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Simultaneously Occurring Elevated Metabolic States Expose Constraints in Maximal Levels of Oxygen Consumption in the Oviparous Snake *Lamprophis fuliginosus*

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Abstract

African house snakes *(Lamprophis fuliginosus)* were used to compare the metabolic increments associated with reproduction, digestion, and activity both individually and when combined simultaneously. Rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production $\dot{V}_{CO_2}$ were measured in adult female (nonreproductive and reproductive) and adult male snakes during rest, digestion, activity while fasting, and postprandial activity. We also compared the endurance time (i.e., time to exhaustion) during activity while fasting and postprandial activity in males and females. For nonreproductive females and males, our results indicate that the metabolic increments of digestion (~3–6-fold) and activity while fasting (~6–10-fold) did not interact in an additive fashion; instead, the aerobic scope associated with postprandial activity was 40%–50% lower, and animals reached exhaustion up to 11 min sooner. During reproduction, there was no change in digestive $\dot{V}_{O_2}$, but aerobic scope for activity while fasting was 30% lower than nonreproductive values. The prioritization pattern of oxygen delivery exhibited by *L. fuliginosus* during postprandial activity (in both males and females) and for activity while fasting (in reproductive females) was more constrained than predicted (i.e., instead of unchanged $\dot{V}_{O_2}$, peak values were 30%–40% lower). Overall, our results indicate that *L. fuliginosus*'s cardiopulmonary system's capacity for oxygen delivery was not sufficient to maintain the metabolic increments associated with reproduction, digestion, and activity simultaneously without limiting aerobic scope and/or activity performance.

Keywords

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Introduction

In vertebrates, the oxygen demands resulting from elevated metabolic states are met by the transport capacity of the cardiopulmonary system (Wagner 1996). Physiologists have traditionally studied the physiological response to elevated metabolic states in isolation (e.g., the physiological response to activity; Jones 1994; Secor 2009). However, in nature, animals may carry out several metabolically demanding activities simultaneously, and it is important to determine how such conflicting demands for oxygen are resolved (Jackson 1987; Bennett and Hicks 2001).

In carnivorous reptiles, the metabolic increments associated with digestion (specific dynamic action) can beequivalent to values measured during strenuous activity (Secor and Diamond 1997; Secor et al. 2000). Consequently, during postprandial activity, the oxygen transport capacity of the cardiopulmonary system may be exceeded, and oxygen delivery may be prioritized either to digestive functions or to active muscles (Hicks and Bennett 2004). Alternatively, if the oxygen transport capacity of the cardio-pulmonary system accommodates the oxygen demands of both active muscles and digestive functions simultaneously, then the resulting $\dot{V}_{O_2}$ response is additive (Bennett and Hicks 2001). For example, in the monitor lizard (*Varanus exanthematicus*), the maximum $\dot{V}_{O_2}$ during postprandial activity was higher than the $\dot{V}_{O_2max}$ during activity while fasting, indicating an additive response (i.e., a summation of the individual $\dot{V}_{O_2}$ responses of activity and digestion; Bennett and Hicks 2001).

In reptiles, reproduction is a less thoroughly studied physiological state that also elevates oxygen demand over resting values. Snakes exhibit viviparous (live-bearing) and oviparous (egg-laying) modes of reproduction, often within the same family (e.g., *Colubridae, Elapidae, Viperidae, Boidae;* Neill 1964). oviparity is considered the ancestral mode of reproduction, but many of the same physiological processes (i.e., vitellogenesis, organ remodeling, and embryogenesis) that increase oxygen demand occur in both oviparous and viviparous species (Stewart 1992; Blackburn 1995). Recently, the patterns of oxygen transport in response to the simultaneous demands of digestion, activity, and reproductive state were measured in the checkered garter snake (*Thamnophis marcianus*), a species with a viviparous mode of reproduction (Jackson et al. 2015). In this species, the metabolic processes associated with digestion and reproduction are temporarily compromised during activity. This indicates that the capacity to deliver oxygen is not sufficient to meet the demands of all the elevated metabolic states, suggesting a prioritization pattern in this species.

The goal of our study was to investigate the patterns of oxygen consumption during reproduction, digestion, and activity both individually and when combined simultaneously in an oviparous species, the African house snake (*Lamprophis fuliginosus;* Broadley 1983). This Old World colubrid, native throughout sub-Saharan Africa, regularly produces multiple clutches of eggs per annum, ingests large meals at frequent intervals, and—as an actively foraging species—has a large capacity for maximal energy production during activity (Fitch 1970; Ruben 1976; Broadley 1983; Ford 2001). Furthermore, *L. fuliginosus* does not require

a hibernation cycle to initiate reproduction, can store sperm for long intervals of time between mating events, and has very modest husbandry requirements (Haagner 1987; Ford and Seigel 2006). On the basis of the potentially lower oxygen demands associated with an oviparous mode of reproduction, we hypothesized that *L. fuliginosus* would exhibit an additive pattern in the $\dot{V}_{O_2}$ response to simultaneous metabolic demands of digestion and activity.

Material and Methods

Ethical Procedures

All procedures involving this species were approved by the University of California–Irvine Institutional Animal Care and Use Committee (protocol 2010–2966 and 2009–2906).

Study Animals and Procedures

Adult *Lamprophis fuliginosus* were purchased from commercial retailers in September 2010 and housed in a large vivarium room at the University of California–Irvine maintained at 25° ± 2°C with a 12L:12D photoperiod. Animals were maintained individually in opaque plastic enclosures (50.2 cm × 34.3 cm × 14.2 cm), each with water ad lib. and a subsurface heating element (Sunbeam [Boca Raton, FL] or Zoo Med Laboratories [San Louis Obispo, CA]). All animals were offered prekilled mice ad lib. approximately once weekly.

Reproductive condition was assessed using ultrasonography (Smith et al. 1989; Stahlschmidt et al. 2011; Van Dyke and Beaupre 2011; Van Dyke et al. 2012). To limit struggling, females were mildly sedated with gaseous isoflurane in a bell jar before all scans. Following mild sedation, a portion of the animal's caudal abdomen was placed in a shallow bath of warm water, and brief (approximately 5–10 min) scans were performed using a 5–10-MHz linear array transducer (CTS 3300, Shantou Institute of Ultrasonic Instruments, Guangdong) as 3–5-cm lateral sections (depending on the magnification) at varying adjustable depths (range = 2–4 cm).

Females recovered from sedation within 5 min following exposure to gaseous isoflurane and were observed to have regained normal behavior (i.e., alertness, behavioral thermoregulation, and feeding without regurgitation) within 12 h of exposure to gaseous isoflurane. The presence of follicles was observable on the basis of the soft-tissue echogenicity of the developing follicles and the boundary differences in echogenicity of the shell parchment versus the surrounding tissues (Stahlschmidt et al. 2011). Coe-lomic palpation was used to confirm visual analyses.

If no follicles were observable using ultrasonography, females were considered to be nonreproductive (fig. 1B); but if enlarged ovarian follicles were observed (fig. 1A) and the individual subsequently laid eggs, the female was considered to be reproductive. Once the reproductive female oviposited her clutch, we determined post hoc the percentage of the reproductive cycle from which our measurements were made, and the reproductive cycle length was considered to be the time elapsed between previous and current oviposition events. For reproductive females, the mean reproductive cycle period was 76 ± 6 d, and the

measurements obtained for the reproductive experimental observations were obtained, on average, 22 ± 13 d before oviposition, which indicates that females were measured during either secondary vitellogenesis or early embryonic development.

Unlike turtles, crocodilians, birds, and tuatara, which oviposit eggs containing embryos at very early stages of development, squamate reptiles retain embryos within the oviduct until stages 25–33 of Hubert and Dufaure (1968)'s developmental scheme (i.e., the limb bud stages of development) before oviposition (Shine 1983; Blackburn 1995). In *L. fuliginosus*, freshly oviposited eggs contain embryos at stage 33 of this developmental scheme, but embryos represents less than 0.01% of the prepartum female's body mass (Boback et al. 2012; A. G. S. Jackson and J. W. Hicks, personal observations). Therefore, our measurements obtained, on average, nearly 3 wk before oviposition were not likely to be strongly influenced by embryonic metabolism.

