

# The effect of elevation and temperature on metabolic intensity of *Eulamprus kosciuskoi* in Kosciuszko National Park

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## Abstract

The effects of size, elevation and temperature on the metabolic intensity of *Eulamprus kosciuskoi* (Kinghorn, 1932) were determined by measuring the metabolic rate of 25 skinks from two locations with a 200 m difference in elevation using closed system respirometers. Skink size did influence metabolic rate, but to a relatively small degree, suggesting other factors also play a role. Skinks from higher elevation had extremely high metabolic intensity at 18°C, possibly as this improves fitness. This would benefit them if they face greater intraspecific competition, more predation, different food availability or diseases specific to their environment. Skinks from the lower elevation increased their metabolic intensity with temperature in accordance with the metabolic theory of ecology. Surprisingly, skinks from the higher elevation followed the opposite trend, possibly to prevent overheating. This study demonstrates that skinks do adapt or acclimate to different environments, which could aid their conservation in the face of climate change. It contributes to our understanding of *E. kosciuskoi* and to the debate surrounding thermal ecology and metabolic cold adaptations in ectotherms. It also paves the way for further research into alpine water skink ecology and the effect of elevational gradients on physiology.

## Key Words

Adaptation, ectotherm, MCA, physiology, thermal ecology

## Introduction

Elevation gradients result in a variety of different environmental conditions, including changes in oxygen content of the air, amount and time of year of snow or rainfall, temperature, air pressure and wind intensity (McCutchan and Douglas 1985). There may also be different types and amounts of vegetation, predators and prey (Colwell and Hurtt 1993). These factors mean animals at different elevations face different selective pressures, which can result in divergent adaptations to their specific environments. Variation in adaptations can even occur within species (intraspecific variation) (Iraeta *et al.* 2008). Alternatively, animals of the same species may exhibit different traits as they acclimatise to separate environments (Zari 2016). Metabolic rate (MR) can vary within species at different elevations and refers to the sum of all metabolic reactions taking place in an organism when it is fasted and at rest; it reflects the minimum energy expenditure required of an animal (Liang *et al.* 2017). Metabolic intensity (MI) accounts for the mass of the organism (Seymour and Lillywhite 2000).

Multiple studies have found that traits including clutch size, rate of growth and maturity, social behaviour and aggressiveness vary between lizards of the same species at different elevations (Ruby and Baird 1994; Iraeta *et al.* 2008). Studies of intraspecific lizard metabolism have indicated that MR is faster at warmer temperatures (which is associated with low elevation), as enzymes can work faster, speeding up biochemical reactions (Zagar *et al.* 2015). However, it has been found that groups of lizards at high elevations adapt to have faster MR than their low elevation counterparts of the same species when measured at the same temperature. Such adaptations compensate for low temperature and ensure they can generate sufficient energy in their environment (Yuni *et al.* 2015). These adaptations are referred to as metabolic cold adaptations (MCA), and are due to altered physiology (specifically greater mitochondrial density facilitating energy transformation). There is some scientific debate as to whether MCA is a genuine phenomenon in ectotherms, with different studies uncovering conflicting evidence (Addo-Bediako *et al.* 2002). However, MCA has been found in numerous species of geckos in New

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Zealand, and in some snake species (Yuni *et al.* 2015). Different elevations are also associated with differences in size (length and mass) of animals, with larger animals generally being found in colder environments. This is because larger lizards may be less able to thermoregulate in a warm climate (Zari 1991). Size also influences MR, as differently sized lizards often have different energy requirements (Hulbert and Else 2003). Large lizards typically have higher MR due to their greater energy requirements (Zari 1991).

The aim of this project was to determine the relationships between elevation, temperature, size and MR in the alpine water skink, *Eulamprus kosciuskoi* (Kinghorn 1932). The alpine water skink is a native Australian species found in the mountainous Kosciuszko region along elevation gradients (at elevations over 1,400 m) (DNRE 2000). It is hypothesised that skinks will have higher MI when warmed to a higher temperature than when at a cooler temperature, as biochemical reactions in their bodies will be quicker (Zagar *et al.* 2015). It is also expected that skinks from a higher elevation will have faster MR than skinks from a lower elevation, as such an adaptation would be necessary to ensure they have enough energy to survive in a cold environment (Yuni *et al.* 2015). Additionally, it is likely that skinks will be larger at higher altitudes, and that larger skinks will have faster MRs (Hulbert and Else 2003).

