied across three seasons. Figures 4a-c are the low altitude in spring, summer, and autumn. Figures 4d-f are the mid altitude in spring, summer, and autumn. Figures 4g-i are the high altitude in spring, summer, and autumn. The diagonal line (axes X/Y) indicates the strength of relationship between body temperature and air temperature (thermoconformity).

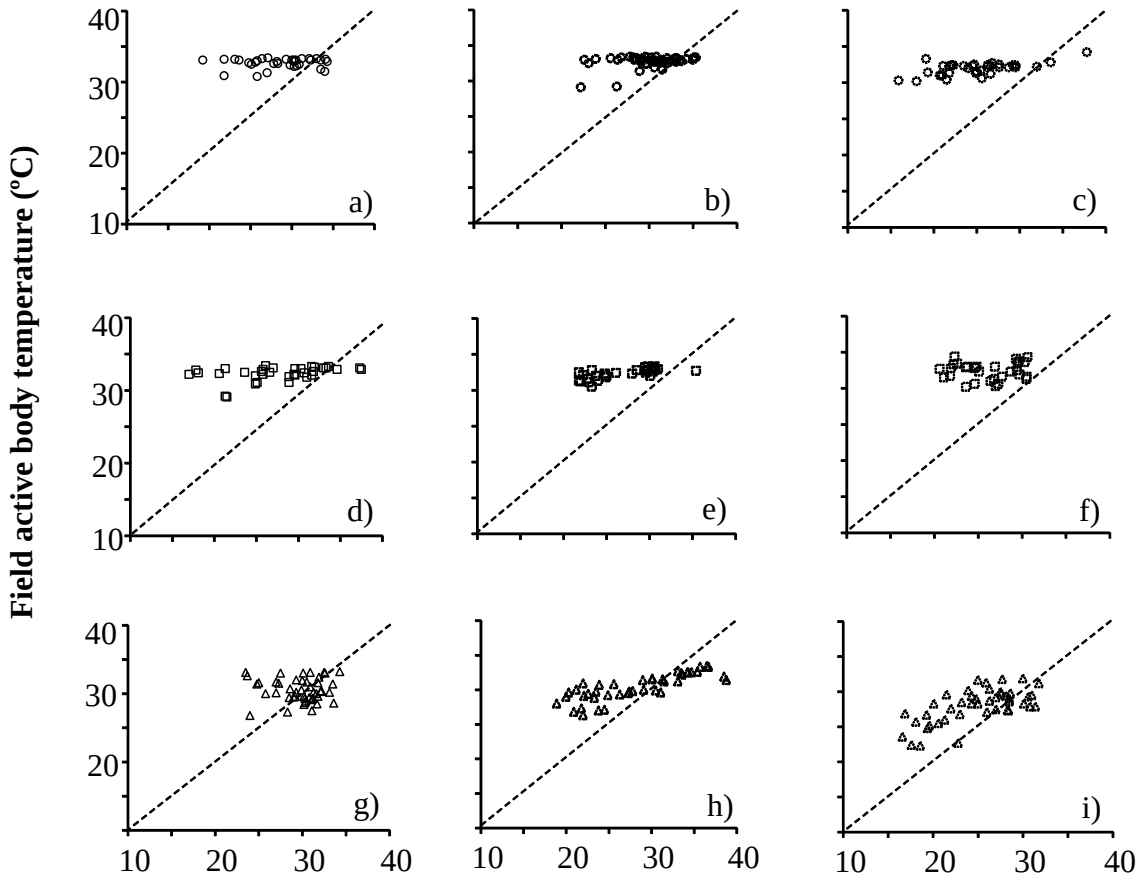

Figure 5. The relationship between the field active body temperatures and the substrate temperatures of the spotted snow skink, *Niveoscincus ocellatus,* from the three sites studied across three seasons. Figures 5a-c are the low altitude in spring, summer, and autumn. Figures 5d-f are the mid altitude in spring, summer, and autumn. Figures 5g-i are the high altitude in spring, summer, and autumn. The diagonal line (axes X/Y) indicates the strength of relationship between body temperature and substrate temperature (thermoconformity).

Table 2. The mean ± S.E. of the lower and upper set of thermal preference of the spotted snow skink, *Niveoscincus ocellatus,* from three sites in Tasmania.

	Low altitude		**Mid altitude**		**High altitude**	
	Lower set	**Upper set**	**Lower set**	**Upper set**	**Lower set**	**Upper set**
Spring	30.16 ± .49	31.48 ± .34	29.63 ± .46	30.85 ± .36	29.12 ± .43	31.03 ± .34
Summer	29.18 ± .22	30.98 ± .61	28.78 ± .36	30.00 ± .50	28.64 ± .33	29.88 ± .24
Autumn	28.56 ± .38	29.58 ± .40	29.63 ± .72	30.88 ± .66	29.68 ± .42	30.88 ± .47

Discussion

We measured active body temperatures in the field and the thermal preference in the laboratory of the widespread spotted snow skink, *Niveoscincus ocellatus,* at three sites along an altitudinal gradient. The study was conducted across the spring, summer, and autumn, representing the full activity period of this species. The mean field active body temperatures of *N. ocellatus* differed across sites; this difference was largely driven by activity at lower body temperatures in the high altitude population. Field active body temperatures were lower in autumn than in spring and summer at the high and low altitudes. The field active body temperatures, however, was consistent across seasons at the mid altitude. We also found that lizards reached their thermal preference in the field, with the exception for the high altitude population. The thermal preference was consistent across sites and seasons in this species.

Niveoscincus ocellatus reached their thermal preferences and maintained a stable active field body temperature at about 30 - 32 ºC across seasons largely independent of environmental air and substrate temperatures at the low and mid altitudes. These temperatures were slightly higher than temperatures they selected in the laboratory (1-2 °C). We were uncertain about the reason of why they selected a slightly lower temperature in the laboratory than in the field. However, although their body temperatures at the field were slightly higher than their thermal preferences, they were 2 – 4 ºC below their upper limit for maximum performance, i.e., within the upper limit of 80% of maximum performance for this species (Yuni et al. 2015 submitted for publication; Chapter 3). Moreover, the active body temperatures maintained by *N. ocellatus* at the low and mid altitudes corresponded with the

optimum temperature for locomotor performance observed for *N. ocellatus* (Yuni et al. 2015 submitted for publication; Chapter 3). Conversely, the lizards at the high altitude had difficulty reaching their thermal preference suggesting either that they were limited in the opportunity to reach their preferred temperature or that costs of thermoregulation restricted their basking (e.g., Huey and Slatkin 1976; Seebacher 2009; Gutierrez et al. 2010). As a result, their field active body temperatures were also more strongly related to their thermal environments than their counterparts from the lower altitudes in part because they were active at lower air temperatures than those from the lower altitude sites. As a result, lizards at the high altitude had their body temperature farther from their thermal preference in the laboratory than those from the lower altitude sites. In addition to geographic variation in the capacity to maintain high body temperatures, we also found seasonal variation in thermoregulation in *N. ocellatus*. In general, their body temperatures were similar in spring and summer, but at the high and low altitude sites body temperatures were lower in autumn than in spring and summer. This downshift was more pronounced at the high altitude (~2.5^{o}C) than at the low altitude (~0.8^{o}C). Populations of a species may respond differently to the variation in their environment (Williams et al. 2014) which appears to be determined by the level of temporal and spatial temperature variation they typically encounter (Janzen 1967; Addo Bediako et al. 2000; Clusella-Trullas et al. 2011; Sunday et al. 2011).

It is important for ectothermic species to maintain an optimally high body temperature because this optimises many key processes, including in *N. ocellatus*. For example, high body temperature maximises embryonic development rate, which is important for offspring survival (Wapstra et al. 1999, 2010; Cadby et al. 2010; Uller et al. 2011). However, as is typical in high altitude sites, restricted thermal opportunity may limit the lizards' ability to maintain a high and stable field active body temperature via behavioural thermoregulation (e.g., Ibarguengoytia et al. 2010; Zamora-Camacho et al. 2013) even with greater intensity of basking (Huey and Slatkin 1976; Vidal et al. 2010). Greater intensity of basking is, however, not without costs. Basking, especially at lower temperatures exposes lizards to higher risk of predation and takes time away from other activities such as foraging (Huey and Slatkin 1976; Angilletta 2009). To counter the costs of extended basking in colder environments or cooler seasons, they may have the ability to be active at a wider range of body temperature (Yuni et al. 2015) or to shift their thermal preferences.

Previous studies in reptiles have demonstrated that individuals from cooler environments (such as high altitudes or latitudes) have adaptations to extend their activity at lower air and

body temperatures (e.g., Van Damme 1989; Addo-Bediako et al. 2000; McConnachie et al. 2009; Bonino et al. 2011). For example, some reptiles elevate their metabolic rate to facilitate physiological processes in order to compensate for the effects of the low temperatures in high altitudes or latitudes (Clarke 1993; Lourdais et al. 2013). In *N. ocellatus*, we observed higher energy expenditure at the high altitude than at the low altitude, which we argued was partly due to metabolic rate elevation to compensate with cold temperature at the high altitude. This elevation in metabolic rate might help *N. ocellatus* to maintain activity while minimising the cost of behavioural thermoregulation to maintain a high body temperature at the high altitude site (Yuni et al. 2015). Related to this, some populations of wide ranging ectothermic species living in cold environments (e.g., high altitude or latitude) have the ability to perform at or near optimal levels at a lower body temperature than those living at the warm environment (e.g., low altitude or latitude) (Van Berkum 1986; Wilson, 2001; Castaneda et al. 2004). This is because they can shift downward the body temperatures at which performance (e.g., sprint speed or endurance) are optimised (Clarke 1993; Angilletta 2009; Lourdais et al. 2013). It has been widely observed that thermal optima for some physiological traits vary (i.e., are labile) across populations of wide ranging ectothermic species, to match activity body temperature they experience in nature (Van Berkum 1986, Castaneda 2004, Moritz et al. 2012). Moreover, there is considerable variability (i.e., lability) in tolerance to low temperatures between lizard species and populations (Munoz et al. 2014), in which can occur over relatively short timescales (~35 generations in lizards *Anolis cristatellus*) (Leal and Gunderson 2012). This is what we found in *N. ocellatus* also; in the high altitude population, both sprint speed and endurance were maximised at a lower body temperature than in the warm lowland population (Chapter 3; Yuni et al. submitted). Based on the our results in this study where body temperatures were at times compromised in the high altitude population, this capacity is undoubtedly important to escape predators (Chapple and Swain 2002; Melville and Swain 2003), for mate searching (Robson and Miles 2000) and for their active foraging (Wapstra and Swain 1996).

While we found that field active body temperature varied across sites and seasons in *N. ocellatus*, there was no evidence that the thermal preference in the laboratory varied across these spatial or temporal scales. This finding is in agreement that thermal preference is often evolutionarily conservative (e.g., Gvozdik and Castilla 2001; Valdecantos et al. 2013) largely because thermoregulatory behaviour provides a strong buffer to environmental temperature heterogeneity (Huey and Bennett 1990; Gvozdik and Castilla 2001). Our results showing that thermal preferences were conserved across sites suggest that the difference in thermal

environment across ~1000 m altitude might be not sufficiently strong to induce a shift in thermal preference in *N. ocellatus*. Our work is, however, in contrast to a recent broader scale interspecific comparison of thermal biology in *Niveoscincus* species. Caldwell et al. (2015) demonstrated that alpine species (*N. microlepidotus* and *N. greeni*) that occur only marginally higher in altitude than our high altitude site had a lower critical thermal minimum (CT_{min}) (and to a lesser degree critical thermal maximum) than the lowland *N. ocellatus*. The differences between our results can potentially be explained by the way selection acts on different thermal traits. Specifically, CT_{min} is under strong selection pressure because lizards have little capacity to behaviourally buffer against its deleterious effects on physiology and fitness-related traits (i.e., at very low temperatures when they emerge to bask they are especially vulnerable to predation). Conversely, preferred body temperature in all of our populations fell well with the body temperatures that can be achieved by active behavioural thermoregulation.

Knowledge of thermal biology of ectothermic species can be used to forecast how they will be affected by the future predicted global warming (Sinervo et al. 2010; Seebacher et al. 2012; Ceia-Hasse et al. 2014; Caldwell et al. 2015). Due to their susceptibility to perturbation in their thermal environment, many ectothermic species have been predicted to face extinction as a result of recent climate changes and future climate changes (Araujo et al. 2006; Sinervo et al. 2010). In Tasmania, temperature is projected to rise by about 2.9 ºC under a high greenhouse gas emissions scenario and by about 1.6 ºC under a low greenhouse gas emissions scenario (Climate Futures for Tasmania 2010). As we found in this study, *N. ocellatus* can achieve higher temperatures closer to their thermal preferences at both the low and mid altitude sites. A modest warming will allow *N. ocellatus* to easily reach their thermal preferences for an extended period before reaching the limit of behavioural buffering (Kearney et al. 2009; Huey et al. 2012; Sunday et al. 2014). In fact at the high altitude site modest warming may allow *N. ocellatus* to spend more time at their preferred body temperatures (and decrease the time at which they are vulnerable to predation when basking). This is confirmed in a recent modelling study on *N. ocellatus* (Caldwell et al. 2015). By incorporating our thermal preference data, they confirmed that the number of days in which the maximum predicted air temperature falls within the thermal preference for *N. ocellatus* is projected to increase by between 10 – 30 % under both high and low emissions scenarios. Further, we suggest that the high altitude population will be more tolerant to the future predicted warming. This is because organisms living in more highly fluctuating temperature regimes are often adapted to a wider range of temperatures (i.e., wider thermal windows) than

those living in relatively uniform temperature regimes (Janzen 1967; Pörtner and Farrell 2008). Although *N. ocellatus* is generally predicted to obtain potential benefits in extended activity from modest future warming (across its range but particularly in the current alpine areas) increases in extreme hot events may result in body temperatures exceeding their critical thermal maxima, which could result in fatalities (Sinervo et al. 2010; Ceia-Hasse et al. 2014; Caldwell et al. 2015). We suggest that the impact will be more pronounced for those already living in warmer environments. A well-developed capacity for behavioural thermoregulation and the availability of shade (e.g., vegetation cover) are essential for the animals because they allow them to shuttle between sun and shady patches in order to maintain their optimal body temperature without the risk of overheating (Chamaille-James et al. 2006; Kearney et al. 2009; Huey et al. 2012). In the field, we observed a bimodal activity that involve periods of avoidance of hot areas in the middle of the day or reduction in activity on hot days which allow them to remain at or near their preferred body temperature (L. Yuni *pers.obs*.). Shaded habitat substantially reduces exposure to extreme conditions (Scheffers et al. 2014) and thus thermoregulation plays a significant role as a buffer to allow *N. ocellatus* to remain at or near their preferred body temperature during the hot weather. More generally, the ability to adjust the short term and long term temperature variation across seasons and sites via thermoregulatory behaviour will act as an important potential buffer to allow *N. ocellatus* to overcome changes in their environmental conditions according to future predicted global warming.

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Chapter Three

Altitudinal and seasonal variation in the relationship between locomotor performance and temperature in a wide ranging spotted snow skink, *Niveoscincus ocellatus*

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Abstract

Ectotherms may be particularly susceptible to perturbations in the thermal environments because most key physiological processes are sensitive to environmental conditions, including their locomotor performance. In this study, the thermal dependence of locomotor performance (sprint speed and endurance) was studied in three populations of the spotted snow skink, *Niveoscincus ocellatus*, living along an altitudinal gradient in different seasons (spring versus summer). The relationship between the locomotory performance and temperature varied along the altitudinal gradient. The thermal optimum (T_{opt}) for both sprint speed and endurance was lower at the colder high altitude. The breadth of performance (B_{80}) for sprint speed and endurance was wider due to a shift in lower limit (L_{80}) of performance breadth at the high altitude. The variation in T_{opt}, B_{80} and L_{80} of sprint speed and endurance is in line with temperature differences of our three study sites and is likely to be important in allowing extended performance at low temperatures in the cold alpine site. The higher limit (H_{80}) for both sprint speed and endurance, however, was consistent across sites which could be related to the effectiveness of thermoregulatory behaviour to buffer *N. ocellatus* from high environmental temperatures across its range. There was evidence of a seasonal shift in the breadth of performance and L_{80} of endurance although the direction of change was contrary to expectations with a slightly lower L_{80} in summer than in spring. It appears that this mechanism is relatively unimportant in allowing *N. ocellatus* to be active at lower temperatures typically experienced in spring versus summer. Our major result is that T_{opt} and B_{80} (driven by a lower L_{80}) of sprint speed and endurance varied among sites which considered in light of the lack of a strong within site seasonal effect suggests the potential for local adaptation.

Keywords: locomotor performance, temperature, lizards, geographic variation

1. Introduction

Ectotherms have a particularly strong relationship to their thermal environment. The body temperatures of ectotherms are a product of thermoregulation and the constraints imposed by the thermal environment (Hertz et al. 1993; Angilletta 2009). In the absence of thermoregulation, their body temperatures depend on the thermal environment. The body temperatures they are able to maintain will affect their physiological and metabolic processes (Isaksson et al. 2011), developmental and growth rates (de Queiroz and Ashton 2004), and locomotor performance (Bonino et al. 2011; Fernandez et al. 2011; Kubisch et al. 2011). As a result, it has been suggested that ectotherms may be particularly susceptible to perturbations in the thermal environment which have led to dire predictions of extinction from global climate change (e.g., Araujo et al. 2001; Warren et al. 2001; Sinervo et al. 2010; Amarasekare and Savage 2012; Huey et al. 2012; Sunday et al. 2014).

The vulnerability of a species to climate change depends on its sensitivity to environmental change, its exposure to that change, its resilience or ability to recover from perturbations, and its potential to adapt to change (Williams et al. 2008). An organism or population may respond to short term changes in the thermal environment by dispersal to more suitable habitat (Gienapp et al. 2008; Buckley et al. 2013) or by coping with local conditions through adaptation or through phenotypic plasticity (Salamin et al. 2010). Phenotypic plasticity is defined as the ability of one genotype to produce more than one phenotype when exposed to different environmental conditions so that individuals are able to modify their behaviour (Beldade et al. 2011), morphology (Price 2006), or physiology (Beldade et al. 2011) in response to environmental conditions (Salamin et al. 2010). When a species is exposed to the new environmental condition and its phenotypic response is a good match with the new environment, the species can adjust to the new condition without a change in genotype (Lynch and Gabriel 1987; Ghalambor et al. 2007; Hoffmann and Sgro 2011). Plasticity, however, may diverge between populations of a species due to differences in environmental heterogeneity faced by those populations (e.g., Husby et al. 2010; Vitasse et al. 2013). For example, up-regulation of hemoglobin in *Daphnia magna* only occurred in some clones acclimatized to lower temperatures, but not in those acclimatized to higher temperature (Yampolsky et al. 2014). The local difference in responding the selection can promote evolutionary processes for local adaption (Kawecki and Ebert 2004; Pigliucci et al. 2006; Hoeksema and Forde 2008). The interplay between plastic response and local adaptation with regard to temperature will be key in determining the fate of species and

populations under climate change (Atkins and Travis 2010; Somero 2010; Refsnider and Janzen 2012; Yampolsky et al. 2014).

In ectotherms, there is a substantial variation in thermal sensitivity of physiological traits among populations of species living in different thermal environments (Helmuth et al. 2005; Peck et al. 2009). Such geographic variation in thermal sensitivity of a physiological traits occurs when there is limited opportunity to adjust thermoregulatory behaviour to local environment conditions and as a result body temperatures varies significantly among climatically distinctive habitats (e.g., van Berkum 1986; Moritz et al. 2012). Conversely, reduced (or no) geographic variation in thermal sensitivity of key traits is expected when the animals can effectively adjust their thermoregulatory behaviour to the local environmental conditions so that the geographic variation in their body temperatures across sites is minimal (e.g., Huang and Tu 2009; Munoz et al. 2014).

In addition to species coping with geographic temperature variation, individuals within populations (rather than among populations) also need to cope with seasonal temperature variation (Janzen 1967; Ghalambor et al. 2006). The importance of seasonal changes in temperature in shaping the thermal sensitivity of organism to different climatic conditions was emphasized by Janzen (1967). He pointed out that a species living in an environment with a relatively uniform temperature regime will be more acclimated and evolutionarily adapted to a narrower (absolute) range of temperatures than one which lives in an environment with highly fluctuating seasonal temperatures (such as temperate regions). Since that work, many studies have been conducted to examine the role of seasonal temperature variation in setting limits on thermal tolerance of physiology and distribution (e.g., Ghalambor et al. 2006; Archibald et al. 2013; Sheldon and Tewksbury 2014). In general, thermal tolerance (e.g., critical thermal maximum – critical thermal minimum) increases with the greater seasonality (Archibald et al. 2013; Sheldon and Tewksbury 2014) and this is hypothesized to be important in allowing species to occur over a wide geographic range. Animals with a wider thermal tolerance than those from a relatively uniform temperature regime also tend to evolve a broader plasticity to optimize their performance in fluctuating seasonal temperatures (Seebacher et al. 2004; Overgaard et al. 2011).

In this study, the thermal dependence of locomotor performance (sprint speed and endurance) in populations of a wide ranging ectothermic species, with particular focus on thermal optimum (T_{opt}) and performance breadth (B_{80}) was investigated. Locomotor performance attributes such as sprint speed and endurance integrate a variety of organismal

traits at physiological, morphological, and ecological levels which influence the fitness (including survival) of an organism (Miles 1994, 2004; Ghalambor et al. 2003; Le Gallard et al. 2004). Sprint speed and endurance are important to capture prey and escape from predators (Van Damme et al. 1989; Husak 2006). In ectotherms, the relationship between body temperature (T_{body}) and locomotor performance is reflected by a thermal performance curve (Fernandez et al. 2011; Huey et al. 2012). Locomotor performance is constrained at low body temperature, then it increases until a maximum is reached at or near the optimal body temperature range, before decreasing precipitously at a higher body temperature (Huey and Stevenson 1979; Hertz et al. 1983). Within a thermal performance curve, thermal optimum (T_{opt}) and performance breadth (B_{80}) are the physiological parameters which are ecologically related to the animal's thermal niche (Hertz et al. 1983). The T_{opt} is the T_{body} at which performance is maximal (Hertz et al. 1983; Amarasekare and Savage 2012), and the B_{80} is the range of T_{body} at which performance is greater than or equal to 80% of the maximal performance (Hertz et al. 1983; Schulte et al. 2011).

Recently, measurements of T_{opt} and B_{80} have been used to predict how an organism will be affected by a warming climate (e.g., Deutsch et al. 2008; Angert et al. 2011; Sunday et al. 2014). Populations with T_{opt} close to the mean maximum daily air temperature in their current range are predicted to be more negatively affected by the predicted future warming (Huey et al. 2009) because increases in environmental temperatures would push their T_{body} outside the optimal range. However, a species with a well-developed capacity for behavioural thermoregulation living in a thermally heterogeneous environment can achieve T_{body} near T_{opt} for an extended period of warming before reaching the limits of behavioural buffering (Huey et al. 2012; Kearney et al. 2009). Furthermore, a narrow B_{80} might decrease fitness (and in extreme cases may lead the population to extinction) because small changes in environmental temperature will mean that performance falls outside 80% of the optimal range (Huey and Kingsolver 1993; Angilletta et al. 2002). Individuals (and therefore population) can alter their performance curve by shifting T_{opt} or by modifying B_{80} in response to the change in temperature (reviewed in Angilletta 2009). Geographic variation in locomotor performance breadth (B_{80}) among populations of ectotherms generally occurs more at the lower limit of body temperature tolerance range than at the higher limit (e.g., Addo-Bediako et al. 2000; Bonino et al. 2011; Munoz et al. 2014). This may be explained by selection for variation in this parameter either via local adaptation or plasticity (Salamin et al. 2010; Moritz et al. 2012) because it is essential for ectotherms living in cold areas to be able to adjust their performance

at low temperatures in order to facilitate their normal activity (i.e., Addo-Bediako et al. 2000; Bonino et al. 2011), otherwise, they may be vulnerable to predation (Crowley 1985).