Measurement of $\dot{V}_{O2}$ and $\dot{V}_{CO2}$

The preferred body temperature for *L. fuliginosus* has previously been determined to be $25° \pm 0.1°C$ (Zurovsky et al. 1987; Lutterschmidt et al. 2002; Lutterschmidt and Hutchison. Therefore, all gas exchange experiments were carried out in a large walk-in environmental chamber maintained at $25° \pm 1°C$. Body mass was obtained to the nearest ± 1 g either before or after all metabolic trials. If possible, experiments were carried out in complete darkness, but some of the trials were carried out under 12L: 12D conditions because of simultaneously occurring experiments that required those conditions.

Oxygen consumption $(\dot{V}_{O2})$ and carbon dioxide production $(\dot{V}_{CO2})$ values in adult male and adult female *L. fuliginosus* were measured using flow-through respirometry. In brief, atmospheric air was pushed by a mass flow controller (Brooks Instruments, model 5841; Emerson Electric, Hatfield, PA; or GF-3 Gas Mixing Flowmeter; Cameron Instruments, Guelph, Ontario) and directed into up to eight metabolic chambers (940 or 1,555 mL, depending on the size of the animal). Incurrent flow rates were determined and then monitored at least once daily using mass flow meters (±1–5 mL/min; GFM17 AALBORG, Orangeburg, NY) and/or Cole-Parmer rotameters (± 5 mL/min; Cole-Parmer, Vernon Hills, IL). At least one of the metabolic chambers remained free from experimental animals and served as a baseline for referencing. Flow rates ranged from approximately 100 to 200 mL/min, depending on the size of the animal and the metabolic state under examination. Excurrent air from each of the metabolic chambers was then subsampled by a separate gas analysis circuit in one of two ways. (1) Excurrent air was carried away from each of the metabolic chambers by a separate line of Tygon tubing, which ended with a syringe barrel that facilitated subsampling. An 18-gauge needle was affixed to each of the syringe barrels, attached to a Cole-Parmer three-way solenoid valve, and selectively drew a subsample of air into the gas analysis circuit. (2) Each of the excurrent Tygon tubing lines from the metabolic chambers fed directly into one of eight Cole-Parmer three-way solenoid valves. Air through the solenoid exited through one of two ports, depending on whether the solenoid valve was activated. If the solenoid valve was not activated, excurrent air flowed freely into the room; if the solenoid line was activated, the chamber's excurrent air flowed through a separate port into a final line of Tygon tubing, into which an 18-gauge needle was inserted. Through the

18-gauge needle, a subsample of this excurrent air was pulled into the gas analysis circuit. By both methods, only one chamber was sampled at a time, in a repeating fashion, without changing the incurrent flow rate experienced by the animal. In both subsampling setups, the sampling time and frequency of measurements were determined by a motorized rotational multiplexer (Industrial Timer, Centerbrook, CT), which electronically activated the eight Cole-Parmer three-way solenoid valves for approximately 4–10 min at a time, once every 80 min for the duration of the trial.

The gas analysis air circuit's flow rate was controlled by a diaphragmatic pump (model R-1, Applied Electrochemistry Technologies, Pittsburg) attached to a Cole-Parmer mass flow controller. The gas analysis equipment was arranged as follows: AEI CD-3A CO_2 sensor/ analyzer $\rightarrow$ soda lime cartridge (remove CO_2) $\rightarrow$ drierite cartridge (remove H_2O) $\rightarrow$ Sable Systems FC-II oxygen analyzer or AEI S-3A oxygen sensor/analyzer. Analog outputs from the S-3A/FC-II and the CD-3A were connected to a Biopac MP-100 hardware system, downloaded to a personal computer at 20 MHz, and quantified using Acknowledge (ver. 3.2.4) software (Biopac, Santa Gotela, CA). The O2 and CO2 analyzers were calibrated regularly throughout the experimental period and—if signal drift occurred between calibrations—verified for consistency in signal linearity by inserting calibration gas with known O2 and CO2 concentrations. To calculate oxygen consumption $(\dot{V}_{O2})$, we used equation (10.1) from Lighton (2008), and we used the Fick equation to calculate carbon dioxide production $(\dot{V}_{CO2})$. The respiratory quotient (RQ) was calculated as the quotient of $\dot{V}_{CO2}$ and $\dot{V}_{O2}$.

Standard Metabolic Rate and Reproduction (REST)

On the day of the experiment, snakes were removed from their enclosures, transported to a walk-in environmental chamber, and placed in opaque plastic metabolic chambers, and gas exchange was measured for 20–48 h. Previous studies in snakes report that the attainment of a postabsorptive state may take as long as 14 d in some species (Secor and Diamond 1995; Secor 2009). Thus, we chose to fast all animals for 14–16 d before the determination of standard metabolic rate or reproductive metabolic rate. Although previous work in juvenile *L. fuliginosus* indicates that large differences exist between photophase and scoto-phase $\dot{V}_{O2}$ values (~2.1–2.7-fold difference; Roe et al. 2004), more recent work in adults indicates that these differences either are not present (reproductive females and males) or are small (1.15-fold, nonreproductive females; Jackson 2014). Thus, the three lowest consecutive $\dot{V}_{O2}$ (and corresponding $\dot{V}_{CO2}$) measurements, regardless of the phase of the photoperiod, were considered to represent the resting or standard metabolic rate. We chose to eliminate the first 4 h of each trial to permit recovery from handling stress and acclimation to the new environment.

Activity While Fasting (ACT)

Females (reproductive and nonreproductive) and males were fasted 17–19 d before the ACT trial. The animals were placed in a fixed-volume glass chamber (either 2.7 or 3.9 L, depending on the size of the animal), which was sealed at the top with a screw-on cap

containing incurrent and excurrent airlines. The airlines protruding into the glass container from the cap were fixed at various lengths to ensure proper air mixing. Incurrent air was pushed from a Brooks mass flow controller (Brooks Instruments 5841-A1A2QG) into the chamber, and excurrent air was forced out through an excurrent air line by the slight positive pressure of the system (less than 1 mm H_2O). Flowrates of air entering and leaving the chamber were constantly monitored via an AALBORG mass flow meter or a calibrated Cole-Parmer rotameter. A subsample of the excurrent air stream was pulled through an 18-gauge needle into the gas analysis circuit described above.

The activity trial and peak $\dot{V}_{O2}$ was measured using an approach that has been previously described (Ruben 1976; Gratz and Hutchison 1977; Ellis and Chappell 1987; Andrade et al. 1997). Briefly, a snake was placed in a metabolic chamber, and the snake was forced to right itself from an investigator-induced rotation. The snake was repeatedly flipped (via rotation of the chamber) until the snake could no longer perform a righting response. The time to exhaustion (time from the first rotation until exhaustion [TTE]) was recorded, and the snake was allowed to recover for 10–20 min. The highest values of the fractional content of expired oxygen (FEO_2) and the corresponding values of the fractional content of expired carbon dioxide ($FECO_2$) during the entire trial were considered to represent peak gas exchange levels.

Digestion (DIG)

Females and males were fasted 20–23 d and then fed a meal equivalent to 20% their body mass (prekilled mice). The snake was transferred from its enclosure to a metabolic chamber in the constant-temperature environmental chamber ($25° \pm 1°C$), and flow rates were adjusted to reflect an expected increase in $\dot{V}_{O2}$ and $\dot{V}_{CO2}$. Values were recorded approximately once every 80 min for 46–60 h (females) and 45 h (males). This time frame encompasses the duration required for $\dot{V}_{O2}$ values to begin to decline following the postprandial peak (fig. A1). The means of the three consecutively highest $\dot{V}_{O2}$ values (and corresponding RQ values) were chosen to represent peak postprandial gas exchange values. The first 4 h of measurements were discarded to account for recovery from handling stress and acclimation to the chamber.

Postprandial Activity (DIG + ACT)

To determine whether there was an interaction between digestion and activity, both males and females (reproductive and nonreproductive) were fasted for 20–23 d and fed a meal equivalent to 20% of their body mass (prekilled mice). After 46–56 h (females) and 47–49 h (males) of digestion (a time frame sufficient to expose peak postprandial $\dot{V}_{O2}$; fig. A1), the snake was placed into the glass activity chamber (mentioned above) and subjected to the same respirometry activity trial as previously described under the same conditions.