The significance of this work is that it will increase our limited understanding of water skinks, and of ectotherms more broadly. Expanding our knowledge may improve our ability to conserve the alpine water skink, which is especially important as the species is critically endangered in Victoria (DNRE 2000). Additionally, understanding the way skink physiology relates to temperature could help us understand their potential to survive climate change (Huey and Kingsolver 1993). This is especially important as ectotherms are often regarded as some of the most vulnerable species to climate change due to their strong dependence on the temperature of their surroundings for survival (Sinervo *et al.* 2010).

## Methods

### Lizard collection and measurement, temperature data

All materials were provided by the Research School of Biology at The Australian National University. The experiment was conducted in Kosciuszko National Park in December 2016. Eight *E. kosciuskoi* were collected from Rainbow Lake (elevation 1,636 m) on a sunny day (around midday). The skinks were caught using dental floss nooses tied to fishing poles – the nooses were lifted around skinks' necks, and the fishing rods were pulled to tighten (preventing escape). Tape was numbered and left on each of the rocks where the skinks were found, and the same numbers were drawn on each bag the skinks were placed into so they could be identified. The skinks were taken back to the ski lodge and were placed in plastic boxes containing water, sand, a roll of bark and cardboard. Each box was numbered according to the number on the skink's bag. This process was repeated at Spencers Creek (elevation approximately 1,830 m), with 22 lizards being collected from this site.

The snout–vent length (SVL) and tail length of each skink were determined by pressing the lizard against a clear ruler and stretching out their bodies. The weight of each skink was determined by placing the skink in a bag and onto the scales, and then weighing the bag separately and subtracting it from the original mass. The sex of lizards can be found by turning each lizard on its back and pushing out its pelvic section. Juveniles which were so young their sex could not be determined were not included in any further study, as any differences in size or metabolism could be due to age rather than elevation.

Temperature iButtons were placed at the two field locations with foil coverings to prevent solar radiation influencing data readings. Three iButtons were used at Spencers Creek which began logging data on the morning of 13 December 2016 (between 12:05 and 12:17am) and finished on 15 December 2016 (between 7:45 and 8:05am). They took temperature recordings every 30 minutes over the time period. One iButton was used at Rainbow Lake. It began logging at 1:00am 13 December 2016 and finished at 7:00am 15 December 2016, taking temperature recordings every 2 hours (different regimes were used at the two locations as the iButtons which were placed for this experiment malfunctioned, requiring the use of backups set by other researchers). However, it is known that elevation is inversely

proportional to temperature, and there was an elevational difference of 200 m between the two sites (McCutchan and Douglas 1985; McIntire *et al.* 2016; Pepin *et al.* 2016). It can hence be confidently stated that the two groups of lizards from different elevations will have experienced different temperatures throughout the year.

## Metabolic rate recordings

MR readings were taken using a FireStingO2 optical oxygen meter. Two large aluminium trays were filled with sand. The program Pyro oxygen logger was opened on a laptop and four glass chambers were plugged into the computer through an adapter. Two chambers were placed on the sand in each tray, so they were all in a line. The program was calibrated for temperature and amount of oxygen in the ambient air, and the settings were changed so that internal temperature of the FireSting box was used for this calibration (as it is deemed more accurate). The air temperature was  $\sim 18^{\circ}\text{C}$  whilst recordings were taken. One lizard was then placed in each chamber (and the number assigned to each lizard was recorded). The lizards were left in the chambers for a few minutes (until they appeared calm) to ensure they were at rest. The button 'log to file' was pressed, and recordings were taken for 15 minutes. After this time period, the data logging was stopped and four new lizards were placed in the chambers. This was repeated until each lizard from both locations had metabolic recordings taken.

These steps were then repeated, but with a heating cord placed under the chambers and a heat lamp over the lizards. The settings on the Pyro oxygen logger were changed so that external temperature was used, and the temperature probe was placed in the sand. The sand reached a temperature of  $\sim 30^{\circ}\text{C}$ . Fifteen minutes was allowed prior to recordings being taken to allow the lizards to warm up inside their chambers. These recordings were taken for the eight lizards from Rainbow Lake, and seven lizards from Spencers Creek which had weights and lengths within the range of those from Rainbow Lake (they were size matched so MR could be compared directly between the two locations). After all metabolic recordings were taken, each skink was returned to the exact location from which they were found.

## Statistical analyses

iButton data were exported from the program OneWire to Microsoft Excel spreadsheets. Only iButtons which had data within a selected time period were included (to ensure locations could be accurately compared). The average for each iButton was taken using Excel formulae, as was the mean 90 per cent range (excluding the top and bottom 5 per cent of temperatures to reduce the effect any unusually high or low values would have had on the mean). Height and weight data were managed using Excel (2016) formulae to determine the average and standard error for the different categories, and bar graphs were made.

