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Standard and digestive metabolism in the banded water snake, *Nerodia fasciata fasciata*

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Abstract

Estimating energy costs by respirometry is fundamental to many studies of the ecology, behavior and evolution of reptiles. However, traditional respirometry procedures seldom incorporate objective techniques for removal of outliers from estimates of metabolic parameters. We demonstrate how computer-automated respirometry equipment, which records many respiratory measurements over short intervals, can be coupled with mathematical procedures to produce robust estimates of pre- and post-prandial metabolism in banded water snakes (*Nerodia fasciata fasciata*). Standard metabolic rate of *N. f. fasciata* was estimated to be 1.21 ml O_2/h (mass = 30.21 ± 0.74 g) at 25 °C. After ingestion of a fish equaling 20% of their body mass, snakes exhibited a fivefold increase in metabolic rate with peak O_2 consumption rate ($\dot{V}O_2$) reaching 6.5 ml O_2/h. Total cost of digestion was 5.44 kJ, equivalent to approximately 21% of the energy in the meal. Repeated measurements of metabolism in the same individuals revealed that our methods yielded similar results, even when individuals exhibited different patterns of $\dot{V}O_2$ variation between respiratory trials. Our results underscore the importance of obtaining many $\dot{V}O_2$ measurements, coupled with objective removal of outlier values from estimates of metabolic rate, especially when metabolic values are to be interpreted in a comparative context.

Keywords: Digestion; *Nerodia fasciata fasciata*; Oxygen consumption; Respirometry; Specific dynamic action; Standard metabolic rate

1. Introduction

Because life histories of reptiles are strongly influenced by energy allocation patterns (Congdon et al., 1982), examining energy costs is central to many studies of the ecology, behavior and evolution of reptiles. Two significant components of a reptile's energy budget are standard metabolic rate (SMR; the metabolic rate of a resting, post-absorptive ectotherm at a specified temperature during the inactive phase of its circadian cycle; Bennet and Dawson, 1976), and specific dynamic action (SDA, the increased energy expenditure associated with digestion, assimilation and biosynthesis; Kleiber, 1975; Secor and Diamond, 2000). Because the costs of SMR and SDA represent a large portion of an individual's total energy budget in snakes (Secor and Nagy, 1994; Peterson et al., 1997; McCue and Lillywhite, 2002), measuring energy costs of SMR and SDA has proven useful in assessing variation in energy allocation patterns associated with many aspects of reptilian biology including foraging ecology (Secor and Diamond, 2000), biogeography (Beaupre, 1995) and exposure to xenobiotics (Hopkins et al., 1999).

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A recurring challenge in the design and implementation of research on animal metabolism is the estimation of metabolic parameters while minimizing physiological artifacts due to handling and other experimental stress, diel or circadian rhythms, or spontaneous activity. Historically, attempts to meet some of these challenges include methods that serve to reduce stress and activity (e.g. placing the animal in the dark, limiting handling stress, increasing acclimation time). However, our previous work (Hopkins et al., 1999, 2002) suggested that some organisms, such as the banded water snake (*Nerodia fasciata fasciata*), continue to exhibit circadian rhythms and spontaneous activity when external cues are removed and conditions are made conducive for less stressful respirometry measurements. Thus, for species such as *N. f. fasciata*, more rigorous data acquisition and interpretation protocols may be required to remove the influence of activity and circadian cycles from estimates of metabolic parameters.

In the current study, we examine the SMR and post-feeding metabolic response of the banded water snake, *N. f. fasciata*. Because previous measures of metabolism in *N. f. fasciata* revealed that $\geqslant 50\%$ of oxygen consumption rate (V_{O_2}) measurements were increased above resting values for some individuals while fasting in the dark (Hopkins et al., 1999, 2002), the goal of this study was to develop an accurate and repeatable methodology for distinguishing increases in metabolism caused by activity and circadian rhythms from estimates of SMR and SDA. We describe a computer-automated method of V_{O_2} data acquisition, coupled with a mathematical procedure for removing outliers that produces consistent estimates of metabolic parameters even when animals exhibit different patterns of V_{O_2} variation during metabolic measurements.

