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RESEARCH ARTICLE

Patterns of oxygen consumption during simultaneously occurring elevated metabolic states in the viviparous snake *Thamnophis marcianus*

Alexander G. S. Jackson[1], Szu-Yun Leu[3,4], Neil B. Ford[2] and James W. Hicks[1,*]

ABSTRACT

Snakes exhibit large factorial increments in oxygen consumption during digestion and physical activity, and long-lasting sub-maximal increments during reproduction. Under natural conditions, all three physiological states may occur simultaneously, but the integrated response is not well understood. Adult male and female checkered gartersnakes (*Thamnophis marcianus*) were used to examine increments in oxygen consumption (i.e. $\dot{V}_{O_2}$) and carbon dioxide production (i.e. $\dot{V}_{CO_2}$) associated with activity (Act), digestion (Dig) and post-prandial activity (Act+Dig). For females, we carried out these trials in the non-reproductive state, and also during the vitellogenic (V) and embryogenic (E) phases of a reproductive cycle. Endurance time (i.e. time to exhaustion, TTE) was recorded for all groups during Act and Act +Dig trials. Our results indicate that male and non-reproductive female *T. marcianus* exhibit significant increments in $\dot{V}_{O_2}$ during digestion (~5-fold) and activity (~9-fold), and that Act+Dig results in a similar increment in $\dot{V}_{O_2}$ (~9- to 10-fold). During reproduction, resting $\dot{V}_{O_2}$ increased by 1.6- to 1.7-fold, and peak increments during digestion were elevated by 30–50% above non-reproductive values, but values associated with Act and Act+Dig were not significantly different from non-reproductive values. During Act+Dig, endurance time remained similar for all of the groups in the present study. Overall, our results indicate that prioritization is the primary pattern of interaction in oxygen delivery exhibited by this species. We propose that the metabolic processes associated with digestion, and perhaps reproduction, are temporarily compromised during activity.

KEY WORDS: Activity, Digestion, Oxygen consumption, Prioritization, Reproduction, *Thamnophis marcianus*

INTRODUCTION

Studies on mammals and birds have shown that maximum levels of oxygen consumption (i.e. $\dot{V}_{O_2,max}$) are attained during intense physical activity (Jones, 1994; Bishop, 1999). However, some species of squamate reptiles (i.e. snakes and lizards) that consume large and protein-rich meals at infrequent intervals exhibit factorial increments in $\dot{V}_{O_2}$ during the specific dynamic action (SDA) (Rubner, 1902) response that can approach values associated with physical activity (Hicks and Bennett, 2004; Secor, 2009). In

squamates, reproduction represents another physiological state that may result in long-lasting (i.e. months) sub-maximal aerobic scopes (Beaupre and Duvall, 1998; Schultz et al., 2008; Van Dyke and Beaupre, 2011). During the first half of the reproductive cycle (i.e. during vitellogenesis), maternal oxygen demand increases as a result of biomolecule synthesis and organ remodeling (i.e. oviductal and liver tissues) (Beaupre and Duvall, 1998; Van Dyke and Beaupre, 2011). After ovulation, maternal gas exchange requirements increase progressively throughout the remainder of the reproductive cycle as embryonic development proceeds (Birchard et al., 1984; Schultz et al., 2008). Females may continue to forage and consume meals throughout the reproductive cycle (Cooper et al., 1990), and it is important to examine how the simultaneous oxygen demands from reproduction, digestion and activity are resolved (Jackson, 1987).

Based on the concepts of prioritization and additivity introduced by Bennett and Hicks (2001), two general hypothetical outcomes for $\dot{V}_{O_2}$ during the simultaneous metabolic challenges of physical activity and digestion are presented in Fig. 1. In the first scenario, if the cardiopulmonary system's maximum capacity for O_2 transport is sufficient, activity and digestive performance are not compromised, and the resulting aerobic scope is a simple summation – termed additivity (Fig. 1C). Alternatively, if the cardiopulmonary system does not have sufficient capacity to support the oxygen demands of both activity and digestion simultaneously, then the performance of one state is scaled down at the expense of the other, and the resultant aerobic scope is unchanged – termed prioritization (Fig. 1D). In addition to these two generalized outcomes, intermediate effects that cannot be demonstrated by this model are also possible; for example, shared prioritization between the competing functional demands (Bennett and Hicks, 2001).