Pyro oxygen logger data were exported to Excel spreadsheets. The oxygen content for each chamber through time and temperature columns were used. Any data outside of the intended time range (15 minutes) was deleted. The first and last three data points for each column were averaged to determine the first and final oxygen percentages (accounting for any slight variation in data due to equipment reading variability). The final oxygen was subtracted from the starting oxygen to determine the difference. The volume of oxygen in the chamber (mL) was determined by subtracting the mass of each lizard (g) from the known volume of an empty chamber (220 mL). The volume of oxygen consumed was determined by dividing the difference in oxygen by 100 and multiplying this by the volume of the chamber. The MR (mL/hour) was calculated by multiplying the volume consumed by four (as recordings were taken for 15 minutes, and MR is measured in mL/h). MR was divided by the mass of the lizard to determine MI ( $\text{mL g}^{-1} \text{h}^{-1}$ ).

MR was plotted against mass on a scatter plot in Excel. Both variables were plotted on a log scale so a linear trend line could be used. MR was used instead of MI so the data could be more easily compared to literature, as MR is more commonly used. Bar graphs were made in Excel using MI data. Unpaired *t*-tests were conducted for size, weight and warm and low MI between the two locations. Paired *t*-tests were conducted for ambient and warm MI comparisons within each location.

## Results

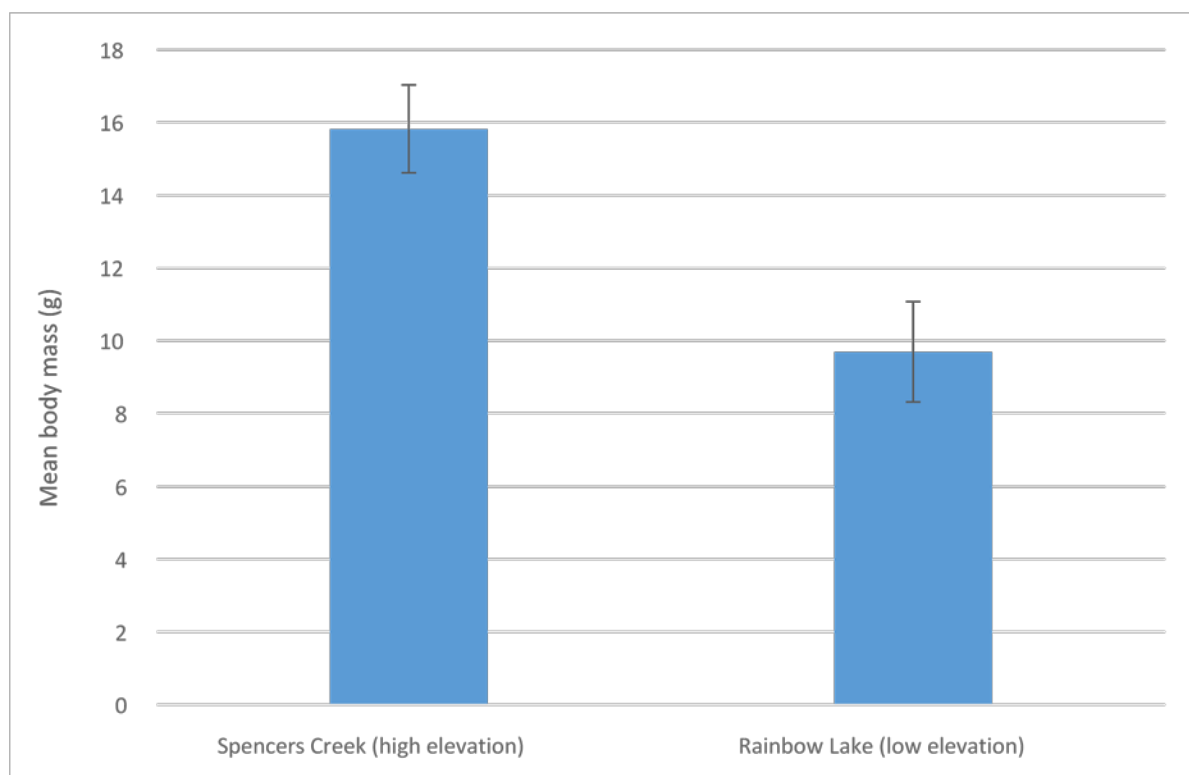
Qualitative Description: Rainbow Lake was a relatively grassy location, with some boulders present and smaller ones along the stream. Spencers Creek was a larger location, with a greater number of rocks present, especially in the sun along the edge of the water. Many skins were observed at both locations.

Spencers Creek had a mean temperature 5°C lower than Rainbow Lake over the days studied (Table 1). The mean 90 per cent temperature range represents the average temperature range excluding the 5 per cent highest and lowest values. Spencers Creek had a larger mean 90 per cent temperature range by 1.76°C (Table 1). Rainbow Lake also experienced higher maximum and minimum temperatures than Spencers Creek.