2. Materials and methods

2.1. Study species

N. f. fasciata is a common species of aquatic snake in the southeastern US that preys upon fish and amphibians (Mount, 1975; Mushinsky and Hebrard, 1977a; Mushinsky et al., 1982; Gibbons and Semlitsch, 1991). The 11 snakes (mass = 30.21 ± 0.75 g; all results reported as the mean $\pm$ 1 S.E.) used in this study were captured from unpolluted areas (Risher Pond ($-81°42'19''$, $33°10'0.1''$) and Ellenton Bay ($-81°44'48''$, $33°13'18''$)) of the Savannah River Site, near Aiken, SC, from 23 March to 21 August, 1999. For the purposes of this study, we intentionally focused on individuals within a very narrow range of body masses (26.6–34.7 g) to reduce the confounding effects of allometric relationships between mass and V_{O_2}. Snakes were housed individually in 38-l aquaria in a temperature-controlled greenhouse (25.0 ± 1.5 °C) where they were exposed to natural photoperiod and could bask under sunlight. Each aquarium was equipped with a hidebox, a 1-l water bowl and a heat source (Flexwatt Heat tape; Flexwatt, West Wareham, MA). Snakes were initially fed live sunfish (*Lepomis* spp.) once per week. When snakes consistently ate under captive conditions, dead sunfish (previously captured and stored frozen) were substituted as their source of food. Snakes were held in the greenhouse for 1–3 months. All snakes were released at their site of capture at the end of the study.

2.2. V_{O_2} measurements

Prior to measurements, snakes were fasted for 10 days. Snakes were allowed to acclimate for 24 h in the same environmental cabinet (constant dark, 25 °C) in which subsequent respiratory measurements were taken. Individual snakes were then weighed and placed in 3.8-l chambers. Several chamber modifications were made (in comparison to previous work, see Hopkins et al., 1999) to reduce experimentally-induced stress. Each chamber contained a circular plastic hidebox (150 mm diameter, 30 mm height) perforated with 23 holes (9 mm diameter) and an opening (30×40 mm^2) that snakes could voluntarily enter for refuge. The hidebox did not restrict the snake's movement or impede air-flow within the chamber. Chambers also contained a paper towel to absorb excreta and a small dish of water (20 ml) attached to the top of the hidebox (with Velcro) to prevent it from capsizing.

We determined metabolic rate indirectly as V_{O_2} (adjusted for standard temperature and pressure) by connecting each chamber to a computer-controlled, closed-system respirometer (MICRO OXYMAX, Columbus Instruments, Columbus, OH, software version 5.35). Up to four snakes (each in individual chambers) were run simultaneously during each trial. Measurements commenced at 12:00–13:00 h, and each chamber was sampled at

alternating intervals of 1.0 and 2.0 h during preprandial measurements, and at alternating intervals of 0.6 and 1.5 h during digestion. After every other sample, chambers were refreshed with ambient air equaling four times the headspace (i.e. the volume of the chamber minus the volume of the snake, hideplate, water bowl and paper towel) of each chamber. Thus, additional time was required between paired samples to allow for complete refreshment of air in the chambers. Every 24 h, water was changed, fecal material was removed and snakes were weighed, so that the chamber headspace could be recalculated (because snake volume changes slightly as food is digested).

SMR was determined for each post-absorptive snake over the initial 48 h period at 25 °C (15 V_{O_2} measurements/individual/day). Twenty-four hours after SMR measurements were completed, each snake (13 days post-absorptive) was fed a single dead bluegill sunfish, weighing approximately 20% (19.71 ± 0.004%) of snake body mass. Oxygen consumption was then measured 22 times/day for 6 days to document changes in V_{O_2} related to digestion, absorption and biosynthesis. For both SMR and digestive metabolic trials, water was withheld from snakes for 24 h prior to the beginning and the end of trials in order to prevent inflationary effects of water ingestion on initial and final snake mass.

To assess the repeatability of our techniques for estimating parameters of SDA, we used six snakes in a second respiratory trial. Seven days after termination of their initial SDA measurement, these six snakes (13 days post-absorptive) were weighed and fed a sunfish weighing approximately 20% (19.04 ± 0.013%) of the snake's body mass. Snakes were returned to their respiratory chamber and metabolic measurements were repeated.