Statistical Analysis

The data collected in this research occurred between May 2011 and September 2012. Over this time, the female *L. fuliginosus* in this study underwent multiple reproductive cycles;

consequently, samples obtained for the experimental conditions of REST, DIG, ACT, and DIG + ACT did not always occur within the same reproductive cycle. Repeated measures (range = 1–5) were obtained from the same individual during more than one reproductive cycle for one or more of the experimental conditions, and these measurements were considered correlated. Thus, a linear mixed model (LMM) was used to evaluate the difference in $\dot{V}_{O2}$, $\dot{V}_{CO2}$, RQ, and TTE between nonreproductive and reproductive states among the four experimental conditions as well as possible interactions between reproductive state and experimental condition. The repeated measures were modeled with a compound symmetry covariance structure in the LMM. Also, because of the variation associated with body mass among individuals in our population (up to a fivefold difference) and the confounding factor associated with the increased mass during reproduction by females (Ellis and Chappell 1987; Angilletta and Sears 2000), the lowest nonreproductive body mass value obtained for each individual during an experimental observation was included in the LMM as a covariate to adjust for the effect of body mass on the outcomes. For males, a separate LMM was applied for each outcome to evaluate the differences among the experimental conditions. The $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ values were log transformed to reduce variation and skewness of the data, and then the fold change was used to describe the differences between the four experimental conditions. Post hoc pairwise comparisons were performed if a significant experimental condition effect or a significant reproductive state and experimental condition interaction was observed. Tukey-Kramer's method was utilized for multiple comparison adjustment. Adjusted P values are presented for the multiple comparison results. All analyses were performed with SAS 9.3 (Cary, NC), and the significance level was set at the 0.05 level.

Results

Descriptive Statistics

There were a total of 17 females and six males on which multiple experimental observations were carried out. Females (reproductive and nonreproductive) had a median of seven (range = 2–12) total observations, and males had a median of four (range = 2–4) total observations. The average body mass of males obtained during observations was 79 ± 20 g. For females, the average body mass obtained while nonreproduc- tive was 247 ± 81 g; for reproductive observations, average female body mass increased to 315 ± 98 g (a factorial increment of 1.2).

Oxygen Consumption $\left(\dot{V}_{O2}\right)$

The results for average whole-animal $\dot{V}_{O2}$ values in females (reproductive and nonreproductive) for all four experimental conditions are reported in figure 2A; $\dot{V}_{O2}$ values for males are reported in figure 3A. The fold change in $\dot{V}_{O2}$ during each of the three treatments over REST for females (nonreproductive and reproductive) and males is reported in table 1.

For nonreproductive females, the peak $\dot{V}_{O2}$ during ACT was significantly higher than both DIG (adjusted $P < 0.0001$, 1.6- fold) and DIG + ACT (adjusted $P = 0.0041$, 1.5-fold) $\dot{V}_{O2}$ values. During reproduction, no differences were identified among DIG, ACT, and DIG + ACT values. When comparing between the reproductive and nonreproductive states, the $\dot{V}_{O2}$ from REST was significantly higher during reproduction compared with the nonreproductive state (adjusted $P < 0.0001$, 1.4-fold). Furthermore, the $\dot{V}_{O2}$ from ACT in the nonreproductive state was significantly higher than while reproductive (adjusted $P = 0.012$, 1.3-fold).

For males, the $\dot{V}_{O2}$ values from ACT (adjusted $P < 0.0002$, 2.1-fold) and DIG + ACT (adjusted $P = 0.021$, 1.5-fold) were significantly higher than DIG. The $\dot{V}_{O2}$ from ACT (adjusted $P = 0.014$, 1.4-fold) was significantly larger than DIG + ACT.

Respiratory Quotient (RQ)

The results for RQ associated with each of the four experimental conditions for females (reproductive and nonreproductive) are reported in figure 2B; RQ results for males are reported in figure 3B. The post hoc pairwise comparisons showed that females in both reproductive and nonreproductive states had RQ values from ACT and DIG 1 ACT that were significantly larger (adjusted $P < 0.0001$) than from both REST and DIG values. No differences were observed in RQ values between ACT and DIG 1 ACT or between REST and DIG. For females, it was shown that the RQ from ACT in the reproductive state was significantly higher than the ACT values obtained during the nonreproductive state (adjusted $P = 0.0001$). For males, significant differences were observed among all four experimental conditions (adjusted $P < 0.003$).

Time to Exhaustion (TTE)

The results for TTE for ACT and DIG + ACT for both males and females (reproductive and nonreproductive) are reported in figure 4A and 4B. For females during the nonreproductive state, the TTE was 11.4 ± 3.2 min longer (adjusted $P = 0.0062$) during ACT compared with DIG + ACT (fig. 4A). There was no significant difference between ACT and DIG + ACT during the reproductive state (adjusted $P = 0.95$) or between reproductive and nonreproductive states for ACT or DIG + ACT (adjusted $P > 0.38$). For males, the TTE during ACT ($n = 6$) was not significantly different from DIG + ACT ($n = 5$, $P = 0.056$; fig. 4B).

Discussion

Under natural conditions, animals respond to a variety of metabolic challenges, often simultaneously, but the integrated response to these challenges remains a relatively unexplored area of research (Jackson 1987; Anderson and Wang 2003). Digestion, activity, and reproduction all independently elevate oxygen consumption; therefore, it is likely that an interaction effect (i.e., additivity or prioritization) occurs when an animal's cardiopulmonary system is simultaneously challenged with multiple metabolic demands (e.g., digestion and activity; Bennett and Hicks 2001).

Squamate reptiles (particularly the snakes) are a particularly useful lineage of organisms to study digestion, activity, and reproduction. With the exception of some sit-and-wait foraging species (e.g., *Python molurus*; Secor and Diamond 1997), the oxygen demand during digestion peaks at values that range from three- to eightfold resting values, and this increment varies with meal composition, size of the meal, and body temperature (McCue 2006; Secor 2009). Likewise, the oxygen demand during unsustainable activity in snakes produces aerobic scopes that range from approximately five- to 12-fold resting values (Ruben 1976; Gratz and Hutchison 1977; Ellis and Chappell 1987; Andrade et al. 1997). Reproduction also results in long-lasting submaximal increments on maternal oxygen demand that range from approximately 1.3–2.9-fold above nonreproductive levels (Ellis and Chappell 1987; Birchard et al. 1984; Van Dyke and Beaupre 2011). In this study, we predicted that reproductive females subjected to postprandial activity would exhibit a summation of the reproductive (i.e., 1.4-fold), digestive (i.e., sixfold), and physical activity (i.e., ~10-fold) O_2 demands. Reproductive and postprandially active females are also 40% heavier, potentially elevating $\dot{V}_{O_2}$ during physical activity by another 40% (Taylor et al. 1980).

In contrast to our predictions, results support a hypothesis for a prioritization response to postprandial activity (DIG + ACT) in males and nonreproductive females, but because DIG + ACT $\dot{V}_{O_2}$ values were 30%–40% lower than those elicited during ACT, the response is more constrained than anticipated (i.e., instead of unchanged $\dot{V}_{O_2}$, values were lower during DIG + ACT; Bennett and Hicks 2001). Reproductive females exhibited a prioritization pattern (i.e., unchanged $\dot{V}_{O_2}$ values) of O_2 delivery during digestion (DIG) and postprandial activity (DIG + ACT) but demonstrated a constrained pattern of prioritization during ACT (i.e., peak $\dot{V}_{O_2}$ values were 30% lower compared with nonreproductive levels).

The prioritization patterns of O_2 delivery exhibited by *Lamprophis fuliginosus* in this study contrast with previous work on *Varanus exanthematicus* and *P. molorus*, which exhibit an additive response to postprandial activity (Secor et al. 2000; Bennett and Hicks 2001). Although we are not aware of previously published cases of constrained prioritization, our results for prioritization are consistent with work on the garter snake *Thamnophis marcianus*, the amphibian *Bufo marinus*, and a number of species of fish, all of which either do not change their maximum $\dot{V}_{O_2}$ and/or performance during DIG + ACT or demonstrate a decrement in performance (Beamish 1974; Aslop and Wood 1997; Anderson and Wang 2003; Thorarensen and Farrell 2006; Fu et al. 2009; Jackson et al. 2015).