**Table 1: Average temperature data for three iButtons from Spencers Creek and one from Rainbow Lake.**

Location	Mean temperature (°C)	Mean 90% temperature range (°C)	Maximum temperature (°C)	Minimum temperature (°C)
Spencers Creek	11.43	15.36	27.63	19.55
Rainbow Lake	17.30	13.60	11.11	0.6

The mean body mass was 15.83 g (with a range of 5.60–22.77 g) for the Spencers Creek lizards and 9.69 g (with a range of 5.10–15.62 g) for the Rainbow Lake lizards. The average mass of lizards from Spencers Creek was significantly greater than those from Rainbow Lake (Figure 1). A *t*-test determined that the *p*-value is 0.0028.



**Figure 1: Mean body mass (g) of alpine water skinks from Spencers Creek (n = 17) and Rainbow Lake (n=8) (Kosciuszko National Park, NSW, Australia). Error bars represent standard error.**

The mean snout–vent length for the lizards from Spencers Creek was 92.58 mm, whereas the mean for Rainbow Lake lizards was 71.35 mm. The lizards from Spencers Creek were significantly longer than those from Rainbow Lake (Figure 2). A *t*-test determined that the *p*-value is 0.0005.

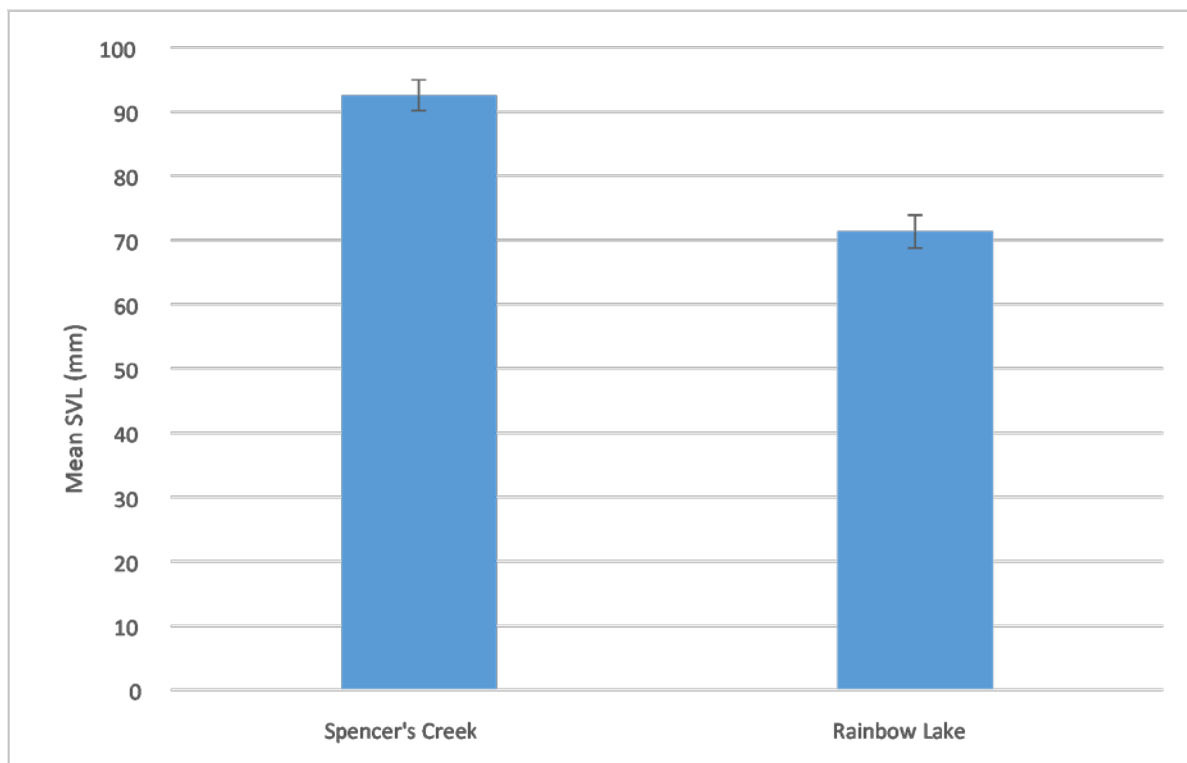


Figure 2: Mean snout-vent length (mm) of alpine water skinks from Spencers Creek ( $n = 17$ ) and Rainbow Lake ( $n = 8$ ). Error bars represent standard error.