2.3. SMR estimation

The V_{O_2} measurements obtained during the first 48 h represented V_{O_2} of snakes during inactivity as well as during varying degrees of activity. Because SMR serves as the baseline from which elevations in metabolism associated with digestion are gauged, accurate SMR estimates are critical to obtaining reliable estimations of SDA parameters. We chose the lower quartile V_{O_2} (the value that marks the boundary between the lowest two quarters of the data set) as our estimate of each snake's SMR. For this data set, the measurement that approximates the lower quartile value was the eighth lowest of 30 values. This choice removed measurements influenced by activity bouts and fell in the modal region of the distribution (Figs. 1 and 2).

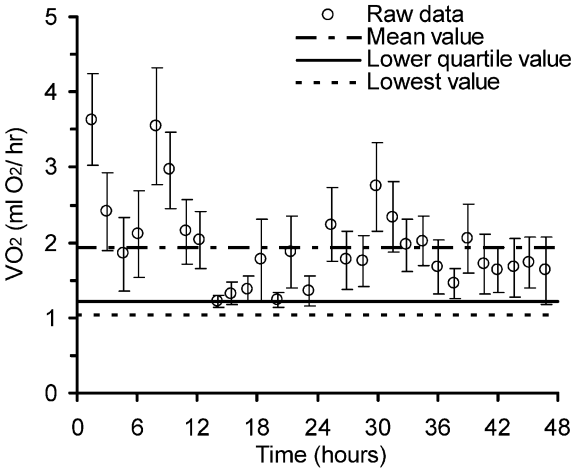

Fig. 1. Mean (±1 S.E.) raw V_{O_2} values for *N. f. fasciata* (N = 11, mean mass = 30.21 ± 0.75 g) during pre-feeding metabolic measurements at 25 °C. Measurements began at 12:00 h (time 'zero'). The three horizontal lines represent different methods of SMR estimation. SMR was calculated separately for each individual snake using the three estimation techniques, and the mean SMR for each method was plotted. We chose the lowest quartile value as the best estimate of SMR for *N. f. fasciata*.

To facilitate interspecific comparisons, we made thermal and allometric corrections of metabolic rate, because experimental temperatures and body mass differ between our study and others. We used standard Q_{10} values from the literature (Q_{10} = 2.23 between 20 and 30 °C; Bennet and Dawson, 1976) to adjust SMR within this temperature range. This Q_{10} was similar to that reported for other *Nerodia* at these temperatures (Gratz and Hutchison, 1977). Allometric corrections were based on SMRs of snakes in the current study, as well as SMRs determined from 19 additional *N. f. fasciata* across a wider range of masses (9.8–370.0 g; Hopkins et al., 1999, 2002). Regression of $\log_{10}$ transformed snake mass and SMR was used to determine the mass coefficient and mass exponent for this species (*sensu* Andrews and Pough, 1985).

2.4. SDA parameter estimation

As with SMR, V_{O_2} values during digestion were assumed to be the sum of several components: (1) SMR, (2) the energetic cost of digestion, absorp-