In this present study, the thermal dependence of sprint speed and endurance in three populations of the scincid lizard, *Niveoscincus ocellatus*, living along an altitudinal (and thus climatic) gradient was investigated in different seasons (spring versus summer). Since the climate at the high altitude site is colder and is more variable than that at the low and mid altitude sites (see Pen et al. 2010; Figure 1), lizards at the high altitude were predicted to have greater tolerance of performance toward low temperatures (i.e., maintain higher performance measures at lower temperatures) than their counterparts at the lower altitudes. Further, we also predicted that the more variable climate at the high altitude might induce variation in locomotor performance between seasons in this population.

2. Materials and Methods

Study species

Niveoscincus ocellatus is a small lizard (4-7 g, 55-75 mm snout vent length) that occurs over a wide geographic and climatic range in Tasmania (Wapstra et al. 1999; Uller et al. 2011; Yuni et al. 2015) and it thus offers an excellent opportunity to study population level responses to environmental variation (e.g., Cadby et al. 2010; Pen et al. 2010; Uller et al. 2011). Across its range, *N. ocellatus* displays distinct life history variation (Wapstra and Swain 2001; Wapstra et al. 2001) which reflects a combination of local adaptation and plastic responses to climate variation (Melville and Swain 2000; Cadby et al. 2010, 2011, 2014; Uller et al. 2011). The effect of climate on life history traits has created divergent selection pressure on basking behaviour in this species: for example, gravid females at high altitudes are more opportunistic in their basking behaviour than those from low altitudes (Uller et al. 2011; Cadby et al. 2014). The opportunistic basking behaviour evolved, in part, to counteract the poor thermal conditions at the high altitude because low body temperatures affect a range of fitness-related traits including offspring traits (notably birth date) and locomotor performance (Cadby et al. 2010; Wapstra et al. 2010; Uller et al. 2011).

Description of study sites

The study was conducted using three populations along the full climatic (and altitudinal) gradient occupied by *N. ocellatus* (warm low altitude: Orford 147°44'E; 42°33'S; 50 m asl, mid altitude: Oatlands 147°45'E; 42°12'S; 550 m asl, and cold high altitude: Central Plateau 146°45'E; 41°55'S; 1050 m asl) in Tasmania, Australia. The low altitude site experiences a warm coastal temperature with a mild winter, while the high altitude site is much cooler where snow or hail can fall in any month (www.bom.gov.au) and the mid altitude site is intermediate between the these extremes. The lowland site was separated from the mid altitude site by 100 km and the mid altitude site was separated from the highland site by 105 km. Monthly mean maximum air temperature (2001 – 2010) at the nearest weather station to each study site from the Bureau of Meteorology Australia is displayed in Figure 1.

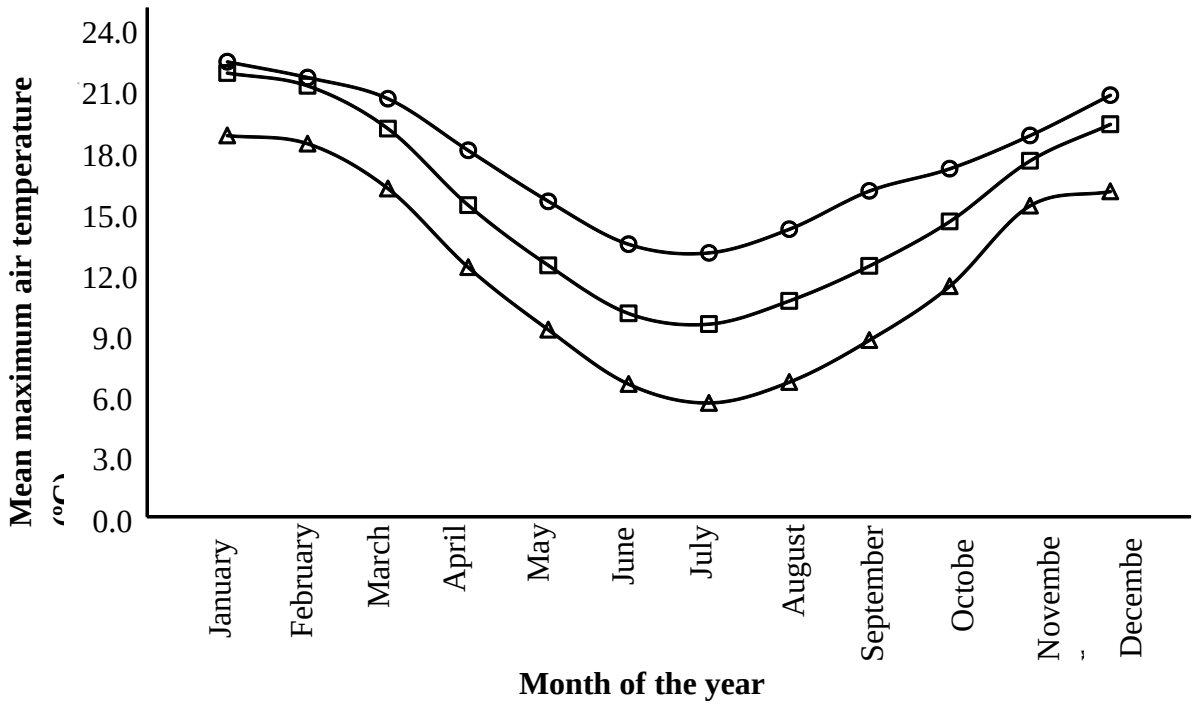

Figure 1. Mean maximum monthly air temperature recorded at the nearest weather station from each study site from 2001 to 2010.

High altitude (CP) △ ; Mid altitude (Oatlands) ▭; Low altitude (Orford) ⬭

Animal collection and husbandry

Male *N. ocellatus* with intact or fully regenerated tails were caught in austral Spring (September) 2010, and Summer (December) 2010 at three study sites by 'fishing' with a mealworm tied to a piece of cotton suspended from a rod. Only males were used in this study because reproduction affects locomotor performance in females of this species (Wapstra and O'Reilly 2001). They were transported from the field site to the Herpetology Laboratory at the School of Biological Sciences at the University of Tasmania on the afternoon of their capture. Lizards were weighed to the nearest 0.001 g using an electronic balance (Sartorius TE 153S) and snout vent length (SVL) was measured to the nearest 1 mm using a plastic ruler. For identification purposes, each lizard was marked on its dorsal surface with a unique number, using a non-toxic silver pen.

The lizards were housed individually in plastic cages measuring 30 x 20 x 10 cm. Each cage contained paper towel as substrate, a piece of wood (10 x 8 x 2 cm) and two terracotta saucers as basking surface and/or as a hiding place. Water was available *ad libitum*. Each lizard was fed two mealworms (tenebrionid larvae) dusted with multi-vitamins (Aristopet repti-vite) and baby food (Heinz, fruity pears or apples) mixed with calcium (Aristopet repti-cal), three times a week. Lizards were kept under a light and temperature regime that resembles their natural environment. Photo period was established by overhead lights (fluorescent tubes) which were set for 10 Light: 14 Dark during spring, and 14 Light: 10 Dark during summer. A 25-watt spotlight providing a basking opportunity was situated above each cage. The availability of the spotlights matched the approximate thermal opportunities available at each of the sites (e.g., Uller et al. 2011; Cadby et al. 2014).

Lizards were housed for two weeks to allow acclimation to captive conditions (Beck and Lowe 1994) before their performance was assessed in order to provide acclimation so the lizards all had similar conditions prior to test. Additionally, this was also to anticipate if the sudden thermal change that the animals experienced (i.e., new thermal condition after their arrival at the laboratory) would affect their performance. This was because there was a strong geographic variation in rapid physiological response (i.e., heat-hardening capacity increase up to 2.6 °C in the upper thermal limit) to sudden temperature change in locomotor function of lizard species in which, within a species, lizards from more predictable and more seasonal thermal environments exhibited greater hardening compared with those from more stochastic and less seasonal habitats (Phillips et al. 2015). Lizards were not fed in the 24 h period prior to any measurement of locomotor performance

because the presence of food can affect performance in reptiles (Melville and Swain 2000; Kelehear and Webb 2006). Locomotor performance tests were conducted at 21 ºC, 23 ºC, 26 ºC, 29 ºC, 31 ºC, 33 ºC and 35 ºC in randomized order, with at least 48 hours recovery period between each test. These temperatures were chosen as they represent the range of the active T_{body} observed at their natural habitat (Yuni, *unpublished data*).

Sprint speed

Sprint speed was tested on a 1.5 m 'race track' (150 x 6 x 10 cm) with a metal bottom, lined with fine sandpaper to allow traction. The track was divided into 20 cm intervals (6 intervals) by a pair of light beams, attached to a digital timer which displays the sprint time (to 0.01 seconds) at each interval. Prior to each sprint test, each lizard was placed in a sealed metal container immersed in a water bath for at least 30 min, by which time the T_{body} had equilibrated to the desired test temperature ± 0.3^{o}C. This was confirmed by inserting a thermocouple probe (K-type, 0.5 mm diameter), connected to a digital micro-processor thermometer (HH 21, OMEGA Engineering, Stamford, CT, USA), 5 mm into the lizard's cloaca. Each lizard was encouraged to run along the race track by gently tapping the tail from behind with an artist's paintbrush. Each lizard was encouraged to run twice without any rest in between trials (Chapple and Swain 2002). The fastest of the two runs over a 20 cm interval was chosen as the maximum sprint speed and it was converted into speed (ms^{-1}) (Melville and Swain 2000; Wapstra and O'Reilly 2001). This distance is representative of a typical distance over which animals would escape predation in the field. Any lizard that refused to run was excluded from the analysis.

Endurance capacity

Endurance capacity was measured by running each lizard on a 50 x 20 cm treadmill fitted with a neoprene belt set at a constant speed of 10 cm s^{-1} (Chapple and Swain 2002). As with the sprint tests, each lizard was first heated to the desired body temperature. A ceramic heat lamp (60 W) was positioned above the treadmill. The height of the bulb was adjusted to maintain the lizard's T_{body} throughout the trial using a mercury thermometer placed near the treadmill surface to ensure the desired temperature was obtained (± 0.3 ºC). Each lizard was then encouraged to run on the treadmill by gently tapping the tail from behind with an artist's paintbrush. The trial was terminated when the lizard stopped and

refused to move after several gentle taps to its tail, and the total time was recorded. Due to the potential impact of running on fatigue, each lizard was tested only once (Perry et al. 2004). Lizards that refused to run were excluded from the analysis.

Data analysis

To characterize the thermal sensitivity of the locomotor performance, a curve was fitted to the data for each lizard and the T_{opt} and the B_{80} were determined from the fitted curves (Hertz et al. 1983; Angilletta et al. 2002). The thermal optimum (T_{opt}) was the temperature at which the maximum performance was achieved (Huey and Stevenson, 1979; Angilletta et al. 2002). The thermal performance breadth (B_{80}) was determined as the range of temperatures over which the lizard performs at 80 % of its best performance (Hertz et al. 1983; Moritz et al. 2012). The lower (L_{80}) bound of B_{80} was the temperature at the lower end of the B_{80}, whereas the upper (H_{80}) was the temperature at the upper end of the B_{80} (Hertz et al. 1983; Moritz et al. 2012). All further statistical analyses were conducted in SAS 9.2. with significance, α, set at 0.05. Prior to the analysis, data were checked for normality using the normal quantile plot. All data were normally distributed, except for the T_{opt} of the endurance data which was log-transformed to achieve normality. A two-way ANOVA was used to determine whether the B_{80}, L_{80}, H_{80} and the T_{opt} varied among sites and seasons. If there was a significant difference in the interaction or main effect, a post hoc (Tukey-Kramer) test was used to isolate the differences. One might expect that since we had 3 populations and 3 altitudes, the differences we found in this study could be because of population effects rather than altitude. One way to address this was the possibility to replicate the three sites, which is beyond the scope of this study. Given we know that temperature drives many processes in ectotherms (rather than altitude per se), it is more parsimonious to accept that the population differences (see later) are in fact likely driven by temperature.

3. Results

Thermal performance curves for sprint speed and endurance of *N. ocellatus* are presented in Figure 2. The shape of the performance curve was similar between the low and mid altitude populations for both sprint speed and endurance. However, the shape of the performance curve at the low and mid altitude population was different to that from the high altitude. The high altitude population had a broader performance breadth (B_{80}) than those from the lower altitudes which was due to the shift in the lower temperature at the lower bound (L_{80}) of the B_{80}. The upper bound (H_{80}) of B_{80}, however, did not vary among populations. The temperature at which the performance was at its maximum level, i.e., the optimal temperature (T_{opt}), was lower at the high altitude than that of at the lower altitudes.

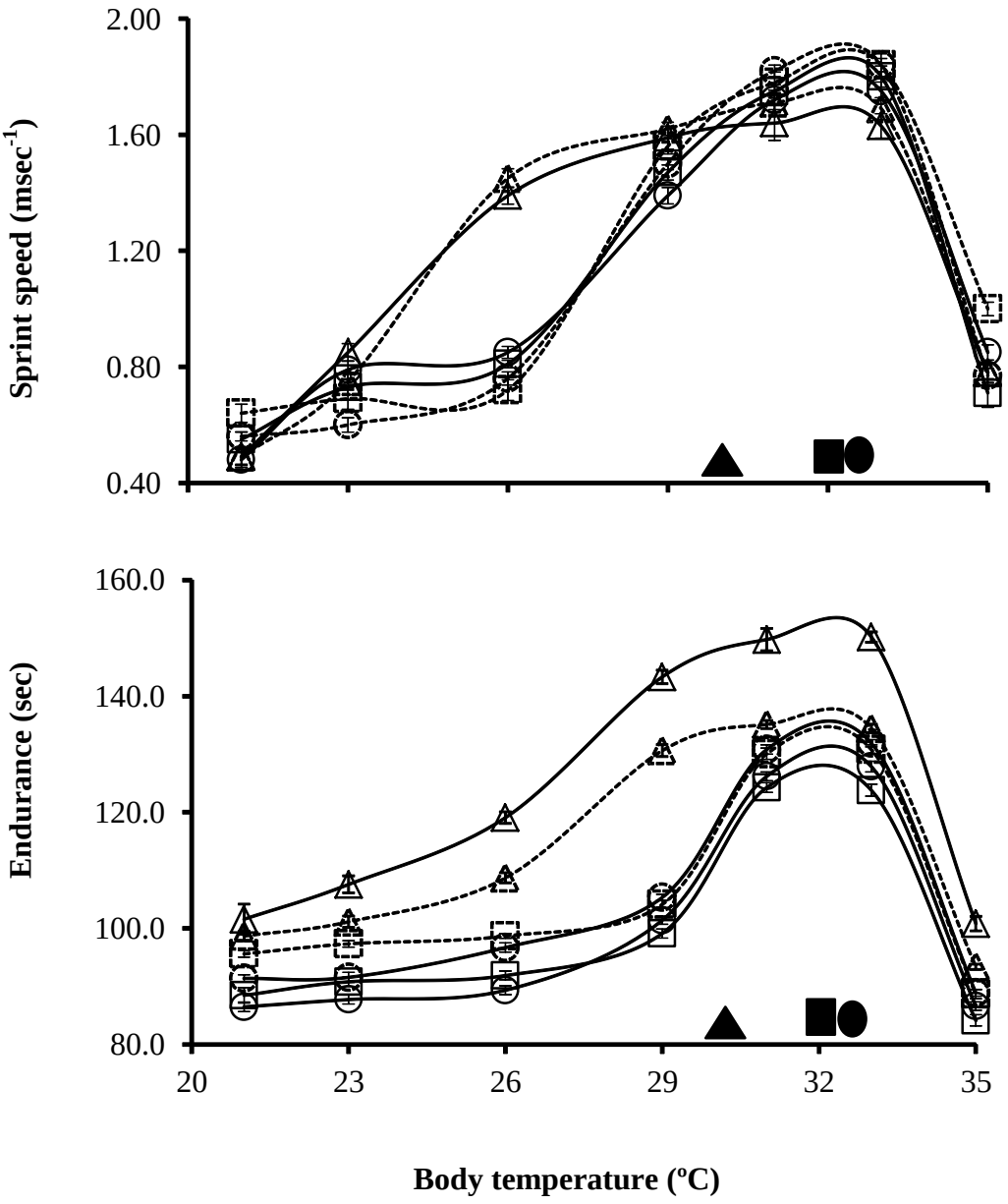

Figure 2. The thermal performance curve of the spotted snow skink, *Niveoscincus ocellatus,* along an altitudinal gradient in Tasmania. Data are presented as the mean (± S.E.) of maximum sprint speed and endurance at each temperature. Low altitude ◯ ; Mid-altitude □ ; High altitude △ ; Spring ——— ; Summer ------- Black triangle, black square and black circle are the mean active body temperature of the spotted snow skink measured at the high, mid, and low altitude sites, respectively (Yuni, *unpublished data*).

Thermal optimum

There was no interaction among sites and seasons for T_{opt} for sprint speed ($F_{2,86} = 0.20$, $P = 0.8192$), and there was no significant difference in T_{opt} between seasons ($F_{1,86} = 0.95$, $P = 0.3327$). However, there was a significant difference between sites ($F_{2,86} = 6.90$, $P = 0.0017$) with the high altitude population having a lower T_{opt} compared with the low and mid altitude populations (Figure 3a).

For endurance, there was no interaction among sites and seasons ($F_{2,87} = 1.05$, $P = 0.3556$) and no significant difference in T_{opt} between seasons ($F_{1,87} = 0.29$, $P = 0.5913$). However, there was a significant difference between sites ($F_{2,87} = 107.12$, $P < 0.0001$) which was due to a lower T_{opt} for the high altitude population compared with the low and mid altitude populations (Figure 3b).

Performance breadth, upper bound, and lower bound of performance breadth

There was no interaction among sites and seasons ($F_{2,86} = 0.34$, $P = 0.7117$) or seasonal differences ($F_{1,86} = 2.77$, $P = 0.0999$) in B_{80} for sprint speed. However, B_{80} for sprint speed differed significantly ($F_{2,86} = 220.24$, $P < 0.0001$) among sites. This difference was due to a lower value for the lower bound (L_{80}) of B_{80} at the high altitude site compared to both the low and mid altitude sites (Figure 4). There was no interaction among sites and seasons for the B_{80} for endurance ($F_{2,87} = 0.41$, $P = 0.6679$); however, B_{80} for endurance differed among sites ($F_{2,87} = 6105.72$, $P < 0.0001$) and seasons ($F_{1,87} = 6.30$, $P = 0.0139$). As with sprint speed, the site difference was explained by a broader B_{80} at the high altitude sites compared to both the low and mid altitude sites (Figure 4). The difference between seasons was due to a broader B_{80} in summer compared to that in spring (Figure 4).

For the upper bound of B_{80} (H_{80}) for sprint speed, there was no interaction among sites and seasons ($F_{2,86} = 0.32$, $P = 0.7260$) and no effect of site ($F_{2,86} = 0.23$, $P = 0.7964$) or season ($F_{1,86} = 1.52$, $P = 0.2215$). A similar pattern was seen for H_{80} for endurance: there was no interaction among sites and season ($F_{2,87} = 0.61$, $P =0.5472$) and no site effect ($F_{2,87} = 0.52$, $P = 0.5937$) or season effect ($F_{1,87} = 0.20$, $P = 0.6575$) (Figure 5a and 5b).

The lower bound of B_{80} (L_{80}) for sprint speed showed no interaction among sites and seasons ($F_{2,86} = 0.41$, $P = 0.6633$) and there was no difference between seasons ($F_{1,86} = 1.72$, $P = 0.1926$). There was, however, a significant difference in L_{80} between sites for sprint speed ($F_{2,86} = 241.23$, $P < 0.0001$). The difference resulted from a lower L_{80} at the

high altitude population compared to the low and mid populations (figure 5c). For endurance, there was no interaction among sites and seasons ($F_{2,87} = 0.11$, $P = 0.8963$), however, L_{80} differed among sites ($F_{2,87} = 6309.50$, $P < 0.0001$) and between seasons ($F_{1,87} = 5.49$, $P = 0.0214$). The difference among sites was due to a lower L_{80} at the high altitude site compared with low altitude site ($t_{2,87} = 13.59$, $p < .0001$ and $t_{2,87} = 13.27$, $p < .0001$ in spring and summer, respectively) and mid altitude site ($t_{2,87} = 13.96$, $p < .0001$ and $t_{2,87} = 12.90$, $p < .0001$ in spring and summer, respectively) (Figure 5d). The difference between seasons was due to a lower L_{80} in summer than in spring (Figure 5d).