Metabolic rate was positively correlated with body mass (Figure 3), with an  $R^2$  value of 0.37. This indicates that 37 per cent of the variation in metabolic rate can be explained by the variation in mass. The remaining 63 per cent is hence due to other variables.

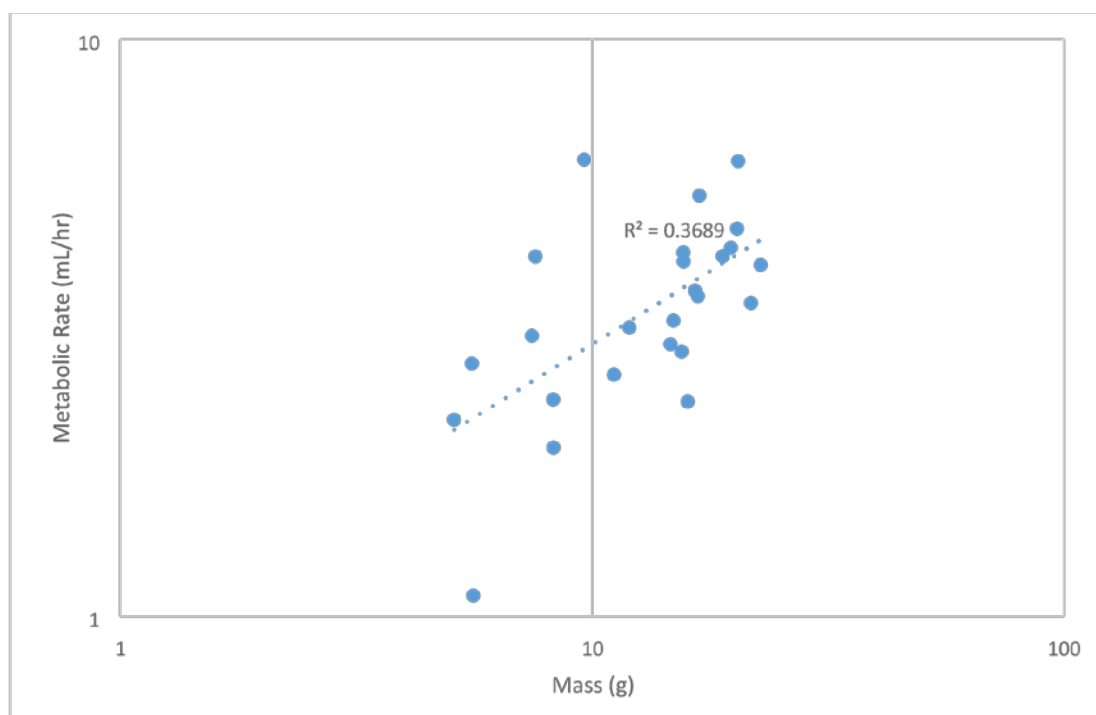


Figure 3: Effect of size (g) on metabolic rate (mL/h) in alpine water skinks ( $n = 25$ ) from Rainbow Lake and Spencers Creek.

At Rainbow Lake, lizards had a significantly higher MI at cooler temperatures than at warmer temperatures ( $t$ -test:  $p = 0.014$ ) (Figure 4). At Spencers Creek, MI was significantly higher at warmer temperatures ( $t$ -test:  $p = 0.027$ ). Comparing the cooler temperatures between the two sites, the  $p$ -value is 0.013 ( $t$ -test), with MI for lizards from Rainbow Lake being significantly higher. At the warm temperature, MI is not significantly different between lizards from the two locations, as the  $p$ -value is 0.085 ( $t$ -test).