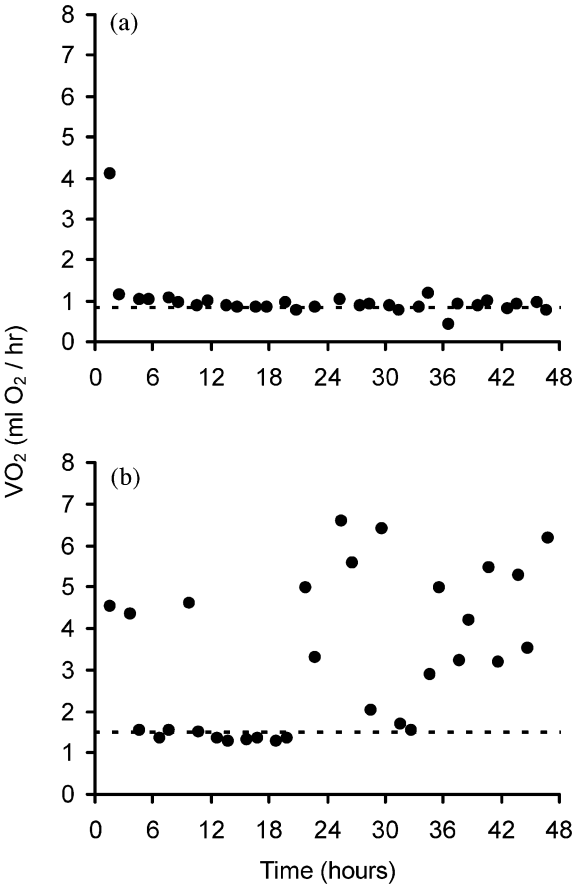

Fig. 2. Raw V_{O_2} values ($N=30$ measurements/snake) obtained over a 48 h period at 25 °C for (A) a *N. f. fasciata* (31.30 g) that showed low V_{O_2} variation and (B) a *N. f. fasciata* (31.88 g) with high V_{O_2} variation. The horizontal dashed line represents the lowest quartile V_{O_2} estimate of SMR for each snake. Note that our estimation technique provided robust estimates of SMR despite individual differences in patterns of V_{O_2} variation.

tion, and biosynthesis (SDA) and (3) increased V_{O_2} due to sporadic activity and circadian rhythms during some of the measured intervals. Therefore, curve smoothing was necessary to exclude measurements substantially influenced by undesired sources of V_{O_2} variation. We report results from a two-part smoothing technique that first uses a moving central minimum of three values (each value replaced by the minimum of itself and the value on either side), followed by a moving central median of 11 measurements (each value replaced by the median of itself and the five values on either side). The moving minimum accounts for occasional sporadic increases in V_{O_2} (likely due to brief periods of sporadic activity), while the moving median of 11 values reduces the influence of more prolonged increases in V_{O_2} (likely resulting from extended periods of activity or circadian rhythms) from digestive metabolic estimates. Moving medians based on fewer measurements had shorter time windows and sometimes failed to smooth through extended activity bouts. In contrast, a moving median based on more measurements underestimated slopes of the SDA curve. The moving median was more effective than other curve smoothing procedures, such as the moving mean.

During digestion, the increased V_{O_2} of each snake was characterized by several parameters. Peak V_{O_2} was estimated as the maximal smoothed V_{O_2}. Time to peak V_{O_2} was the starting time of the 30-min sample interval during which time snakes exhibited peak V_{O_2}. The metabolic range was the difference between the peak V_{O_2} and SMR. Digestive scope was the maximum factorial increase in V_{O_2} (Peak V_{O_2}/SMR). The rate of return to SMR was characterized as the elapsed time from peak V_{O_2} to 50, 25 or 10% of the range. We do not report rate of return below 10% of the range because estimates beyond this were highly variable and in some cases snakes did not return to their initial SMR within 6 days. SDA was estimated as the integral of V_{O_2} from feeding until the end of day 6 minus the integral of SMR over the same period. To calculate the SDA coefficient, a standard of comparison of digestive metabolism among species (the cost of SDA as a percentage of total ingested energy; Jobling and Davies, 1980; Jobling, 1981; Secor and Diamond, 2000), we first converted SDA to kilojoules expended (19.8 J/ml O_2 consumed; Secor and Diamond, 2000). Whole bluegill sunfish ($N=20$) were then lyophilized, homogenized and analyzed for energy content using bomb calorimetry (4.34 ± 0.70 kJ/g wet mass). The SDA coefficient was calculated as the percentage of ingested energy used during digestion.

