Morphological and physiological determinants of performance and associations to thermoregulatory preferences in an alpine skink, *Eulamprus kosciuskoi*

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Abstract

Performance is dependent on a combination of morphological and physiological features in the context of present environmental factors. Sprint speed is important in the fitness of many animals and is used to represent performance. This study explored the effects of temperature and features of body condition on performance of an Australian alpine skink, *Eulamprus kosciuskoi*, along with reproductive costs, using sprint speed to represent performance. Further, the effect of size on preferred body temperature was analysed to investigate the determinants of behavioural thermoregulatory responses that are associated with performance. A sample of 21 lizards consisting of juveniles, males and gravid and non-gravid females were caught in December in the Kosciuszko National Park, Australia. Lizards were raced at cold and hot temperature treatments, temperature preference tests were conducted and morphological features measured. Performance was significantly greater at higher temperature and correlated positively with body mass but not with relative tail length. Gravidity incurred no significant performance cost, and no significant correlation was found between preferred body temperature and size. Understanding the determinants of performance, along with temperature regulation effects, has conservation implications in the context of rising temperatures associated with climate change.

Key Words

Alpine water skink, performance, sprint speed, thermoregulation

Introduction

Sprint speed is of capital importance to the fitness of many animals (Zamora-Camacho *et al.* 2015). Speed is relevant in many predator-prey interactions, where an individual might flee from a predator or use speed to catch prey (Lima 2002). Further, speed can affect social dominance (Garland *et al.* 1990) and has been related to mating success (Husak *et al.* 2006; Byers *et al.* 2010).

The alpine water skink, *Eulamprus kosciuskoi*, is one species in which speed could play an important role in fitness. *Eulamprus kosciuskoi* is a lizard endemic to Australia, inhabiting regions of Victoria and southern New South Wales in alpine and subalpine environments near water sources (Department of Sustainability and Environment 2003). Sprint speed is used in this study as an indicator of performance, where performance is assumed to be reflected in fitness.

Performance of an individual is dependent on the combination of its morphological and physiological features in the context of the present environment (Bauwens *et al.* 1995; Moore and Hopkins 2009). In the context of sprinting, for example, performance is dependent on the anatomical composition of the body and muscles, and the metabolic processes that fuel muscle activity (Bauwens *et al.* 1995). Metabolic rates are highly dependent on temperature due to thermal kinetic effects on the chemical reactions taking place, being faster at higher temperatures (Angilletta 2009; Angilletta *et al.* 2010), and therefore sprint speed is dependent on body temperature according to the metabolic affects. This is a relationship that is consistent across ectothermic and endothermic organisms, including lizards (Van Damme and Vanhooydonck 2001), and the basis for this study on *E. kosciuskoi*.

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Reproduction is one life history event which can incur great performance costs, and this has been seen in lizards to be exhibited in substantially slower sprint speeds and stamina (Cooper Jr *et al*. 1990). *Eulamprus kosciuskoi* are viviparous (bear live young) however, like other related skinks, they display a reproductive strategy in which they do not bear offspring every breeding season in which they are fertile (Schwarzkopf 1993; Department of Sustainability and Environment 2003).

Lizards are ectotherms and, like other thermoregulating ectotherms, have an optimal temperature at which their performance peaks (Stapley 2006; Clusella-Tullas and Chown 2014). As a result of their body temperature being dependent on the external environment, lizards have developed efficient thermoregulatory responses to achieve and maintain optimal, or preferred, body temperatures (PBT) in different thermal environments for performance purposes (Stevenson 1985). Whilst preferred body temperature exhibits interspecific variation, interestingly, intraspecific variation within given populations can also occur (Stapley 2006). Differences in thermal preferences can be associated both with trade-offs between the thermal sensitivities of different physiological processes and also variation in environmental circumstances; for example, high temperatures might facilitate faster locomotion but incur greater metabolic costs, with PBT thus varying with relative abundances of food and predators (Stapley 2006). Stapley (2006) also explored effects of body size on performance in *Pseudemoia entrecasteauxii*, an alpine skink of Australia inhabiting similar areas to *E. kosciuskoi*, but found there to be no correlation in this species.