Reproduction

The 1.4-fold increment exhibited by reproductive *L. fuliginosus* likely represents the maternal costs of allocating yolk to developing follicles (i.e., vitellogenesis) and the cost of extensive remodeling of the maternal reproductive tract and the liver (Dessauer and Fox 1959; Masson and Guillette 1987; Van Dyke and Beaupre 2011). Similar increments have also been demonstrated by the oviparous *Python regius* and by multiple viviparous species

of snakes, measured during the vitellogenic phase of reproduction (Ellis and Chappell 1987; Beaupre and Duvall 1998; Van Dyke and Beaupre 2011; Jackson et al. 2015).

During vitellogenesis, the maternal liver upregulates the synthesis of the yolk precursor molecule vitellogenin, very low density lipoproteins, and a number of vitamin/mineral binding proteins, which are then transported by the bloodstream to the developing ovarian follicles and incorporated into the cells via receptor-mediated endocytosis (Wallace 1985; White 1991). During vitellogenesis, the maternal liver increases in mass (more than twofold in some species; Dessauer and Fox 1959; Gavaud 1986), and much of this increase may be associated with the storage of protein and the proliferation of organelles responsible for protein and lipid processing (Dessauer and Fox 1959; Hahn 1967; Gavaud 1986; Santos et al. 2007). Simultaneously, follicular and oviduct tissues undergo substantial growth and remodeling, with concomitant angiogenesis (Masson and Guillette 1987; Blackburn 1998; Parker et al. 2010). Together, these processes may increase the rate of whole-animal protein synthesis, an energetically demanding process that has been demonstrated to correlate linearly with increments in $\dot{V}_{O2}$ (Houlihan 1991).

Digestion (DIG)

Our results indicate that the ingestion of a meal equivalent to 20% of body mass was accompanied by a large increment in $\dot{V}_{O2}$ over standard values: approximately threefold in males, fourfold in reproductive females, and sixfold in nonreproductive females (figs. 2A, 3A). In this study, the lower digestive aerobic scope by reproductive females is principally due to an elevated baseline, not because of a lower absolute $\dot{V}_{O2}$, suggesting that either reproduction and digestion are not additive or the magnitude of the metabolic increments of reproduction (1.4-fold) to digestion (approximately sixfold) is relatively too small to observe any statistically significant difference. our results are consistent with previously published $\dot{V}_{O2}$ factorial increments in snakes (following 20% body mass meals), which range from 3.7-fold in *Crotalus durissus* to 8.6-fold in *P. molurus* (Andrade et al. 1997; Toledo et al. 2003; Wang et al. 2003; Hopkins et al. 2004; Roe et al. 2004).

Activity While Fasting (ACT)

Our values for aerobic scope during ACT in nonreproductive (9.6-fold) and reproductive (fivefold) females and our values for males (5.9-fold) all fall within the ranges previously published for snakes. For example, *P. regius, P. molurus, Coluber constrictor, Crotalus viridis*, and *Mastocophis flagellum* all exhibit aerobic scopes between five-and 12-fold resting values (Ruben 1976; Ellis and Chappell 1987; Secor et al. 2000). In this study, we hypothesized that reproductive females would exhibit an additive response (i.e., there would be a summation of rest, reproductive, and digestive metabolic increments). However, our results do not support this hypothesis. Instead, peak $\dot{V}_{O2}$ was 30% lower, reproductive females did not change their time to exhaustion, and RQ values increased from 1.3 to 1.7.

During reproduction, the presence of developing ovarian follicles increases female body mass by 40% over nonreproductive levels. Therefore, this species would need to increase the force necessary to perform bodily movements (e.g., an increase in the cost of locomotion by

10% for each 10% increment in body mass; Taylor et al. 1980; Moon and Gans 1998; Shine 2003). Furthermore, in some oviparous snake species, protein catabolism during reproduction results in considerable epaxial musculature loss and subsequent reductions in strength (Lourdais et al. 2004, 2013). These factors—in addition to other competing and/or confounding physiological processes associated with reproduction (e.g., partial collapse of regions of the lungs due to the presence of enlarged ovarian follicles or compressed abdominal vasculature; Munns et al. 2004; Munns and Daniels 2007)—may act concomitantly to constrain oxygen delivery. These factors may consequently result in a greater overall reliance on anaerobic metabolism in order to produce force, hence a more profound metabolic acidosis (manifesting as higher RQ values and a lower aerobic scope; Gleeson and Bennett 1982). In this study, the effects of reproduction were not sufficient to decrease endurance time (TTE), a finding that contrasts with previous work in snakes (Seigel et al. 1987; Brodie 1989) and lizards (Shine 1980, 2003; Cooper et al. 1990; Sinervo et al. 1991). In these previously published studies, reproduction resulted in 20%–50% decrements for both endurance and speed.

Postprandial Activity (DIG + ACT)

Contrary to our predictions, this study's data support a prioritization pattern of O_2 delivery for males and nonreproductive females during DIG + ACT. However, this prioritization response is more constrained than predicted by Bennett and Hicks's (2001) model. That is, instead of unchanged aerobic scopes between ACT and DIG + ACT, male and nonreproductive female *L. fulignosus* exhibit a 30%–40% decrease in peak $\dot{V}_{O_2}$ values during DIG + ACT (figs. 2A, 3A). In addition to this constraint in aerobic scope, nonreproductive females also exhibit shorter endurance times during DIG + ACT when compared with ACT (i.e., TTE reduced by 11 min; fig. 4A). A similar pattern was also observed for males (i.e., TTE reduced by 4 min), although it was not statistically significant.

During DIG + ACT, reproductive female *L. fuliginosus* exhibits unchanged peak $\dot{V}_{O_2}$ values from ACT, and peak $\dot{V}_{O_2}$ values during DIG + ACT did not differ between reproductive conditions (fig. 2A). These results indicate a prioritization pattern of O_2 delivery. During reproduction, female *L. fuliginosus* did not experience any significant detriments in endurance time (i.e., TTE) between ACT and DIG + ACT, nor was TTE reduced between reproductive conditions during DIG + ACT (fig. 4A). Therefore, peak levels of gas exchange are not more constrained for DIG + ACT during reproduction.

Previous work on squamate reptiles demonstrates either an additive $\dot{V}_{O_2}$ response to the combined challenges of digestion and activity *(Varanus exathematicus* and *P. molurus)* or a prioritization pattern of oxygen delivery *(T. marcianus).* The additive response in *V. exathematicus* and *P. molurus* was hypothesized to occur because of increased tissue O_2 extraction, evidenced by an elevated arterial-venous O_2 difference (Secor et al. 2000; Hicks and Bennett 2004). In *P. molurus*, this additive $\dot{V}_{O_2}$ response occurs in spite of a 47%–81% decrease in blood flow to the gastrointestinal tract (Secor and White 2010). Our results in *L. fuliginosus* are more similar to the prioritization response exhibited by the snake *T. marcianus* and a number of fish species (Jackson et al. 2015). For example, rainbow trout

(*Oncorhynchus mykiss*), Chinook salmon (*Oncorhynchus tshawytscha*), and largemouth bass (*Micropterus salmonoides*) all demonstrate a prioritization response to postprandial activity, which manifests as a decrement in activity performance (Aslop and Wood 1997; Thorarensen and Farrell 2006). The decreased TTE by 11 min between ACT and DIG + ACT exhibited by nonreproductive female *L. fuliginosus* are also similar to the decrements in speed and endurance exhibited by the postprandially active (nonreproductive) garter snakes *Thamnophis elegans* and *T. marcianus* (Garland and Arnold 1983; Ford and Shuttlesworth 1986).

