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## Effect of sloughing and digestion on metabolic rate in the Australian carpet python, *Morelia spilota imbricata*

Graham G. Thompson<sup>A</sup> and Philip C. Withers<sup>B</sup>

<sup>A</sup>Centre for Ecosystem Management, Edith Cowan University, Joondalup Drive, Joondalup, WA 6027, Australia. Email: g.thompson@cowan.edu.au.

<sup>B</sup>Department of Zoology, University of Western Australia, Nedlands, WA 6907, Australia. Email: philip.withers@uwa.edu.au

### Abstract

The standard metabolic rate for juvenile carpet pythons, *Morelia spilota imbricata*, with a mean body mass of 129.6 g (range 57.7–253 g) increased from  $6.75 \pm 0.96$  (s.e.) mL h<sup>-1</sup> to  $42.6 \pm 12.40$  (s.e.) mL h<sup>-1</sup> in 48 h after ingesting mice equal to approximately 23% of their body mass, at a temperature of 30°C. Sloughing increased metabolic rate to approximately 146% of standard metabolic rate at 30°C. Metabolic rate is elevated before the eyes become opaque and other visual signs indicate that a slough is imminent. The implications of these two factors when measuring standard metabolic rate are discussed.

### Introduction

Recent technical and methodological advances as well as interest in metabolic respirometry has made precise measurement of standard metabolic rate ( $\dot{V}_{O_{2std}}$ ) a routine laboratory procedure. It is important when measuring  $\dot{V}_{O_{2std}}$  that the 'standard' conditions are clearly defined and adhered to (Andrews and Pough 1985; Hayes *et al.* 1992). One of the standard conditions for reptiles is a post-absorptive state (Andrews and Pough 1985). For example, the metabolic rate of juvenile Burmese pythons, *Python molurus*, increases up to 45 times over fasting  $\dot{V}_{O_{2std}}$  within 48 h of feeding (Secor *et al.* 1994; Secor and Diamond 1995) as a result of specific dynamic action (SDA), the increased metabolic rate associated with digestion, assimilation and biosynthesis of ingested food (Brody 1945; Kleiber 1975; Jobling 1981).

Although the process of sloughing in reptiles has been well documented (Maderson 1965; Maderson and Licht 1967; Roth and Jones 1967, 1970; Landmann 1979, 1986; Maderson *et al.* 1998), nothing is known of a possible metabolic cost of sloughing. Given the biochemical and physical processes involved in sloughing (Maderson 1966, 1967; Maderson and Licht 1967; Chiu and Maderson 1975; Maderson *et al.* 1998), the metabolic rate ( $\dot{V}_{O_2}$ ) might be expected to increase during the sloughing process.

The objective of this study was to investigate the influence of feeding and sloughing on  $\dot{V}_{O_2}$  for the Australian carpet python, *M. s. imbricata*.

### Methods

*Morelia spilota* is a medium-sized python found throughout continental Australia except in southern Victoria and the arid central and western areas (Cogger 1992). *M. spilota imbricata* is one of three subspecies (the others being *M. s. spilota* and *M. s. variegata*; Cogger 1992); it occurs in the south-west of Western Australia.

Six *M. s. imbricata* were hatched in captivity from a captive pair. Metabolic measurements were made for these six *M. s. imbricata* from when they were about 9 months of age over a period of approximately 6 months. These pythons were maintained under near-identical conditions and fed on mice of varying sizes. Before the commencement of digestion and sloughing studies, all pythons were fasted for a period of at least 14 days to ensure that they were post-absorptive (Secor 1995; Secor and Diamond 1995). Water was available at all times during routine maintenance and for at least 3 h prior to experimentation. Pythons had

access to an ambient temperature of 30°C for a minimum of 8 h per day for at least 14 days before experimentation to ensure that meals had been fully digested. Except during the feeding trial, all pythons were offered food at least every 2 weeks, and usually weekly. Some pythons occasionally refused to eat, presumably because of incipient sloughing, but none went without feeding for more than 5 weeks. All pythons were healthy and gained weight; none died during or after the experiments.