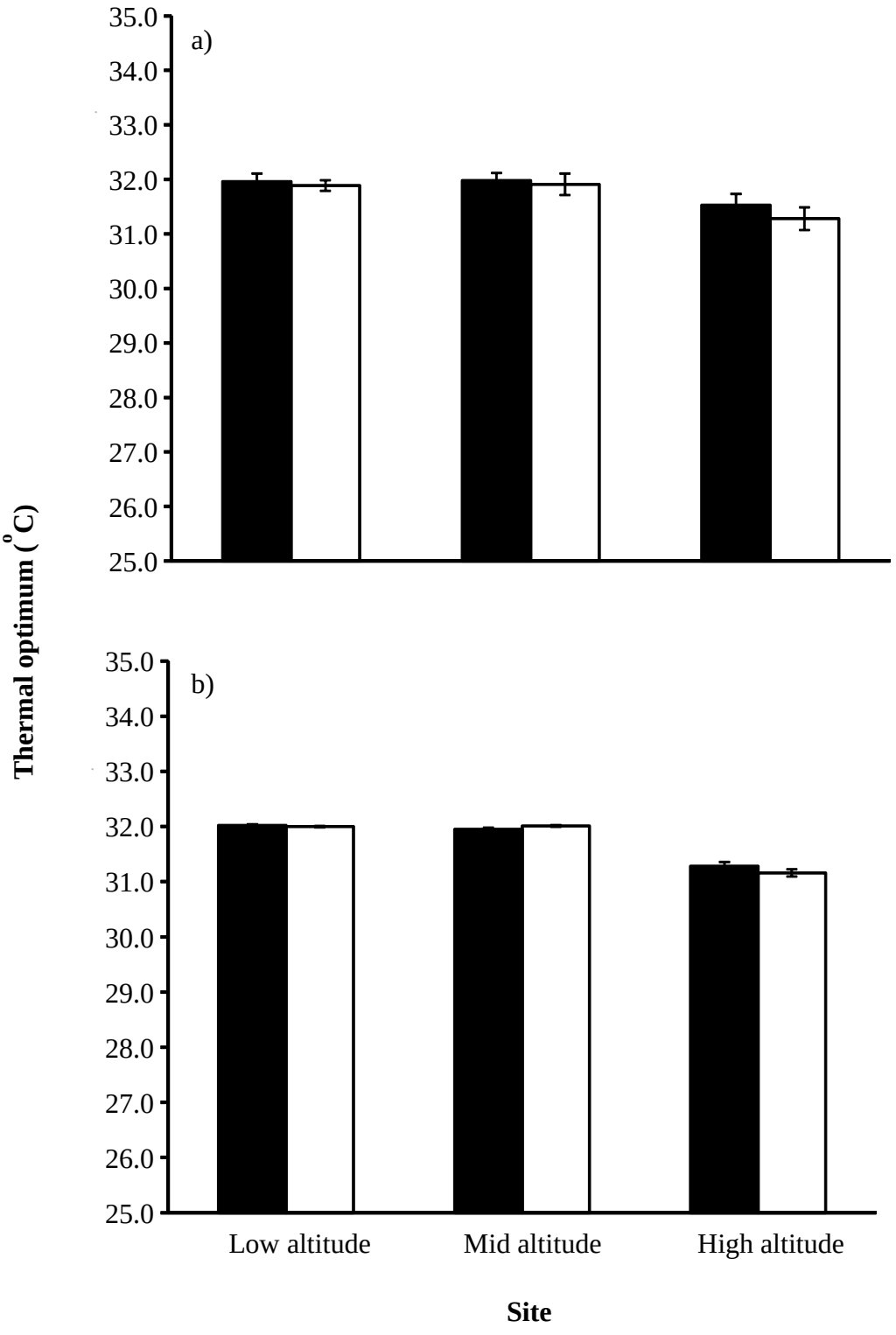

Figure 3. Thermal optimum (T_{opt}) in locomotor performance of the spotted snow skink, *Niveoscincus ocellatus*, living along an altitudinal gradient in Tasmania. Data presented as mean ± S.E. Spring ■ ; Summer □ ; a) is sprint speed; b) is endurance

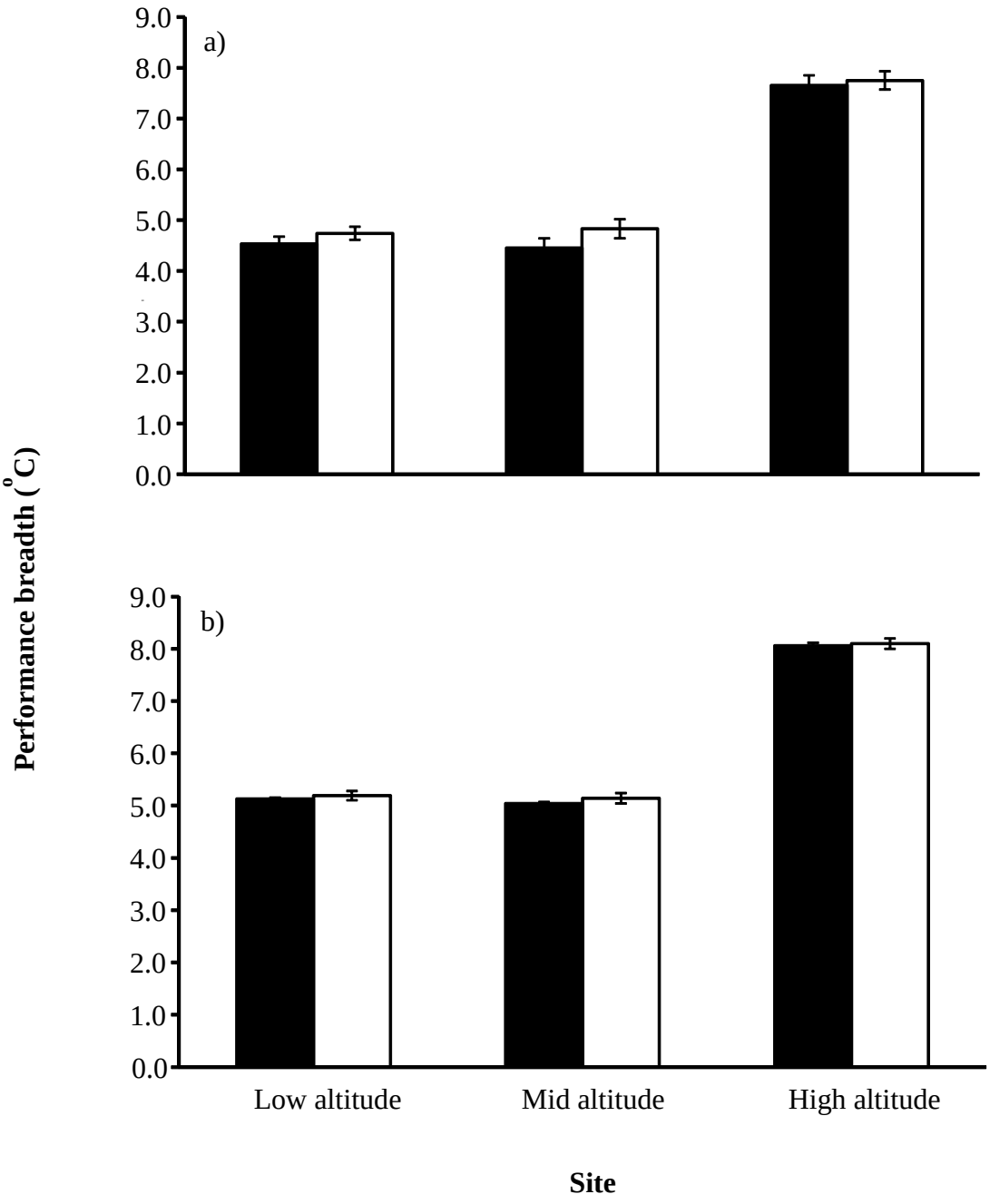

Figure 4. The performance breadth (B_{80}) in locomotor performance of the spotted snow skink, *Niveoscincus ocellatus*, living along an altitudinal gradient in Tasmania. Data presented as mean ± S.E. Spring ■ ; Summer □ ; a) is sprint speed; b) is endurance.

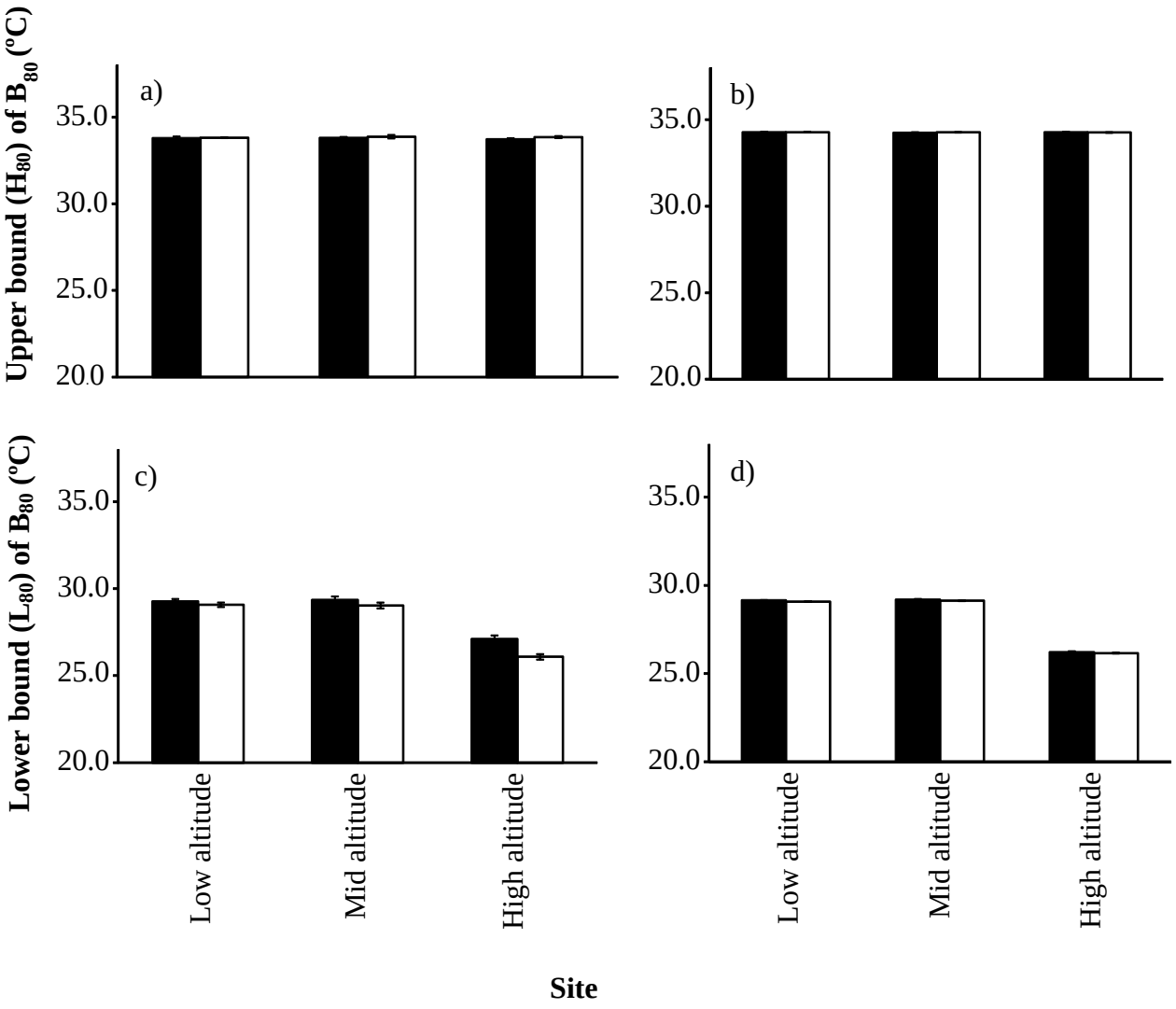

Figure 5. The upper bound (H_{80}) and the lower bound (L_{80}) of the performance breadth (B_{80}) of the sprint speed and the endurance of three populations of the spotted snow skink, *Niveoscincus ocellatus,* living along an altitudinal gradient in Tasmania. Data presented are mean ± S.E. Spring ■ ; Summer □ ; a) is higher limit of B_{80} of sprint speed; b) is higher limit of B_{80} of endurance; c) is lower limit of B_{80} of sprint speed; d) is lower limit of B_{80} of endurance.

4. Discussion

In the spotted snow skink, *Niveoscincus ocellatus*, as in most reptiles, locomotory performance (sprint speed and endurance) is strongly temperature-dependent. Importantly, the relationship between locomotory performance and temperature varied along an altitudinal gradient. The T_{opt} of both sprint speed and endurance was lower for the high altitude population compared to T_{opt} for individuals from the low and mid altitudes. The high altitude population also had a wider B_{80} for both sprint speed and endurance than the low and mid altitude populations due to differences in $L_{80.}$ The H_{80}, however, remained consistent among populations. Evidence for a seasonal shift was also found in L_{80} of endurance in this species. While the differences between sites in T_{opt} and B_{80} for sprint speed and endurance could be due to either local adaptation or developmental plasticity (see below), the seasonal shift demonstrates potential for within individual plasticity to current environmental conditions.

The findings in this study are in agreement with previous studies that geographic variation in temperature dependent processes occur more at the lower limit of temperature tolerance range than at the higher thermal limit (e.g., Addo-Bediako et al. 2000; Salamin et al. 2010; Bonino et al. 2011; Moritz et al. 2012; Hoffmann et al. 2013; Munoz et al. 2014). As with other species (e.g., Clusella-Trullas et al. 2011), the variation in T_{opt}, B_{80} and L_{80} of sprint speed and endurance is in line with temperature differences of our three study localities and is likely to be important in allowing extended performance at low temperatures in the cold alpine site. It is crucial for *N. ocellatus* to have a capacity to sprint at low temperatures because sprint speed is essential to escape from predators (Chapple and Swain 2002; Melville and Swain 2003), especially when they emerge they will always be cool and will be vulnerable to predators. Maintaining an enhanced endurance at low temperatures is also important for *N. ocellatus* at the high altitude since endurance is important for mate searching (Robson and Miles 2000) and because they have an active foraging strategy (Wapstra and Swain 1996).

While T_{opt} and B_{80}/L_{80} varied geographically for both sprint speed and endurance, H_{80} for these two performance traits did not vary. The lack of geographic variation in H_{80} may be explained by the effectiveness of thermoregulatory behaviour to buffer *N. ocellatus* from high environmental temperatures across its range (e.g., Huang and Tu 2008; Sunday et al. 2011, 2014; Grigg and Buckley 2013; Munoz et al. 2013). In many lizards, activity is bimodal with periods of inactivity in the heat of the middle of the day or reduction in

activity on hot days (Winne and Keck 2004; Nicholson et al. 2005; Vidal et al. 2010). *Nivesoscincus ocellatus* follows this pattern with distinct bimodal activity patterns avoiding the heat in the middle of the day (e.g., shade under the canopy or in small gaps between boulders wall) after initial periods of basking, which allow them to remain at or near their T_{opt} for locomotor performance or other functions and avoiding temperatures near the maximal limit.

There are two potential explanations for the geographic variation in the relationship between temperature and locomotor performance traits in *N. ocellatus*; it could reflect a plastic response to the different thermal environment of the populations or it could reflect local adaptation to their local environment. There is evidence from this and related studies that both potential explanations are relevant. Seasonal effects on the thermal dependence of performance within each site was specifically examined in this study because evidence of a seasonal shift would suggest the potential for plastic responses to contemporary thermal conditions and would provide evidence that such effects might contribute to between site differences. Plasticity in responses to changes in the thermal environment is well established in *Niveoscincus ocellatus* in both the laboratory and in the field (e.g., Wapstra et al. 2004, 2009, 2010; Pen et al. 2010; Uller et al. 2011; Cadby et al. 2010, 2014). Such plasticity has been suggested as an important mechanism to cope with both seasonal and geographic variation in thermal environment (Wapstra et al. 1999; Melville and Swain 2000; Wapstra and Swain 2001; Wapstra et al. 2009; Cadby et al 2010, 2014; Pen et al. 2010; Uller et al. 2011). However, plasticity does not preclude local adaptation. Female *N. ocellatus* express geographical differences in basking behaviour, with females from the cold alpine regions being more plastic in their basking behaviour than those from the warmer region (Uller et al. 2010; Cadby et al. 2014). In this study, we found only limited evidence for plasticity as evidenced by only a small seasonal effect in L_{80} of endurance (which also resulted in an effect on B_{80}) and the direction of change was contrary to expectations with a slightly lower L_{80} in summer than in spring. Typically such seasonal effects are explained by a downward shift in physiological performance to lower temperature during the colder season (e.g., Seebacher et al. 2003). These effects can be an important mechanism to maximize performance and activity in colder seasons. It appears that this mechanism is relatively unimportant in allowing *N. ocellatus* to be active at lower temperatures typically experienced in spring versus summer. However, it remains possible that such effects are more pronounced than we observed very early in the season post

hibernation or very late in the season just prior to hibernation when temperatures are at their most extreme. Our major result is that T_{opt} and B_{80} (driven by a lower L_{80}) of sprint speed varied among sites which considered in light of the lack of a strong seasonal within site suggests local adaptation. Recent phylogeographic work in this species revealed strong evidence of strong population affinities (i.e., evidence of restricted gene flow between isolated populations) within a larger widespread clade from which out three populations were chosen (Cliff et al. 2015) further supporting the potential for local adaptation (see also Cadby et al. 2014).

The B_{80} and the T_{opt} have been used to predict how a species will be affected by a warming climate (e.g., Deutsch et al. 2008; Kearney et al. 2008; Angert et al. 2011; Sunday et al. 2014). Organisms with a wider B_{80} are predicted to be more tolerant of changing climate than those with narrower B_{80} (Huey and Kingsolver 1993; Deutsch et al. 2008; Pörtner and Farrell 2008). The position of the higher thermal limit (e.g., H_{80}) toward environmental temperature can also be used to indicate how the terrestrial ectotherms may be affected by the rising global temperature (Hoffmann et al. 2013). Organisms that currently live in a habitat where the mean maximum daily temperature is close to their T_{opt} (such as at low latitude or in the tropics) have a small thermal safety margin and are predicted to be vulnerable to climate warming (Deutsch et al. 2008; Huey et al. 2009). In this study, the position of the higher thermal limit (H_{80}) where locomotor performance drops is well in excess of the typical shade temperatures observed at all sites. Specifically, the higher limit of performance breadth (H_{80}) for locomotor performance of *N. ocellatus* was 34 ± 0.3°C at all three sites, whereas the highest mean environmental temperature recorded (as in Figure 1) was 22.3 ºC, 21.8 °C, and 18.7 ºC at the low, mid, and high altitude sites, respectively. The difference between the higher thermal maximum of an ectotherm and the current climatological temperature of its habitat can indicate the average amount of environmental warming that an ectotherm can tolerate (i.e., warming tolerance) (Deutsch et al. 2008). Moreover, all *N. ocellatus* populations in this study live in habitats that are considerably cooler than their T_{opt} which provides this species with a wide safety margin. Thermal safety margin measures the difference between an organism's thermal optimum and its current climate temperature (Deutsch et al. 2008). For *N. ocellatus*, the distance of their current habitat temperatures (as in Figure 1) was 9.59 – 12.83 °C below their thermal optimum. Our results thus support the idea that species living in a temperate climate, including *N. ocellatus*, have the benefit of having a wider thermal safety than

those living in the tropics or at low latitudes (Tewksbury et al. 2008; Asbury and Angilletta 2010; see also a similar argument by Caldwell et al. 2015 for CT_{min} and CT_{max} for *N. ocellatus*).

Given the T_{opt} of locomotor performance in this species is located below the mean daily maximum temperatures across their range, modest warming that induces increases in their T_{body} may enhance their fitness as they can achieve T_{body} near their T_{opt} for an extended period of warming before reaching the limit of behavioural buffering (Huey et al. 2012). Modest warming will also benefit the high altitude population that has wider B_{80} due to variation in L_{80} because extensive basking (with its associated costs of predation) may not be required to reach body temperatures within their $B_{80.}$ Moreover, a warming climate will potentially extend their activity into the cooler months (Kearney et al. 2009), enabling high altitude populations of *N. ocellatus* to be active for a longer period than at present. Longer activity period near their thermal optimum will optimise basic physiological processes such as digestion, foraging and performance in this population, without the demand to increase the energetic cost of maintenance as at present (Yuni et al. 2015). This finding is in contrast with Sinervo et al. (2010) prediction that the warming climate will restrict the activity time of lizards, which most likely will undermine the growth rates and raise the extinction risk. Furthermore, the extended growing season at high altitudes may also affect life history traits, potentially including age and size at maturity with concomitant effects on reproductive output (see Wapstra et al. 2001; Wapstra and Swain 2001).

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Chapter Four

Energy expenditure of the spotted snow skink, *Niveoscincus ocellatus,* at two climatic extremes of its distribution range

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ABSTRACT

The study of energy expenditure between populations of a wide ranging ectothermic species may provide an insight into how organisms respond to variation in environmental conditions. In this study, the energy expenditure of male spotted snow skinks, *Niveoscincus ocellatus,* living at the two extremes of the species' distribution range (warm lowland versus cold alpine site) was measured using the doubly labelled water method. Males at the cold alpine site expended more energy per gram per hour compared to their counterparts living at the warm lowland site. Lizards living at high altitude were active at lower temperatures compared with those at the low altitude site, which resulted in a longer activity time for the highland population. However, the differences in energy expenditure cannot be explained only by these differences in activity time. We further suggest that at the cold alpine site, lizards compensated for the low temperatures by elevating their metabolism which subsequently increased their energy expenditure. An elevated metabolic rate combined with modified thermoregulatory behaviour is likely an important mechanism allowing *N. ocellatus* to cope with the cold environments at high altitude sites.

Keywords: energy expenditure, doubly labelled water, lizards, activity time, metabolism

1. Introduction

Studying energy expenditure and energy allocation by an animal in nature helps us to understand how organisms have adapted to the environmental conditions. Such studies can also provide insights into how species respond to temperature differences between different latitudes or altitudes (Porter 1989; Angilletta 2001a). This is especially important for ectotherms in which life processes, including metabolism (DeVries et al. 2013), are strongly affected by the thermal environment (de Queiroz and Ashton 2004; Fernandez et al. 2011).

An animal's metabolism, often measured as standard or resting (in ectotherms) or basal (in endotherms) metabolic rate, reflects the cost of maintenance of an animal's energy expenditure (Hulbert and Else 2000). Energy expenditure of an animal comprises of several components including those associated with maintenance, growth, activity, and reproduction (Nagy et al. 1984; Pontzer et al. 2010). Among these, maintenance comprises the largest proportion of an animal's energy expenditure (Angilletta 2001b; Clarke 2003). Accordingly, basal metabolic rate is generally related to energy expenditure in endotherms (e.g., Pontzer et al. 2010; Dalton et al. 2014), and standard or resting metabolic rate in ectotherms (e.g., Beaupre and Duvall 1998; Angilletta and Sears 2000) because standard or resting metabolism relates to the energy required for maintenance (Pörtner et al. 2000; Homyack et al. 2010). Energy expenditure (and metabolic rate) of ectotherms is typically related to activity patterns (e.g., Sears 2005; Smith et al. 2008). This is because higher level of activity is typically associated with higher body temperatures: thus, within species, energy expenditure varies with season or geographic variation in altitudes or latitudes (Karasov and Anderson 1984; Patterson and Davies 1989; Grant and Dunham 1990; Clarke 1991, 2003; Hare et al. 2010; Powers and Anderson 2010; Schaefer and Walters 2010). The relationship between energy expenditure and activity patterns is likely to be especially pronounced in ectotherms compared to endotherms because of the stronger relationship between activity patterns and higher body temperatures in ectotherms. In general, ectotherms living in warmer environments have higher resting metabolic rates because the speed of chemical reactions, including aerobic respiration, increases exponentially with temperature (Clarke and Fraser 2004; Martinez del Rio and Karasov 2010). For example, eastern fence lizards, *Sceloporus undulatus*, living in a warmer environment (lower latitude) had higher resting metabolic rates than those living in colder environment (higher latitude) (Angilletta 2001b). Similarly, energy expenditure was higher in a population of the same species that experienced a longer activity time at their preferred temperature than

in a population with a shorter activity time (Angilletta 2001a). In addition, the population with the higher energy expenditure also had a significantly higher metabolizable energy intake than those with the lower energy expenditure (Angilletta 2001a).