With performance being a multifaceted phenomenon determined by a wide range of influencing factors, this study aims to explore the morphological and physiological determinants of performance on *E. kosciuskoi*, particularly in the context of temperature. Using sprint speed to represent performance, the effects of temperature on sprint speed, according to physiological effects, was tested along with analysis of the relationship between sprint speed and morphological traits and the costs of reproduction. To provide context and further understanding of temperature responses, the effects of size on behavioural temperature regulation responses were analysed. We predicted that the lizards will run faster at higher temperatures and the conditional measurements of body mass and relative tail length (tail length to snout ventral length ratio) will positively correlate with sprint speed. We also predicted a performance cost to be associated with gravidity in the form of slower sprint speeds, and PBT to show variation depending on body mass.

Studying the effects of temperature on performance, and how temperature is regulated to maximise performance, is important in understanding how organisms are going to be influenced by increasing temperatures associated with climate change and how they might respond. This is particularly relevant to *E. kosciuskoi* which is adapted to an environment that experiences temperature extremes.

Methods

All lizards for this study were caught alongside Spencers Creek in the Charlotte Pass village (36.43°S, 148.33°E) in Kosciuszko National Park, Australia, in the month of December by method of a dental floss noose attached to a fishing rod or stick; 21 skinks were caught in total. The lizards were kept in labelled holding containers at room temperature throughout the study period. Particular morphological features were recorded: snout ventral length (SVL; tip of snout to cloaca at tail base), and tail length (cloaca to tail tip) were measured using a ruler; head length and width, using Vernier calipers; and body mass, using scales. The sex and maturity of each lizard were determined, and the conditional features of tail state (original/regrown) and gravidity (for females; gravid or not) were recorded. To test sprint speed, lizards were coaxed to run along a walled 1 m track by chasing them with a rectangular (to fit the track) object moving at a consistent speed. Infrared gates recorded the time taken for the lizard to run between 25 cm intervals, with the lowest time (i.e. fastest) from each test recorded. Lizards were run at cold (~18°C) and hot (35°C) temperatures, with three runs per trial and two trials at both treatments. The cold treatment was achieved by racing the lizards with their body temperatures at ambient temperature, and the hot treatment after leaving the lizards to warm in an oven at 35°C for approximately 1 hour. Thermal preference tests were conducted using a thin chamber with a temperature gradient ranging from approximately 18 to 40°C. The temperature gradient was achieved using heat tape along a metal base; where it was lacking in one end, had one strip through the middle, and was doubled over to make two strips at the other end. Lizards were placed in the middle of the chamber and then left undisturbed for 20 minutes to position themselves along the temperature gradient as desired; at 20 minutes their body temperature and the temperature of their final position were recorded.

The recorded sprint times were converted into a speed by the following formula to give a speed in m/s:

$$sprint\ speed\ \left(\frac{m}{s}\right) = \frac{0.25m}{recorded\ time\ (s)} \qquad (1)$$

Data analysis

To analyse performance at different temperatures, a paired *t*-test was conducted to test for difference in sprint speed between the cold and hot treatments. Similarly, an independent t-test between sprint speeds of gravid and non-gravid females was conducted to test for the difference in performance due to gravidity. To analyse the effects of body size on sprint speed, body condition on sprint speed, and body size on PBT, regression analyses between these variables were conducted. Statistical analyses were conducted using Microsoft Excel 15.0.4971.1000 (Microsoft Corporation, 2012) and JMP 12.2.0 (SAS Institute Inc. 2015).

Results

Eulamprus kosciuskoi had significantly higher sprint speeds in the higher temperature treatment than the cold temperature (Figure 1), with lizards running an average 1.207 m/s faster in the high temperature treatment (n = 21, paired *t*-test: $t = 9.624$, $p < 0.001$).