For measurements of SDA, the  $\dot{V}_{O_2}$  of each python was measured during the night preceding a meal. On the following day, each python consumed mice of mean mass approximately 23% ( $\pm 4.6$ , s.d.) of pre-feeding body mass. We selected a meal mass of approximately 25% of the python's body mass as this is similar to the relative prey sizes used by Secor (1995), Secor and Diamond (1995) and Bedford (1996), and because it approximated the meal size reported by Bedford (1996). Secor and Diamond (1997) have subsequently shown that SDA increases with meal size beyond 25% of body mass in some pythons. Following ingestion of the meal, we measured  $\dot{V}_{O_2}$  of each python nightly until  $\dot{V}_{O_2}$  returned to pre-feeding values. During these measurements, pythons were maintained at approximately 23.5°C for 12 h during the day and transferred to a metabolic chamber for approximately 12 h at night and maintained at 30°C. Water was available during the day.

For the sloughing trials,  $\dot{V}_{O_2}$  was measured for the six pythons for 3–5 days after their eyes had turned opaque and one night after the python had shed its skin. To demonstrate the change in  $\dot{V}_{O_2}$  during the sloughing period, the  $\dot{V}_{O_2}$  of two pythons was measured every night from the first signs of sloughing (opaque eyes or darkening and dullness of the skin colour indicating that a slough would occur within the next 14 days). These two specimens were maintained at a constant 30°C day and night, and were given access to water during the day but were not fed.

#### *Measurement of standard metabolic rate*

$\dot{V}_{O_2}$  was measured using a flow-through respirometry system and each python was measured separately. A python was weighed before being placed in an opaque plastic cylinder. Cylinder size was selected so that it confined, but did not restrict, movement. The cylinder was placed in a controlled-temperature chamber at 30°C ( $\pm 1^\circ\text{C}$ ). All  $\dot{V}_{O_2}$  values were adjusted for the  $<1^\circ\text{C}$  offset to a temperature of exactly 30°C, using a  $Q_{10}$  value of 2.5. A  $Q_{10}$  of 2.5 was selected because it is the mid-point of 2–3, the range of temperature coefficients for reptile metabolism (Bennett and Dawson 1976).  $\dot{V}_{O_2}$  was measured between 0000 (midnight) and 0800 hours, with the python being placed in the opaque plastic cylinder before 2000 hours on the previous day. This provided a minimum of 4 h for body temperature ( $T_b$ ) to equilibrate with ambient air temperature before measurement; we assumed that the  $T_b$  of all pythons was the same as the ambient air temperature ( $T_a$ ). On most occasions, the lowest  $\dot{V}_{O_2}$  recording was achieved between 0400 and 0800 hours, thus providing at least 8 h for body temperature to equilibrate with ambient air temperature.

Ambient air was pumped through the chamber at 100 mL min<sup>-1</sup> (Brooks mass-flow controller), which maintained an excurrent O<sub>2</sub> content greater than 20.0%. A 2-m length of copper tube in the incurrent air-flow line, located in the controlled-temperature chamber, ensured that the temperature of the air passing into the cylinder containing the python was within  $\pm 0.5^\circ\text{C}$  of the air temperature within the chamber. A humidity-temperature probe (Vaisala HMP 35B) in the excurrent air flow measured the temperature of the air ( $T_a$ , °C) in the metabolic chamber. A Drierite column removed water and an Ascarite column removed CO<sub>2</sub> from the excurrent air before it passed through one channel of a paramagnetic O<sub>2</sub> analyser (Servomex OA184). A microcomputer recorded the differential output of the O<sub>2</sub> analyser (ambient air – excurrent air) via a Thurlby digital volt-meter and a RS232 interface, and calculated the STPD  $\dot{V}_{O_2}$  every 60 s (after Withers 1977) commencing at 0000 hours. We calculated the standard  $\dot{V}_{O_2}$  from the average of the lowest continuous period of O<sub>2</sub> consumption (20-min duration) between 0000 and 0800 hours. Body mass was taken as the pre-meal mass in order to standardise mass-specific measurements.