However, it may not be as straightforward as previously thought. Ectotherms primarily respond to the the variation in their thermal environment by behaviourally regulating their body temperatures (Seebacher et al. 2004; Kearney et al. 2009; Zamora-Camacho et al. 2013; Sunday et al. 2014), although it may be limited by several factors, e.g., thermal quality of the environment (Scheers and Van Damme 2002; Besson and Cree 2010), predation risk (Gutierrez et al. 2010), and time allocation for activities other than thermoregulatory behaviour (Huey and Slatkin 1976; Sinervo et al. 2010). When thermoregulatory opportunities are limited due to a trade-off between multiple costs and benefits (Huey and Stevenson 1979; Seebacher 2009), ectotherms may shift their physiological and biochemical rates in order to allow an optimum physiological or physical performance (Seebacher 2005; Beldade et al. 2011). Individuals from cooler environments such as high altitudes or latitudes may have adaptations that extend their daily activity (e.g., Van Damme 1989; Addo-Bediako et al. 2000; McConnachie et al. 2009; Bonino et al. 2011), therefore affecting energy expenditure. For example, to be active at low temperatures, ectotherms can shift the body temperatures downward to allow basic physiological processes, such as performance (e.g., sprint speed or endurance) are optimised at low body temperatures (Clarke 1993; Angilletta 2009; Lourdais et al. 2013). A downward shift in lower limit of performance curve and thermal optima in locomotor performance have been documented in many ectotherms species (reviewed in Angilletta 2009). Basic physiological processes at low body temperatures can be achieved by elevating standard or resting metabolic rates at specific temperatures in populations living in cold environments compared to those from warm environments (e.g., Hare et al. 2010; Schaefer and Walters 2010). This is known as metabolic cold adaptation or MCA (Clarke 1991, 2003; Hare et al. 2010; White et al. 2012). Metabolic cold adaptation (MCA) reflects compensation to cold temperatures (Clarke 1991). It has been demonstrated within populations of eurythermal species living in the cold environment especially where the environment is highly variable, but not in stenothermal species due to stable temperatures (Pörtner 2006). MCA occurs via adjustments of mitochondrial density and capacity according to the environmental temperature regime (Pörtner et al. 2000, 2008). For example, an elevation of mitochondrial density causes a rise in energy expenditure due to

the cost of mitochondrial maintenance (Pörtner et al. 2000; White et al. 2012). Elevated standard or resting metabolic rates have been associated with a downward shift of critical temperatures in cold adapted populations of a species (Sommer and Pörtner 2002; Pörtner 2006). Metabolic cold adaptation has been observed in many species, including in reptiles species. One example of evidence of MCA was found in 'cold populations' of several species (*Hoplodactylus maculatus*, *H. chrysosireticus*, and *H. stephensi)* of nocturnal geckos from New Zealand where they had a higher standard and resting metabolic rate than diurnal 'warm' populations (Hare et al. 2010). Similarly, a northern (cold) population of cottonmouth snakes (*Agkistrodon piscivorus leucostoma),* had a higher standard metabolic rate at low temperatures than a southern (warm) population (Zaidan 2003).

Niveoscincus ocellatus are small viviparous lizards (adult body mass 3-12 g) living over a wide geographic and climatic range in Tasmania with concomitant effects on life history traits (Wapstra and Swain 2001; Wapstra et al. 2001; Atkins et al. 2007; Cadby et al. 2010, 2014; Pen et al. 2010; Uller et al. 2011). Individuals are considerably larger in body size at maturity (and larger overall) at the colder extremes (65 mm versus 55 mm) of the species' range (Wapstra et al. 2001) where the active season is reduced to approximately October to April, compared to their counterparts that are active approximately from September to May at the warmer extreme of the species' range (Wapstra et al. 1999). Individuals reach maturity at three years old at the warm lowland sites compared to four to five years old at the cold alpine sites (Wapstra et al. 2001; Pen et al. 2010). Key reproductive events such as mating and parturition occur approximately one month later at cold highland sites compared with lowland warmer sites (Wapstra et al. 1999; Pen et al. 2010; Uller et al. 2011). The effect of climate on life history traits, especially on embryonic development and the associated effects on the offspring traits such as on offspring birth date, size and sex, has created divergent selection pressures on basking behaviour in this species (Wapstra et al. 1999, 2004, 2009; 2010; Pen et al. 2010; Cadby et al. 2014). Divergence in basking behaviour has been demonstrated in a laboratory study where females from high altitude populations thermoregulated more actively (i.e. bask more) than low altitude lizards especially under reduced thermal opportunities (Uller et al. 2011; Cadby et al. 2014) presumably because of past selection to maximise the use of the limited opportunities that occur in the colder environments. *Niveoscincus ocellatus* at the high altitude site are probably active at lower temperatures than those at the lower altitudes: at the high altitude site, opportunistic basking has been under selection in order

to ensure the offspring's optimal development and potentially offspring fitness (Atkins et al. 2007; Uller et al. 2011; Cadby et al. 2014). It is clear that the thermal environment can create selection on basking and presumably other thermoregulatory behaviour, such behaviour that can certainly translate to males if the trait is under selection in females only because it is also likely that her offspring inherit the traits (Cadby et al. 2014). Although *N. ocellatus* has been extensively studied, the physiological flexibility that underpins the observed geographic variation in life history traits has not yet been studied.

In this study, we investigated the energy expenditure of *Niveoscincus ocellatus,* under natural living conditions at the two climatic extremes of its distribution range: a warm lowland site versus a cold alpine site. Both cold alpine and warm lowland sites are located near the upper and lower limit of this species distribution ranges (Jones et al. 1997; Cadby et al. 2010). Accordingly, this species presents ideal opportunity to understand how a wide ranging ectothermic species has adapted to the environmental conditions they live in. The climate at the cold alpine site is colder than at the warm lowland site (Figure 1; see Pen et al. 2010). *Niveoscincus ocellatus* are typical temperate shuttling heliotherms that actively bask in the morning (when weather permits) and then use shade and sun intermittently to maintain temperatures at or near their optimum body temperature. During these periods of activity *N. ocellatus* is an active forager (Wapstra et al. 1996). On warmer days (especially in the warmer months), activity is distinctly bimodal with active basking only in the mornings and later afternoon. Typically, studies have found that ectotherms living in cold environments, such as at high altitudes or latitudes, have more restricted activity times than those living at lower altitudes or latitudes, which is due to more limited thermal opportunity. However, we suggested that it is not as straightforward as this because lizards at the cold alpine site might have physiological adaptations that allow them to cope with cold environments. This resulted in a higher energy expenditure in this population than that at the warm lowland site.

Energy expenditure was estimated using the doubly labelled water (DLW) method which allowed us to calculate the cost of living in the wild (Hulbert and Else 2000). Only male lizards were used in this study because during the spring and summer months female snow skinks are at various stages of pregnancy (Jones et al. 1997; Wapstra et al. 1999) which affects both basking behaviour and energy expenditure in lizards (e.g., Beuchat and Vleck 1990; Schwarzkopf and Shine 1991; DeMarco 1993; Robert and Thompson 2000; Schultz et al. 2008; Dupoue and Lourdais 2014). To determine whether the energy

expenditure was related to activity in this species, we estimated the potential available period of time that the lizards were capable of being active (actively basking or foraging) at each of the study sites.

2. Materials and methods

2.1. Study sites

The warm lowland site is located near Orford (147°44'E; 42°33'S; 50 m asl), and the cold alpine site is located at Miena on the Central Plateau (146°45'E; 41°55'S; 1050 m asl). Monthly mean minimum and maximum air temperature (2008 – 2012) at the nearest weather station to each study site were obtained from the Bureau of Meteorology Australia (Figure 1). The cold alpine site has lower mean minimum (Figure 1a) and maximum (Figure 1b) air temperatures than the warm lowland site. The difference in thermal characteristics between sites has profound effects on daily activity patterns and the length of the activity season of lizards. At the warm lowland site, lizards are active from August to May, with occasional emergence during winter in warmer days. At the cold alpine site, however, their activity is restricted in early spring (September) and during autumn (April-May) (Wapstra and Swain 1996). It is beyond the scope of this study to present more fine-scaled detail on annual activity patterns based on the data obtained in this study because there would be significant annual variation that would be hard to capture and present. Despite the difference in their thermal characteristics, the availability of substrate for basking sites is similar at both study sites. *Niveoscincus ocellatus* is a saxicolous (rock-dwelling) species in which their occurrence is typically associated with rocky outcrops which are used as basking sites (Melville and Swain 2000).

2.1. Estimation of the length of active time

Prior to the doubly-labelled water experiment, the potential active time of *N. ocellatus* was determined by measuring the field active body temperatures of captured male *N. ocellatus* and the corresponding air temperatures at the time of capture over several seasons (Summer 2009/2010, Autumn 2010, Spring 2010, Summer 2010/2011, and Autumn 2011 at each study site; N = 164 and N = 221 body temperature measurements for the warm lowland and cold alpine site, respectively). Body temperature was measured to ± 0.1°C using a thermocouple probe (K-type, 0.5 mm diameter) connected to a digital micro-

processor thermometer (HH 21, OMEGA Engineering, Stamford, CT, USA), which was inserted 5 mm into the lizard's cloaca immediately after capture. The air temperature (in the micro-environment) was measured 1 m above the ground in the shade using an air thermometer (Humidity/temperature pen, ProSciTech E800012). After body temperature was taken, and prior to their release at their site of capture, each lizard was marked on its back with a non- toxic silver pen for a temporary identification as a precaution against repeated measurements on the same individual on several consecutive days.

We then estimated active time during the DLW study using these body and environmental (air) temperatures combined with weather characteristics obtained from the nearest Bureau of Meteorology stations of the sites (rain occurrence, air temperature, time of sunrise and sunset) during the period of the DLW study at each study sites. The ibuttons were previously placed within the study sites to record the air temperature. However, the computer program malfunctioned so the air temperature data from the Bureau of Meteorology weather station were used. The data for the warm lowland site were obtained from Cambridge weather station (45 km from the study site), and the data for the cold alpine site were obtained from the Liawenee weather station (10 km from the study site). These weather stations were chosen because they both represent a close approximation of the weather at our specific field sites and have been used before to model lizard activity and responses to climate (Cadby et al. 2010). There is a Bureau of Meteorology weather station within 7 km of the site at the warm lowland site but it is limited in the data available (maximum and minimum temperature, rain only) and does not have the fine-scale 30 minute resolution. When these two east coast weather data were compared, they were closely matched. Both are on the lower east coast of Tasmania, at a similar altitude and experience the same weather patterns.

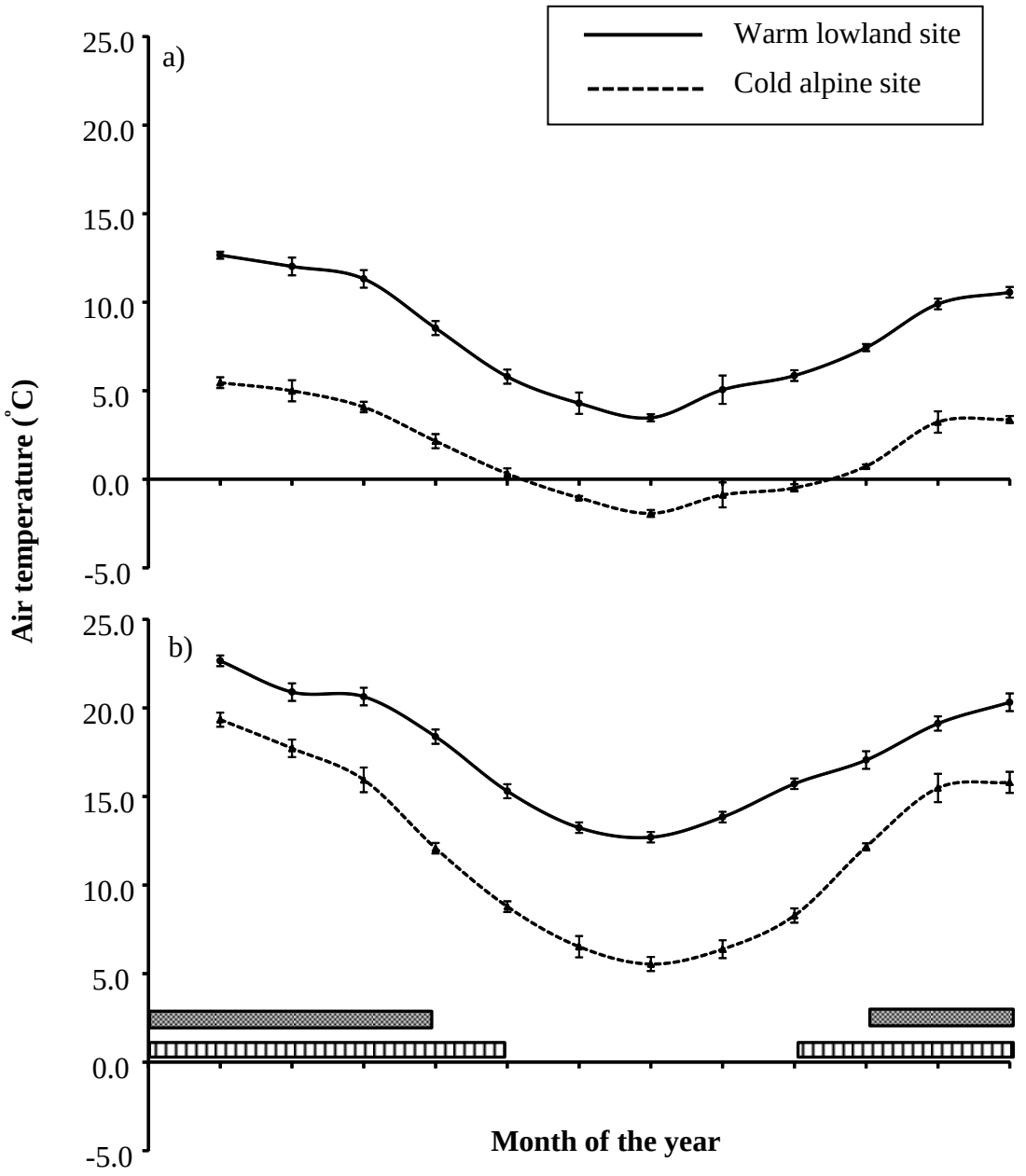

Figure 1. Mean minimum (a) and maximum (b) monthly (± S.E.) air temperature recorded at the nearest weather station from each study site from 2008 to 2012. ▬▬ Cold alpine site; ▬▬ Warm lowland site; ▥ Activity period of lizards at warm lowland site; ▩ Activity period of lizards at cold alpine site.

In order to estimate the length of potential active time available for each population, assumptions were set as follows:

a. The maximum length of active time estimated for each lizard was based on the time the DLW solution was injected into the lizard to the time when the second blood sampling was collected (see below).
b. The maximum period over which the lizards could be active during this time was calculated based on the time of sunrise and sunset during the DLW study. This species usually emerged about 2 hours after sunrise and retreated about 2 hours prior to sunset at both study sites (L. Yuni *pers.obs*); therefore, the maximum active period was set between two hours after sunrise and two hours prior to sunset at both study sites.
c. We also took into account the specific weather experienced during the DLW study to refine our estimate of activity. The lizards were assumed to be active when there was no precipitation during the active period and the air temperature was a minimum of 10.3 $^{\circ}$C for the cold alpine site and 15.2 ºC for the warm lowland site. These minimum air temperatures represent the lowest air temperatures at which lizards were captured from Summer 2009/2010 to Autumn 2011 at each of these sites (see Results). A tolerance range of -0.5 $^{\circ}$C from the minimum air temperature recorded at each study site was added because lizards may have already been active below the minimum air temperature recorded before they were located and caught.

2.2. Measurement of energy expenditure using doubly labelled water

Energy expenditure was estimated using DLW for a period of 7 (± 2) days in Summer 2011/2012. The study was conducted at the warm lowland site from 20 to 29 December 2011, and at the cold alpine site from 14 to 22 January 2012. The approximately one month time difference between the studies took into account the documented differences in the timing of annual activity cycles and reproductive events at the cold alpine site compared with the warm lowland site (Wapstra et al. 1999; Cadby et al. 2010; Uller et al. 2011). All key reproductive events occur approximately one month later at the cold site including vitellogenesis and parturition in females and spermatogenesis in males (see Wapstra et al.1999; Atkins et al. 2007; Uller et al 2011; Cadby et al. 2010, 2014). In males, testes begin to increase in volume in December with a peak in testis size in January in the warm lowland population, with a corresponding increase in January with a peak in February in

the cold alpine population (Wapstra et al. 1999). The DLW method involves injecting the animal with stable isotopes of Hydrogen (^{2}H) and Oxygen (^{18}O) and measuring the decline in activity of these isotopes in blood samples over time (Nagy 1988; Southwood et al. 2006). Prior to the field experiment, a solution of DLW was prepared in the laboratory, such that the lizards could be injected with a solution containing 0.15 µl ^{18}O-water (97 % ^{18}O) per g of lizard body weight and 0.06 µl D_2O (99.9% deuterium) per g of lizard body weight (Metabolic Solutions protocol modified from Speakman 1997). The dosage of the DLW solution was prepared to allow a 7 (± 2) days recapture period in which to obtain the second blood sample: the second blood sample must be taken before the injected isotopes are washed out from the animal's body. A 100 µl sample of the DLW solution was placed into a 2 ml capped glass vial; this solution was kept separate from the bulk solution used for injection. It provided the baseline reading when the blood plasma containing the DLW solution was analysed by Isotope Ratio Mass Spectrometry (IRMS). The DLW solutions were stored at room temperature (20 ºC) away from direct sunlight until used in the field.

For each population, male *N. ocellatus* were collected in the field by fishing with a mealworm tied onto a piece of cotton. Forty-one and forty-six lizards were injected at the warm lowland site and at the cold alpine site respectively. Each lizard was toe-clipped, weighed to the nearest 0.1 g, and a round piece of cloth tesa-tape ® was stuck on its back to assist recognition from a distance. A piece of marking tape with the lizard's number on it was stuck to the area of substrate closest to the site of capture to assist in the re-location of individuals at release and recapture. Each lizard was injected with 0.21 µl (0.15 µl ^{18}O-water and 0.06 µl D_2O) per gram of lizard body weight by intra-peritoneal injection using a previously calibrated 10 µl syringe (*Hamilton*). For example, a 5 g animal was injected with 1.05 µl of DLW solution (Metabolic Solutions protocol modified from Speakman 1997). The exact time of injection was recorded, and each animal was left for approximately 2.5 hours to allow the DLW solution to become equilibrated with their body water. During this period, they were kept in the shade (air temperature less than 20 ºC) in a large plastic container (80 x 40 x 60 cm) layered with paper pellets and terracotta saucers for cover. At the end of this period, 50 - 60 µl of blood was collected from the lizard's sub orbital sinus using a 75 µl heparin-coated capillary tube. The exact time of the blood collection was recorded, and each blood sample was placed into a heparin-coated eppendorf tube. These tubes were stored in a portable cooler at 4 - 8 ºC until they were returned to the laboratory. In addition, a sample of 50 - 60 µl of blood was collected from

three uninjected male lizards: these blood samples were required to provide the baseline reading of plasma without DLW solution in the mass spectrometry analysis. We collected the blood samples for baseline reading from three different uninjected males since we could not bled individual lizard three times because of their small sizes. After the blood sampling, all lizards were released at their point of capture. On returning to the laboratory, the blood was centrifuged for 5 minutes at 3000 rpm. The separated plasma was transferred into a labelled cryogenic storage tube and stored in a freezer.

The injected lizards were recaptured within 5 to 9 days after the day of injection; a sample of 50 - 60 µl of blood was collected from the sub orbital sinus using a 75 µl heparin-coated capillary tube and processed as described above. Each lizard was re-weighed to the nearest 0.1 g prior to bleeding and the time of blood collection was recorded. All lizards were re-released at their point of capture. Twenty-three injected lizards were recaptured at warm lowland site, and twenty-five were recaptured at the cold alpine site. However, only those plasma samples with a volume more than 30 µl could be used for analysis because this is the minimum volume required for the IRMS analysis by Metabolic Solutions, Inc. (United States of America). Thus, for each site, 28 frozen plasma samples (14 individuals x 2 samples) were later transported on dry ice to Metabolic Solutions, Inc. (United States of America) for IRMS analysis.

The rate of CO_2 production was estimated using the Lifson and McClintock (1966) equation $rCO_2 = (N/2)\ (k_{18} - k_2)$ which assumes that the total body water pool (N) is a homogeneous compartment that remains constant during observation. This equation is:

$rCO2 = (N/2)\ (k_{18} - k_2)$

where rCO_2 is the rate of CO_2 production (in ml g^{-1} hr^{-1}), N is the total body water pool, k_{18} is the rate of elimination of ^{18}O and k_2 is the rate of deuterium (D_2O) elimination. The total body water was obtained by plotting a decay curve for each isotope, D_2O and ^{18}O. The decay data were linearised by taking the logarithm of the ^{18}O and deuterium data. By the extrapolation to time zero, the total body water was obtained. *Niveoscincus ocellatus* is primarily insectivorous (Wapstra and Swain 1996), so the rate of CO_2 production was converted to energy expenditure based on a thermal equivalent of 25.7 J per ml CO_2 for a diet of insects (Nagy 1983). Therefore, energy expenditure (in J g^{-1} hr^{-1}) = (CO_2 in ml g^{-1} hr^{-1}) (25.7 Joules).

Lizards have a very low metabolism when they are cold and inactive, which is only 11 to 17 % of total daily energy expenditure (Bennett and Nagy 1977). Accordingly, the result obtained from the above calculation (in J g^{-1} hr^{-1}) was adjusted according to the active time estimated for individuals at each study site to provide an estimate of total energy expenditure when active (in J g^{-1} hr^{-1}). The adjustment was made by dividing the energy expenditure calculated for the entire period of DLW study (in J g^{-1} hr^{-1}) with the potential active time available for the lizards at each site. In addition to the energy expenditure calculation, we also calculated the water flux rate of the lizards by using Nagy and Costa (1980) equation for a constant body water based on the elimination rate of Deuterium data to determine the rate of energy intake in these populations.

2.3. Statistical analysis

All statistical analyses were conducted in SAS 9.2. with significance α set at 0.05. Prior to the analysis, the data for the energy expenditure and length of active time were checked for normality using a normal quantile plot. Both data sets were normally distributed, so transformation was not required. The energy expenditure of *N. ocellatus* (J g^{-1} hr^{-1}) and the active time (hours) were compared between sites using one-way ANOVA. Similarly, a one-way ANOVA was used to compare the water flux rate (ml g^{-1} hr^{-1}) between sites. The field active body temperature and the air temperature data were descriptively analysed to determine the range (minimum and maximum) of temperature at which the lizards were active at each study site (see above). Field active body temperature data were compared between populations using two-way ANOVA to determine whether their body temperature varied with season across the two sites (Summer 2009/2010, Autumn 2010, Spring 2010, Summer 2010/2011, and Autumn 2011) at the climatic extremes of their distributional range. When the full models were significant, planned comparison tests were used to isolate significant interactions between sites and seasons as well as the main effect of site and seasons. A one-way repeated ANOVA was used to determine whether the body masses of lizards changed during the course of DLW study. A simple correlation data analysis was also conducted to determine the relationship between energy expenditure and body size between sites. One might expect that since we had 2 populations and 2 altitudes, the differences we found in this study could be because of population effects rather than altitude. One way to address this was the possibility to replicate the two sites, which is beyond the scope of this study. Given we know that temperature drives many processes in

ectotherms (rather than altitude per se), it is more parsimonious to accept that the population differences (see later) are in fact likely driven by temperature.