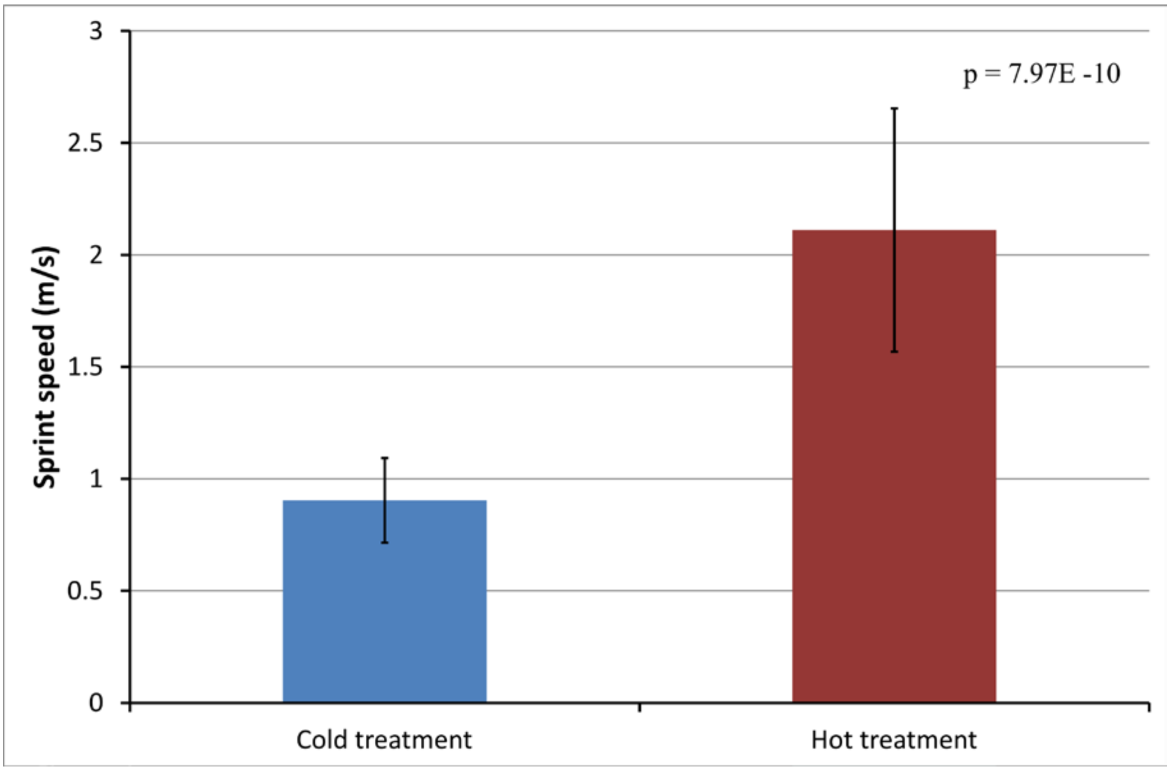

Figure 1: The average maximum sprint speeds (m/s) of *E. kosciuskoi* at cold (18°C) and hot (35°C) temperatures, with the probability value (*p*) from the associated t-test included. Error bars represent ± 1 SD.

There was a significant positive correlation of moderate strength between sprint speed and body mass, indicating faster sprint speeds for larger lizards (Figure 2) (n = 21, $r = 0.539$, $p = 0.006$). However, there was no significant correlation between sprint speed and relative tail length, which was our measure of body condition (Figure 3) (n = 21, $r = 0.184$, $p = 0.212$). Gravid females were not significantly slower than non-gravid females (Figure 4) (n = 11, 2-sample t-test: $t = 0.659$, $p = 0.268$).