The prioritization pattern of oxygen delivery combined with the lack of change in TTE for most groups (with the exception of nonreproductive females) may indicate two trends: (1) priority of O_2 delivery was accorded to actively contracting skeletal muscles, and (2) weight-related increase in energy demand during locomotion may be met by increased levels of anaerobic glycolysis (i.e., larger RQ values; Gleeson and Bennett 1982; Bennett and Hicks 2001). Moreover, direct biomechanical constraints placed on convective steps of oxygen transport by reproductive, digestive, and locomotor processes may limit oxygen uptake and delivery sufficiently to produce the constrained prioritization patterns observed in male (i.e., DIG + ACT), nonreproductive female (i.e., ACT + DIG), and reproductive female (ACT, ACT + DIG) *L. fuliginosus.* The highly distensible lungs of squamate reptiles can be distorted and compressed by the gastrointestinal tract and ovarian follicles (Munns 2013). In three species of lizards, lung volume decreases by 30%–50% during reproduction (Gilman et al. 2013; Munns 2013). The ingestion of a meal equivalent to 20% of body mass is likely to elicit a similar effect. In lizards, physical activity results in both ventilatory (i.e., conflicts between locomotor and ventilation recruitment of hypaxial musculature) and circulatory (i.e., increased intra-abdominal pressure resulting in decreased venous return) constraints that may limit $\dot{V}_{O_2}$ (Carrier 1990; Munns et al. 2004). The combination of all three functional states may synergistically compound the above-mentioned effects, resulting in the 30%–40% reduction in $\dot{V}_{O_2}$ exhibited by *L. fuliginosus* in this study.

Future Directions

Measurements on gas exchange and activity performance serve as inferences about patterns of oxygen delivery during simultaneously occurring functional states. However, alterations in any one or more points in the oxygen transport cascade may have resulted in the prioritization patterns that were measured in this study. Future work that repeats the experiments described in this study—while also measuring cardiopulmonary parameters (e.g., heart rate, stroke volume, breathing frequency, tidal volume, venous and arterial blood gas levels) and regional blood flow distribution patterns (e.g., superior and inferior mesenteric arterial blood flow)—would help to identify the limiting factors in O_2 transport. Moreover, the consequences of visceral organ compression (i.e., increased cost of ventilation, decreased venous return) have been examined only in the lizards *Tiliqua rugosa, Crotaphytus collaris,* and *Gambelia wislizenii* during reproduction (Gilman et al. 2013; Munns 2013) and in *Varanus exanthematicus* following the injection of saline (Munns et al. (2004), but not for snakes. Therefore, additional measurements of the cost of ventilation, central venous/arterial blood pressure, and venous return would help to elucidate whether

these factors contributed to the constrained prioritization pattern of O_2 delivery exhibited by *Lamprophis fuliginosus* in this study.

overall, our work contributes to a growing body of literature on how oxygen demand is resolved between simultaneously occurring functional states. Unfortunately, most work to date has been carried out on a limited number of species of fish and squamate reptiles (e.g., Beamish 1974; Aslop and Wood 1997; Secor et al. 2000; Hicks and Bennett 2004; Thorarensen and Farrell 2006; Fu et al. 2009; Jackson et al. 2015). Understanding how animals resolve conflicts in oxygen demand during simultaneously occurring functional states is an important topic not only for reptiles but also for species of all lineages, because animals rarely experience just one functional state in isolation under natural conditions (Jackson 1987). For example, in some species of mammals (e.g., *Mus musculus* and *Callospermophilus lateralis*), lactation results in elevations in maternal metabolism by as much as sevenfold above nonreproductive levels, during which time females simultaneously continue to consume frequent meals (Kenagy et al. 1990; Hammond and Diamond 1992). Thus, we hope that future work focuses on patterns of interaction during simultaneously occurring functional states in a variety of vertebrate and invertebrate species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Literature Cited

Anderson JB and Wang T. 2003 Cardiorespiratory effects of forced activity and digestion in toads. Physiol Biochem Zool 76:459–470. [PubMed: 13130426]

Andrade DV, Cruz-Neto AP, and Abe AS. 1997 Meal size and specific dynamic action in the rattlesnake Crotalus durissus (Serpentes: Viperidae). Herpetologica 53:485–493.

Angilletta MJ and Sears MW. 2000 The metabolic cost of reproduction in an oviparous lizard. Funct Ecol 14:39–45.

Aslop DH and Wood CM. 1997 The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (Oncorhynchus mykiss). J Exp Biol 200:2337–2346. [PubMed: 9320259]

Beamish FWH 1974 Apparent specific dynamic action of largemouth bass, Micropterus salmonoides. J Fish Res Board Can 31:1763–1769.

Beaupre SJ and Duvall D. 1998 Variation in oxygen consumption of the western diamondback rattlesnake (Crotalusatrox): implications for sexual size dimorphism. J Comp Physiol B 168:497–506.

Bennett AF and Hicks JW. 2001 Postprandial exercise: prioritization or additivity of the metabolic responses? J Exp Biol 204: 2127–2132. [PubMed: 11441054]

Birchard GF, Black CP, Schuett GW, and Black V. 1984 Influence of pregnancy on oxygen consumption, heart rate and hematology in the garter snake: implications for the "cost of reproduction" in live bearing reptiles. Comp Biochem Physiol 77:519–523.

Blackburn DG 1995 Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. J Theor Biol 174:199–216. [PubMed: 7643614]

Blackburn DG 1998 Structure, function, and evolution of the oviducts of squamate reptiles, with special reference to viviparity and placentation. J Exp Zool 282:560–617. [PubMed: 9867504]

Boback SM, Dichter EK, and Mistry HL. 2012 A developmental staging series for the African house snake, Boaedon (Lamprophis) fuliginosus. Zoology 115:38–46. [PubMed: 22206643]

Broadley DG 1983 FitzSimmons' snakes of Southern Africa. Delta, Johannesburg.

Brodie ED 1989 Behavioral modification as a means of reducing the cost of reproduction. Am Nat 134:225–238.

Carrier D 1990 Activity of the hypaxial muscles during walking in the lizard Iguana iguana.J Exp Biol 152:453–470. [PubMed: 2146360]

Cooper WE Jr., Vitt LJ, Hedges R, and Huey RB. 1990 Locomotor impairment and defense in gravid lizards (Eumeces laticeps): behavioral shift in activity may offset costs of reproduction in an active forager. Behav Ecol Sociobiol 27:153–157.

Dessauer HC and Fox W. 1959 Changes in ovarian follicle composition with plasma levels of snakes during estrus. Am J Physiol 197:360–366. [PubMed: 13816197]

Ellis TM and Chappell MA. 1987 Metabolism, temperature relations, maternal behavior, and reproductive energetics in theball python (Python regius). J Comp Physiol B 157:393–402.

Fitch HS 1970 Reproductive cycles of lizards and snakes. University of Kansas Museum of Natural History, Lawrence.

Ford NB 2001 Reproduction in the brown house snake, Lamprophis fuliginosus. Afr J Herpetol 50:31–34.

Ford NB and Seigel RA. 2006 Intra-individual variation in clutch and offspring size in an oviparous snake. J Zool 268: 171–176.

Ford NB and Shuttlesworth GA. 1986 Effects of variation in food intake on locomotory performance of juvenile garter snakes. Copeia 1986:999–1001.

Fu SJ, Zeng LQ, Li XM, Pant X, Cao ZD, Peng JL, and Wang YX. 2009 The behavioral, digestive and metabolic characteristics of fishes with different foraging strategies. J Fxp Biol 212:2296–2302.

Garland T Jr., and Arnold SJ. 1983 Effects of a full stomach on locomotory performance of juvenile garter snakes (Thamnophis elegans). Copeia 1983:1092–1096.

Gavaud J 1986 Vitellogenesis in the lizard Lacerta vivipara jacquin. Gen Comp endocrinol 63:11–23. [PubMed: 3770443]

Gilman CA, Candelaria G., Gershman. B, Norenberg. JP, and Wolf. BO 2013 Respiratory biology during gravidity in Crotaphytus collaris and Gambelia wislizenii. J Herpetol 47:262–269.

Gleeson TT and Bennett AF. 1982 Acid-base imbalance in lizards during activity and recovery. J ExpBiol 98:439–453.

Gratz RK and Hutchison VH. 1977 Energetics for activity in the diamondback water snake, Natrix rhombifera. Physiol Zool 50:99–114.

Haagner GV 1987 Reproductive data on the brown house snake (Lamprophisfuliginosus) (Boei 1827). J Herpetol Assoc Afr 33:9–12.

Hahn WF 1967 Fstradiol-induced vitellogenesis and concomitant fat mobilization in the lizard Uta stansburiana. Comp Biochem Physiol 23:83–93. [PubMed: 6075170]

Hammond KA and Diamond J. 1992 An experimental test for a ceiling on sustained metabolic rate in lactating mice. Physiol Zool 65:952–977.