The principal goal of the present study was to expand upon previous work (i.e. Bennett and Hicks, 2001) by examining the interaction effects between not only activity and digestion but also reproduction. Previous work in viviparous snakes indicates that reproduction results in a 1.3- to 2.9-fold increment in maternal $\dot{V}_{O_2}$, with the largest increments being reported during mid to late embryogenesis (e.g. Birchard et al., 1984; Schultz et al., 2008). During the digestion of a large meal, snakes exhibit peak factorial increments in $\dot{V}_{O_2}$ of ~3- to 5-fold above standard metabolic rate (SMR) (e.g. Britt et al., 2006; Christian et al., 2007). Moreover, snakes routinely exhibit peak factorial increments in $\dot{V}_{O_2}$ of ~2- to 12-fold above SMR during intense physical activity (e.g. Ruben, 1976; Ellis and Chappell, 1987).

To investigate the interactive effects of simultaneously occurring metabolic challenges, we chose the checkered gartersnake (*Thamnophis marcianus*), a viviparous (live-bearing) species native to southwestern North America (Rossman et al., 1996). Gartersnakes undergo a reproductive cycle that can be divided into two distinct physiological phases: vitellogenesis (i.e. yolk allocation) and embryogenesis (i.e. embryonic development)

[1]Department of Ecology and Evolutionary Biology, School of Biological Sciences, University of California, Irvine, Irvine, CA 92627, USA. [2]Department of Biology, University of Texas at Tyler, Tyler, TX 75799, USA. [3]Institute for Clinical and Translational Science, University of California, Irvine, Irvine, CA 92687, USA. [4]Department of Pediatrics, University of California, Irvine, School of Medicine, Orange, CA 92868, USA.

*Author for correspondence (jhicks@uci.edu)

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(Dessauer and Fox, 1959; Garstka et al., 1985; Ford and Karges, 1987), but it is not well understood how the phase of the reproductive cycle may influence the metabolic costs of digestion and activity. *Thamnophis marcianus* is particularly well suited for such examinations because females continue to consume large meals throughout much of the reproductive cycle and, as an actively foraging species, these snakes exhibit a high propensity for physical activity (Ford and Shuttlesworth, 1986; Seigel et al., 1987).

We predicted that reproduction (i.e. vitellogenesis and embryogenesis), digestion and activity would all result in significant increments in $\dot{V}_{O_2}$; and during reproduction, the $\dot{V}_{O_2}$ increment would be largest during embryogenesis. We hypothesized that the capacity for oxygen transport in these animals would be sufficient to meet the oxygen demands of these elevated metabolic states (activity, digestion and reproduction) simultaneously. Consequently, the resulting $\dot{V}_{O_2}$ would be the sum of SMR, reproduction $\dot{V}_{O_2}$, digestion $\dot{V}_{O_2}$ and activity $\dot{V}_{O_2}$ (i.e. an additive response).

MATERIALS AND METHODS
Ethical procedures
All procedures involving this species were approved by the University of California, Irvine, Institutional Animal Care and Use Committee (IACUC protocol numbers 2010-2966 and 2009-2906).

Animal husbandry
A captive-bred population of adult male (*N*=14) and female (*N*=20) checkered gartersnakes, *Thamnophis marcianus* (Baird and Girard 1853), was obtained from the Ophidian Research Colony (University of Texas at Tyler) in July 2012. Snakes were maintained in a large vivarium at the University of California, Irvine, at 27±2°C with a 12 h light:12 h dark photocycle. Snakes were housed in individual enclosures (50×34×14 cm, females; 33×23×13 cm, males) lined with newsprint bedding and each enclosure was equipped with (1) a subsurface heating element, (2) two appropriately sized hide-boxes and (3) a water dish. To facilitate fertilization, males were cycled among the females multiple times weekly. Between experimental treatments, snakes were offered thawed mice approximating 2–10% body mass, multiple times weekly.

Experimental treatments (detailed below) were carried out on females in the vitellogenic, embryogenic and non-reproductive treatments. The same experimental treatments were also carried out on males. For females, the phase of the reproductive cycle was assessed using ultrasonography (e.g. Stahlschmidt et al., 2011; Van Dyke and Beaupre, 2011; Van Dyke et al., 2012). Briefly, a portion of the female's caudal abdomen was placed into a warm water bath and 4.2 cm lateral sections were imaged using a 5.0–10.0 MHz linear array transducer (CTS 3300, Shantou Institute of Ultrasonic Instruments, Guangdong, China). A representative scan from each individual was then photographed using the built-in software from the ultrasound machine, and saved to a portable USB drive.