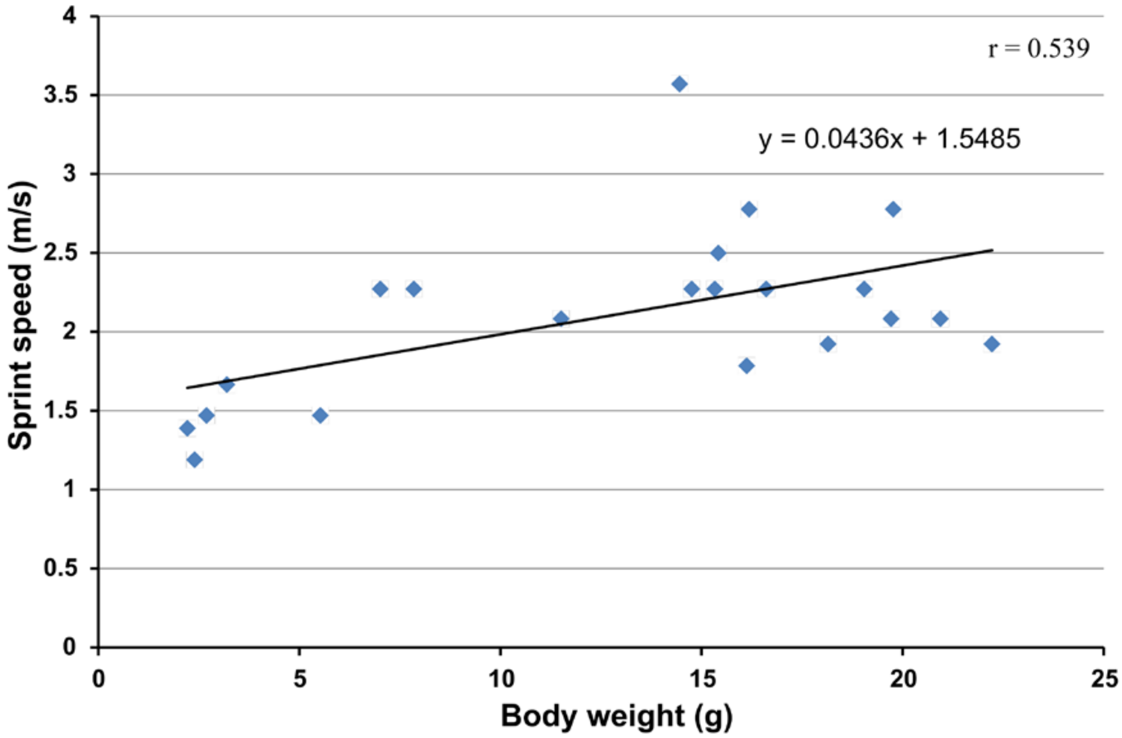

Figure 3: The relationship between maximum sprint speed (maximum speed at optimal temperature) (m/s) and body mass (g) in *E. kosciuskoi*, with the trend-line and Pearson's correlation value (*r*) included.

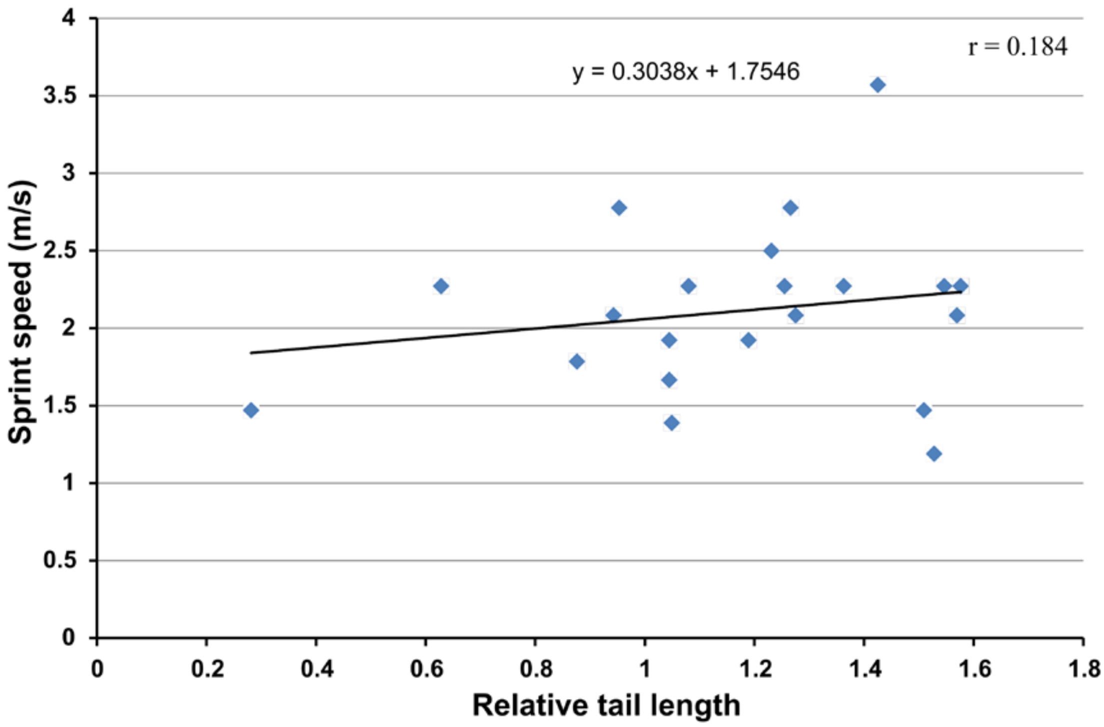

Figure 3: The relationship between maximum sprint speed (maximum speed at optimal temperature) (m/s) and relative tail length in *E. kosciuskoi*, where relative tail length is representative of body condition, with the trend-line and Pearson's correlation value (*r*) included.