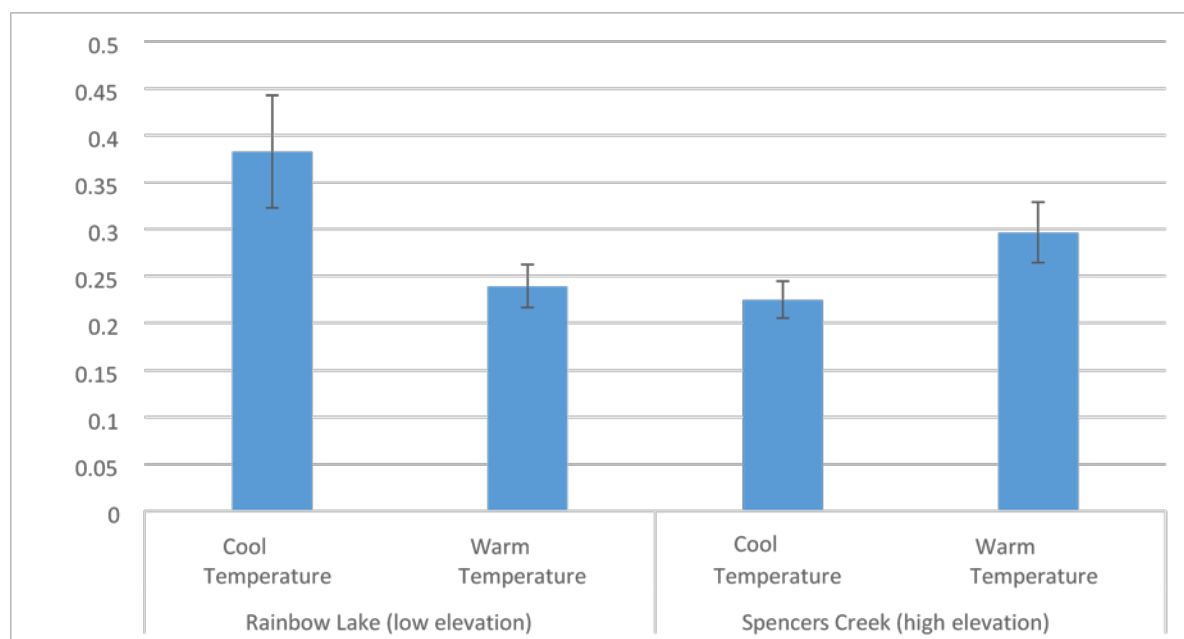


Figure 4: Metabolic intensity of size-matched alpine water skinks from Spencers Creek ( $n = 7$ ) and Rainbow Lake ( $n = 8$ ) at cooler ( $\sim 18^{\circ}\text{C}$ ) and warmer temperatures ( $\sim 30^{\circ}\text{C}$ ). Error bars represent standard error.

## Discussion

### Effect of elevation on size of alpine water skinks

The results indicate that lizards from the lower elevation location weigh significantly less than the lizards from the higher elevation (Figure 1), and are significantly shorter (as illustrated by their SVL in Figure 2). Additionally, it should be noted that the weight range of the Rainbow Lake skinks is 5.1–15.6 g, whereas that of Spencers Creek skinks is 5.6–22.8 g. The weight ranges suggest that there is selective pressure against large lizards at low elevations which is causing the difference in average size. The sample size is 25 lizards (8 from Rainbow Lake, 17 from Spencers Creek), so this result can be stated with a moderate degree of confidence. This finding is in accordance with Bergmann's rule, which states that animals are larger in colder environments (Jin and Liao 2015). At warm temperatures, a lower surface to volume ratio can be beneficial in thermoregulation, as heat is dissipated faster (McNab and Auffenberg 1976). A large lizard in a hot climate may struggle to lose excess heat if required. In contrast, the ability of large lizards to lose heat slowly may prevent cold stress in cooler environments. It should be noted that literature on other lizard species does not offer a single clear relationship between size and elevation, with a significant number following the opposite trend (having large lizards at warm temperatures) (Jin and Liao 2015). Therefore, an understanding of size clines in *Eulamprus* skinks contributes to our understanding of lizard size clines as a whole.

### Effect of size on metabolic rate of alpine water skinks

Overall, the trend in Figure 3 suggests that larger skinks have faster MR than smaller lizards. The  $R^2$  value suggests that 37 per cent of the variation in MR can be explained by size. The  $R^2$  value is fairly low, suggesting that there are other factors which are also influencing MR (e.g. sex of the lizards or gravidity, which were not considered in this study). However, the trend of larger lizards within species

having greater MR is strongly supported by the literature (Wood *et al.* 1978; Zari 1990). This can be explained by the greater amount of energy which is required to sustain a large organism necessitating faster MR (Steyermark *et al.* 2005). Also, large lungs and other organs facilitate greater oxygen uptake in big animals (Garland and Else 1987).

## Effect of elevation on metabolic intensity of alpine water skinks

It was expected that skinks at higher elevation would have higher MI. This adaptation would allow skinks to meet the greater energy requirements they would face in cold environments. Previous studies have referred to such phenomena as metabolic cold adaptation (MCA), in which greater mitochondrial density at high elevation increases energy uptake (Yuni *et al.* 2015). However, there was no significant difference between the average MI for the two populations at the warm temperature and the opposite trend was found at the lower temperature, with skinks from Rainbow Lake having significantly higher MI. Overall, it is apparent that the MI of low elevation skinks from Rainbow Lake is significantly higher than expected in relation to heat and relative to skinks from higher elevations. This suggests there is a difference between the two locations (aside from temperature), causing different MI at the lower temperature.