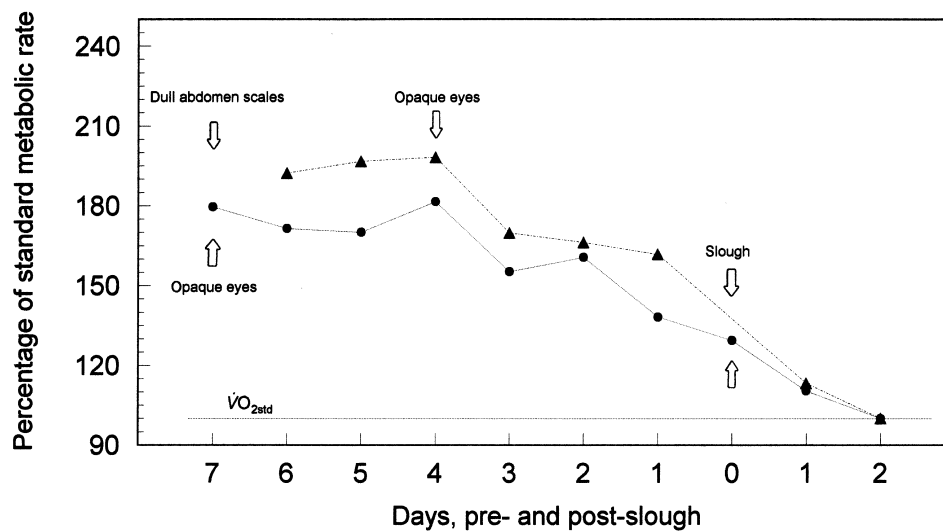
Means are reported with  $\pm 1$  s.e., and sample size, unless otherwise stated. Confidence limits, where presented, are 95%.

#### **Results**

The standard (pre-feeding)  $\dot{V}_{O_{2\text{std}}}$  was 6.75 ( $\pm 0.958$ , s.e.) mL h<sup>-1</sup> for six pythons with a mean body mass of 129.6 g (range 57.7–253.3 g); the coefficient of variation for  $\dot{V}_{O_2}$  was 34.8%. All six pythons were induced to feed under the experimental conditions. The  $\dot{V}_{O_{2\text{std}}}$  increased approximately 4.8-fold within the first 24 h and peaked at 6.3 times standard at 48 h post-feeding (Table 1). After 48 h,  $\dot{V}_{O_2}$  declined and returned to pre-feeding levels, generally within 7 days (Table 1).

**Table 1. Oxygen consumption pre- and post-feeding for *M. s. imbricata* at 30°C**  
 $\dot{V}_{O_2}$  data for individual pythons were excluded when they returned to predigestion levels. Values are shown  $\pm$  s.e.,  $n$  = sample size

	$n$	Body mass (g)	$\dot{V}_{O_2}$ (mL h <sup>-1</sup> )	Factorial increase on $\dot{V}_{O_{2std}}$
Pre-feeding ( $\dot{V}_{O_{2std}}$ )	6	129.6 ( $\pm 37.89$ )	6.75 ( $\pm 0.958$ )	
Post-feeding day 1	6	129.6 ( $\pm 37.89$ )	32.5 ( $\pm 6.39$ )	4.8
Post-feeding day 2	6	129.6 ( $\pm 37.89$ )	42.6 ( $\pm 12.4$ )	6.3
Post-feeding day 3	6	129.6 ( $\pm 37.89$ )	31.7 ( $\pm 11.2$ )	4.7
Post-feeding day 4	6	129.6 ( $\pm 37.89$ )	21.61 ( $\pm 8.65$ )	3.2
Post-feeding day 5	6	129.6 ( $\pm 37.89$ )	13.93 ( $\pm 4.52$ )	2.1
Post-feeding day 6	6	129.6 ( $\pm 37.89$ )	10.97 ( $\pm 3.31$ )	1.6
Post-feeding day 7	4	110.9 ( $\pm 44.70$ )	7.54 ( $\pm 2.24$ )	1.2
Post-feeding day 8	2	163.2 ( $\pm 80.80$ )	10.38 ( $\pm 2.55$ )	1.3
Post-feeding day 9	1	244.0	11.47	1.2