The vitellogenic phase of the reproductive cycle (6–7 weeks prior to parturition) was identified based on the presence of large, highly echogenic ovarian follicles (Fig. 2) (Van Dyke and Beaupre, 2011). The embryogenic phase of the reproductive cycle (3–4 weeks prior to parturition) was identified by the presence of (1) a low echogenic amnion/allantois fluid-filled space and (2) highly echogenic embryonic somatic tissue within the ovarian follicles (Fig. 2) (Van Dyke and Beaupre, 2011). The baseline, non-reproductive state (1–2 weeks following parturition) was verified by the lack of vitellogenic follicles (Fig. 2), and follow-up scans (2–4 weeks later) confirmed that a subsequent reproductive cycle was not initiated.

Resting metabolic rate
On the day of the experiment, snakes were removed from their vivarium enclosures and transported to a walk-in environmental chamber, maintained at 29±1°C, the preferred body temperature of *T. marcianus* (Rosen, 1991). Snakes were weighed to the nearest 0.1 g (Mettler Toledo model SB8001, Columbus, OH, USA) and placed into individual metabolic chambers. Each chamber was covered by a thick black tarpaulin to minimize disruption by investigators.

Oxygen consumption (i.e. $\dot{V}_{O_2}$) and carbon dioxide production (i.e. $\dot{V}_{CO_2}$) were measured using flow-through respirometry. Briefly, atmospheric air
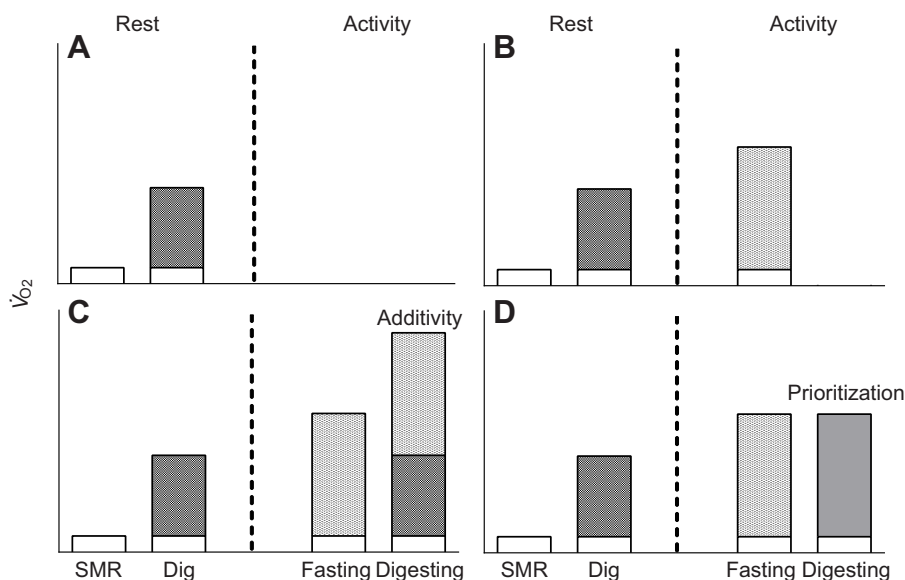
Fig. 1. A hypothetical framework demonstrating a reptile's standard metabolic rate, and the peak $\dot{V}_{O_2}$ increments associated with digestion, physical activity, and the combined effects of physical activity and digestion. Adapted from Bennett and Hicks (2001). (A) Under this framework, the expected $\dot{V}_{O_2}$ values for an animal measured under standard conditions (standard metabolic rate, SMR) and also following the consumption of a large meal (digestion, Dig) are depicted. (B) The expected peak $\dot{V}_{O_2}$ value for a fasting animal that is subjected to an activity trial. If the animal is subjected to the same activity trial while digesting a large meal, one of two general outcomes may occur: (C) an additive pattern, whereby the $\dot{V}_{O_2}$ increment associated with digestion is maintained, and the resulting peak $\dot{V}_{O_2}$ value is a summation of the two responses; or (D) a prioritization pattern, whereby one or both $\dot{V}_{O_2}$ responses are curtailed and peak levels are constrained.
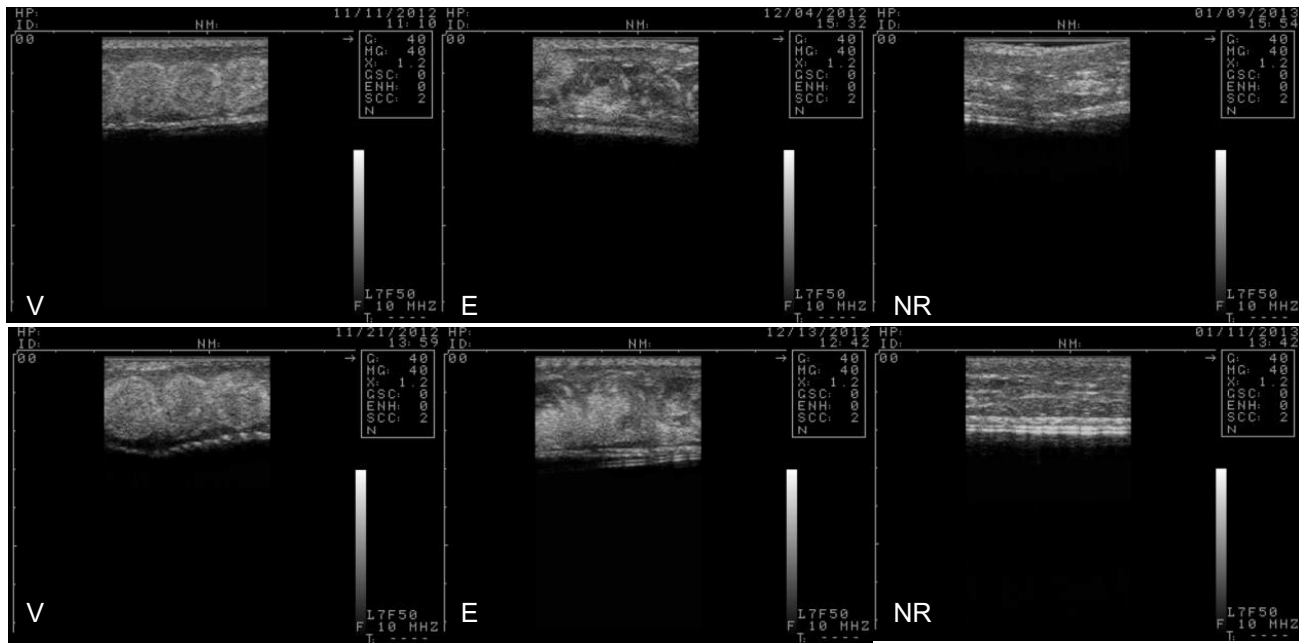
Fig. 2. Ultrasound images of two representative female *Thamnophis marcianus*, imaged during the vitellogenic (V), embryogenic (E) and non-reproductive (NR) conditions. Images were obtained using a 5–10 MHz linear array transducer, and represent approximately 4.5 cm sections (with a depth of approximately 2–3 cm) of a portion of the posterior coelemic abdomen.