To assess the repeatability of SDA estimates, we compared SDA parameters between feeding trials using repeated measures ANCOVA, with snake body mass as the covariate (Proc Mixed Model, SAS V 8.1, SAS Institute, 1999). Because SDA parameters are not independent of one another, we used a sequential Bonferroni correction to maintain an experiment-wide error rate of $P=0.05$. Because SMR was not determined for snakes

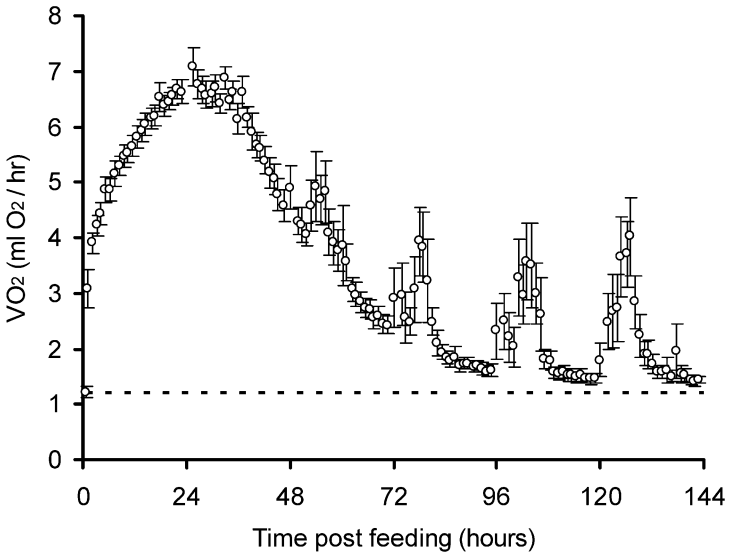

Fig. 3. Mean (±1 S.E.) raw V_{O_2} values for *N. f. fasciata* ($N=11$, mean mass = 30.21 ± 0.75 g) during digestion of a fish equal to 20% of the snake's body mass at 25 °C. Measurements began at 1200 (time 'zero'). Dashed lines represented the mean SMR of snakes based on the lower quartile value.

on trial II, SMR from trial I was used to calculate SDA parameters for the second feeding.

3. Results

Snakes exhibited circadian cycles in V_{O_2} before and during digestion (Figs. 1 and 3). Oxygen consumption rates were highest between 18:00 and 20:00 h and lowest from 02:00 to 12:00 h. Fasted animals exhibited factorial increases in V_{O_2} 2.3–2.9 above SMR during the active phase of the circadian cycle (Fig. 1). Increased V_{O_2} during the active phase of the circadian cycle was not obvious during the first 2 days of digestion, but beginning on day 3, circadian cycles returned. During active periods, snakes exhibited factorial increases in V_{O_2} of 1.3, 1.9, 2.2 and 2.4 above V_{O_2} of inactive periods on days 3, 4, 5 and 6 of post-prandial measurements (Fig. 3).

Based on the lowest quartile value, we estimate SMR of *N. f. fasciata* to be 1.21 ± 0.10 ml O_2/h (range = 0.86–1.77 ml O_2/h; Fig. 1, Table 1).

Table 1
Mean (± S.E.) metabolic parameters for *N. f. fasciata* before and during digestion of single bluegill sunfish weighing approximately 20% of snake body mass at 25 °C

Parameter measured (units)	Trial I	Trial II
N	11	6
Mass (g)	30.21 ± 0.75	31.88 ± 1.06
SMR (ml O_2/h)	1.21 ± 0.10	ND
Peak V_{O_2} (ml O_2/h)	6.50 ± 0.17	6.35 ± 0.43
Digestive scope (peak V_{O_2}/SMR)	5.64 ± 0.38	5.72 ± 0.58
Time to peak V_{O_2} (h)	22.9 ± 1.6	21.3 ± 2.0
Time to 50% decrease from peak (h)	52.7 ± 1.6	51.9 ± 2.7
Time to 75% decrease from peak (h)	68.4 ± 2.1	67.2 ± 1.0
Time to 90% decrease from peak (h)	83.7 ± 2.8	81.7 ± 1.9
SDA		
Total ml O_2 consumed (ml)	275 ± 9	281 ± 18
Total kJ expended (kJ)	5.44 ± 0.17	5.56 ± 0.35
SDA coefficient (% ingested E utilized)	21.1 ± 0.6	21.5 ± 1.3

SMR was not determined (ND) for trial II.