One of the major factors which influences MI is seasonal climatic variation (Burton *et al.* 2011). At warmer times of the year, the lizards may have more plentiful food available due to greater insect numbers or snowmelt (so conservation of energy will become a lesser priority). Additionally, an organism's energy requirements vary throughout the year depending on the timing of breeding and growth (Zari 2016). MR is associated with rate of growth, as the faster an organism can absorb and assimilate food into tissues, the quicker it will grow (Alvarez and Nicieza 2011). Studies have indicated that some animals increase their MR during favourable periods to allow them to grow faster (Burton *et al.* 2011). Focusing on growth while conditions are good allows lizards to concentrate energy on functions necessary for survival during the rest of the year. However, seasonal effects often vary with elevation. Higher elevation locations may have a shorter proportion of the year which is very warm, for instance (with warm periods potentially starting later) (Moser *et al.* 2009; Sheldon *et al.* 2015). If the alpine water skinks increase their MI during favourable conditions, it is possible that the skinks from Rainbow Lake are currently in such a period (with summer having just begun at the time the study was conducted). However, at the higher elevation, it is possible that the temperature is not warm enough for skinks to put large amounts of energy into growth (although this may occur slightly later in the year if temperatures increase later in summer).

MI can also be influenced heavily by competition. Animals with higher MI are more aggressive. This gives them an advantage when competing for resources, including for mates and territories (Artacho *et al.* 2013). The alpine water skinks are highly territorial, and fight each other to obtain the best territories possible (Done and Heatwole 1977). The density of lizards at the two locations is unknown; however, higher density may see selection for higher MI as there would be more lizards in competition with each other. It is possible that this is the case at Rainbow Lake. The number of suitable territories in the two locations is also unknown; however, from qualitative analysis, it appears that Spencers Creek had a larger number of rocks (including in the sunlight). Having more appropriate territories at the creek may reduce competition, and may explain why lizards at Spencers Creek do not have such high MI. Previous studies have noted that limiting resources can result in different elevational metabolic trends than would otherwise be expected (Cano and Nicieza 2006).

Interactions with other species may also contribute to the MR of skinks (Reuman *et al.* 2013). It has been found that animals with faster MR generally have faster sprint speed, so MR has implications for fitness (Frappell and Butler 2004). This is believed to be because fast MR is often due to large heart and lungs, a physiological adaptation related to heightened maximum sprint speed (Biro and Stamps 2010). If there are a larger number of predatory animals (such as birds which eat lizards) at Rainbow Lake, this could explain the higher MI at a cooler temperature (although the number of animals which prey on the lizards at each location is unknown).

The amount and quality of food available during early stages of growth and development (as a juvenile or even in utero) can also contribute significantly to the future MI of an organism (Burton *et al.* 2011).

This can be because the amount of nutrients available in youth will determine how much energy an organism can allocate for growth. Animals with high nutrient access can have larger organs, which generally results in faster, more efficient metabolism, and can also have different enzyme physiology (Burton *et al.* 2011). It is possible that differences in MI between Rainbow Lake and Spencers Creek are not due to selection or adaptation, but are instead due to better access to food at Rainbow Lake as juveniles. This is plausible, as elevation is very likely to have an effect on insect density (the lizards' prey) (Hodkinson 2005). Another explanation is the small possibility that some of the skinks from Rainbow Lake may be fighting a viral, bacterial or parasite infection (Freitak *et al.* 2003; Burton *et al.* 2011). Infection would cause an increase in oxygen consumption as the skinks require additional energy for the immune response or to accommodate for nutrients being lost (Burton *et al.* 2011).

### **Effect of warming on metabolic intensity in alpine water skinks**

Lizards from Spencers Creek had greater MI when they were in warm conditions than when at cooler temperatures (Figure 4). This result is as expected, and is supported by most literature which suggests that warmer temperatures see a greater number of enzymes reach activation energy, speeding up biochemical reactions (Yuni *et al.* 2015). This is covered under the metabolic theory of ecology, under which it is suggested that MR increases with temperature, and has been demonstrated using a wide variety of animals, including ectotherms (Marshall and McQuaid 2011). However, while this theory is widely supported, extremely few studies have focused on whether ectotherms from extreme environments conform to this theory, leading scientists to question its validity (Marshall and McQuaid 2011). Skinks from Rainbow Lake showed the opposite metabolic trend to those from the higher elevation, having significantly higher MI at cooler temperatures than when warmed. While this trait has been observed infrequently, it has been found in *Echinolittorina* snails (Marshall and McQuaid 2011). Marshall and McQuaid (2011) suggest that this is because the evolutionary life strategy of the snail requires that it prioritises conserving energy over fast metabolism. The skinks from Rainbow Lake appear to have quite high MIs at low temperature. The faster the MR, the greater the amount of heat produced as a by-product (Ruf and Fiedler 1999). As such, it is possible that the skinks have adapted lower MRs at high temperatures to prevent overheating.