Across all lizards, the mean PBT was 30.84 ± 3.44°C (n = 21). All sizes of lizards preferred a similar temperature, with no notable correlation between body mass and PBT (Figure 5) (n = 21, r = 0.054, p = 0.817).

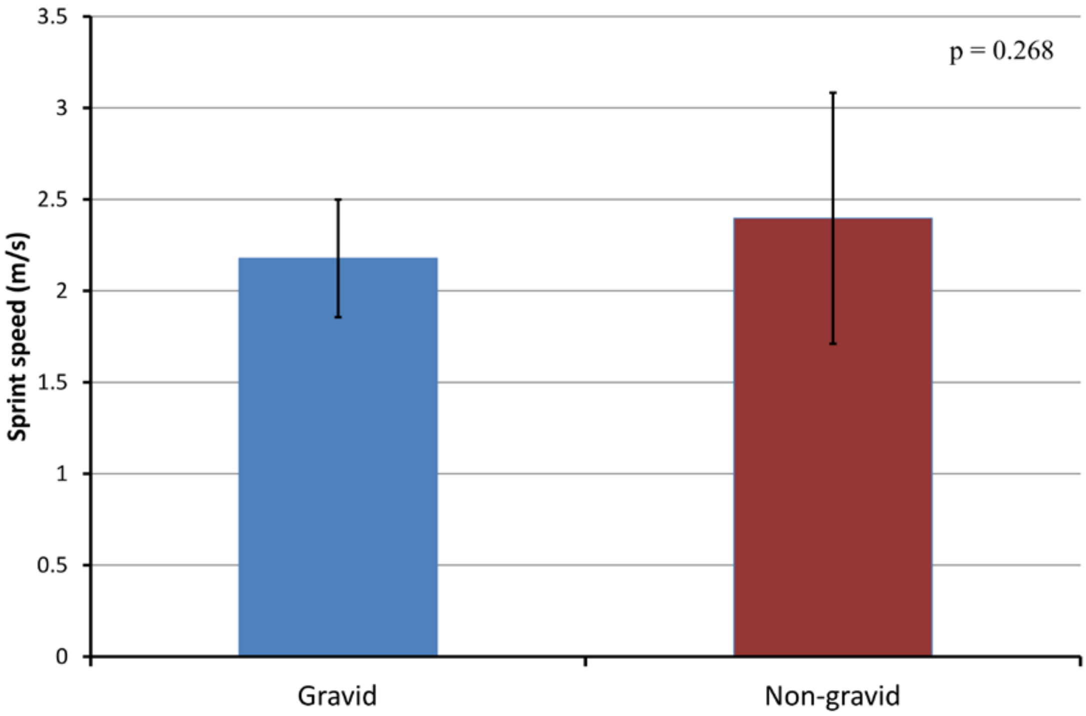

Figure 4: The mean maximum sprint speeds (m/s) of gravid and non-gravid female *E. kosciuskoi*, with the probability value (*p*) from the associated *t*-test included. Error bars represent ± 1 SD.