was pushed by a mass-flow controller (GF-3 Gas Mixing Flowmeter, Cameron Instruments Inc., Guelph, ON, Canada) into the metabolic chambers, and the incurrent flow rate to each chamber was determined by a mass-flow meter (± 1 ml min^{-1}; GFM17, Aalborg, Orangeburg, NY, USA). Incurrent flow rates ranged from 50 to 240 ml min^{-1}. At least one chamber remained free from snakes and served as a baseline for reference.

A sub-sample of the excurrent air from each of the metabolic chambers was pulled into a gas analysis circuit at 20–50 ml min^{-1} (depending on the excurrent flow rate) by a mass-flow controller (Brooks Instrument, Hatfield, PA, USA), once every 80 min, for 6–11 min at a time. The gas analysis circuit consisted of the following equipment in this order: AEI Technologies CD-3A CO_2 sensor/analyzer→soda lime cartridge (to remove CO_2)→Drierite cartridge (to remove H_2O)→ AEI Technologies S-3A O_2 sensor/analyzer. Analyzers were calibrated repeatedly throughout the experimental period. If baseline drift occurred during a trial, calibration gas with known O_2 and CO_2 values was used to confirm linearity of the signal. $\dot{V}_{O_2}$ was calculated using eqn 10.1 from Lighton (2008), $\dot{V}_{CO_2}$ using the Fick equation, and respiratory quotient (RQ) as the quotient of $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$.