SMR was also calculated using two alternative estimation techniques (mean of all V_{O_2} values and the lowest V_{O_2} value for each snake), but among the three estimation techniques, the lowest quartile V_{O_2} produced estimates that best represented V_{O_2} of all snakes during time periods when V_{O_2} was lowest (Fig. 1). To illustrate that the lowest quartile provides robust SMR estimates regardless of a snake's degree of V_{O_2} variation, pre-prandial V_{O_2} measurements and the lowest quartile SMR estimate for two snakes, one that exhibited high V_{O_2} variation and one with little V_{O_2} variation, are illustrated in Fig. 2.

Based on regression of SMR and body mass of 30 snakes, SMR in *N. f. fasciata* increased with increasing body mass according to the following equation: $\log_{10}\text{SMR} = 0.7047 \times \log_{10}\text{body mass} - 0.8097$ ($r^2 = 0.70$, $P < 0.001$). Based on this relationship, the mass coefficient and mass exponent for *N. f. fasciata* are 0.15 and 0.70, respectively, values that fall within the range reported for other snakes (Andrews and Pough, 1985).

After ingesting prey, V_{O_2} increased to peak values within 24 h, then steadily decreased to 10% of the range in approximately 3.5 days. Metabolic parameters from both digestive trials are presented in Table 1. Repeated digestive trials on the same individuals indicate that our methods produced similar estimates even when different patterns of V_{O_2} variation occurred between trials (Fig. 4, Table 1). Some snakes that were inactive on the first trial were active on the second, and vice-versa. However, after applying our estimation procedures, snakes with high SDA values on the first trial typically had high SDA values on the second trial, with a similar trend for snakes with low values. There was no significant effect of respiratory trial on any of the SDA parameters (in all cases, $0.03 \leqslant F_{1,5} \leqslant 0.61$; $0.47 \leqslant P \leqslant 0.86$).

4. Discussion

Our study provides a clear example of problems associated with estimating SMR and SDA in relatively active organisms. Even when experimental conditions are designed to minimize stress, elevations in metabolism can still occur due to sporadic activity and circadian cycles. The daily fluctuations in metabolism for *N. f. fasciata* were likely endogenous (i.e. circadian rhythms), because oscillations in V_{O_2} persisted while snakes were in dark chambers. The highest V_{O_2} occurred around sunset (18:00–20:00) and remained elevated for a few hours afterwards, which is consistent with the summer nocturnal behavior observed in *N. fasciata* (Mushinsky and Hebrard, 1977b). The magnitude of the increase in V_{O_2} during active phases of the circadian cycle for *N. f. fasciata* (1.4–2.9 times higher than inactivity) was within the range of increases in other squamates (1.5–5 times higher than inactivity; Waldshmidt et al., 1987), and consistent with other *Nerodia* (1.6–2.4 times higher than inactivity; Gratz and Hutchison, 1977; Blem and Killeen, 1993). Data acquisition techniques that involve the collection of few measurements per day may not easily discriminate desired values (e.g. SMR) from those influenced by such activity and circadian cycles, particularly for species that tend to be more active. However, collecting frequent V_{O_2} or V_{CO_2} measurements, followed by objective identification and removal of outliers, can minimize these problems.

Although a wide variety of SMR measurement and estimation techniques have been used in other studies, some techniques may not adequately describe patterns of V_{O_2} variation. For instance, the most commonly employed techniques for estimating SMR are based on 1–4 measurements collected over varying amounts of time (from a few hours up to 5 days) and the lowest measurement obtained is reported as SMR (Ruben, 1976; Secor and Diamond, 2000; Secor, 2001; Overgaard et al., 2002). Such techniques, potentially (1) overestimate SMR if the animal is active or experiencing a circadian-dependent elevation in resting metabolism during this lowest measurement, or (2) under-estimate SMR if the single lowest measurement is in error due to experimental or instrumentation problems (MaNab, 2003), or is generated from an animal experiencing long episodes of apnea (Andrade et al., 1997). Since many squamates exhibit circadian cycles in metabolic rate or remain sporadically active even under constant light or dark laboratory conditions (current study; Bennet and Dawson, 1976; Andrews and Pough, 1985; Blem and Killeen, 1993; Beaupre and Zaidan, 2001), more reliable estimations of SMR could be generated by a larger number of metabolic measurements made over at least one full day, coupled with a mathematical technique to remove outliers. Our use of a standardized percentile of the V_{O_2} data as an SMR estimate is not unprecedented (Muusze et al., 1998; Litzgus and Hopkins, in press). While the use of the lowest