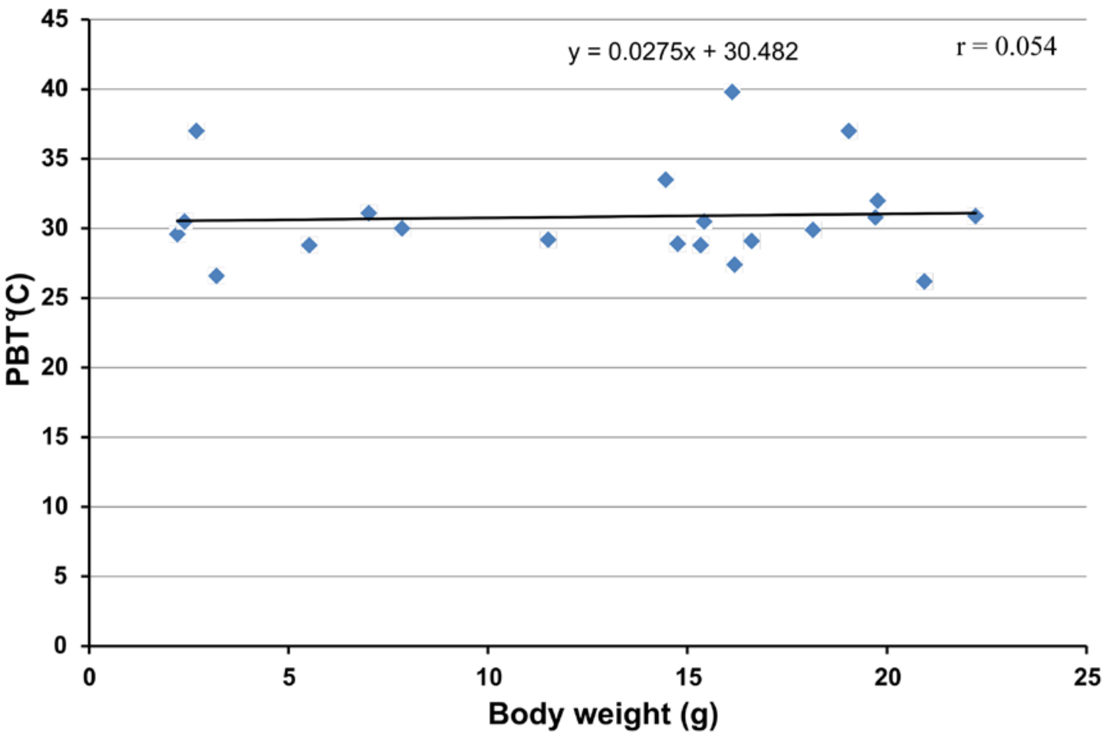

Figure 5: The relationship between body mass (g) and preferred body temperature (PBT, °C) in *E. kosciuskoi* measured after 20 minutes of warming from suboptimum body temperature, with the trend-line and Pearson's correlation value (*r*) included.

Discussion

Temperature directly links to performance through interaction with morphological and physiological features. *Eulamprus kosciuskoi* performance was greater at higher temperatures and in general increased with body condition, whilst gravidity seemingly did not manifest in a significant performance cost. While morphology can define the dynamics of heat balance, thermoregulatory behaviour showed little variation among individuals according to the morphological feature of size, with no relationship between temperature preferences and body mass found.

The results of this experiment demonstrate a varied response to the range of expected relationships associating performance and its determinants. Firstly, with sprint speed representing performance, *E. kosciuskoi* performed better at higher temperature (Figure 1). This result matches the hypothesis and also conforms with literature regarding the effect of temperature on sprint speeds in lizards and the notion of optimal temperature for animal function (Van Damme and Vanhooydonck 2001; Stapley 2006; Clusella-Tullas and Chown 2014). Higher temperatures increase metabolic rate which in turn facilitates greater energy expenditure (Angellitta *et al*. 2010). This is the limit of the extent to which the relationship between performance and temperature can be inferred from this study, however; as sprint speeds were only tested at two temperatures, this study cannot suggest an optimal temperature. This would require a larger array of tested temperatures, including those at which sprint speed decreases. This is a limitation of the study; however, it also provides a feasible opportunity for future research that has significance in the context of rising temperatures associated with climate change.

In this study, condition of the lizard was represented in two measures; body mass and relative tail length. These exhibited varying levels of certainty in their appropriateness at representing performance; however, this is likely a reflection of their suitability at representing lizard condition in this context and not necessarily a trade-off scenario between the two regarding performance.

As expected and in accordance with research by Van Damme and Vanhooydonck (2001), larger lizards ran faster (Figure 2); further, this effect was more pronounced at the higher (closer to optimal) temperature. This is important to note, as it could suggest a relationship where being bigger is better under optimal conditions but can be a burden when conditions fall below optimum, where effects of larger size such as greater metabolic rate (Andrews and Pough 1985) and decreased relative locomotor capabilities (Garland 1985) incur additional performance costs. This would have ecological significance in being a driver of size selection depending on environmental conditions. Another factor that is likely to contribute to this trend is that the difference in sprint speed between optimum (at higher temperatures) and suboptimum (at lower temperatures) conditions was less, in absolute terms, for smaller lizards. The metabolic rate to mass ratio gives an indication of mass-specific metabolic costs, so by comparing this to sprint speed we could determine the potential costs of larger body size under different conditions. This would allow us to explore whether the observed trend is due to the latter effect.