3. Results

A wider range of field active body temperature measurements from Summer 2009/2010 to Autumn 2011 were observed in the cold alpine population (18.1 to 34.2 ºC; N = 221) compared with the warm lowland population (29.1 to 34.5 ºC; N = 164) (Figure 2). The body temperatures were collected when lizards were active at air temperatures between 10.3 to 26.6 ºC at the cold alpine site and 15.2 to 29.4 ºC at the warm lowland site. The field active body temperature of *N. ocellatus* showed significant interaction among sites and seasons ($F_{4,384}$ = 9.44, P < 0.0001) with a strong site ($F_{1,384}$ = 202.83, P <.0001) and seasonal ($F_{4,384}$ = 6.60, P <.0001) main effect also. The mean active body temperature recorded was the lowest in autumn compared to in spring and summer at both sites (in Autumn 2011 at the warm lowland site and in Autumn 2010 at the cold alpine site). The field active body temperature of the high altitude population (33.48 ± 0.26 ºC, 33.67 ± 0.13 ºC, 32.7 ± 0.12 ºC, 32.74 ± 0.13 ºC, 31.92 ± 0.14 in Summer 2010, Autumn 2010, Spring 2010, Summer 2011 and Autumn 2011, respectively) was significantly lower than those at the low altitude (28.87 ± 0.58 ºC, 27.27 ± 0.74 ºC, 30.53 ± 0.25 ºC, 30.4 ± 0.29 ºC, and 28.02 ± 0.36 ºC in Summer 2010, Autumn 2010, Spring 2010, Summer 2011 and Autumn 2011, respectively) in all seasons observed (Summer 2010 $F_{1,56}$ = 34.22, P <.0001; Autumn 2010 $F_{1,78}$ = 126.79, P <.0001; Spring 2010 $F_{1,79}$ = 14.67, P = 0.0002; Summer 2011 $F_{1,87}$ = 19.32, P <.0001; Autumn 2011 $F_{1,80}$ = 49.01, P <.0001).

The time from when the lizards were injected with the DLW solution to the time when the second blood samples were taken for the DLW study was similar in both populations (8-9 days or 151.74 ± 5.4 hrs and 7-8 days or 158.13 ± 3.7 hrs for the warm and cold site respectively; $F_{1,26}$ = 0.98, P = 0.3316). In total, we had 13 and 14 plasma samples for IRMS analysis from the warm lowland and the cold alpine sites, respectively. Energy expenditure was significantly higher at the cold alpine site than at the warm lowland site (8.96 ± 0.29 J g^{-1} hr^{-1} and 6.31 ± 0.37 J g^{-1} hr^{-1} respectively; $F_{1,26}$ = 32.25, P < 0.0001) (Figure 3a). No significant changes in the mean body mass of lizards occurred during the DLW study (4.80 ± 0.24 g versus 4.68 ± 0.22 g (t value= 0.36, P = 0.7238) and 5.38 ± 0.82 g versus 5.29 ± 0.93 g (t value = 0.27, P = 0.7852)) at the warm and cold sites respectively. A significant

($F_{1,12}$ = 6.08, P = 0.03) but weak (r^2 = 0.36) relationship between the energy expenditure and body mass was found in the warm lowland population. However, no significant relationship was found in the cold alpine population ($F_{1,13}$ = 0.20, P = 0.67, r^2 = 0.02). In addition, there was no significant difference in water flux rate between the warm lowland and cold alpine populations ($F_{1,26}$ = 2.49, P = 0.1275). The mean water flux rate was 5.35 ± 0.64 ml g^{-1} hr^{-1} at the warm lowland site while 4.33 ± 0.19 ml g^{-1} hr^{-1} at the cold alpine site.

The estimated activity time during the DLW study was significantly longer in the cold site (71.79 ± 1.3 hrs) than the warm site (62.51 ± 2.2 hrs) ($F_{1,26}$ = 14.01, P = 0.001). In part this was because during the DLW study there were three rain periods restricting lizard activity at the warm lowland (day 5 (120 mins), day 6 (120 mins), day 7 (30 mins)) whereas there was no rain restricting activity at the cold site. The temperature profile and the rain periods are presented in Figure 4. When the rate of energy expenditure took into account the activity time of each lizard, the rate of energy expenditure remained higher in cold alpine population (19.70 ± 0.6 J g^{-1} hr^{-1}) than in warm lowland population (15.33 ± 0.9 J g^{-1} hr^{-1}) ($F_{1,26}$ = 15.80, P = 0.0005; (Figure 3b).

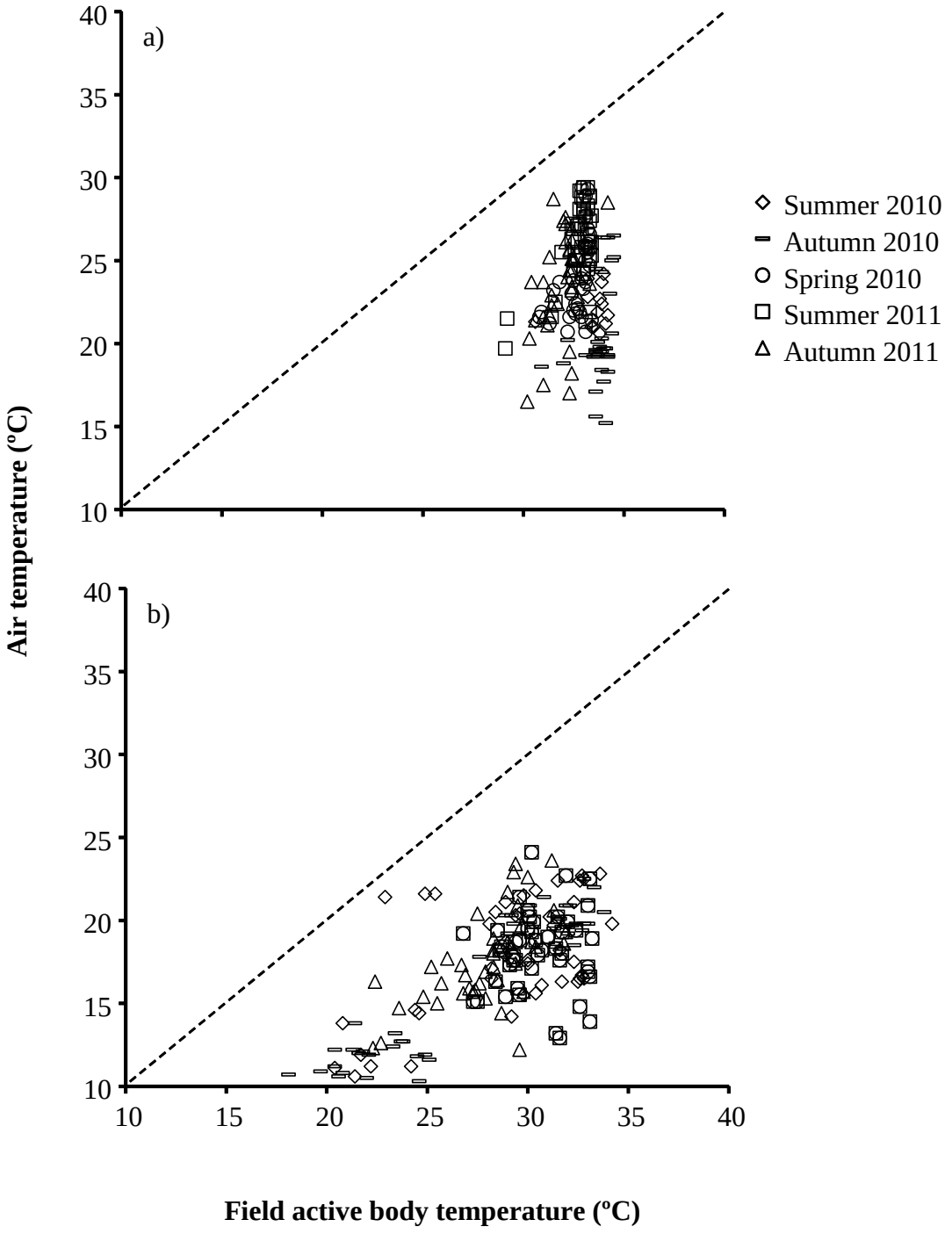

Figure 2. The field active body temperature of *Niveoscincus ocellatus* and air temperature collected from Summer 2009/2010 to Autumn 2011) at warm lowland site (a) and cold alpine site (b) in Tasmania. ---------- Conformity line.

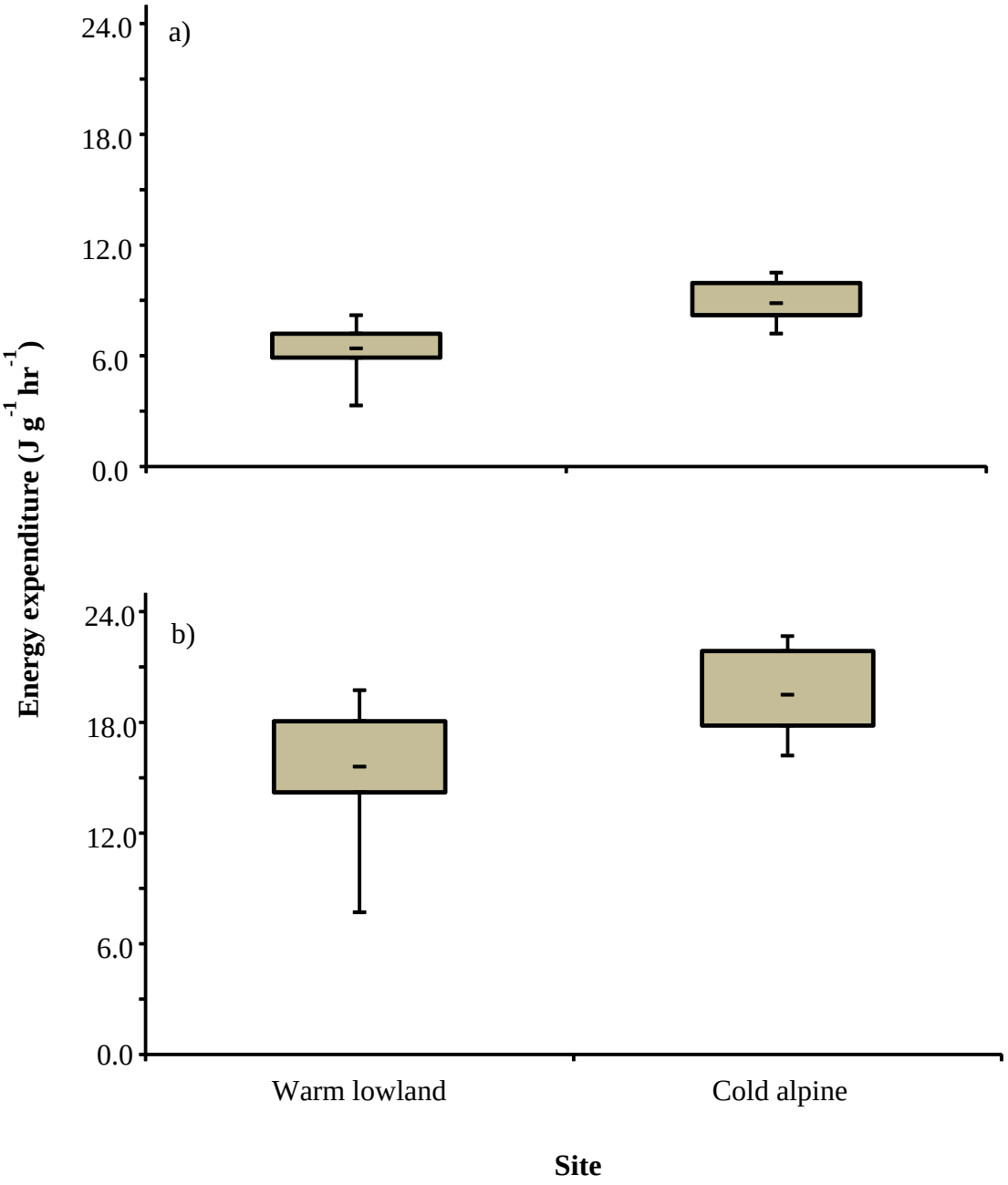

Figure 3. Box plot of the energy expenditure (J g^{-1} hr^{-1}) of male *Niveoscincus ocellatus* from the warm lowland site and the cold alpine site in Tasmania over (a) the whole period when the DLW study was conducted (b) the potential activity time available for each population.

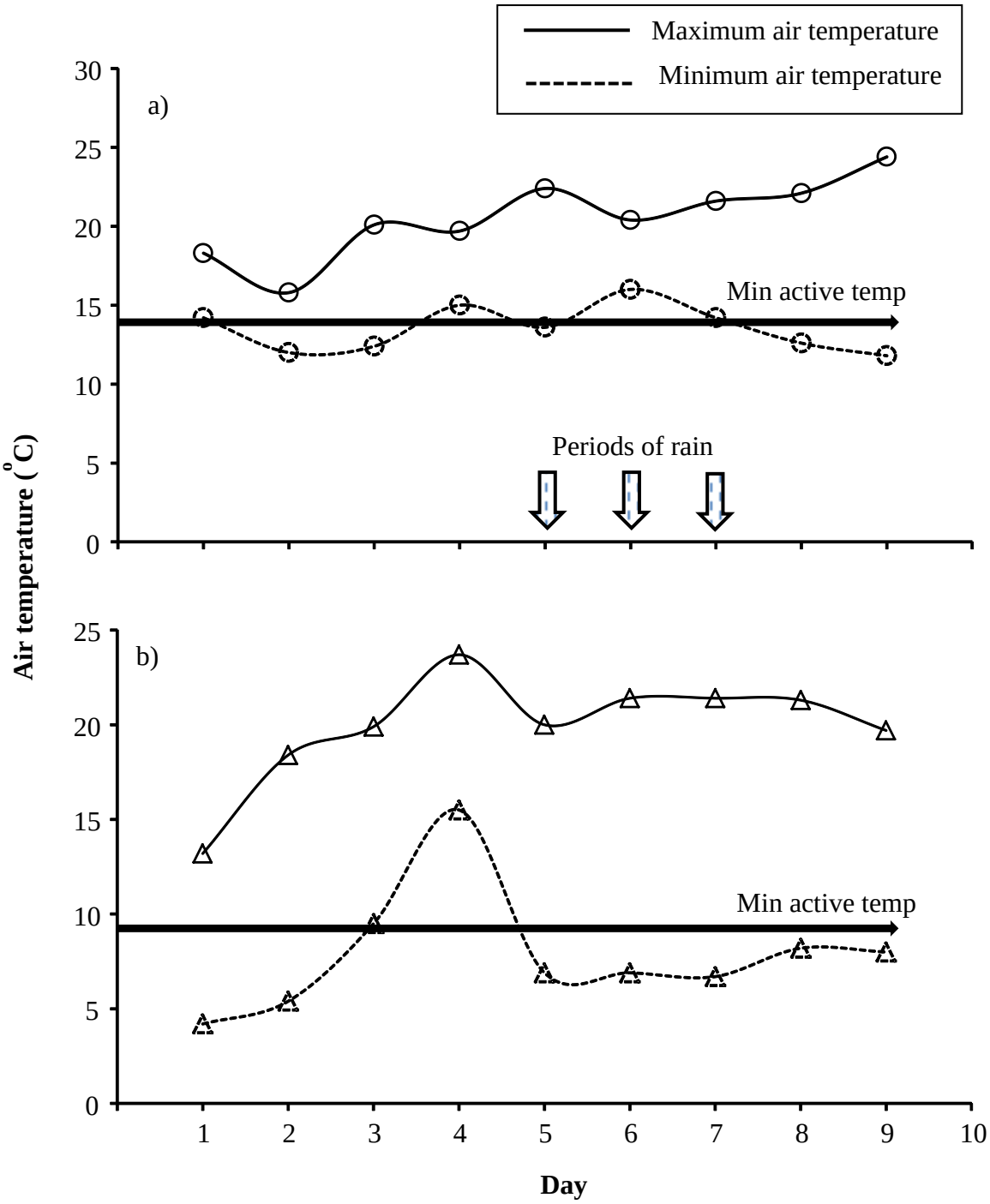

Figure 4. Minimum and maximum temperature profile and rain occurrence at warm lowland site (a) and cold alpine site (b) during the doubly labelled water study. ⇩ Rain occurrence ——— Maximum air temperature; - - - - - - - Minimum air temperature. Data were collected from the nearest weather station from each study site (10 km away at warm lowland site; 45 km away at cold alpine site).

4. Discussion

We measured the energy expenditure in two populations of a widespread lizard, the spotted snow skink, *Niveoscincus ocellatus,* using the DLW method. We also estimated the potential activity time of the lizards at both study sites based on an analysis of their activity patterns in relation to air temperatures. Male *N. ocellatus* at the cold alpine site expended a higher rate of energy per gram per hour than at the warm lowland site. Previous studies in a range of endotherms (e.g., Pontzer et al. 2010; Zelova et al. 2011) and ectotherms (e.g., Angilletta 2001a; Naya et al. 2008; Smith et al. 2008) found that energy expenditure is primarily associated with activity period, although other factors, such as reproductive stage (Bennett and Nagy 1977; Schultz et al. 2008), Oxygen levels (Frappell et al. 1992), predation levels (Congdon 1989), or prey densities (Brown et al, 1992; Christian et al. 2007) could also play a role in differences in energy expenditure across populations. In endotherms, higher energy expenditure results from increased energy use during periods of activity (e.g., Pontzer et al. 2010; Zelova et al. 2011). In ectotherms, in addition to the energy used during periods of activity (e.g., Angilletta 2001a; Smith et al. 2008; Powers and Anderson 2010), a higher energy expenditure also reflects a higher cost of maintenance because cost of maintenance comprises the largest component of energy expenditure (e.g., Angilletta 2001b; Pörtner et al. 2008).

There are potentially two explanations for the differences in energy expenditure between the two populations of *N. ocellatus*. The first explanation is the difference in activity times of lizards between sites during the DLW study. Individuals from the cold alpine site had a longer active time than those from the warm lowland site. This finding is in agreement with other energy expenditure studies conducted in many species: individuals with a longer activity time have a higher energy expenditure than their counterparts with a shorter activity time, either within population e.g., variation between seasons (Christian et al. 2003; Smith et al. 2008) or between populations (Powers and Anderson 2010). Typically, ectotherms living in cold environments, such as at high altitudes or latitudes, have more restricted active times than those living at lower altitudes or latitudes due to more limited thermal opportunity (e.g., Gutierrez et al. 2010; Ibarguengoytia et al. 2010; Valdecantos et al. 2013). However, we found the opposite: over the period when the DLW study was conducted, *N. ocellatus* at the cold alpine site had a longer activity time than those at the warm lowland site. Some of this variation was because there were short periods of rain which restricted the lizards' activities at the warm site – while we attempted

to ensure the weather was as comparable as possible (by offsetting our experiments at the two sites by ~ 1 month), some geographic variation in weather patterns are inevitable in the temperate climate of Tasmania. However, the largest contributor to the longer active time at the cold alpine site was that the lizards were able to be active at lower minimum air temperatures (~ 10 °C) than those at the warm lowland site (~ 15 °C). The cold alpine lizards were also active at a lower, and a greater range, of body temperatures (18.1 – 34.5 ^{o}C) than the warm lowland lizards (29.1 – 34.5 ^{o}C). The range of body temperatures at which the lizards were active at the cold alpine site was ~ 14 ^{o}C higher than their Critical Thermal Minimum (Cadwell et al. 2015. *In press*.). In addition, thermal preference of the cold alpine lizards were 27.3 – 28.4 ^{o}C which indicated that they were able to be active at ~ 10 ^{o}C below their thermal preference (Uller et al. 2011, Cadby et al. 2014). No geographic variation, however, was observed in thermal preference in this species (L. Yuni, *unpublished*). The ability to be active at lower air and body temperatures suggests a potential adaptation toward lower temperatures in the cold alpine populations of *N. ocellatus* which allows these lizards to extend their active time.

In order to maintain a high and stable body temperature when thermal opportunities are restricted (e.g., cool seasons or at high altitudes), within species comparisons reveal that to maintain a high and stable body temperature when thermal opportunities are restricted (e.g., cool seasons or at high altitudes), lizards can increase their basking intensity by up 50% compared to those at lower altitudes (Huey and Slatkin 1976; Vidal et al. 2010). This is in agreement with past work on *N. ocellatus* which suggests strong selection on basking in highland populations, i.e., they bask more than low altitude lizards when exposed to poor reduced thermal opportunities to maximize the use of limited thermal opportunities (Uller et al. 2011; Cadby et al. 2014). However, increased basking intensity is not without costs via an increase in the risk of predation, increased costs of metabolism and potentially decreased time available for other activities such as foraging, especially for actively foraging lizards (Huey and Slatkin 1976; Wapstra and O'Reilly 2001; Sears 2005; Angilletta 2009; Isaksson et al. 2011). To some extent these costs can be avoided by being active (i.e., foraging rather than basking) at lower and at a wider range of body temperatures as we found, i.e., they cease basking at a lower temperature than conspecifics from warmer areas.

While the higher energy expenditure at the cold subalpine site can be explained in part by their extended daily activity period, it does not fully explain the differences. When we

took the differences in activity time between sites into account, individuals from the cold site still had a higher mass specific energy expenditure. Some ectotherms elevate their standard or resting metabolic rate to facilitate physiological processes in order to compensate for the effects of the low temperatures in high altitudes or latitudes: this is known as metabolic cold adaptation (MCA) (Clarke 1993; Lourdais et al. 2013). Metabolic cold adaptation had been observed in many species of ectotherms living in highly variable cold environments, and has been used to explain wide thermal tolerances in these zones (e.g., reviewed by Addo-Bediako et al. 2002; Cano and Nicieza 2006; Pörtner 2006; Lourdais et al. 2013), and this could further explain our results in *N. ocellatus* at the high altitude. For *N. ocellatus* from the cold alpine site, MCA may provide a mechanism for this population to compensate for the effect of low and variable temperatures to maintain their physiological processes at an optimal rate. Previous studies have revealed that the elevated metabolic rate occurs via adjustments of mitochondrial density and capacity that results in higher temperature-dependent maintenance costs, and subsequently in higher energy expenditure because cost of maintenance comprises the major portion of energy expenditure (Pörtner et al. 2000, 2008; Homyack et al. 2010). A similar phenomenon had been observed in the lizard *Sceloporus undulatus* (Angilletta 2001b) in which a higher maintenance metabolism resulted in higher energy expenditure in a cold population compared to a warm population. Another example of MCA was also demonstrated in fish blackspotted topminnow (*Fundulus olivaceus)* in which the northern (cold) population had a higher mass specific metabolic rate compared to a southern (warm) population at set temperatures (Schaefer and Walters, 2010). This explanation for *N. ocellatus* is supported by their ability to be active at lower body temperatures and is further supported by population differences in temperature dependent performance. In the highland population, both sprint speed and endurance have a wider performance breadth and are maximised at a lower body temperature than in the warm lowland population (L. Yuni *unpublished*). It has been understood that elevated metabolic rates due to MCA have been associated with observations of a downward shift of critical temperatures in cold adapted populations of the Polychaete worm (*Arenicola marina* L.) (Sommer and Pörtner 2002; reviewed in Pörtner 2006). The combination of elevated metabolic rate and modified thermoregulatory behaviour is likely an important mechanism allowing *N. ocellatus* to cope with the cold environments at high altitude sites.

To support the higher energy expenditure at the cold alpine site than at the warm lowland site, a higher energy intake would be required by this population. We also calculated the water flux rate of the lizards by using Nagy and Costa (1980) equation for a constant body water based on the elimination rate of Deuterium to determine the rate of energy intake in these populations and found there were no significant difference in their energy intake rate between sites during our study period. This does not preclude higher energy expenditure at other times of the year to support their higher energetic requirements. In a recent detailed laboratory study examining the diet of this species in these two populations using the geometric framework, we found little evidence for differences in diet preference but strong evidence for greater intake by cold site lizards (Yeoh et al. unpublished). The ability to be active at low temperatures at the cold alpine site provides a mechanism for this population to forage at a wider range of temperatures to fulfil their high energy expenditure. Resource availability is high at both cold alpine and warm lowland sites during the summer months indicated by large fat reserves in late summer/early autumn (Wapstra and Swain 2001). *Niveoscincus ocellatus* exhibits an active foraging behaviour. They take a wide variety of terrestrial arthropods opportunistically, including cryptic and hidden prey in accordance with a wide ranging foraging strategy (Wapstra and Swain 1996).