**Fig. 1.**  $\dot{V}_{O_{2std}}$  for two *M. s. imbricata* held at 30°C in the days leading up to a slough. The ▲ and ● indicate the two individual pythons measured.

The mean pre-sloughing  $\dot{V}_{O_2}$  of 15.0 ( $\pm 5.96$  s.e.) mL h<sup>-1</sup> for six *M. s. imbricata* (mean body mass 170.7  $\pm$  72.1 (s.e.) g, range 48–441 g) was significantly higher [repeated measures *t*-test using mass-corrected (mass<sup>0.75</sup>) values;  $t_5 = 3.49$ ] by about 46% (using mass-corrected values) than the post-sloughing  $\dot{V}_{O_2}$  of 9.4 ( $\pm 3.09$ , s.e.) mL h<sup>-1</sup>. For the two *M. s. imbricata* measured nightly from the first signs that sloughing was imminent, an elevated  $\dot{V}_{O_2}$  was evident before opaque eyes were noticed (see Fig. 1). Metabolic rate generally declined from when the eyes were opaque until a day after sloughing (Fig. 1).

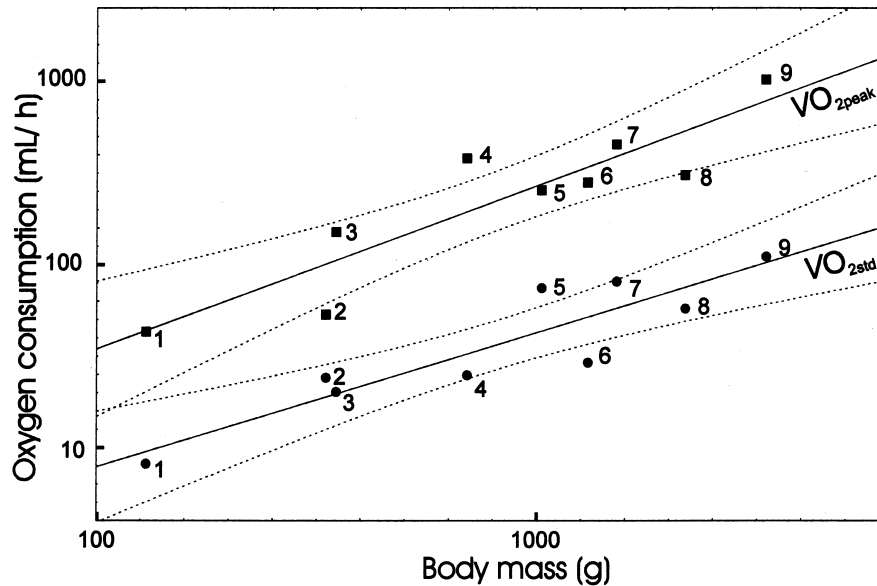
## Discussion

Digestion and fasting have a profound effect on metabolic rate of carpet pythons, as expected. The pioneering studies of Benedict (1932) were the first to suggest that pythons fasted over a long period had a 'depressed' metabolic rate. Benedict (1932) reported peak metabolic rate ( $\dot{V}_{O_{2peak}}$ ) of digesting *P. molurus* to be approximately 8 times pre-feeding values after eating a meal equal to 11% of its body mass, and of *Constrictor constrictor* to be slightly less but variable depending on the size of the meal and the body temperature while digesting prey. Post-feeding metabolic rate increased and peaked after two days for *M. s. imbricata* and then declined, as has also been reported by Secor (1995), Secor and Diamond (1995) and Bedford (1996) for other pythons. The factorial increase in post-feeding  $\dot{V}_{O_2}$  was 6.3 for *M. s. imbricata*, which is within the range of values reported by Bedford (1996) for *Liasis childreni* (2.2), *Aspidites melanocephalus* (3.4), *Morelia s. variegata* (5.3), *M. s. spilota* (5.6), *Antaresia stimsoni* (7.5), *Liasis olivaceus* (9.2) and *Liasis fuscus* (9.6), but lower than the 17-fold increase that Secor and Diamond (1995) reported for sub-adult *Python molurus* and the 11.5-fold increase for neonates of the same species (Secor 1995) even though the relative size of the meal was approximately the same. Using the available  $\dot{V}_{O_2}$  and  $\dot{V}_{O_{2peak}}$  values for *Aspidites melanocephalus*, *Antaresia