Hicks JW and Bennett AF. 2004 Eat and run: prioritization of oxygen delivery during elevated metabolic states. Respir Physiol Neurobiol 144:215–224. [PubMed: 15556104]

Hopkins WA, Roe JH, Philippi T, and Cogdon JD. 2004 Standard and digestive metabolism in the banded water snake, Nerodia fasciata fasciata. Comp Biochem Physiol A 137:141–149.

Houlihan DF 1991 Protein turnover in ectotherms and its relationships to energetics. Adv Comp Environ Physiol 7:1–43.

Hubert J and Dufaure JP. 1968 Table de développement de la vipère aspic: Vipera aspis L. Bull Soc Zool France 93: 135–148.

Jackson AGS 2014 The interaction of elevated metabolic states in snakes. PhD diss. University of California, Irvine.

Jackson AGS, Leu SY, Ford NB, and Hicks JW. 2015 Patterns of oxygen consumption during simultaneously occurring elevated metabolic states in the viviparous snake Thamnophis marcianus. J Exp Biol 218:3570–3579, doi:10.1242/jeb.115477. [PubMed: 26417014]

Jackson DC 1987 Assigning priorities among interacting physiological systems Pp. 310–326 in Feder MF, Bennett AF, Burggren WW, and Huey RB, eds. New directions in ecological physiology. Cambridge University Press, New York.

Jones JH 1994 Comparative vertebrate exercise physiology: phyletic adaptations. Academic Press, San Diego, CA.

Kenagy GJ, Massman D, Sharbaugh SM, and Nagy KA. 1990 Energy expenditures during lactation in relation to litter size in free-living golden-mantled ground squirrels. J Anim Ecol 59:73–88.

Lighton JRB 2008 Measuring metabolic rates: a manual for scientists. Oxford University Press, New York.

Lourdais O, Brischoux F, DeNardo D, and Shine R. 2004 Protein catabolism in pregnant snakes (Epicrates cenchria maurus, Boidae) compromises musculature and performance after reproduction. J Comp Physiol B 174:383–391. [PubMed: 15088116]

Lourdais O, Lorioux S, and DeNardo DF. 2013 Structural and performance costs of reproduction in a pure capital breeder, the children's python Antaresia children. Physiol Biochem Zool 86:176–183. [PubMed: 23434777]

Lutterschmidt DI and Hutchison VH. 2003 Influence of thyroid hormones on the thermal selection of African house snakes (Lamprophis fuliginosus). J Therm Biol 28:167–173.

Lutterschmidt DI, Lutterschmidt WI, Ford NB, and Hutchison VH. 2002 Behavioral thermoregulation and the role of melatonin in a nocturnal snake. Horm Behav 41:41–50. [PubMed: 11863382]

Masson GR and Guillette LJ. 1987 Changes in oviductal vascularity during the reproductive cycle of three oviparous lizards (Eumeces obsoletus, Sceloporus undulatus, and Crotaphytus collaris). J Reprod Fertil 80:361–371. [PubMed: 3656274]

McCue MD 2006 Specific dynamic action: a century of investigation. Comp Biochem Physiol A 144:381–394.

Moon BR and Gans C. 1998 Kinematics, muscular activity and propulsion in gopher snakes. J Exp Biol 201:2669–2684. [PubMed: 9732322]

Munns S 2013 Gestation increases the energetic cost of breathing in the lizard Tiliqua rugosa. J Exp Biol 216:171–181. [PubMed: 22972896]

Munns S and Daniels C. 2007 Breathing with big babies: ventilation and oxygen consumption during pregnancy in the lizard Tiliqua rugosa. Physiol Biochem Zool 80:35–45. [PubMed: 17160878]

Munns SL, Hartzler LK, Bennett AF, and Hicks JW. 2004 Elevated intra-abdominal pressure limits venous return during exercise in Varanus exanthematicus. 207:4111–4120.

Neill WT 1964 Viviparity in snakes: some ecological and zoogeographical considerations. Am Nat 97:35–55.

Parker SL, Manconi F, Murphy CR, and Thompson MB. 2010 Uterine and placental angiogenesis in the Australian skinks, Ctenotus taeniolatus, and Saiphos equalis. Anat Rec 293:829–838.

Roe JH, Hopkins WA, Snodgrass JW, and Congdon JD. 2004 The influence of circadian rhythms on pre and postprandial metabolism in the snake Lamprophis fuliginosus. J Biochem Physiol A 139:159–168.

Ruben JA 1976 Aerobic and anaerobic metabolism during activity in snakes. J Comp Physiol 109:147–157.

Santos X, Arenas C, Llorenta GA, and Ruiz X. 2007 Exploring the origin of egg protein in an oviparous water snake (Natrix maura). Comp BiochemPhysiol A 147:165–172.

Secor SM 2009 Specific dynamic action: a review of the post-prandial metabolic response. J Comp Physiol B 179:1–56. [PubMed: 18597096]

Secor SM and Diamond J. 1995 Adaptive responses to feeding in Burmese pythons: pay before pumping. J Exp Biol 198:1313–1325. [PubMed: 7782719]

Secor SM and Diamond J. 1997 Determinants of the postfeeding metabolic response of Burmese pythons, Python molurus. Physiol Zool 70:202–212. [PubMed: 9231393]

Secor SM, Hicks JW, and Bennett AF. 2000 Ventilatory and cardiovascular responses of pythons (Python molurus) to exercise and digestion. J Exp Biol 203:2447–2454. [PubMed: 10903159]

Secor SM and White SE. 2010 Prioritizing blood flow, cardiovascular performance in response to the competing demands of locomotion and digestion for the Burmese python, Python molurus. J Exp Biol 213:78–88. [PubMed: 20008365]

Seigel RA, Huggins MM, and Ford NB. 1987 Reduction in locomotor ability as a cost of reproduction in gravid snakes. Oecologia 73:481–485. [PubMed: 28311962]

Shine R 1980 "Costs" of reproduction in reptiles. Oecologia 45:92–100.

Shine R 1983 Reptilian reproductive modes: the oviparity-viviparity continuum. Herpetologica 39:1–8.

Shine R 2003 Locomotor speeds of gravid lizards: placing "costs of reproduction" within an ecological context. Funct Ecol 17:526–533.

Sinervo B, Hedges R, and Adolph SC. 1991 Decreased sprint speed as the cost of reproduction in the lizard Sceleporus occidentalis: variation among populations. J Exp Biol 155: 323–336.

Smith CR, Cartee RE, Hathcock JT, and Speake DW. 1989 Radiographic and ultrasonographic scanning of gravid eastern indigo snakes. J Herpetol 23:426–429.

Stahlschmidt ZR, Brashears JB, and DeNardo DF. 2011 Assessing the accuracy of ultrasonographic estimation of reproductive investment and effort in pythons. Biol J Linn Soc 103:772–778.

Stewart JR 1992 Placental structure and nutritional provision to embryos in predominantly lecithotrophic viviparous reptiles. Am Zool 32:303–312.

Taylor CR, Heglund NC, McMahon TA, and Looney TR. 1980 Energetic cost of generating muscular force during running. J Exp Biol 86:9–18.

Thorarensen H and Farrell AP. 2006 Postprandial intestinal blood flow, metabolic rates, and exercise in Chinook salmon (Oncorhynchus tshawytscha). Physiol Biochem Zool 79:688–694. [PubMed: 16826495]

Toledo LF, Abe AS, and Andrade DV. 2003 Temperature and meal size effects on the postprandial metabolism and energetics in a boid snake. Physiol Biochem Zool 76:240–246. [PubMed: 12794677]

Van Dyke JU and Beaupre SJ. 2011 Bioenergetic components of reproductive effort in viviparous snakes: costs of vitellogenesis exceed costs of pregnancy. Comp Biochem Physiol A 160:504–515.

Van Dyke JU, Beaupre SJ, and Kreider DL. 2012 Snakes allocate amino acids acquired during vitellogenesis to off-spring: are capital and income breeding consequences of variable foraging success? Biol J Linn Soc 106:390–404.

Wagner PD 1996 Determinants of maximal oxygen transport and utilization. Annu Rev Physiol 58:21–50. [PubMed: 8815793]

Wallace RA 1985 Vitellogenesis and oocyte growth in non-mammalian vertebrates Pp. 127–177 in Browder LW, ed. Developmental biology, a comprehensive synthesis. Plenum, New York.

