The effect of body mass on the mass specific metabolic rate of *Pseudemoia entrecasteauxii* and *Eulamprus quoyii*

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Abstract

Measuring metabolic rate is important to understand energy acquisition, transfer and expenditure within an organism. Mass specific metabolic rate is also known as metabolic intensity. The metabolic intensities of *Pseudemoia entrecasteauxii* and *Eulamprus quoyii* were measured within a closed system through the amount of oxygen depleted when at rest. There was found to be a negative correlation between the mass of the skink and the metabolic intensity, or the oxygen consumption per hour. The average mass of *P. entrecasteauxii* was found to be lower than *E. quoyii*, and *P. entrecasteauxii* was found to have a lower metabolic intensity than *E. quoyii*, which follows Kleiber's law. The difference in metabolic intensity may be influenced by the climate of the microhabitats that the skinks live in.

Introduction

All animals have chemical processes within them that keep them alive. Such processes include cell growth, brain and nerve function and respiration (Parry 1983). Metabolism is the term used to describe these processes of energy transactions that occur throughout the body and how the energy is transformed within the organism and the environment (Gillooly 2001).

The mass of an animal affects the rate of metabolism and this relationship is known as the mass specific metabolic rate, or metabolic intensity. This relationship is quantified by Kleiber's law (Kleiber 1947), which proposes that larger sized animals use less energy per cell to sustain their needs (Kalra et al. 2013). Kleiber's law states that metabolic rate of the organism increases at a rate proportional to its mass to the power of 0.75, which means that larger animals have a lower metabolic rate per unit mass compared to smaller animals. Thus, there is a negative correlation between the metabolic rate per unit mass, also known as metabolic intensity, and mass (Kleiber 1947). This means that smaller organisms have a higher metabolic rate per unit mass compared to larger organisms.

The measurement of metabolic intensity, and hence metabolic rate, is important to describe energy acquisition, transfer or expenditure within an organism. This can be done through measuring oxygen consumption (Weir 1949). Measuring oxygen consumption is a common method to measure metabolic rate. Within all vertebrates respiration is mainly aerobic, meaning they consume molecular oxygen for cellular respiration. The assumption is made that oxygen consumption is an estimate of the rate of energy metabolism (Schmidt-Neilsen 1990; Speakman 2013).

To measure the oxygen consumption, and hence the metabolic rate, a closed system is often used as the decline in oxygen is easily measureable. The organism is measured at rest to find out the standard metabolic rate (or basal metabolic rate in endotherms). Standard metabolic rate is what the minimum metabolic rate is to sustain the organism's life at the certain temperature.

Ectotherms have their standard metabolic rate dependent upon the surrounding temperature. Skinks are an example of an ectothermic animal. Skinks can be found in a wide range of habitats, including the Australian alpine region. Two such species that are found in the Australian alpine region are *Pseudemoia entrecasteauxii* (mountain log skink) and *Eulamprus quoyii* (eastern water skink). *Pseudemoia entrecasteauxii* can be found in a variety of preferably sunny habitats, such as on rocks and north-west aspects (Jellinek et al. 2004), whereas *E. quoyii* are commonly found nearby creeks or moist areas (Law and Bradley 1990). The unique microhabitats that these species live in reflect their optimal temperatures, at which their metabolic rates are at their highest. It would be assumed that *E. quoyii* has a lower optimal temperature based upon its microhabitat of near moist areas, as they would be cooler. Similarly, based upon the microhabitat of *P. entrecasteauxii*, it would be assumed that their optimal temperature is higher than that of *E. quoyii*.

The main aim of the experiment is to compare the metabolic intensities of the two skink species, *P. entrecasteauxii* and *E. quoyii*. This is explored by investigating the effect body size has upon the metabolic intensity as well as how the habitats of the skinks affect their optimal temperatures and therefore their metabolic intensities at a given temperature.

The predicted comparison of metabolic intensities of *P. entrecasteauxii* and *E. quoyii* is that *P. entrecasteauxii* should have a lower metabolic intensity compared to *E. quoyii*. This is because they are smaller organisms and their optimal temperature may be further from the ambient experimental temperature than that of *E. quoyii*.

Methods

The skinks were all caught around Rainbow Lake in Kosciuszko National Park on 9 and 11 December 2015. There were eight *Pseudemoia entrecasteauxii* and seven *Eulamprus quoyii*. The *P. entrecasteauxii* were caught on 9 December whereas the *E. quoyii* were caught on 11 December, both around 9 am to 12 pm. The weather on both days was sunny and slightly windy; however, it should be noted that 8 December had severe rain while 10 December had pleasant weather. The experiments were completed the day after capture and the skinks were placed back where they were caught on the day when experiments were completed. The skinks were caught by using mealworms to entice them within range of capture by hand. The skinks were housed separately in containers (~8 cm \times 13 cm \times 25 cm) with mulch, some water in a bottle cap and a cardboard tube for shelter. For the duration of the experiments, the skinks were not given any food.