childreni*, *Liasis fuscus*, *Liasis olivaceus*, *Antaresia stimsoni*, *Morelia spilota spilota*, *M. s. variegata*, *Python molurus* and *M. s. imbricata* from Bedford (1996), Bedford and Christian (1998), Secor and Diamond (1997) and this study, the inter-specific regression equation for  $\dot{V}_{O_{2std}}$  is  $\log_{10} \dot{V}_{O_2} \text{ (mL O}_2 \text{ h}^{-1}) = -0.574 (\pm 0.3742) \log_{10} + 0.734 (\pm 0.1273) \log_{10}(\text{mass, in g})$ , and for  $\dot{V}_{O_{2peak}}$  after ingesting a meal of approximately 25% of the body mass the inter-specific regression equation is  $\log_{10} \dot{V}_{O_2} \text{ (mL O}_2 \text{ h}^{-1}) = -0.241 (\pm 0.4544) \log_{10} + 0.890 (\pm 0.1546) \log_{10}(\text{mass, in g})$ . There was no significant difference between the slopes of these two regression lines ( $t_{14} = 0.78$ ) but, as expected, there was a significant difference between the elevations ( $t_{15} = 9.42$ ). At the mean body mass of 1206 g,  $\dot{V}_{O_{2peak}}$  was 6.5 times higher than  $\dot{V}_{O_{2std}}$  (Fig. 2).

Peak metabolic rate as a result of SDA is proportional to the size of the prey item relative to the animal's body mass (Secor and Diamond 1997). Secor (1995) and Secor and Diamond (1995, 1997) report higher peak post-feeding values (11.5, 17, 15.3 times) for *P. molurus* than reported by either Bedford (1996) or ourselves for pythons consuming similar-sized prey. This difference could be attributed to interspecific differences or from using a depressed metabolic rate (e.g. Benedict 1932) for comparison with  $\dot{V}_{O_{2peak}}$ , reducing the denominator for calculating factorial scope.

Secor and Diamond (1995) and Secor (1995) reported that the elevated metabolic rate of adult *P. molurus* held at 30°C declined to pre-feeding values by Day 8, and by Day 6 for neonate *P. molurus* held at 30°C. Bedford (1996) reported that metabolic rate returns to pre-feeding rates after 7 days at 24 and 27°C, 6 days at 30°C and 4 days at 33°C. Subsequently, Secor and Diamond (1997) reported that larger prey items take longer to digest than smaller ones. If digestion time is defined as the duration of elevated metabolism above  $\dot{V}_{O_{2std}}$ , then higher temperatures hasten the rate of digestion (Bedford 1996). For *M. s. imbricata* in this study at 30°C, there was some variability in when pythons returned to the predigestion metabolic rate, but it was generally by 7 days.