Wang T, Zaar M, Arvedsen S, Vendel-Smith C, and Overgaard J. 2003 Effects of temperature on the metabolic response to feeding in Python molurus. Comp Biochem Physiol A 113:519–527.

White HB 1991 Maternal diet, maternal proteins and egg quality Pp. 1–15 in Deeming DC and Ferguson MWJ, eds. Egg incubation: its effects on embryonic development in birds and reptiles. Cambridge University Press, Cambridge.

Zurovsky Y, Laburn BH, and Mitchell D. 1987 Pyrogens fail to produce fever in the snakes Psammorphis phillipsii and Lamprophis fuliginosus. Comp Biochem Physiol A 87:911–914. [PubMed: 2887389]
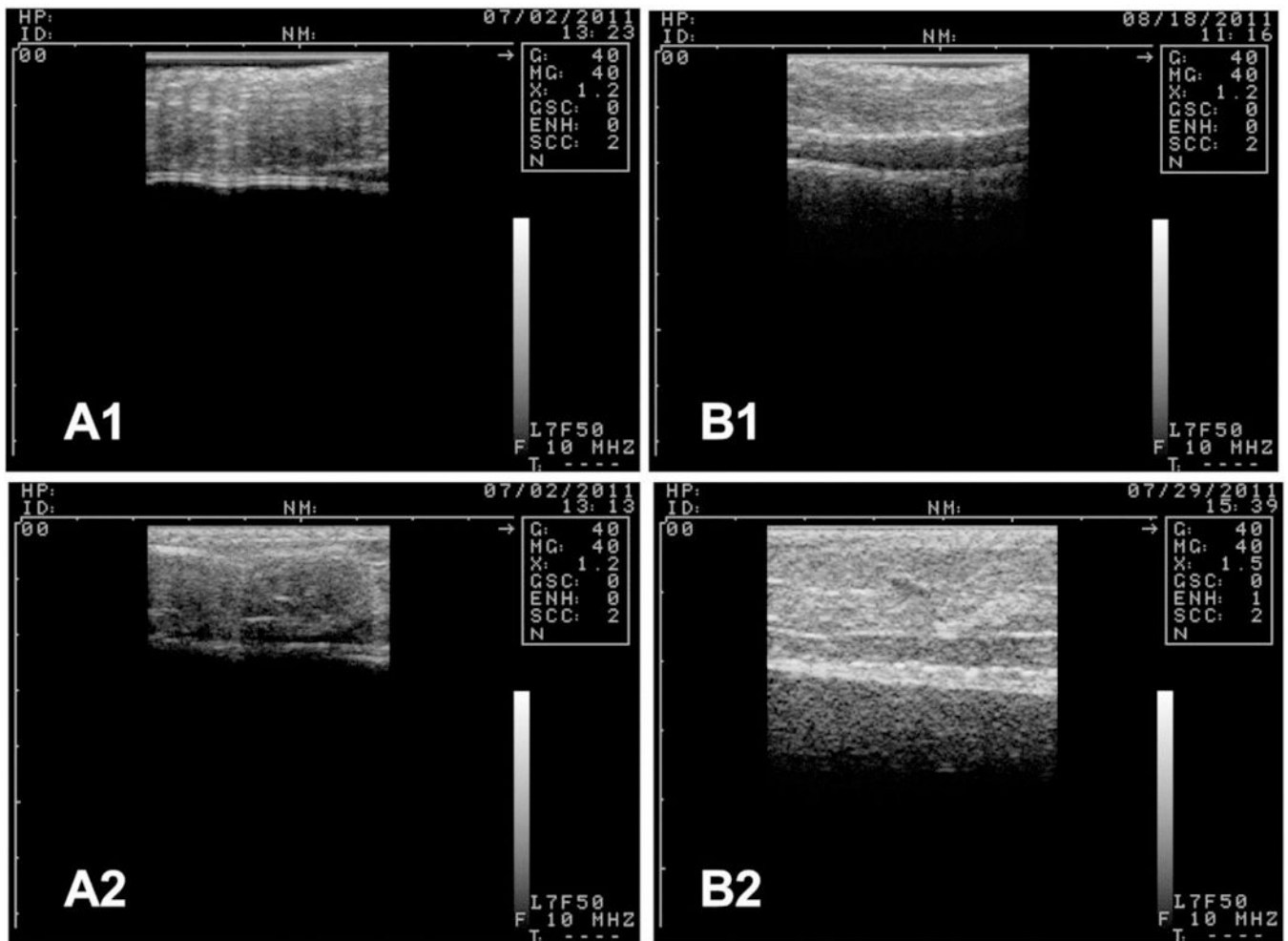
Figure 1.
Representative ultrasound images of two *Lamprophis fuliginosus* females measured while reproductive (*A1, A2)* and nonreproductive (*B1, B2).* Images represent the lateral aspect of one or more ovarian follicles within the posterior coelemic cavity (*A1, A2*) or a lateral aspect within the posterior coelemic cavity in the nonreproductive condition (*B1, B2*). Images were obtained using a 5–10-MHz linear array transducer (SIUI 3300) at a zoom level of × 1.2 or × 1.5 (approximately 4-cm section) at varying adjustable depths (range = 2–3 cm). Discrepancies in apparent size of the photographs are due to variable zoom levels (e.g., × 1.2 vs. × 1.5) and depth.
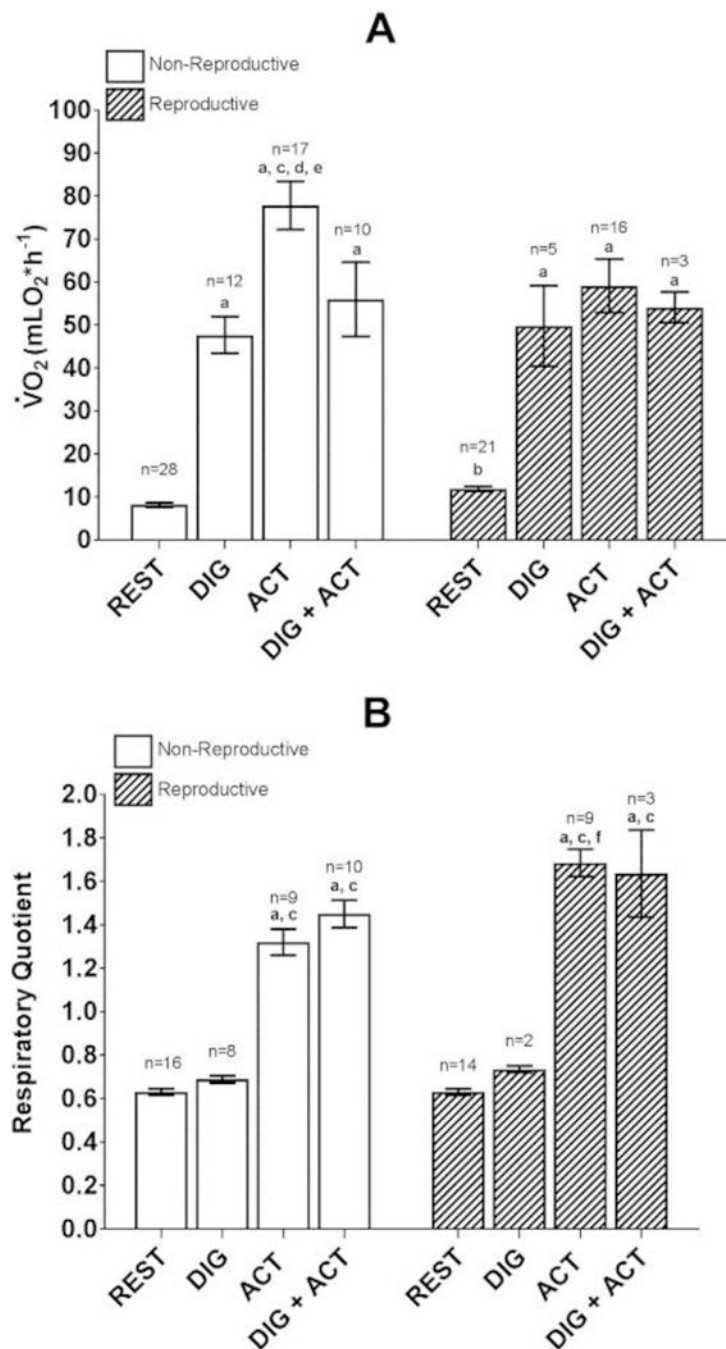
Figure 2.
Female whole-animal $\dot{V}_{O2}$ (*A*) and respiratory quotient (*B*) values for each of the