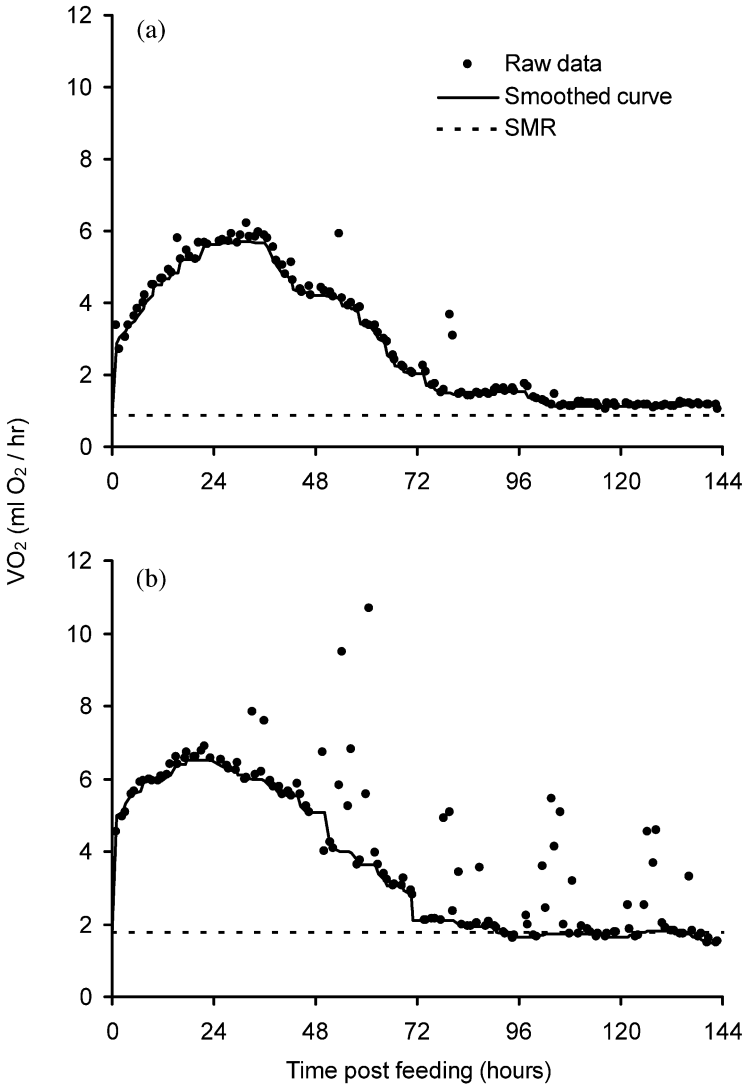

Fig. 4. Raw V_{O_2} data obtained over a 6-day period used to estimate the SDA of a 27.73 g (A) and a 30.49 g (B) *N. f. fasciata* at 25 °C after ingesting a fish weighing 20% of the snake's body mass. The horizontal line represents the lowest quartile V_{O_2} estimate of SMR. The solid line running through the V_{O_2} data represents our estimate of metabolism during digestion after outliers were removed by the two-part smoothing technique. Note that this estimation technique provided robust estimates of SDA despite inter- and intra-individual differences in patterns of V_{O_2} variation.

quartile V_{O_2} value as an SMR estimate was effective for *N. f. fasciata*, other outlier removal techniques may prove more or equally effective for species that exhibit different patterns of V_{O_2} variation. For instance, using the lower 5 and 30% of V_{O_2} data was considered as an effective means of estimating SMR for cichlids (Muusze et al., 1998) and mud turtles (Litzgus and Hopkins, in press), respectively.