To summarize, the variation in energy expenditure between populations measured in this study adds to the evidence of how a wide range of ectothermic species, exemplified by *N. ocellatus*, respond to variation in environmental conditions. Differences in the active time between the cold alpine and warm lowland populations, potentially coupled with MCA, resulted in higher energy expenditure at the cold highland population than the warm lowland population. Strong evidence for population-level differences in temperature-dependent metabolism should next be addressed in the laboratory by examining metabolic rate under standard conditions in a range of populations along an altitudinal gradient at a range of different ambient temperature.

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Chapter Five

Climate variation does not induce population-specific responses in date of birth in the climatically widespread spotted snow skink, *Niveoscincus ocellatus*

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Abstract

Parturition date (or date of birth) is largely driven by thermal conditions experienced by females in viviparous lizards because temperature during gestation influences the rate at which her embryos develop. In this study, a long-term data set (14 years) on date of birth of the viviparous spotted snow skink, *Niveoscincus ocellatus*, was used to investigate the relationship between date of birth and environmental temperature experienced by females during their gestation period at the climatic extremes of the species distribution (warm lowland versus cold alpine populations). Geographic and annual variation in date of birth was observed in this species with earlier date of birth corresponding strongly to mean maximum air temperature during gestation period both between sites and among years within sites. There was no evidence of site-specific differences in the relationship between annual variation in birth dates and annual variation in mean maximum temperatures. Thus, we found no evidence of site-specific buffering of annual variability in weather by females. However, given the significant differences in temperatures experienced at the sites and the similarity in gestation lengths, our results do suggest site-specific rates of physiological optima for embryonic development. Broadly our results suggests that females have limited capacity to buffer variation in environmental conditions during gestation and thus directional climate change are likely to result in a directional (and strong) effects on birth dates in the future with potential concomitant effects on life history and demographic processes.

Keywords: lizards, date of birth, environmental temperature, thermoregulation, physiological adjustment

1. Introduction

Climatic conditions vary geographically and over time and they can have direct and indirect effects on natural populations including effects on physiology, phenology or timing of annual events, and on distribution of natural populations in both endotherms (e.g., Moritz et al. 2008; Visser et al. 2006) and ectotherms (e.g., Calosi et al. 2008; Sinervo et al. 2010). Phenological effects (the timing of life cycle events) are important because they affect a range of fitness related events and thus can affect demographic processes (Reed et al. 2012). In seasonal environments, the timing of annual events such as annual migration and annual reproduction are typically scheduled to coincide with favourable conditions. In many regions, these favorable periods are shifting as the climate changes (Parmesan and Yohe 2003; Root et al. 2003). However, the shifts among species are often asynchronous which is resulting in phenological mismatches among interacting species and may pose consequences for the persistence of ecological communities across an array of ecosystems (Walther et al. 2002; Both et al. 2006, 2009; Ibanez et al. 2010; Reed et al. 2012;Ogawa-Onishi and Berry 2013). One well described example is the trophic mismatch between the breeding seasons of great tits (*Parus major)* with the caterpillar (*Opheroptera brumata)* abundance on oak (*Quercus robur)* to feed their offspring (Visser et al. 1998, 2006; Visser and Holleman 2001; Both and Visser 2001, 2005). In great tits, several components of individual fitness are affected by individual mismatch (i.e., late breeding relative to the caterpillar food peak date), including the probability of double-brooding, fledging success, offspring recruitment probability and the number of recruits (Visser et al. 1998, 2006; Visser and Holleman 2001; Both and Visser 2001, 2005; Reed et al. 2012).

One approach to understand the potential impacts of directional climate change is to understand how populations respond to climate variation. For example, those populations with wider tolerance to climate variability are predicted to be more tolerant to changing climate than those with narrower tolerance (Huey and Kingsolver 1993; Deutsch et al. 2008; Pörtner and Farrell 2008). In order to avoid the risk of fatalities, there are two possible responses toward short term variation in its thermal environment, namely dispersal to more suitable habitat (Gienapp et al. 2008; Buckley et al. 2013) or coping with local conditions through adaptation or through phenotypic plasticity (Salamin et al. 2010). Phenotypic plasticity is defined as the ability of one genotype to produce more than one phenotype when exposed to different environmental conditions so that individuals are able

to modify their behaviour (Beldade et al. 2011), morphology (Price 2006), or physiology (Beldade et al. 2011) in response to environmental conditions (Salamin et al. 2010). Plasticity has a clear and ubiquitous role in promoting phenotypic changes in response to climate variation and it is predicted to mitigate some of the negative biotic consequences of climate change (reviewed in Urban et al. 2013). Many studies then have been conducted in order to reveal how populations respond to climate variability, which later can be used to understand the potential impacts of directional climate on natural populations (e.g., Stillman 2003; Calosi et al. 2008; Angert et al. 2011; Naya et al. 2011; Huey et al. 2012).

While there are literally hundreds of papers on effects of climate variation or climate change on phenology, there is a strong taxonomic bias in our understanding of these effects in natural populations (reviewed in Walther et al. 2002; Ogawa-Onishi and Berry 2013) with much of the work focussed on birds (e.g., reviewed in Dunn 2004; Reed et al. 2012), butterflies (e.g., Polgar et al. 2013) and certain flowering plants (e.g., Primack et al. 2009; Bartomeus et al. 2013; Straka and Starzomski 2014). Phenological responses in reptiles are, however, poorly understood. As ectotherms, reptiles have a particularly strong relationship to their thermal environment and may be particularly susceptible to perturbations in the thermal environments (Amarasekare and Savage 2012). Understanding phenological responses relies on long term data where life cycle events can be linked to underlying weather and climate effects (Visser and Both 2005; Polgar et al. 2013) and reptiles are particularly poorly represented by such approaches (e.g., Brown and Shine 2007) perhaps because key events are harder to capture than flowering in plants or hatching in birds (especially when many of the best examples of long-term bird studies take advantage of nest boxes). The taxonomic bias can result in understanding bias of how climate affects key life cycle events because responses among species with fundamentally different physiology (e.g., ectotherm vs endotherm taxa) or reproductive ecology (e.g., oviparous vs viviparous) are likely to differ.

Even when phenology of a species is well studied, it is rare that more than one population is studied and this can complicate our effort to predict phenological responses to ongoing climate change because responses may be population-specific (Helmuth et al. 2005; Rubolini et al. 2007; Peck et al. 2009; Ibanez et al. 2010). One example is demonstrated in the long term study of cherry trees (*Prunus xyedoensis*) in Japan that showed that flowering times in urban, suburban, and rural sites at each city gradually began to diverge (Primack et al. 2009). Population-specific response toward climate

variation has also been observed among populations of European migratory birds in their first arrival dates from long migration (Rubolini et al. 2007; Moller et al. 2008). Divergent responses among populations within a species may relate to variation in thermal environment in which they live (Helmuth et al. 2005; Peck et al. 2009; Seebacher et al. 2012) and are locally adapted. Local adaptation may then constrain responses to climate variation that falls outside the conditions they normally experience; therefore, it is important to incorporate population-specific responses to build reliable predictions of the fate of a species during the future predicted climate change (Rubolini et al. 2007).

In this study, we used a rare long-term data set (14 years) on lizard birth dates from two populations of the viviparous spotted snow skink, *Niveoscincus ocellatus*. We investigated the relationship between date of birth and environmental temperature experienced by females during their gestation period at the climatic extremes of the species distribution (warm lowland versus cold alpine populations). *Niveoscincus ocellatus* is a viviparous lizard occurring over a wide geographical and climatic range in Tasmania (Atkins et al. 2007; Cadby et al. 2010; Uller et al. 2011; Yuni et al. 2015). In viviparous lizards, parturition date is largely driven by thermal conditions experienced by females because temperature during gestation influences the rate at which her embryos develop (Schwarzkopf and Shine 1991; Doughty and Shine 1998; Wapstra 2000). Accordingly, pregnant females experiencing higher temperature such as at warm lowland sites or in warm years are predicted to give birth earlier than those experiencing lower temperature in cold sites or cold years (e.g., Wapstra et al. 2001; Pen et al. 2010; Itonaga et al. 2012; Lorioux et al. 2013). However, there are number of processes that influence the relationship between thermal environments and the date of birth within populations (i.e. among years) and among populations, including selection on basking behaviour to buffer the poor thermal conditions (Gvozdik and Castilla 2001; Gvozdik 2002; Cadby et al. 2010, 2014; Uller et al. 2011; DeNardo et al. 2012; Lorioux et al. 2013), their body temperatures (Le Galliard et al. 2003; Wang et al. 2014), and potentially shifts in optimum temperature to facilitate physiological processes (Seebacher 2005; Lourdais et al. 2008; Beldade et al. 2011; Schwarzkopf et al. 2016). Specifically, in our study species there is strong selection on basking behaviour in females from high altitude populations which means they thermoregulate more actively (i.e. bask more) than low altitude lizards especially under reduced thermal opportunities (Uller et al. 2011; Cadby et al. 2014), even to the extent that counterintuitively they target higher body temperature than their lowland counterparts.

Divergence in basking behaviour has evolved in *N. ocellatus* presumably because of past selection to maximise the use of the limited opportunities that occur in the colder environments (Uller et al. 2011; Cadby et al. 2014). As a result, the effect of temperature on date of birth might be predicted to be less at the cold alpine site than at the warm lowland site because they behaviourally compensate for poorer weather by increasing basking frequency and raising their preferred body temperatures more than those from warm lowland site.

2. Materials and Methods

Study species

Niveoscincus ocellatus are small lizards (adult body mass 3-12 g) living over a wide geographic and climatic range in Tasmania with concomitant effects on life history traits (Wapstra and Swain 2001; Wapstra et al. 2001; Atkins et al. 2007; Cadby et al. 2010, 2014; Pen et al. 2010; Uller et al. 2011). Individuals are considerably larger in body size at maturity (and larger overall) at the colder extremes (65 mm versus 55 mm snout vent length) of the species' range (Wapstra et al. 2001) where the active season is reduced to approximately October to April, compared to their counterparts that are active approximately from September to May at the warmer extreme of the species' range (Wapstra et al. 1999). Individuals reach maturity at three years old at the warm lowland sites compared to four to five years old at the cold alpine sites (Wapstra et al. 2001; Pen et al. 2010). Females reproduce annually with larger litter size at the cold alpine site than that at the warm lowland site (Wapstra and Swain 2001; Pen et al. 2010).

Study sites

Since Austral summer of 2000/2001, data on the timing of birth was collected from two populations of individually marked *N. ocellatus* at the climatic extremes of the species' distribution. The warm lowland site is located near Orford (147^{o}87'E; 42^{o}55'S; 30 m asl), and the cold alpine site is located at Lake Augusta on the Central Plateau (146^{o}53'E; 41^{o}86'S; 1200 m asl) in Tasmania. Each site contains a largely self-contained population of skinks with little immigration or emigration (Wapstra et al. 2009; 2010; Pen et al. 2010; Uller et al. 2011) with the majority of skinks permanently marked making it possible to track individuals from year to year. The lowland site experiences a warm coastal

temperature with a mild winter, while the cold alpine site is much cooler where snow or hail can fall in any month (www.bom.gov.au). Monthly mean maximum air temperature (2000 – 2014) at the nearest weather station to each study site was obtained from the Bureau of Meteorology Australia and they are displayed in Figure 1. At the warm lowland site, the active season begins in early September and ovulation occurs near the beginning of October (Wapstra 2000; Wapstra et al. 2004). Gestation is a minimum of 3 months long depending on the thermal conditions of the year (Wapstra et al. 2004; Wapstra et al. 2009). At the cold alpine site, emergence from hibernation occurs later and there is a concomitant shift in the ovulation and the onset of gestation until approximately October 15 while the period of gestation is a minimum of 3 months as in the warm site. Thus, the gestation period in *N. ocellatus* was assigned as 1 October – 31 December for the lowland population and as 15 October – 15 January for the cold alpine population (see Figure 1) (Wapstra 2000; Wapstra et al. 2004; Wapstra et al. 2009; Cadby et al. 2010).

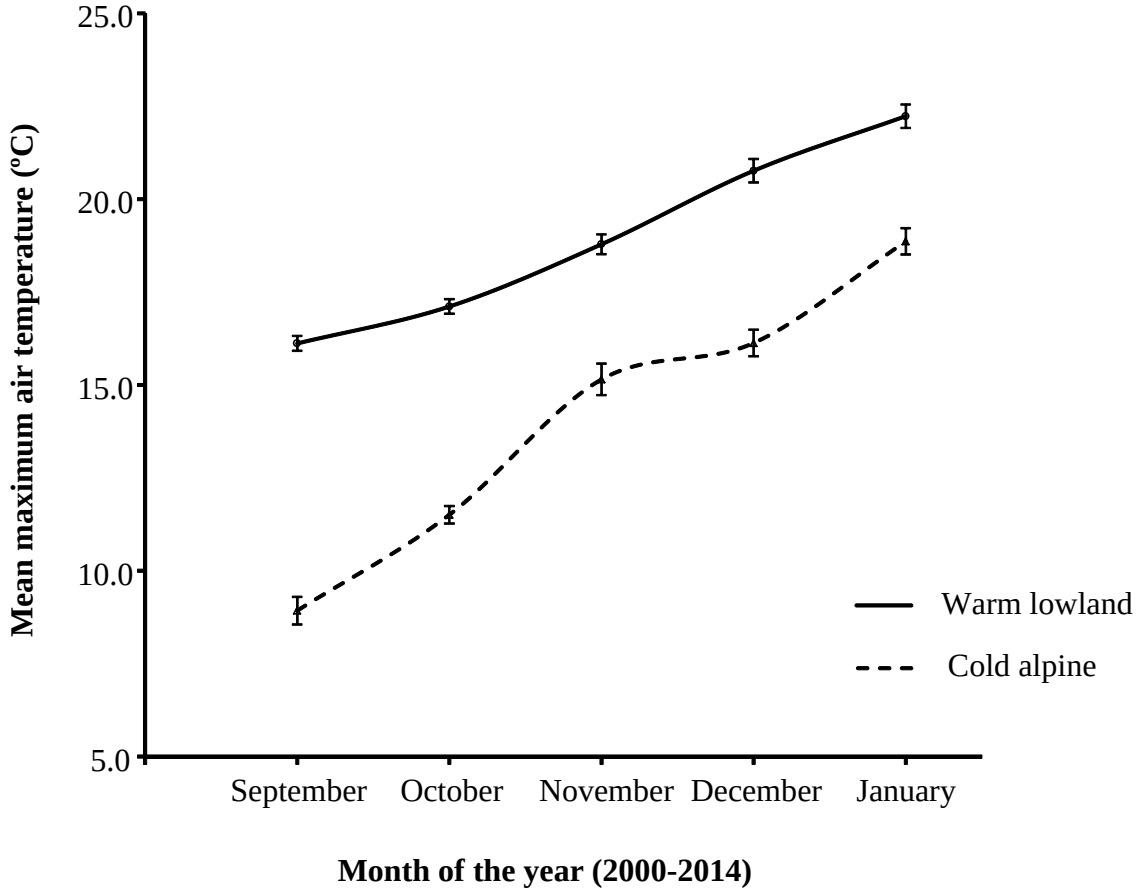

Figure 2. Mean maximum (± S.E.) air temperature at the warm lowland site and at the cold alpine site recorded at the nearest weather station from each study site from 2000 to 2014.

Lizard collection, climate data and analysis of date of birth

At both populations, most gravid females (~ 90–95%) from the study sites were collected at the end of gestation each year (late December at the lowland site and mid to late January at the cold alpine site). These months were chosen so that females experienced as much of the pregnancy as possible in natural environmental conditions. Upon capture, females were identified individually using toe clips when present, else a new toe-clip was assigned, and their position was recorded to ± 5 m within the study site using a handheld global positioning system. Females were transported to a laboratory at the University of Tasmania.

At the laboratory, females were measured for body mass and snout vent length. They were housed in plastic terraria (30 x 20 x 20 cm) with animal bedding, each containing cover and a basking light. They were exposed to 16 $^{\circ}$C ambient temperatures and had access to strong overhead lights to mimic daylight (14 DL: 10 DD) and access to heat lamps from 8.00 am to 6.00 pm (allowing them to thermoregulate within a thermal gradient in their cages of 16 – 40 $^{\circ}$C). Water was provided *ad libitum* and lizards were fed three times per week on live insects and crushed fruit. All lizards were maintained under these conditions until parturition. Terraria were checked twice daily for neonates. At birth, offspring were measured for their mass (± 0.1 mg) and snout vent length (± 0.01 mm), and was given unique code via toe clipping. Females were also weighed and measured following birth. Mothers were released at the original site of capture, and offspring were released randomly at one of 12 locations throughout the field site from where their mothers originated. Offspring were released at random locations within the study site to prevent interpretation of offspring growth and survival being compromised by some mothers occupying potentially advantageous microhabitats and litter mates being released at the same locations (see Wapstra et al. 2010).

For comparison between years and between sites, birth dates were standardised from the earliest recorded birth. The earliest birth date was recorded on the 17th of December 2007 at the warm lowland site, therefore 17 December is considered day 1 for each year. In order to visualize the geographic and inter-annual differences in birth date, the frequency of females giving birth per week at each site then was graphed. Weekly birthing intervals starting from 17th December (week one) to the 14th March (week thirteen) was used.

In order to measure the thermal conditions (basking opportunity) experienced by the gravid females while in the field, the mean maximum daily air temperature data were obtained from the Bureau of Meteorology station situated close to the study sites. The data for the warm lowland site was obtained from Orford weather station (1.0 km from study site), and the data for the cold alpine site was obtained from the Liawenee weather station (10 km from the study site). The daily maximum temperature was used as a measure of the thermal environment experienced by gravid female skinks in the field because it consistently used in examining the effect of maximum temperature as a predictor of offspring phenotypic traits in the wild in reptiles (Bull and Burzacott 2002; Chamaille-James et al. 2006; Wapstra et al. 2009; Cadby et al. 2010). Specifically, in *N. ocellatus* it represents an excellent proxy for opportunities for activity (and hence preferred and high body temperatures) (Cadby et al. 2010). The mean maximum daily air temperature experienced by gravid *N. ocellatus* in the field was determined as follows: the ovulation date is set in part by temperatures after winter emergence. At the warm lowland site, the active season begins in early September and ovulation occurs near the beginning of October (Wapstra 2000; Wapstra et al. 2004). At the cold alpine site, emergence from hibernation occurs later and there is a concomitant shift in the ovulation and the onset of gestation until approximately October 15 while the period of gestation is a minimum of 3 months as in the warm site. The timing of ovulation is highly synchronized among females and consistent between years (assumed to be 1 October in each year as in Wapstra 2000, Wapstra et al. 2004, Wapstra et al. 2009). Such consistency that may be driven by spatial variation in thermal quality of the home range, as female *N. ocellatus* are relatively sedentary (Wapstra et al. 2009) and the relatively short time period between emergence from hibernation and ovulation. In relation to that, the mean of daily maximum air temperature data used was the mean maximum air temperature within the gestation period in *N. ocellatus* which was assigned as 1 October – 31 December for the lowland population and as 15 October – 15 January for the cold alpine population.

During the 14 year study period (from 2000/01 to 2013/14), 2392 birth events were recorded which produced 6903 offspring in the laboratory from the two study sites. Firstly, we evaluated the geographic difference in date of birth and spread of birth date of lizards at the warm lowland and cold alpine site. In addition, the inter-annual variation in date of birth in each site was also evaluated. We conducted a general linear mixed model to evaluate the geographic and annual difference in date of birth. Site was set as the fixed

effect, whilst year of collection was set as the random factor because each site experienced a different climate each year. Additionally, female identity was also included as a random repeated factor because some females appeared in the data for more than one year. Secondly, we evaluated whether the date of birth in *N. ocellatus* was determined by the temperature experienced during gestation. Accordingly, the date of birth was plotted against the mean maximum air temperature during the gestation period at each site to determine the slope of their relationship in each site. We used a linear regression with mean date of birth for each year as the dependent variable and temperature during gestation as the independent variable (N = 14 years for each site). Finally, to determine the variation in the spread of birth within years of collection and between sites, we calculated the coefficient of variation in date of birth (e.g., CoV) for each year of collection at both sites. A one way ANOVA was conducted to determine the geographic variation in spread of birth between two sites. Subsequently, we used a linear regression with spread of birth date (CoV) for each year as the dependent variable and temperature during gestation as the independent variable (N = 14 years for each site). One might expect that since we had 2 populations and 2 altitudes, the differences we found in this study could be because of population effects rather than altitude. One way to address this was the possibility to replicate the two sites, which is beyond the scope of this study. Given we know that temperature drives many processes in ectotherms (rather than altitude per se), it is more parsimonious to accept that the population differences (see later) are in fact likely driven by temperature. All statistical analyses were conducted in SAS 9.2. with significance α set at 0.05.

3. Results

Within the 14 years (2000/01 – 2013/14) of study, 1327 and 1065 birth events were recorded in *N. ocellatus* at the warm lowland and cold alpine sites, respectively (Table 1). The date of birth in *N. ocellatus* showed significant interaction between sites and among years ($F_{13,2392} = 40.89$, $P < 0.0001$). Within a site, there was inter-annual variation in date of birth at both sites (Figure 2). At the warm lowland site, the earliest birth date was in 2000/01 (8 January ± 0.60 days) and the latest was in the following year in 2001/02 (24 January ± 0.90 days). At the cold alpine site, the earliest date of birth occurred in 2009/10 (28 January ± 0.53 days) and the latest occurred almost a month later in 2001/2002 (26 February ± 0.73 days). Mean date of birth was significantly and positively related to the mean maximum air temperature during the gestation period at both the warm lowland ($F_{1,14} = 7.90$, $P= 0.0157$, $r^2 = 0.3969$) and the cold alpine ($F_{1,14} = 35.39$, $P < 0.0001$, $r^2 = 0.7468$) sites. The slope of regression was similar (t value = -0.39, $P = 0.6992$) between the warm lowland and the cold alpine sites, with a 1 degree change in temperature resulting in a shift of 6.26 and 5.49 days at the warm lowland and cold alpine site, respectively (Figure 3). The variation in date of birth (e.g., CoV) was significantly higher at the warm lowland site than at cold alpine site ($F_{1,28} = 167.76$, $P < 0.0001$). The relationship between the CoV of birth dates and the temperature during gestation was significant and strong at the warm lowland site ($P = 0.02$, $F_{1,14} = 6.66$, $r^2 = 0.3570$, Figure 4a). In contrast, there was no significant relationship between CoV and temperature during gestation at the cold alpine site ($P= 0.5643$, $F_{1,14} = 0.35$, $r^2 = 0.0285$, Figure 4b).

Table 1. Birth events of *Niveoscincus ocellatus* recorded at the warm lowland and cold alpine sites from 2000/01 to 2013/14.