experimental treatments. The reproductive condition is indicated by the pattern key. REST = rest, DIG = digestion, ACT = activity while fasting, DIG + ACT = postprandial activity. Lowercase letters represent a statistically significant difference: a = significantly larger than REST values within the reproductive condition; b = significantly larger than nonreproductive REST values; c = significantly larger than DIG values within the reproductive condition; d = significantly larger than DIG 1 ACT values within the reproductive condition; e =

significantly larger than reproductive ACT values; f = significantly larger than nonreproductive ACT values. n = sample size for each treatment. All values represent means ± SEM.
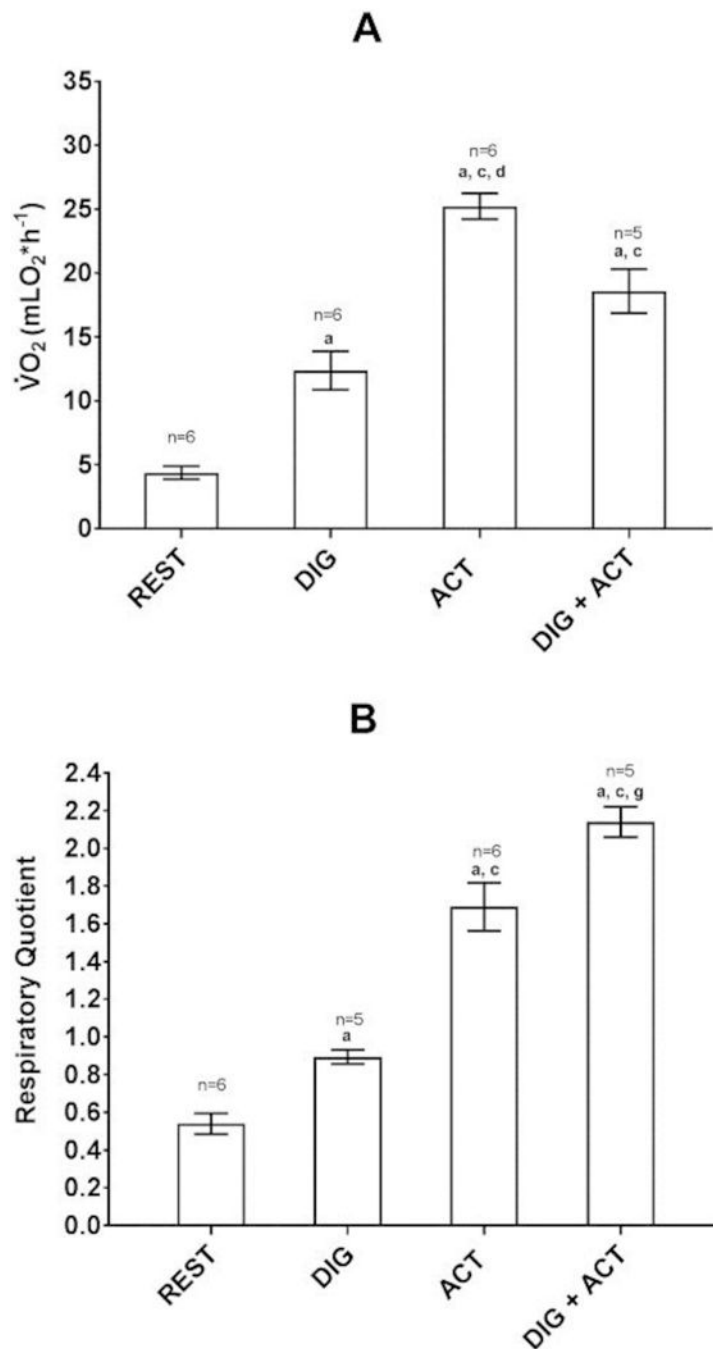
Figure 3.

Male whole-animal $\dot{V}_{O_2}$ (*A*) and respiratory quotient (*B*) values for each of the experimental treatments. REST = rest, DIG = digestion, ACT = activity while fasting, DIG + ACT = postprandial activity. Lowercase letters represent a statistically significant difference: a = significantly larger than REST values within the reproductive condition; c = significantly larger than DIG values within the reproductive condition; d = significantly larger than DIG + ACT values within the reproductive condition; g = significantly larger than ACT values. *n* = sample size for each treatment. All values represent means ± SEM.
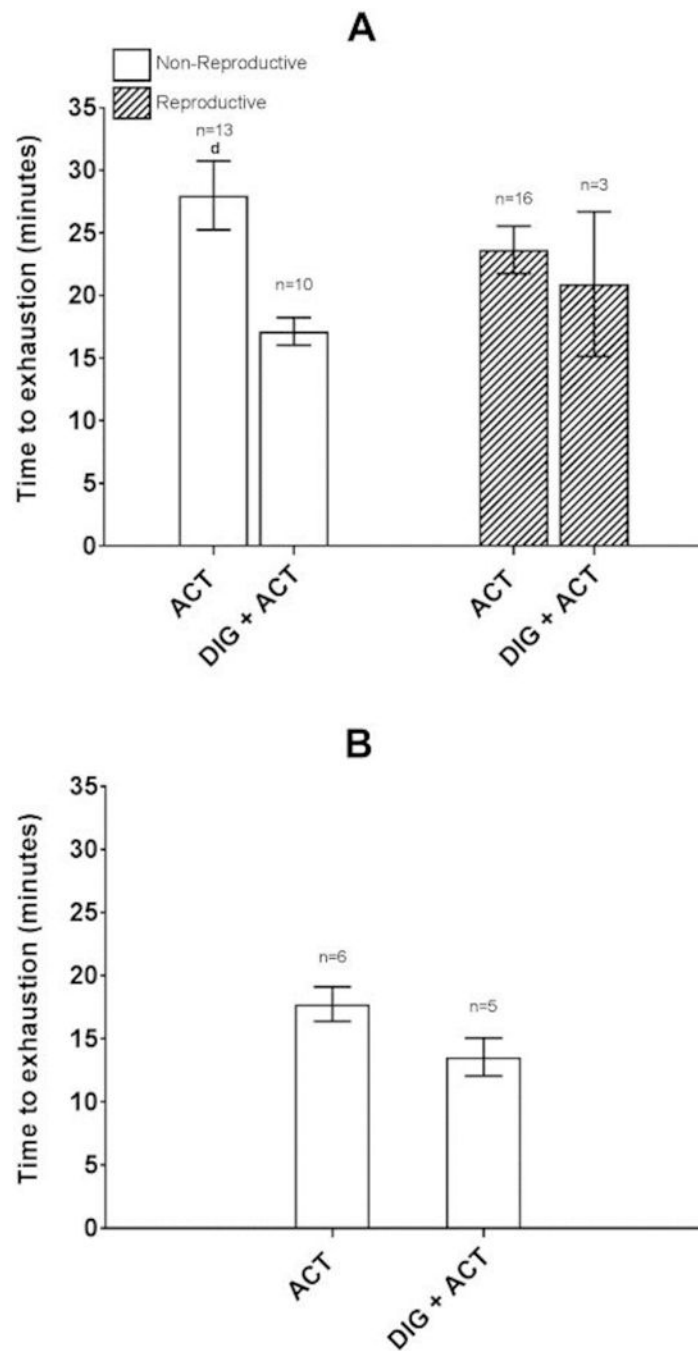
Figure 4.
Time to exhaustion values for females (*A*) and males (*B*) associated with activity while fasting (ACT) and postprandial activity (DIG + ACT). Lowercase letters represent a statistically significant difference: d = significantly larger than DIG + ACT values within the reproductive condition. *n*= sample size for each treatment. All values represent means ± SEM.

Table 1.

Fold change in $\dot{V}_{O2}$ during each of the three treatments over rest (REST) values for females (nonreproductive and reproductive) and males

	ACT	DIG	Postprandial activity (DIG + ACT)
Nonreproductive females	9.6	6.1	6.6
Reproductive females	5.0	4.1	4.7
Males	5.9	2.8	4.3

Note. All values are significantly elevated over REST ($P > 0.0001$). ACT, activity while fasting; DIG, digestion.