Traditional techniques for determining changes in metabolism associated with feeding can also fail to adequately describe and account for patterns of V_{O_2} variation. For instance, the most common techniques rely on linear interpolation of periodic measurements (1–6 measurements/day) of V_{O_2} over 5–15 days (Hailey and Davies, 1987; Sievert and Andreadis, 1999; Secor, 2001; Overgaard et al., 2002, but see Andrade et al., 1997). Just as estimates of SMR are sensitive to increases in metabolism resulting from activity and circadian cycles, SDA parameter estimates can also be greatly influenced by these sources of V_{O_2} variation. In

fact, estimating digestive metabolism is further complicated because researchers must differentiate between increases in metabolism attributed to SDA vs. increases attributed to activity.

By using a curve smoothing process to reduce the impact of spikes in metabolic rate caused by activity and circadian cycles, we produced repeatable estimates of SDA parameters, even when individuals exhibited different patterns of V_{O_2} variation between trials. Other smoothing techniques have been effectively used previously (Janes and Chappell, 1995; Andrade, 1997; Powell et al., 1999) and new techniques may be required in future investigations, but their efficacy will depend upon the frequency and method of data acquisition, as well as the behavior of the study species. For example, in one of the only other attempts to objectively remove activity peaks from SDA estimates, Powell et al. (1999) continuously measured V_{O_2} for 121 h following prey ingestion in the horned frog, *Ceratophrys cranwelli*. The researchers eliminated activity-related V_{O_2} values from their estimates by eliminating means of paired values exceeding two times the average of its leading and trailing neighbors (Mansfield-Jones, personal communication). Similar to our methods, the methods employed by Powell et al. (1999) accounted for individual variation in activity level.

Estimates of SMR and SDA for *N. f. fasciata* fall within the range of values reported for other snakes, but direct comparisons to other studies should be exercised cautiously due to differences in methodologies among studies. SMR of *N. f. fasciata* (estimated SMR of a 350 g *N. f. fasciata* is 14.6 ml O_2/h at 30 °C) is between the extremes described in a synopsis of eight species of phylogenetically and ecologically diverse snake species (7.2–34.6 ml O_2/h at 30 °C allometrically corrected to 350 g; Secor and Diamond, 2000). Overall, digestive parameters in *N. f. fasciata* fed 20% of their body mass more closely resemble responses of frequent feeding species than infrequently-feeding species fed 25% of their body mass (Secor and Diamond, 2000). For instance, the digestive scope (5.6) and duration of the digestive metabolic response (3.5 days) in *N. f. fasciata* were more similar to frequent feeders (5.4–8.0 digestive scope, 4–5 days) than infrequent feeders (9.9–18.5 digestive scope, 8–12 days; Secor and Diamond, 2000). The SDA coefficient of *N. f. fasciata* (21%), was higher than that reported for frequent feeders (13–15%), but within the range reported for infrequent feeders (18–33%; Secor and Diamond, 2000). However, comparisons of SDA coefficients between our study and others are confounded by differences in meal type. We fed *N. f. fasciata* a low-energy-density fish meal (4 kJ/g), while Secor and Diamond (2000) fed snakes high-energy-density rodent meals (e.g. 8 kJ/g; Secor and Diamond, 2000). Differences in a meal's energy content will influence calculations of SDA coefficient. Comparisons to other studies are also confounded by different methods of calculating the SDA coefficient. We calculated SDA coefficient as a percentage of total ingested energy, whereas others have calculated SDA coefficient based on assimilated energy, a function of ingested energy and assimilation efficiency (Andrade et al., 1997). Thus, the biological significance of our results might only be elucidated after phylogenetically-diverse species are examined using similar methodologies.

In conclusion, our results underscore the importance of methodologies that can detect and account for subtle differences in metabolic rate due to circadian cycles in metabolism, as well as more drastic differences induced by activity, when estimating metabolic parameters in active species. Recent technological advances in respirometry enable investigators to address such problems by obtaining many metabolic measurements over multiple days. Using such techniques and objectively removing subsequent outliers produced repeatable estimates of metabolic parameters even when individual snakes exhibited different patterns of V_{O_2} variation. Such protocols for reducing errors are important to account for among-individual variation in activity or circadian cycles, especially when examining the phylogenetic or ecological basis for variation in metabolic parameters.

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