Year of collection	**Warm lowland site**		**Cold alpine site**	
	Number of births	Number of offspring	Number of births	Number of offspring
2000/01	93	199	88	373
2001/02	101	227	114	438
2002/03	90	205	83	294
2003/04	104	211	102	351
2004/05	92	220	99	318
2005/06	111	284	97	321
2006/07	92	223	85	261
2007/08	73	174	74	245
2008/09	96	240	60	209
2009/10	102	249	45	168
2010/11	104	253	45	163
2011/12	96	233	49	177
2012/13	89	197	52	187
2013/14	81	205	71	278
Total	**1327**	**3120**	**1065**	**3783**

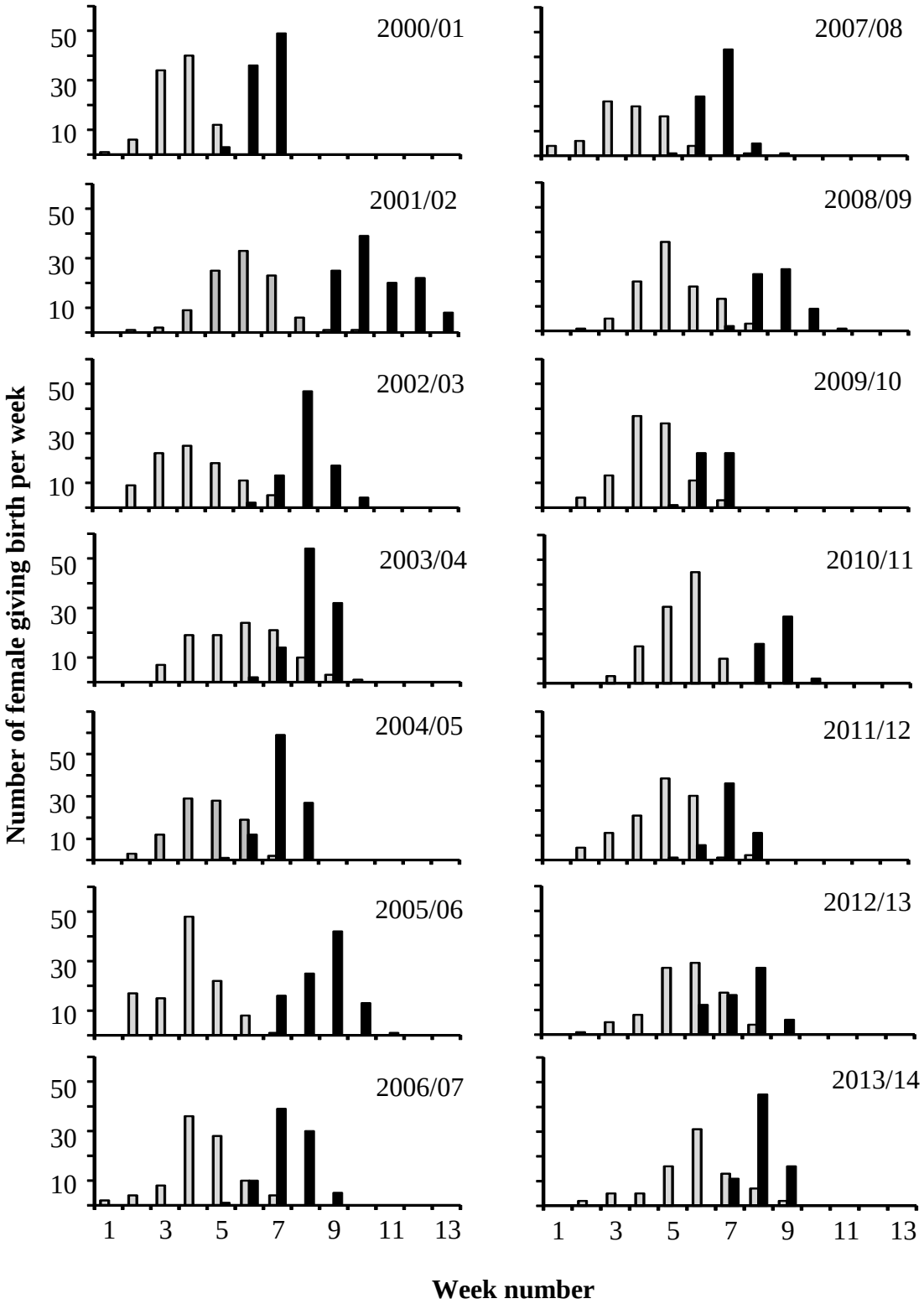

Figure 2. Number of female *Niveoscincus ocellatus* giving birth per week from 2000/01 to 2013/14. The lighter shading represents the lowland population; the darker shading represents the cold alpine population.

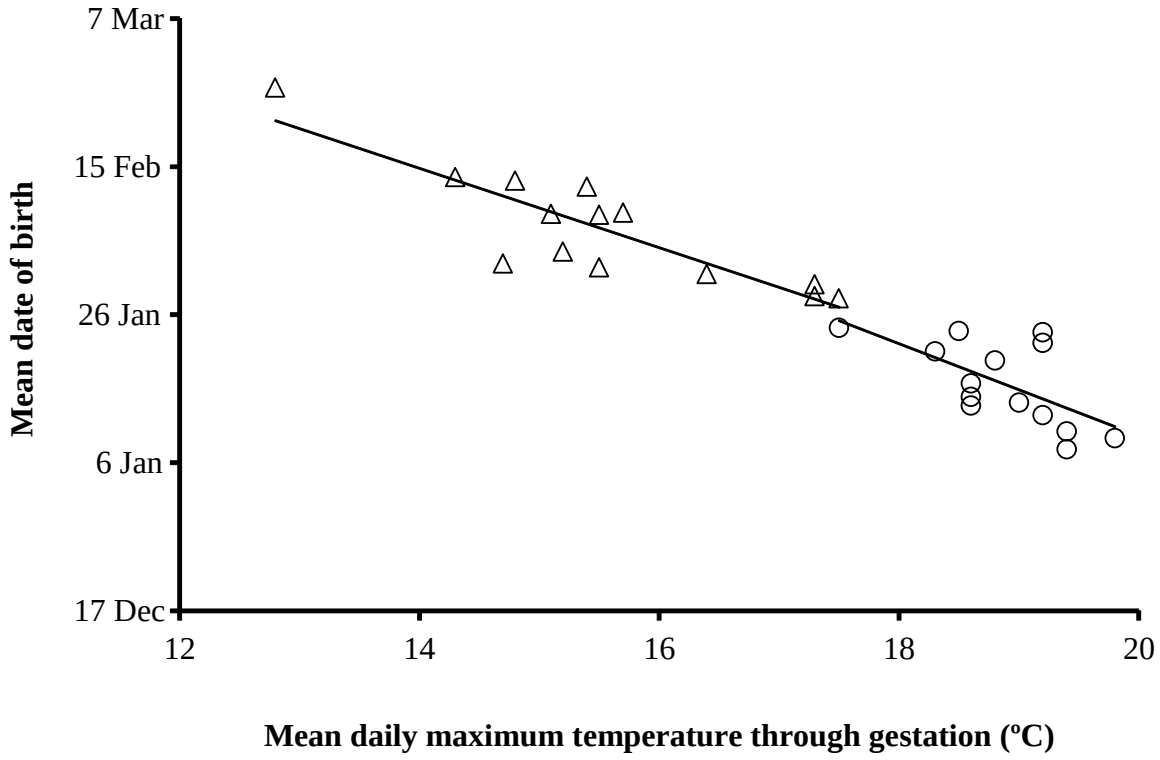

Figure 3. The relationship between the mean daily maximum temperature through gestation with the mean date of birth in *Niveoscincus ocellatus*. The circle marker represents the warm lowland population; the triangle marker represents the cold alpine population.

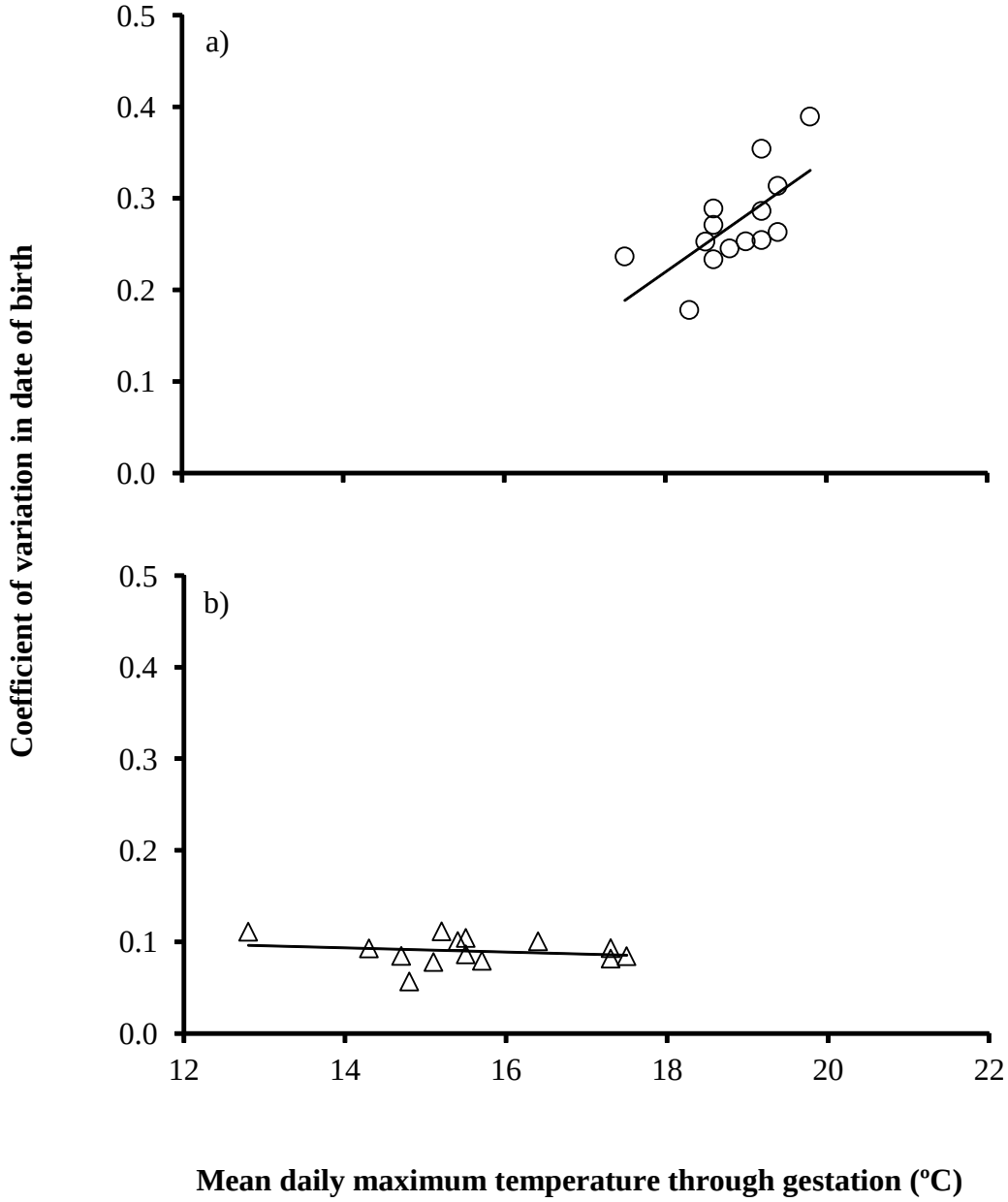

Figure 4. The relationship between the spread of birth and the temperature during gestation in *Niveoscincus ocellatus* at the warm lowland site (a) and at the cold alpine site (b).

4. Discussion

We determined the relationship between the date of birth and the temperature during gestation in two populations of *N. ocellatus* by using a long term data set (14 years) at the climatic extremes of the species distribution (warm lowland versus cold alpine populations). Geographic and annual variation in date of birth was observed in this species with earlier date of birth corresponding with warmer temperature experienced during gestation. Both populations responded in a similar way to the change in temperature. For every 1 degree of temperature increase during gestation, mean date of birth was advanced by approximately 6 days at the warm lowland and by 5 days at the cold alpine sites, respectively. However, the spread of birth date was higher at the warm lowland site than their counterparts at the cold alpine site.

The relationship between thermal conditions and date of birth between sites and among years within sites is explained by the effect of temperature on embryonic development in viviparous lizards (Schwarzkopf and Shine 1991; Doughty and Shine 1998; Wapstra 2000; Wapstra et al. 2001; Caley and Schwarzkopf 2004; Pen et al. 2010; Itonaga et al. 2012; Lorioux et al. 2013; Gibson et al. 2015; Schwarzkopf et al. 2016). However, the relationship between environmental temperature and gestation length is not necessarily straightforward. One of the key advantages of viviparity is the control of developmental conditions it provides to females (Shine 1995; Andrews 2000; Lambert and Wiens 2013). For example, in cold conditions, females can increase their basking to enhance the development of their embryos (e.g., Cadby et al. 2010, 2014; Gibson et al. 2015) while under warm or hot conditions females can protect their developing embryos from detrimentally high temperatures (Shine 1995; Qualls and Andrews 1999; Andrews 2000). As a result, if females substantially alter their basking in response to current environmental conditions, effects of temperature variation on embryonic development rate (and thus gestation length and date of birth) could be reduced and even potentially non-existent. If, as argued in the introduction, there has been different selection on female basking behaviour in different populations experiencing historically different thermal conditions (Gvozdik and Castilla 2001; Gvozdik 2002; Cadby et al. 2010, 2014; Uller et al. 2011; DeNardo et al. 2012; Lorioux et al. 2013), then the effect of variation of annual temperature experienced during gestation could have very different effects in our two populations. However, we found firstly that there were strong effects of temperature variation on gestation length in both populations suggesting limited female capability to

buffer temperature variation among years and secondly we found no evidence for a population-specific effects suggesting females respond to temperature variation in the same way.

Our result is thus surprising because past work in this species has shown strong selection on basking behaviour because females are expected to maximise embryonic development (Atkins et al. 2007; Uller et al. 2011; Cadby et al. 2010, 2014). Certainly, female thermoregulatory behaviour contributes in a major way in determining their optimal body temperature because it can contribute up to four to five times greater than those provided by physiological mechanisms (Stevenson 1985; Hertz and Huey 1981; Crowley 1985; Van Damme 1989). Further, it is well accepted that females do alter their basking behaviour when gravid, in part to optimise embryonic development rate (and potentially offspring phenotypic traits) and to reduce their own costs of pregnancy (e.g., Schwarzkopf and Shine 1991; Ladyman et al. 2003; DeNardo et al. 2012; Schwarzkopf and Andrews 2012; Lorioux et al. 2013). However, increased female basking could be limited by several factors, e.g., thermal quality of the environment (Scheers and Van Damme 2002; Besson and Cree 2010), predation risk (Gutierrez et al. 2010; Lorioux et al. 2013), and time allocation for activities other than thermoregulatory behaviour (Huey and Slatkin 1976; Sinervo et al. 2010). Thus, our results could reflect similar optima for female thermoregulatory responses to temperature variation balancing costs and benefits in ways we have so far underappreciated in this system.

In addition to behaviourally regulating their body temperature, lizards can modify their physiological and biochemical rates to shift their optimal temperatures for physiological and physical performance (Seebacher 2005; Angilletta 2009; Beldade et al. 2011; Schwarzkopf et al. 2016). From laboratory work, we understood that *Niveoscincus ocellatus* can only partially compensate to variation in environmental temperature (Uller et al. 2011; Cadby et al. 2014). In our related work on *Niveoscincus ocellatus*, we showed that in cold subalpine populations they compensated for the low temperatures by elevating their metabolism which subsequently increased their energy expenditure (Yuni et al. 2015). The metabolic elevation might provide a mechanism for *N. ocellatus* at the cold alpine site to compensate for the effect of low and variable temperatures to maintain their physiological processes at an optimal rate. Certainly, gestation lengths are similar in the two populations (see also Wapstra et al. 1999) despite very different climatic conditions (see Figure 1) suggesting mechanisms to maintain relatively high developmental rates in

the colder conditions. These mechanisms are likely to reflect both local adaptation and plasticity in key physiological processes (e.g., Melville and Swain 2000; Cadby et al. 2010, 2011, 2014; Uller et al. 2011; Yuni et al. 2015).

Although both populations responded in the same way to the change of temperature (at the mean level), the spread of birth was lower at the cold alpine site than that at the warm lowland site. It is important for *N. ocellatus* at the cold alpine site to ensure that their offspring were not born late, i.e., too close to the end of their active season (Olsson and Shine 2002; Atkins et al. 2007; Wapstra et al. 2010). Some ectothermic species even have ability to defer their parturition if subjected to adverse environmental conditions near the end of gestation as a strategy to ensure parturition occur in suitable conditions for newborns, as well as to enhance offspring fitness (Cree and Guillette 1995; Girling et al. 2002; Atkins et al. 2007; Waltrick et al. 2012). Further, we also found that geographic variation in the relationship between spread of birth and temperature variation across years in this species. The spread of birth was strongly related to the temperature variation across years at the warm low land site, whilst there was no significant effect of temperature variation across years on spread of birth at the cold alpine site. In previous work on this system we have suggested there may have been stronger canalisation on developmental processes in the cold population (Uller et al. 2011) which would act to reduce variation in birth dates, however, this argument is difficult to reconcile with the strong effect of temperature among years. The reduced variation in birth dates at the cold site could alternatively reflect lower heterogeneity in microclimates at the cold subalpine site compared to the lowland site. While both sites are largely composed of boulder screes, there is more vegetation and slopes at the lowland site potentially resulting in greater variation thermal microclimates with concomitant effects on variation in birth date within this site.

The variation in birth date across sites and years within sites in response to temperature during gestations has implications for understanding how *N. ocellatus* might respond to future warming climate. It has been forecasted that temperature in Tasmania is projected to rise by at least 2.9 ºC under a high greenhouse gas emissions scenario and by about 1.6 ºC under a low greenhouse gas emissions scenario (Climate Futures for Tasmania 2010). As demonstrated in this study, date of birth will be enhanced by ~ 6 days and ~ 5 days for every 1 ºC increase in temperature at the warm lowland and the cold alpine sites, respectively. Accordingly, a modest warming will potentially advance birth

dates in *N. ocellatus* by 1-2 weeks at these sites. In the short term, earlier birth dates could enhance offspring survival as has been demonstrating with this species previously (Wapstra et al. 2010) and in other reptile taxa more generally (Schwarzkopf and Shine 1991; Doughty and Shine 1998; Itonaga et al. 2012). In the longer term, shifts in phenological may continue to be positive provided there is no mismatch with phenological processes with other species on which *N. ocellatus* are reliant. Specifically, in other systems there have been strong fitness cost to earlier births with a trophic mismatch (Both and Visser 2001, 2005; Marshall et al. 2007); as yet we have little data on the how well matched births are to specific prey abundances. Potentially, negative effects may be felt less in a species such as *N. ocellatus* that take a wide variety of terrestrial arthropods opportunistically, including cryptic and hidden prey in accordance with a wide ranging foraging strategy (Wapstra and Swain 1996) and that is able to actively forage for a variety of prey type from birth. Further work is also required to model the potential demographic and life history responses that result from earlier birth and higher growth rates (e.g., potentially altering age and size at maturity; Wapstra et al. 2001). This study adds to the evidence of how a wide range of ectothermic species, exemplified by *N. ocellatus*, respond to variation in environmental conditions in which potentially could be used to understand the potential impacts of directional climate change to natural populations.

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Chapter 6

General Discussion

Thermal environments vary geographically and over time and they can have direct and indirect effects on natural populations of both endotherms (e.g., Porter et al. 2000; Bicudo et al. 2010; Carrascal et al. 2012) and ectotherms (e.g., Adolph and Porter 1993; Angilletta et al 2002b; Angilletta 2009). The effects might be stronger in ectotherms because they rely on their thermal environment to maintain their body temperature via thermoregulation, and their body temperature subsequently will affect their physiological processes, e.g., metabolic rate (Schaefer and Walters 2010; DeVries et al. 2013), energy expenditure (Christian et al. 2003, 2007), developmental and growth rates (Wapstra et al. 2001; Sears and Angilletta 2003; de Queiroz and Ashton 2004; Sears 2005) and locomotor performance (Angilletta et al. 2002a; Fernandez et al. 2011). Since body temperatures in ectotherms can be affected by their thermal environment, their body temperatures may vary across thermally different areas (e.g., different latitudes or altitudes); this variation in body temperature has been linked to variation in the life history between populations in a wide-ranging ectothermic species (Grant and Dunham 1990; Adolph and Porter 1993; Rohr 1997; Sears and Angilletta 2003; Sears 2005).

In this thesis, an integrated approach has been taken to determine the flexibility in thermal biology of a wide ranging ectothermic species, the spotted snow skink, *Niveoscincus ocellatus*. Specifically, I studied three populations of this species along an altitudinal gradient and across seasons to understand and compare population-specific responses to different environmental conditions. I also investigated the species' capacity to respond to seasonal changes in temperature which reflect their capacity to respond to short term changes in environmental temperature (i.e., seasonal changes). Further, studies of the thermal biology of a species can provide fundamental knowledge that allows us to understand species' response to future climate change (e.g., Kearney et al. 2009; Sinervo et al. 2010; Huey et al. 2012; Ceia-Hasse et al. 2014). I used the knowledge gained in my study as a base to predict some of the potential effects of future climate change on this species and on ectotherms in general.

Below, I revisit figure 1 from the General Introduction chapter to demonstrate how the studies described in this thesis integrates to add to our knowledge of the thermal biology of

a widely distributed ectothermic species allowing the species to occupy divergent habitats, and how that knowledge can be used as a base to predict some of the potential effects on future climate change on the species. The dashed square represents the studies conducted to answer the first broad aim of this thesis. As is well known, thermal environments strongly affect ectotherms through physiology (chapters 2, 3, 4), phenology (chapter 5) or distribution. The animals may respond to the variation of thermal environment through adaptive process. To avoid the risk of fatalities, ectotherms may respond to variation in thermal environment either through thermoregulatory behaviour and/or through physiological adjustment to optimize their performance and/or physiological processes. The knowledge obtained through those studies (the dashed square) then is used as a base to predict some of the potential effects of future climate change, namely on physiology and phenology on the study species, as well as on ectotherms in general (chapters 2, 3 4, 5).

I began by investigating variation in the thermal biology (field active body temperature and thermal preference in the laboratory) across seasons in three populations of the spotted snow skink, *Niveoscincus ocellatus*, collected at three sites along an altitudinal gradient in Tasmania (Chapter 2). Since body temperature is related to many physiological processes in ectotherms, the following studies were focused on other key physiological traits, namely locomotor performance (Chapter 3), energy expenditure (Chapter 4, Yuni et al. 2015), and phenology (represented by date of birth) (Chapter 5), that could be affected by body temperature in this species. By using a combination of laboratory and field studies, I showed that the different thermal environments experienced by populations living along the altitudinal gradient had significant influences on these key physiological traits in *N. ocellatus.* Field active body temperature (Chapter 2), the relationship between locomotor performance and temperature (Chapter 3), energy expenditure (Chapter 4, Yuni et al. 2015), and phenology (Chapter 5) all varied geographically between populations in this widespread species. Specifically for the locomotor performance (Chapter 3), there was geographic variation in the performance breadth (B_{80}) due to variation in the lower bound of B_{80} (L_{80}) in *N. ocellatus*. Conversely, I found that the upper bound of B_{80} (H_{80}) remained stable across sites.