$\dot{V}_{O_2}$  might increase during the sloughing process due to elevated cellular activity (Maderson 1966, 1967; Maderson and Licht 1967; Chiu and Maderson 1975; Maderson *et al.* 1998). Indeed, for six *M. s. imbricata* for which  $\dot{V}_{O_2}$  was measured after their eyes went opaque (indicating incipient sloughing), there was an increase of about 46% above  $\dot{V}_{O_{2std}}$ .  $\dot{V}_{O_2}$  decreased from the opaque-eyes stage until a couple of days after the slough in the two pythons (Fig. 1). The  $\dot{V}_{O_2}$  is measurably elevated even before there are any visual signs that the python is beginning the sloughing process (Fig. 1). *M. s. imbricata* held at 25–30°C generally take 5–12 days to shed from the first visual signs (darkening of the skin followed by opaque eyes) that a slough is imminent. The increase in metabolic rate prior to the first detectable signs that a python is going through the sloughing process means that pythons need to be held for an



**Fig. 2.** Standard and peak metabolic rates while digesting a meal for nine species of python.  $\dot{V}O_{2\text{peak}}$  data for seven species of python [*Aspidites melanocephalus* (5), *Antaresia childreni* (2), *Liasis fuscus* (6), *Liasis olivaceus* (9), *Antaresia stimsoni* (3), *Morelia spilota spilota* (7), *Morelia spilota variegata* (8)] come from Bedford (1996),  $\dot{V}O_{2\text{std}}$  for these species come from Bedford and Christian (1998),  $\dot{V}O_{2\text{peak}}$  and  $\dot{V}O_{2\text{std}}$  for *Python molurus* (4) with a meal size equal to 25% of body mass come from Secor and Diamond (1997), and  $\dot{V}O_{2\text{peak}}$  and  $\dot{V}O_{2\text{std}}$  for *Morelia spilota imbricata* (1) come from this study. Values are means for  $\dot{V}O_{2\text{peak}}$  (■) and  $\dot{V}O_{2\text{std}}$  (●) with 95% confidence limits represented by dotted lines.

extended period after their  $\dot{V}O_{2\text{std}}$  is measured to ensure that they are not in a pre-slough condition. The duration of the sloughing period is probably temperature dependent as it is based on biochemical processes (Landmann 1986; Maderson *et al.* 1998).

Our results, and those of previous studies, suggest some necessary conditions for measuring  $\dot{V}O_{2\text{std}}$ . Sloughing and especially digestion elevate  $\dot{V}O_2$ . Pythons should be held at 25–30°C for at least 14 days after the measurement of metabolic rate to ensure that they are not in the pre-slough stage when metabolic rate is measured. Food should be withheld for approximately 14 days if the body temperature is held at 25–30°C (or longer at a lower temperature or where the last meal was greater than 25% of total body mass) to ensure that  $\dot{V}O_2$  has not been elevated by digestion. However, pythons should be fed regularly, say weekly before the 14-day fast immediately prior to measuring metabolic rate to minimise the possibility of obtaining a lower than ‘standard’  $\dot{V}O_2$  because the snake has fasted for an extended period and has a reduced metabolic rate. Long periods without food may result in a state of metabolic depression. The time taken for a fasting python to reduce its metabolic rate below its ‘standard’ rate is unknown and is possibly temperature dependent (Benedict 1932). This aspect of metabolic depression warrants further investigation.