For resting metabolic rate (RMR, Rest) treatments, gas exchange measurements were obtained once every 80 min for 6–11 min at a time, for a total of 8–25 h (females) and 16–38 h (males). The mean of the three lowest consecutive $\dot{V}_{O_2}$ (and corresponding RQ) values was chosen to represent the Rest condition. To ensure that the snakes were in a post-absorptive state prior to measurements, females were fasted for at least 7 days, and males for at least 13 days (Britt et al., 2006; Bessler et al., 2010).

Fasting exhaustive activity and recovery

Following the Rest trial, one snake at a time was placed into a fixed-volume (2.7 l, males; 3.9 l, females) glass metabolic activity chamber. The activity chamber was sealed with a screw cap, into which two incurrent and two excurrent air lines of varying lengths and diameters (to ensure proper air mixing) were inserted. The incurrent flow rate was set by a Brooks mass-flow controller, and then monitored constantly using an Aalborg mass-flow meter. Incurrent flow rates to the activity chamber ranged from 120 to 250 ml min^{-1} (depending on the size of the animal). A subsample of the excurrent airstream was pulled through an 18-gauge syringe at 50 ml min^{-1} into the gas-analysis circuit described above.

We used an exhaustion protocol similar in design to previous work on snakes (Ruben, 1976; Gratz and Hutchison, 1977; Ellis and Chappell, 1987; Andrade et al., 1997). Briefly, the snake was continually forced to right itself from an investigator-induced rotation. The regimen was maintained until the animal could no longer perform the righting response. The elapsed time between the first rotation and the point of exhaustion was considered to represent the time to exhaustion (TTE). The lowest fraction of expired oxygen ($F_{E_{O_2}}$) and corresponding fraction of expired carbon dioxide ($F_{E_{CO_2}}$) values were chosen to represent peak gas exchange levels.

Following the trial, each snake was removed from the activity chamber and placed back into the above-mentioned flow-through respirometry metabolic chamber. Recovery time was considered to be the amount of time required before $\dot{V}_{O_2}$ returned to within 10% of RMR values, and was estimated by obtaining gas exchange values once every 80 min for 5–18 h. If $\dot{V}_{O_2}$ did not return to within 10% of RMR values by the end of the recovery trial, data were not considered for analysis.

Digestion

Following recovery, snakes were removed from the metabolic chambers, placed into their normal enclosures and offered water *ad libitum*. Within 1 day, snakes were offered a meal of thawed rodent equivalent to 10% of their body mass, and within 4 h of voluntary ingestion they were returned to the flow-through respirometry metabolic chambers for post-prandial gas exchange measurements. Gas exchange values were obtained once every 80 min for 6–11 min at a time, for up to 1 day following the time of ingestion, a time frame sufficient to expose peak values following ingestion (Britt et al., 2006; Bessler et al., 2010). The mean of the three highest consecutive $\dot{V}_{O_2}$ (and corresponding $\dot{V}_{CO_2}$) values obtained during this trial period was considered to represent peak post-prandial gas exchange levels.

Post-prandial exhaustive activity

Following the achievement of peak post-prandial gas exchange levels (i.e. 24–28 h following ingestion), we repeated the above-mentioned activity trial. Although gas exchange values had begun to decline by 24–28 h following ingestion, the decline was relatively minor (see Fig. S1; Britt et al., 2006; Bessler et al., 2010). Therefore, the post-prandial exhaustive activity trials in the present study likely suffice to expose snakes to near-maximum interactions between the demands of activity and digestion.

Statistical analysis

To evaluate the difference in $\dot{V}_{O_2}$, RQ and TTE between the three reproductive conditions and the four metabolic states in females and the metabolic states in males, a linear mixed model (LMM – a linear model capable of constructing correlations among repeated measurements from the same animal) was applied. Body mass was considered as a confounder and thus the body mass of males and non-reproductive females was included in the LMM. To use the non-reproductive body mass was due to a significant fraction of the reproductive body mass increment (up to 1.3-fold in this study) is associated with the mass of the ovarian follicles, which contain mostly water and metabolically inert biomolecules throughout much of the reproductive cycle (Ellis and Chappell, 1987; DeMarco and Guillette, 1992; Angilletta and Sears, 2000).

Post hoc pairwise comparisons were performed if a significant difference was observed for reproductive condition, metabolic state or their interaction. Tukey–Kramer's method was utilized for multiple comparison adjustment and an adjusted *P*-value is presented when appropriate. Direct statistical comparisons between male and female *T. marcianus* were not carried out in the present study. The mixed model was performed using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The level of statistical significance was set at 0.05. Values are reported as means±s.e.m.