### **Non-adaptive explanations for both metabolic results across elevation and temperature gradients**

It is possible that the metabolic results of this study are not reflective of an adaptation of the alpine water skinks to their respective environments. Some studies on small ectotherm populations have noted that inbreeding can cause maladaptive traits (Cano and Nicieza 2006). The population size of skinks at Rainbow Lake is not known (or the amount of gene flow between populations), so this cannot be ruled out as a theory, especially given the unexpected results. Additionally, some studies have detailed the effect of stress on MR in skinks, especially as they are removed from their natural habitat and placed in enclosures. While the skinks were given time to adjust to their enclosures before being tested, it is difficult to eliminate all effects of handling stress in animal studies (Langkilde and Shine 2006). It should be noted that this study does have limitations, particularly in regards to small sample size (with 15 lizards being used in total for temperature/elevation comparisons). Furthermore, it was conducted over a short time period, although MI may vary throughout the year.

### **Significance and suggestions for further research**

Whilst many questions are raised as to the biological pressures behind the results of this experiment, the findings are important. To date, the current research on elevational gradients on lizard size has been quite limited. As such, any data which contributes to our knowledge of the effect of elevation on physiology is of importance in increasing our understanding. It has been noted that modulating body size is a key response by many species to climate change (Carey and Sigwart 2014). Based on the finding that skinks are smaller in warm, low elevation environments, it is possible that warming in the alpine environment caused by climate change may see a morphological shift towards smaller skinks. From a conservation perspective, the differences in skink morphology at different elevations and temperatures is positive, as it demonstrates the ability of the skink to adapt to its environment, which is



also a vital trait for surviving climate change (Huey and Kingsolver 1993; Hoffman and Sgro 2011). The metabolic findings also suggest the ability of skinks to adapt to their environment, and demonstrate the importance of understanding the selective pressures which lead to common biological trends (Huey and Kingsolver 1993). However, it should be noted that there is still concern as to whether the skinks will be able to adapt to rapid climate change fast enough protect them from the risk extinction (Sinervo *et al.* 2010). The results of this paper contribute to our understanding of, and debate surrounding, thermal ecology and MCA (Yuni *et al.* 2015).

Future research into water skink ecology should be conducted with regards to competition, diet, activity periods, seasonal variation and predation. Knowledge of how these differ with elevation would greatly improve our ability to find a fitting explanation for the metabolic trends found in this study. Research could expand upon this project by testing the MI of alpine water skinks from a greater number of locations along an elevation gradient to see if the trend of decreasing MR with elevation is consistent. This could be interesting as some studies find that this trend holds true to a point, but then reverses at extreme elevation (Yuanning and Liao 2015). MR could also be tested across a greater range of temperatures, or the effect of different environmental factors which vary with elevation could be tested (such as oxygen pressure). Future research could focus on determining whether differences in metabolism are due to phenotypic plasticity or local adaptation. This can be determined by raising skinks in a common environment and seeing if there is still a difference between MI of high and low elevation animals. If the difference persists, it is indicative of adaptation (genetic change causing a difference), otherwise, it indicates plasticity (no genetic difference between groups at different elevation) (Sears *et al.* 2003).

## Conclusions

This report has shown that size, elevation and temperature do affect the MI of *E. kosciuskoi*. Whilst some of the results are as expected, there have also been surprising findings which warrant further research. There is a positive correlation between size and elevation, as predicted, and the MI of alpine water skinks from Spencers Creek increases with temperature. However, skinks from Rainbow Lake have extremely high MI at cooler temperatures, countering expectations regarding temperature and elevation. This demonstrates the complexity of biological relationships between organisms and their environments and points to the role of different selective pressures in determining phenotype. Overall, this study has added to our currently limited understanding of skink physiology at different elevations and contributed to discussion of elevational selective pressures and adaptation.

## Acknowledgements

I would like to thank Phillipa Beale for her support and assistance throughout this project. I would also like to acknowledge Adrienne Nicotra, Rachel Slatyer and Sonya Geange, and the other staff who attended the Field Studies in Functional Ecology Course (especially Wes Keys for organising the equipment). I would like to thank everyone who assisted in catching and measuring lizards for this project: Hannah Zurcher, Scott Keogh, Jack Keogh, Cameron McArthur, Tobias Gruber, Luke Bavin, Shoshana Rapley, Christiana McDonald-Spicer, Sarah Hazell Pickering, Tenzin Norzin, Isaac Bell, Matt Rolfe, Emily Dickey, Barry Kerton, Edmund Ransome Gilding and Phoebe Worth. I would also like to acknowledge Kosciuszko National Parks Australia for the opportunity to conduct the experiment in the National Park.

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