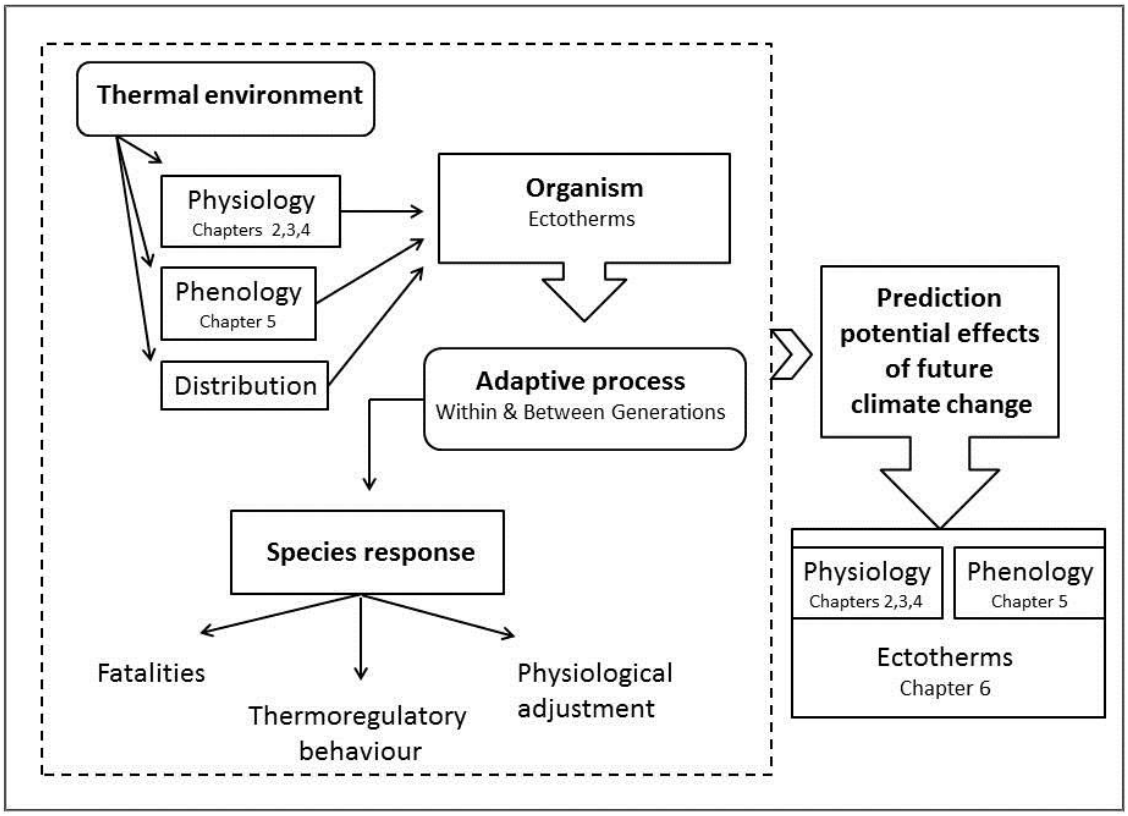

Figure 1 (revisit from Chapter 1). This diagram demonstrates how the components of this thesis (enclosed by the dashed square) adds to our knowledge of thermal flexibility of a widely distributed ectotherm allowing the species to occupy divergent habitats, and shows how that knowledge can be used as a base to predict some of the potential effects (e.g., on physiology, phenology) of future climate change on the study species, as well as on ectotherms in general.

To avoid the risk of fatalities due to variation in their thermal environment, ectotherms may respond to the variation through thermoregulatory behaviour (Seebacher et al. 2004; Blouin-Demers and Nadeau 2005; Kearney et al. 2009; Beldade et al. 2011; Zamora-Camacho et al. 2013; Sunday et al. 2014). How ectotherms are then affected by the temperature variation depends on the extent to which temperature variation can be buffered via behavioural thermoregulation (reviewed by Clusella-Trullas and Chown 2014). Active field body temperature and physiological optima in locomotor performance in *N. ocellatus* varied across sites (Chapter 2 and 3). This variation could be related to the ability of this species to buffer the temperature variation across their sites via thermoregulatory behaviour. *Niveoscincus ocellatus* are strong thermoregulators since they were able to reach their preferred body temperature, including those individual living at the high altitude site (Chapter 2). A higher thermal opportunity at the lower altitudes allowed *N. ocellatus* to achieve a high body temperature in all seasons via behavioural thermoregulation. However, the situation is different for those at the high altitude since the lizards struggled to reach their preferred body temperatures, especially in autumn (Chapter 2). Restricted thermal opportunity such as at the high altitude site may constrain the lizards' ability to achieve a high and stable field active body temperature via behavioural thermoregulation (e.g., Ibarguengoytia et al. 2010; Zamora-Camacho et al. 2013) unless they bask extensively in order to achieve a high and stable body temperature (Huey and Slatkin 1976; Vidal et al. 2010). In the field, however, increased basking intensity is not without cost, including increased risk of predation, increased costs of metabolism, and potentially decreased time available for other activities such as foraging (Huey and Slatkin 1976; Wapstra and O'Reilly 2001; Sears 2005; Angilletta 2009; Isaksson et al. 2011).

To avoid the additional costs and potential risks of the extensive basking intensity, animals may adjust their physiology to shift their optimal temperatures for physiological and physical performance (Seebacher 2005; Angilletta 2009; Beldade et al. 2011). To be active at low temperatures, in ectotherms living in cold environments (high altitude/latitude), basic physiological processes such as digestion, foraging, and performance can be optimised at lower temperatures (Clarke 1993; Lourdais et al. 2013). It has been suggested that colder environments, such as at the high altitudes or latitudes, should lead to the evolution of lower thermal optimum; and more variable environments, such as at the high latitudes, should lead to the evolution of a wider tolerance of performance breadths (Lynch and Gabriel 1987; Angilletta 2009). A tolerance to low

temperatures is also understood to evolve faster than tolerance to high temperatures in lizards (Munoz et al. 2014), which evolves in about 35 generations in tropical lizards (Leal and Gunderson 2012). In this study, the optimal temperature for locomotor performance down-shifted at the high altitude site (Chapter 3), that had a colder and more variable environmental temperature than those at the lower altitude sites (Chapter 2,3,4,5, see also Pen et al. 2010). I also demonstrated that *N. ocellatus* has the ability to be active at lower and at a wider range of body temperatures at the high altitude site than at the lower altitude sites (Chapter 2 and 4; Yuni et al. 2015). This can be achieved by lizards from colder environments by maintaining a higher metabolic rate at low temperatures compared to their counterparts (Clarke 1993; Lourdais et al. 2013): this phenomenon is known as metabolic cold adaptation (MCA) (Clarke 1991, 2003; Hare et al. 2010; Schaefer and Walters 2010; White et al. 2012). I demonstrated that MCA may occur in *N. ocellatus.* Male lizards from the extreme cold of their distribution expended a higher rate of energy per gram per hour than animals at the warm lowland site, even after accounting for differences in activity patterns (Chapter 4; Yuni et al. 2015). The ability to perform in cold temperatures would be under strong selection in *N. ocellatus* since it is crucial in assisting the lizards in at least two different ways. Firstly, the broader tolerance to low temperature will increase the lizards' survival through predator avoidance. It is always cold when the lizards emerge out from the ground, and they will be more vulnerable to predator during this period. A broader tolerance to low temperature will allow the lizards to sprint to escape from predators without the need of prolonged basking to reach their optimal body temperature. In *N. ocellatus*, the lower bound of locomotor performance was at ~ 26 ºC whilst their optimum temperature for locomotor performance was at ~ 31 ºC (chapter 3). Secondly, tolerance to cold temperature will allow the lizards to increase their period of activity so that more time will be available for foraging and other activities. I observed that broader tolerance to low temperatures allow *Niveoscincus ocellatus* at the high altitude site to be active for a longer period of time than their counterparts at the low altitude site (chapter 2, 3 and 4; Yuni et al. 2015).

In contrast to the ability of *N. ocellatus* to adjust their optimal performance at low temperatures, there was no evidence for differences in thermal dependence of performance between sites at the higher temperatures (e.g., the upper bound of B_{80} (H_{80}) for locomotor performance was stable across sites) (Chapters 3). The lack of flexibility at high temperature in this species is likely related to their ability to avoid high temperatures via

thermoregulatory behaviour (see also Bonino et al. 2011; Grigg and Buckley 2013; Hoffmann et al. 2013). As is typical of shuttling heliothermic lizards, I observed that *N. ocellatus* displayed distinct bimodal activity patterns, reducing their activity in the middle of the day on warm/hot days and microhabitat shifting between sunny and shady patches. Thus, as they can avoid the high temperatures via their thermoregulatory behaviour, the ability to adjust to high temperature would be under weaker selection across their range than adjustments of performance at low temperature (e.g., Munoz et al. 2014).

In addition to *N. ocellatus* coping with geographic temperature variation, individual lizards may also need to respond to short term temperature change within their site (i.e., across seasons). There was evidence of seasonal shifts in the breadth of performance and L_{80} of endurance (chapter 3), as well as in active field body temperature (chapter 2) toward the change of temperature within site. When a species is exposed to new environmental conditions and its phenotypic response is a good match with the new conditions, the species can adjust to the new conditions without a change in genotype (Lynch and Gabriel 1987; Ghalambor et al. 2007; Hoffmann and Sgro 2011). The response of body temperature toward the short term temperature variation was in agreement to expectations with more tolerance toward low temperature during colder season (autumn versus spring and summer) (chapter 2). Those results suggest the potential for plastic responses to contemporary thermal conditions, and provide evidence that such effects could contribute to between site differences in active field body temperature in *N. ocellatus*.

Plasticity, however, may diverge between populations of a species due to differences in environmental heterogeneity faced by those populations (e.g., Husby et al. 2010; Vitasse et al. 2013; Yampolsky et al. 2014): such differences can promote evolutionary processes for local adaptation (Kawecki and Ebert 2004; Pigliucci et al. 2006; Hoeksema and Forde 2008). I only found limited evidence of seasonal effects on L_{80} as a result of seasonal effects on B_{80} of endurance, and the direction was contrary to my expectations that there would be greater tolerance to lower temperature during the colder season (e.g., Seebacher et al. 2003). This result indicated that plasticity is a relatively unimportant mechanism in allowing *N. ocellatus* to perform at lower temperatures. The stronger site effect than season effect in locomotor performance displayed by this species, however, suggests the potential for local adaptation. By being locally adapted to the environmental conditions they consistently experience, the lizards can reduce the cost of directional selection of temperature changing (e.g., extensive basking) at their distribution site (Chapter 2,4,5,

Yuni et al. 2015, see also Cadby et al. 2014). The potential for local adaptation was supported by a recent phylogeographic work in *N. ocellatus* that revealed strong evidence of strong population affinities (i.e., evidence of restricted gene flow between isolated populations) within a larger widespread clade from which my three populations were chosen (Cliff et al. 2015). Plasticity and local adaptation therefore may play a significant role in allowing *N. ocellatus* to cope in changing environmental conditions. This study provides evidence that a combination of local adaptation and plastic responses allows *N. ocellatus* to cope with the climate variation across their range (Melville and Swain 2000; Cadby et al. 2010, 2011, 2014; Uller et al. 2011; Yuni et al. 2015).

Geographic variation in body temperature and energy expenditure have been linked to variation in life history in ectothermic species (Adolph and Porter 1993; Rohr 1997; Sears and Angilletta 2003; Sears 2005). Body temperature is positively correlated with growth rate, which subsequently results in geographic variation in body size and age of first reproduction within species (Sinervo and Adolph 1989; Grant and Dunham 1990; Sinervo 1990; Niewiarowski 2001). The geographic variation in the field active body temperature and energy expenditure found in this study links to the distinct life history variation previously observed in *N. ocellatus* across different climatic regions: individuals are considerably larger in body size at maturity (and larger overall) at the colder extremes of the species' range (Wapstra et al. 2001). *Niveoscincus ocellatus* exhibited different growth patterns at the warm lowland and cold alpine sites; there were few differences in growth rate early in life, then it was started to diverge at around three years of age which consistent with the age at which individuals from the warm site mature, whereas individuals from the cold alpine site continue to grow rapidly prior to maturity 1-2 years later (Wapstra et al. 2001). Animals with lesser access to thermal opportunity allocate more energy for growth than their counterparts with more access to thermal opportunity (Niewiarowski 2001). For *N. ocellatus*, the active season is shorter by about 2 months in the high altitude due to more limited thermal opportunity than in the low altitude (Wapstra et al. 1999); thus the lizards at the high altitude have more limited opportunity to reach their preferred temperature (Chapter 2). I suggest that the elevated metabolism at low temperatures achieved via metabolic cold adaptation (Chapter 4; Yuni et al. 2015) could also maximize growth and developmental processes at the lower temperatures experienced by the high altitude population (see also Clarke 1993; Lourdais et al. 2013).

Metabolic cold adaptation helps to facilitate physiological processes in ectotherms, but the rise in metabolic rate represents a cost to the organism (Clarke 1993; Lardies et al. 2004; Cano and Nicieza 2006; Pörtner et al. 2008), including the generation of the reactive oxidative metabolites (ROM) as a by-product of normal metabolism (Speakman 2005; Janssens and Stoks 2013). ROM production is a process regulated by energy metabolism that may be associated with the ageing, lifespan processes, fitness, and survival in free-living populations (Speakman 2005; Nussey et al 2009; Barreto and Burton 2013; Janssens and Stoks 2013). Plasma antioxidant (OXY) is generated to neutralize the ROM production (Isakkson et al. 2011), although any increase in investment in the antioxidant system can only come at cost to investment elsewhere, i.e. reduced investment in reproduction or increased risk of death (Monaghan et al. 2009; Janssens and Stoks 2013). A study to explore how OXY activity and ROM was associated with the animals' life history was conducted in *N. ocellatus* from the high altitude site (Isaksson et al. 2011). Interestingly, they found no significant associations between female reproductive investment (litter size or litter mass) or parturition date and the oxidative (ROM) and OXY status. In this species, parturition date is significantly related to gestation temperature (chapter 5) which is driven by variation in female basking behaviour (Wapstra 2000; Wapstra et al. 2010). Consequently, increased basking will increase the temperature-dependent metabolic rate, as well as enhancing embryonic development. However, Isaksson et al. (2011) found no link between the parturition date and the oxidative stress parameters. Their study, therefore, does not support the suggestion that increased metabolism (independent of fecundity) or high fecundity (independent of basking behaviour) imposes an increased oxidative stress. Accordingly, I would suggest that the high metabolic rate at the high altitude does not impose any significant cost related to reproductive investment in *N. ocellatus*.

My study on the flexibility in thermal biology of *Niveoscincus ocellatus* allow an understanding of population specific responses to different environmental conditions which may be important in allowing the species to occupy divergent thermal habitats. Further, the knowledge obtained can be used as a base to predict some of the potential effects of future climate change on this species and on ectotherms in general (e.g., Sinervo et al. 2010; Huey et al. 2012; Ceia-Hasse. 2014). For example, animals with operative body temperature close to their critical thermal maximum (CT_{max}) limit are predicted to be prone to the increasing air temperature related to climate warming unless they have the capacity to behaviourally thermoregulate to buffer the increase of air temperature (Sunday et al.

2014). Physiological data on population response to different thermal conditions are also required to predict the impact of climate change in animal distribution via mechanistic ecophysiological models (Peck et al. 2009; Kearney et al. 2009).

Climate Futures for Tasmania (2010) reported that Tasmanian temperatures had risen since the 1950s, but at a slower rate (0.10 °C per decade) than mainland Australia (0.16 °C per decade). Daily minimum temperatures in Tasmania have risen more than daily maximum temperatures, which is consistent with other regions of the globe. Tasmanian temperature is projected to rise by about 2.9 °C under high greenhouse gas emissions scenario and by about 1.6 °C under low greenhouse gas emissions scenario (Climate Futures for Tasmania 2010). The mean maximum air temperature at which *N. ocellatus* were active were 26.1 ± 0.3 °C, 24.6 ± 0.3 °C, and 20.2 ± 0.5 °C at the low, mid, and high altitudes, respectively. If the air temperature increases as it is predicted in the future, the mean air temperature even in summer will be still below the upper thermal limit (H_{80}) for locomotor performance (Chapter 3) and the critical temperature maximum (CT_{max}) for *N. ocellatus* which was 41.7 °C (Caldwell et al. 2015).

Since *N. ocellatus* are strong thermoregulators (chapter 3), the increasing air temperature as predicted by Climate Futures for Tasmania (2010) will benefit this species in at least three ways. Firstly, it will facilitate *N. ocellatus* reaching their thermal preferences for extended periods, especially at the high altitude, before reaching the limit of behavioural buffering (Kearney et al. 2009; Huey et al. 2012). This prediction has been confirmed by a recent modelling study on *N. ocellatus* (Caldwell et al. 2015). By incorporating thermal preference data from my study (see chapter 2), they confirmed that the number of days in which the maximum predicted air temperature falls within the thermal preference for *N. ocellatus* is projected to increase by between 10 – 30 % under both high and low emissions scenarios. The modest warming will benefit the high altitude population that has a wider B_{80} of locomotor performance due to variation in L_{80} over the short term because extensive basking (with its associated costs of predation) may not be required to reach body temperatures within their B_{80} (see chapter 3). Secondly, a warming climate will potentially extend their activity into the cooler months (Kearney et al. 2009; Caldwell et al. 2015), enabling high altitude populations of *N. ocellatus* to be active for a longer period than at present. Longer activity periods near their thermal optimum will optimise basic physiological processes such as digestion, foraging and performance in this population, without the demand to increase the energetic cost of maintenance as at present (Yuni et al. 2015; chapter 4). The extended growing season at high altitudes may also

affect life history traits; potentially including age and size at maturity with concomitant effects on reproductive output (see Wapstra et al. 2001; Wapstra and Swain 2001). Thirdly, a modest warming will potentially advance birth dates in *N. ocellatus* by 1-2 weeks at both cold alpine and warm lowland sites. In the short term, earlier birth dates could enhance offspring survival as has been demonstrated with this species previously (Wapstra et al. 2010) and in other reptile taxa more generally (Schwarzkopf and Shine 1991; Doughty and Shine 1998; Itonaga et al. 2012).

Although *N. ocellatus* is predicted to potentially obtain benefit from the modest future warming, increases in extreme hot events may result in body temperatures exceeding their CT_{max}, which could result in fatalities (Sinervo et al. 2010; Ceia-Hasse et al. 2014; Caldwell et al. 2015) especially for those that already living in warmer environments (i.e., lower altitude sites). The fatalities, however, could be avoided if a species has a well-developed capacity for behavioural thermoregulation which is supported by the availability of shade (e.g., vegetation cover) so that animals can shuttle between sun and shady patches to maintain their optimal body temperature without the risk of overheating (Adolph and Porter 1993; Chamaille-James et al. 2006; Kearney et al. 2009; Huey et al. 2012). *Niveoscincus ocellatus* displayed a distinct bimodal activity that involved periods of avoidance of hot areas in the middle of the day or reduction in activity on hot days, which allowed them to remain at or near their preferred body temperature (L. Yuni *pers.obs*.). Shaded habitat substantially reduces exposure to extreme conditions (Scheffer et al. 2014). Thus thermoregulation plays a significant role as a buffer to allow *N. ocellatus* to remain at or near their preferred body temperature during the warmest times.

To summarize, the influence of temperature on the physiological characteristics of the spotted skink, *Niveoscincus ocellatus*, across sites and seasons was examined in this study. Populations of *N. ocellatus* responded differently to the variation in their environment which appears to be determined by the difference in the level of temporal and spatial temperature they typically encounter. The combination between thermoregulatory behaviour and physiological adjustments, namely downward shifts in field active body temperature and physiological optima for locomotor performance, wider tolerance toward low temperatures, and elevated metabolic rate, act as important buffers that allow *N. ocellatus* to overcome changes in their environmental conditions. Both thermoregulatory behaviour and physiological adjustment are likely important mechanisms that allow *N. ocellatus* to occupy a wide geographic area, especially in populations coping with the cold

at high altitudes. This study also indicates that *N. ocellatus* will potentially benefit from the modest future predicted climate change. Therefore, I suggest that the effect of climate change on temperature dependent species could be different to the dire predictions made for other reptiles in tropical and desert areas (e.g., Sinervo et al., 2010; Huey et al. 2012).

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Appendix 1. Description of study area

The study was conducted near Orford (147°44'E; 42°33'S; 50 m asl), at Tooms lake near Oatlands (147º45'E; 42º12'S; 550 m asl), and at the Central Plateau (146°45'E; 41°55'S; 1050 m asl), which were chosen because they represent the full climatic (and altitudinal) gradient occupied by *N. ocellatus*. Orford has a warm coastal temperature with a mild winter. The dolorite scree slope lining with a shallow soil and numerous rocky outcrops dominates this area. The vegetation is dry sclerophyll forest with an open canopy (photo 1), including the White peppermint gum *Eucalyptus pulchella*, the White gum *E. viminalis* and the Silver wattle *Acacia dealbata*. Understorey vegetations are sparse, including spreading Swordsedge *Lepidosperma longitudinale*, Sagg *Lomandra longifolia*, Kangaroo grass *Themeda triandra*, Native cranberry *Astroloma humifusum* and the Pink mountain berry *Cyathoides parviflora*. Others herpetofauna found during the study period were the metallic skink *Niveoscincus metallicus*, White's skink *Egernia whitii*, Blotched bluetongue *Tiliqua nigrolutea*, She-oak *Cyclodomorphus casuarinae*, Eastern three-lined skink *Bassiana duperreyi* and Tiger snake *Notechis ater*.

The Central Plateau study site is situated at the Miena Dam by the Great Lake in the Central Highlands of Tasmania. It is a cold temperate inland subalpine site, at which snow or hail can fall in any month. The study site was composed of large outcrops of dolorite boulders forming a boulder-wall (photo 2). The vegetation consists only of patchy grasses *Poa* sp. along the boulder-wall. Others herpetofauna found were the metallic skink *N. metallicus* and white-lipped snake *Drysdalia coronoides*.

Tooms lake near Oatland is intermediate in temperature between Orford and the Central Plateau. The dolorite scree slope lining with a shallow soil and numerous rocky outcrops dominates this area (photo 3). The vegetation is dry sclerophyll forest with an open canopy, including the Silver wattle *Acacia dealbata*, the Sallow wattle *A. mucronata*, the Australian blackwood *A. melanoxylon*, the Silver banksia *Banksia marginata*, the Black peppermint *Eucalyptus amygdalina*, the Tasmanian blanket leaf *Bedfordia salicina*, and the Native olive *Notelaea ligustrina*. Understorey vegetations are sparse, including the Bracken fern *Pteridium esculentum*, the Sword sedge *Lepidospermum laterale*, the Sagg *Lomandra longifolia*, and the Manuka myrtle *Leptospermum scoparium*. Others herpetofauna found during the study period were the metallic skink *Niveoscincus*

metallicus, White's skink *Egernia whitii*, Eastern three-lined skink *Bassiana duperreyi* and Tiger snake *Notechis ater*.

Photo 1. Study site at Orford (the low altitude site or warm extreme site).

Photo 2. Study site at Miena, the Central Plateau (the high altitude site or cold extreme site).

Photo 3. Study site at Tooms lake, near Oatland (the mid altitude site or intermediate temperature site).