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## References

- Andrews, R. M., and Pough, F. H. (1985). Metabolism of squamate reptiles: allometric and ecological relationships. *Physiological Zoology* **58**, 214–231.
- Bedford, G. S. (1996). Metabolic physiology, digestive efficiency and energetics of some Australian pythons. M.Sc. Thesis, Northern Territory University, Darwin.
- Benedict, F. G. (1932). The physiology of large reptiles with special reference to the heat production of snakes, tortoises, lizards and alligators. Publication No 425, Carnegie Institute, Washington.
- Bennett, A. F., and Dawson, W. R. (1976). Metabolism. In 'Biology of the Reptilia. Vol. 5'. (Eds C. Gans and W. R. Dawson.) pp. 127–223. (Academic Press: London.)
- Brody, S. (1945). 'Bioenergetics and Growth.' (Reinhold: New York.)
- Chiu, K. W., and Maderson, P. F. A. (1975). The microscopic anatomy of epidermal glands in two species of gekkonine lizards, with some observations on testicular activity. *Journal of Morphology* **147**, 23–40.
- Cogger, H. G. (1992). 'Reptiles and Amphibians of Australia.' (Reed: Sydney.)
- Hayes, J. P., Speakman, J. R., and Racey, P. A. (1992). Sampling bias in respirometry. *Physiological Zoology* **65**, 604–619.
- Jobling, M. (1981). The influences of feeding on the metabolic rate of fishes: a short review. *Journal of Fish Biology* **18**, 385–400.
- Kleiber, M. (1975). 'The Fire of Life.' (Krieger, Huntington: New York.)
- Landmann, L. (1979). Keratin formation and barrier mechanisms in the epidermis of *Natrix natrix* (Reptilia: Serpentes): an ultrastructural study. *Journal of Morphology* **162**, 93–126.
- Landmann, L. (1986). Epidermis and dermis. In 'Biology of the Integument. 2. Vertebrates'. (Eds J. Bereiter-Hahn, A. G. Matoltsy and K. S. Richards.) pp.150–187. (Springer-Verlag: Berlin.)
- Maderson, P. F. A. (1965). Histological changes in the epidermis of snakes during the sloughing cycle. *Journal of Zoology (London)* **146**, 98–113.
- Maderson, P. F. A. (1966). Histological changes in the epidermis of the tockay (*Gecko gecko*) during sloughing cycle. *Journal of Morphology* **119**, 39–50.
- Maderson, P. F. A. (1967). The histology of the escutcheon scales of *Gonatodes* (Gekkonidae) with a comment on the squamate sloughing cycle. *Copeia* **4**, 743–752.
- Maderson, P. F. A., and Licht, P. (1967). Epidermal morphology and sloughing frequency in normal and prolactin-treated *Anolis carolinensis* (Iguanidae: Lacertilia). *Journal of Morphology* **123**, 157–172
- Maderson, P. F. A., Rabinowitz, T., Tandler, B., and Alibardi, L. (1998). Ultrastructural contributions to an understanding of the cellular mechanism involved in lizard skin shedding with comments on the function and evolution of a unique Lepidosaurian phenomenon. *Journal of Morphology* **236**, 1–24.
- Roth, S. I., and Jones, W. A. (1967). The ultrastructure and enzymatic activity of the boa constrictor (*Constrictor constrictor*) skin during the rest phase. *Journal of Ultrastructure Research* **18**, 304–323.
- Roth, S. I., and Jones, W. A. (1970). The ultrastructure of epidermal maturation in the skin of the boa constrictor (*Constrictor constrictor*). *Journal of Ultrastructure Research* **32**, 69–93.
- Secor, S. M. (1995). Digestive response to the first meal in hatchling Burmese pythons (*Python molurus*). *Copeia* **1995**, 947–954.
- Secor, S. M., and Diamond, J. (1995). Adaptive responses to feeding in Burmese pythons: pay before pumping. *Journal of Experimental Biology* **198**, 1313–1325.
- Secor, S. M., and Diamond, J. (1997). Determinants of the postfeeding metabolic response of Burmese pythons, *Python molurus*. *Physiological Zoology* **70**, 202–212.
- Secor, S. M., Stein, E. D. and Diamond, J. (1994). Rapid upregulation of snake intestine in response to feeding: a new model of intestinal adaptation. *American Journal of Physiology* **266**, G695–705
- Withers, P. C. (1977). Measurement of  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , and evaporative water loss with a flow-through mask. *Journal of Applied Physiology* **42**, 120–123.

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