Similarly, lizards with better tail condition were faster (Figure 3); however, the correlation between these variables was insufficient for this relationship to be said with confidence. In particular, the relationship being non-significant means that it cannot be said that the correlation has not occurred due to chance, and could be due to a confounding variable. Whilst it could be inferred that tail condition does not affect sprint speed, this result is likely due to limited variation in tail condition within the sample, i.e. too few individuals with each of the tail states (where it can be original, half-regrown or recently dropped, for example). Variability was observed in the measurement of tail and body lengths by different individuals; incorporation of this variability into the results may have also had an effect, but not likely influenced the overall relationship. The overall trend was again stronger at the higher temperature, this with potential implications as discussed above.

Gravid females were on average slower than non-gravid females; however, comparably to relative tail length, the result was not statistically significant (Figure 4). A performance cost of reproduction in *E. kosciuskoi*, exhibited in the form of slower sprint speeds, can therefore not be suggested from this study, as has been reported in other lizards by Cooper Jr *et al*. (1990). A high performance cost was expected in *E. kosciuskoi* as a possible reason for their inconsistent breeding cycle (not always annually) (Schwarzkopf 1993); evidently, further study into the drivers of this reproductive strategy is needed.

Lack of significant effect may be associated with late seasonal effects causing the lizards to be early in their gestation periods and thus suffering less pronounced effects of carrying young when the study was conducted.

In regards to temperature preferences of *E. kosciuskoi*, no notable correlation was found between body mass and PBT, a result that was not expected but which confirms the null hypothesis. Variation associated with size was expected on the reasoning that a smaller surface area to volume ratio accompanying greater size would cause temperature regulation to be slower (Kleiber 1972). Accordingly, larger lizards might opt to maintain a higher (closer to optimum) body temperature to account for what is effectively a slower response time in terms of temperature regulation and thus give them the best chance at maximising performance; consistent with the effect of thermoregulating organisms to limit exposure to suboptimal temperatures (Bogert 1949). The negative result is also comprehensible, however, given resemblance in the trait exhibited by the *P. entrecasteauxii* that shares a similar niche (and direct habitat) (Stapley 2006). While thermal preferences may not be dependent upon size, there may be association with another variable feature that can contribute to performance. It should be noted that the result may incorporate significant error based upon the procedure of the temperature preference tests; the tests relied on the assumption that the lizards would not be disturbed throughout the test duration; however, this could not always be verified due to feasibilities of the study facility. Understanding the time taken for lizards to heat up would also allow better experimental design and application of the thermal preference test method.

The temperature effects explored in this study have relevance in the current context where climate change is causing temperatures to increase (Collins *et al.* 2013). According to the effects of temperature on performance observed in this study, with increasing temperatures it may be easier for *E. kosciuskoi* to reach and maintain optimum body temperatures; however, this study does not explore at what temperatures the effect will be detrimental. In suggesting this potential performance 'benefit', due to inhabiting alpine environments, *E. kosciuskoi* could be adversely affected by increasing temperatures by being limited geographically; they cannot migrate to higher elevations to negate the temperature increase effect (where temperature decreases with elevation). Understanding the ability for adaptation to warmer temperatures is important in understanding how *E. kosciuskoi* will handle pending temperature increases. Further research exploring the temperature effects and adapted responses on sister species in the *Eulamprus* genus, such as *E. quoyii*, that inhabit environments of higher temperatures in northern Queensland (O'Connor and Moritz 2003), could elucidate important information on the potential responses of *E. kosciuskoi* in the alpine/subalpine environment with implications in managing conservation approaches. Future research into the costs of producing offspring would be valuable in understanding the specific reproductive strategy of *E. kosciuskoi*; a potential area for research where this study was limited.

Conclusions

Rising temperatures pose a threat to organisms globally. With performance so linked to temperature, studying temperature effects and behavioural responses is critical in understanding the future ecology of *E. kosciuskoi*. In this study, performance was greater at higher temperatures and in general increased with body condition, whilst female reproductive costs did not exhibit an effect on performance. In regards to thermal regulation preferences, which performance can be dependent upon, size did not have an effect. Whilst this study confirms the application of some conventional concepts of performance determinants to this understudied species, uncertainty and inconclusiveness of other results prompts further study of the temperature effects and responses of *E. kosciuskoi*, with significance in conservation interests within the current context of environmental change.

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