RESULTS
Descriptive statistics

Overall, 10 out of the original 20 females successfully completed a reproductive cycle and were included in the study; 12 out of the original 14 males were included in the study. Female average snout–vent length (SVL) was 61.8 cm (range 51.5–71 cm) and male average SVL was 42.7 cm (range 37.5–47.5 cm). Average female body mass was 199.1 g (range 125.2–269.4 g) in the non-reproductive state. Female body mass increased to 241.6 g (range 177–324.7 g) during vitellogenesis and then to 262 g (range 208.4–358.3 g) during embryogenesis. Average male body mass was 49 g (range 40.3–73.5 g).

$\dot{V}_{O_2}$

The average oxygen consumption ($\dot{V}_{O_2}$) values associated with RMR (Rest), fasting exhaustive activity (Act), digestion (Dig) and post-prandial exhaustive activity (Act+Dig) for females (all reproductive conditions) are presented in Fig. 3A. There was a

significant difference between metabolic states ($F_{3,102}$=275.38, $P<0.0001$) and a significant reproductive condition by metabolic state interaction ($F_{6,102}$=10.44, $P=0.0005$), but no significant difference was identified between reproductive conditions ($F_{2,102}$=0.57, $P=0.57$).

In all reproductive conditions, the $\dot{V}_{O_2}$ at Rest was significantly lower (adjusted $P<0.0001$) than for all the other metabolic states, with the mean difference (±s.e.m.) ranging between 38.9±2.1 and 82.9±5.0 ml O_2 h^{-1}. The factorial scopes (i.e. fold-change in $\dot{V}_{O_2}$ relative to that at Rest) were calculated for each snake and the means±s.e.m. are reported in Table 1.

In the non-reproductive condition, the $\dot{V}_{O_2}$ values associated with Act and Act+Dig were both significantly larger than those for Dig (mean difference of 33.6±5.5 and 44.0±4.9 ml O_2 h^{-1}, respectively; adjusted $P<0.0001$), but not significantly different between Act and Act+Dig (adjusted $P=0.48$). There was no significant difference between Dig, Act and Act+Dig during vitellogenesis (adjusted $P>0.18$). However, during embryogenesis, the $\dot{V}_{O_2}$ values associated with Act+Dig were significantly larger than those for Dig (mean difference of 23.7±5.3 ml O_2 h^{-1}; adjusted $P=0.001$) but not Act (mean difference of 15.4±4.9 ml O_2 h^{-1}; adjusted $P=0.16$).

Relative to the non-reproductive condition, the Rest $\dot{V}_{O_2}$ was significantly higher during vitellogenesis (mean difference of 6.3±1.5 ml O_2 h^{-1}; adjusted $P=0.0037$) and embryogenesis (mean difference of 5.4±1.0 ml O_2 h^{-1}; adjusted $P<0.0001$); the Dig $\dot{V}_{O_2}$ was also significantly higher during vitellogenesis (mean difference of 25.8±3.9 ml O_2 h^{-1}; adjusted $P<0.0001$) and embryogenesis (mean difference of 5.4±1.0 ml O_2 h^{-1}; adjusted $P<0.0001$) than in the non-reproductive condition. There was no significant difference in Act $\dot{V}_{O_2}$ or Act+Dig $\dot{V}_{O_2}$ between reproductive conditions. Table 2 shows the fold-change of $\dot{V}_{O_2}$ in females during vitellogenesis and embryogenesis over the non-reproductive condition for each of the metabolic states.