The equipment used for measuring oxygen consumption was the Firesting Oxygen fibre-optic oxygen meter with Pyro-Science Oxygen Logger Software version >3.2. Four custom-built chambers were used in each trial and had Pyro-Science Contactless Fibre-Optic Oxygen Sensor Spots inside. Pyro-Science Basic Spot Adapters were attached on top of the spots using sticky tape and a needle was used to make a hole for the sensor. The chambers were calibrated to a stable amount of oxygen, which was as close to 21 per cent as possible, and the ambient temperature during experiments was kept at 17.5°C to 18.5°C through the use of a heat lamp. At night, the lamps were turned off to simulate the night environment and to keep the skinks less active. RESEARCHING FUNCTIONAL ECOLOGY IN KOSCIUSZKO NATIONAL PARK

The lizards were numbered in order of capture and the order in which they would be placed in the chambers was randomised using the random number generator implemented in Microsoft Excel. The chamber sizes used were 220 mL and 380 mL. The 220 mL chambers were used for *P. entrecasteauxii* and the 380 mL were predominantly used for the *E. quoyii*.

The lizards' oxygen consumption was measured at rest inside the chambers for 15 minutes. The measurement started when the skink was no longer breathing heavily, and if the oxygen percentage within the chamber lowered by 3 per cent or more within the 15-minute timeframe, the experiment would be stopped for that chamber due to a lack of oxygen. Furthermore, the skinks' behaviours were noted while in the chambers and during the transfer between the containers and chambers.

Three experiments were conducted for each lizard and their masses weighed. The data were averaged and the metabolic intensity calculated. The units used for metabolic intensity are mL of oxygen/gram/hour. The metabolic intensities (MI) of the skinks was calculated using the following formula:

 $\frac{MI = ((Start O_2\% - Finish O_2\% \times 100) \times ((Chamber Volume - Skink Mass) \times 4))}{(Mass of Skink)}$

Results

We found a negative correlation between the mass of the skink and the average oxygen consumption (Figure 1). Pseudemoia entrecasteauxii does not have a strong negative correlation between its mass and the oxygen consumption (Figure 1c), particularly when compared with Eulamprus quoyii, which has a stronger negative correlation between the mass and oxygen consumption. Furthermore, the average mass of E. quoyii is higher than that of *P. entrecasteauxii* (Figures 1a and 1b). When compared logarithmically, the correlation between the mass of *P. entrecasteauxii* and its metabolic intensity is not as strong as the correlation of mass and metabolic intensity of E. quoyii (Figure 1c). The strength of the correlations can be seen through the R-squared values (Figure 1c). Pseudemoia entrecasteauxii has an R-squared value of 0.7084 whereas *E. quoyii* has a value of 0.91823. A logarithmic comparison of the metabolic intensities between the two skinks shows that E. quoyii has a higher metabolic intensity than that of P. entrecasteauxii. In addition to this, the P. entrecasteauxii has a trend line lower than that of the *E. quoyii* (Figure 1c).

Skinks were observed to be heavily breathing and some were still moving around during the experimentation, which were often signs of stress. The skinks with a larger mass were observed to have a tendency to escape when the enclosures were opened.



Figure 1: Average oxygen consumption of a) *Pseudemoia entrecasteauxii*, and b) *Eulamprus quoyii* as a function of body mass, c) log of the mass of the skinks +1 against the log of oxygen consumption +1 at 18°C.

Note: The blue diamond plots are for *E. quoyii* and the red square plots are *P. entrecasteauxii*. All the data points had 1 added to them to give positive values.

Source: Authors' data.

Discussion

The results show a trend for larger *P. entrecasteauxii* and *E. quoyii* to have a lower metabolic intensity than smaller-sized *P. entrecasteauxii* and *E. quoyii* (Figures 1a and 1b). This is seen through the negative correlation between the oxygen consumption and the mass of the skink. The comparison of both *P. entrecasteauxii* and *E. quoyii* on a logarithmic scale (Figure 1c) shows that *P. entrecasteauxii* has a lower metabolic intensity than *E. quoyii* at the ambient temperature of approximately 18°C. The average body size of *P. entrecasteauxii* can also be seen to be less than that of *E. quoyii* within Figure 1c. The results agree with Kleiber's law, which states that there should be a negative correlation between metabolic rate per unit against body size (Kleiber 1947). The lower metabolic intensity and the smaller average body size of *P. entrecasteauxii* agrees with Kleiber's law that states smaller body sizes correlates to a lower metabolic intensity (Kleiber 1947). Furthermore, the lower metabolic intensity of P. entrecasteauxii may suggest that they have a higher optimal temperature, reflected by their habitats of sunnier, warmer areas when compared to E. quoyii.

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There were many limitations encountered when gathering and processing the field data. These include time constraints when allowing the skinks to rest before chambers were closed to begin the timing of oxygen depletion. Chambers were closed when the skinks were still heavily breathing to reduce the time needed for each trial. This could have led to skewed results because the skinks are inhaling more oxygen than if they were at rest.

The many assumptions required were also a limitation when processing the data. These assumptions include that the skinks were fasting, were not gravid, were of good health, were all similar ages, that the ambient temperature was consistently kept between 17.5°C and 18.5°C and that the same amount of oxygen results in the same amount of ATP. Obvious amendments to the method of the experiment would be doing the experiments in better-controlled areas of temperature and have consistent health of the skinks. The stage of fasting within the skinks could be controlled by having the skinks captive for a longer period of time, ensuring that the skinks are fed the same diet for a set amount of time before fasting them.

There have been no previous measurements of the metabolic intensities measured through oxygen depletion using a closed system for *P. entrecasteauxii* and *E. quoyii*. Further research to explore the metabolic intensities of the skinks includes investigating the microhabitats in which *P. entrecasteauxii* and *E. quoyii* are found, which can assist in determining the optimal temperatures for species based upon their habitats. Similarly, a study of the skinks' enzymatic activities can be done to further investigate the optimal temperatures within *P. entrecasteauxii* and *E. quoyii*.

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