The average $\dot{V}_{O_2}$ values associated with Rest, Act, Dig and Act+Dig for males are presented in Fig. 4A. As for females, the $\dot{V}_{O_2}$ values for males at Rest were significantly lower (adjusted $P<0.0005$) than for the other three metabolic states, with the mean difference ranging between 13.3±1.1 and 30.9±3.9 ml O_2 h^{-1}. The
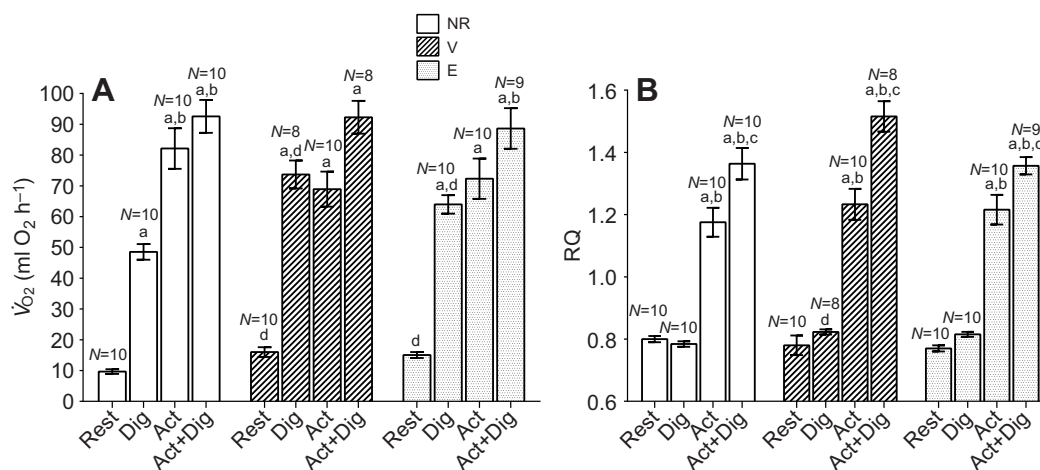
Fig. 3. Female *T. marcianus* whole-animal $\dot{V}_{O_2}$ and respiratory quotient values for each experimental treatment. (A) Whole-animal $\dot{V}_{O_2}$; (B) respiratory quotient (RQ). Mean±s.e.m. data were obtained at rest (resting metabolic rate, RMR), and during post-prandial digestion (Dig), fasting exhaustive activity (Act) and post-prandial exhaustive activity (Act+Dig). Reproductive condition is indicated in the key (NR, non-reproductive; V, vitellogenic; E, embryonic). Lowercase letters above the values represent a statistically significant difference based on *post hoc* pairwise comparisons from a linear mixed model: a, significantly larger than Rest values within the same reproductive condition; b, significantly larger than Dig values within the same reproductive condition; c, significantly larger than Act values within the same reproductive condition; d, significantly larger than NR values for the same experimental treatment. Sample sizes are located above each value.

Table 1. Fold-change in $\dot{V}_{O_2}$ over Rest values during each of the three treatments for female and male *Thamnophis marcianus*

Group	Fasting activity (Act)	Digestion (Dig)	Post-prandial activity (Act+Dig)
Females			
Non-reproductive	9.0±1.0 (*N*=10)	5.2±0.29 (*N*=10)	10.1±0.99 (*N*=10)
Vitellogenic	4.7±0.57 (*N*=10)	5.1±0.35 (*N*=8)	6.4±0.41 (*N*=8)
Embryogenic	5.0±0.59 (*N*=10)	4.4±0.27 (*N*=10)	6.3±0.78 (*N*=9)
Males	9.3±0.91 (*N*=12)	4.5±0.20 (*N*=12)	8.8±0.56 (*N*=10)

All values represent means±s.e.m.

fold-change over Rest is shown in Table 1. In addition, the $\dot{V}_{O_2}$ values associated with both Act and Act+Dig were significantly larger than values elicited during Dig (mean difference of 17.7±3.7 and 15.9±2.3 ml O_2 h^{-1}, respectively; adjusted $P<0.0001$).

RQ
The results for RQ for females (vitellogenic, embryonic and non-reproductive conditions) are presented in Fig. 3B; RQ results for males are presented in Fig. 4B. There was a significant difference between metabolic states ($F_{3,102}$=144.6, $P<0.0001$) but not between reproductive conditions ($F_{2,102}$=3.08, P=0.0502), and no significant interaction was observed ($F_{6,102}$=1.91, P=0.087).

The *post hoc* comparisons showed that female RQ values from Act and Act+Dig were significantly larger than both Rest and Dig RQ values during all reproductive conditions (adjusted $P<0.0001$), with a mean difference of between 0.39±0.036 and 0.72±0.063. Also, female RQ values from Act+Dig were elevated over Act RQ values during all reproductive conditions (adjusted $P<0.05$), with a mean difference of between 0.16±0.044 and 0.29±0.055. There was no difference in female RQ values between Rest and Dig. For males, the RQ values from Act and Act+Dig treatments were significantly larger than Rest and Dig RQ values (adjusted $P<0.0001$), with a mean difference of between 0.43±0.028 and 0.50±0.040. There was no difference in male RQ values between Rest and Dig or between Act and Act+Dig.

Time to exhaustion
There was a significant difference in TTE between reproductive conditions ($F_{2,50}$=4.18, P=0.021) for females, but not between Act and Act+Dig for either females ($F_{1,50}$=0.85, P=0.36) or males ($F_{1,19}$=0.17, P=0.68; Fig. 5). The TTE of females for Act was significantly shorter during vitellogenesis than when non-reproductive (mean difference of 13.9±4.2 min; adjusted P=0.02).

Recovery from activity
Following exhaustion from fasting activity, females returned to within 10% of RMR by an average of 5.8 h (range 3–8.8 h) during vitellogenesis, 8.7 h (range 5.2–14.9 h) during embryogenesis and

9.8 h (range 4.3–16.5 h) in the non-reproductive state. The duration of recovery between the vitellogenic and embryogenic conditions was significantly different (adjusted P=0.0014). Males returned to within 10% of RMR by an average of 5.5 h (range 3–9 h). Upon recovery, female RQ values ranged from 0.67 to 0.93 and male RQ values ranged from 0.58 to 0.92, similar to the RQ at Rest, which ranged between 0.68 and 0.86.

DISCUSSION
This work provides one of the first quantitative examinations of the interaction effects between reproduction, digestion and activity in a viviparous squamate. Our results indicate that *T. marcianus* exhibits substantial metabolic increments during both digestion (∼4- to 5-fold) and activity (∼9-fold), and that a smaller metabolic increment occurs during reproduction (∼1.6- to 1.7-fold). Contrary to our predictions, males and non-reproductive females exhibited a prioritization pattern of oxygen delivery during Act+Dig because $\dot{V}_{O_2}$ values were similar to those elicited by Act. Interestingly, females exhibited a more-than-additive pattern of oxygen delivery during Dig (i.e. summation of the $\dot{V}_{O_2}$ increments, and an extra 10–20 ml O_2 h^{-1} auxiliary cost), but a prioritization response to Act and Act+Dig (i.e. similar $\dot{V}_{O_2}$ increments to these same treatments during the non-reproductive condition). Our results for prioritization contrast with previous work on post-prandially active squamate reptiles (Secor et al., 2000; Bennett and Hicks, 2001) and are more consistent with the responses exhibited by species of anurans and fish (Aslop and Wood, 1997; Andersen and Wang, 2003; Thorarensen and Farrell, 2006).

Reproduction
Reproductive Rest measurements were obtained during secondary vitellogenesis and approximately midway through embryonic development (Fig. 2) (Dessauer and Fox, 1959; Van Dyke and Beaupre, 2011). Consistent with our prediction, the vitellogenic and embryogenic phases of the reproductive cycle were associated with increments in maternal $\dot{V}_{O_2}$ (i.e. 1.6- to 1.7-fold); however, there was no difference between the two phases of the reproductive cycle (Fig. 3A). We did not find any differences in RQ values between the reproductive conditions (Fig. 3B). The reproductive metabolic increments reported in the present study are consistent with previously published values for multiple species of viviparous snakes measured during the vitellogenic (i.e. ∼1.4- to 1.6-fold increments) (Beaupre and Duvall, 1998; Van Dyke and Beaupre, 2011) and embryogenic (∼1.5- to 2.9-fold increments) (Birchard et al., 1984; Schultz et al., 2008; Van Dyke and Beaupre, 2011) phases of the reproductive cycle.

The metabolic increment associated with the vitellogenesis may be caused by increased rates of whole-animal protein synthesis (Garlick et al., 1976; Houlihan, 1991) due to biomolecule synthesis by the liver [i.e. vitellogenin, very low density lipoproteins (VLDLs) and vitamin and trace mineral binding proteins; Wallace, 1985; White, 1991] and organ remodeling (i.e. oviduct and follicular tissues) (Masson and Guillette, 1987; Blackburn, 1998). Following vitellogenesis, the mature ova are fertilized

Table 2. Fold-change in $\dot{V}_{O_2}$ of the reproductive condition over the non-reproductive condition for each of the four metabolic states in female *T. marcianus*

Reproductive condition	Rest	Fasting activity (Act)	Digestion (Dig)	Post-prandial activity (Act+Dig)
Vitellogenic	1.67±0.11 (*N*=10)	0.87±0.08 (*N*=10)	1.52±0.11 (*N*=8)	0.96±0.07 (*N*=8)
Embryogenic	1.63±0.16 (*N*=10)	0.89±0.06 (*N*=10)	1.33±0.06 (*N*=10)	0.98±0.07 (*N*=9)

All values represent means±s.e.m.
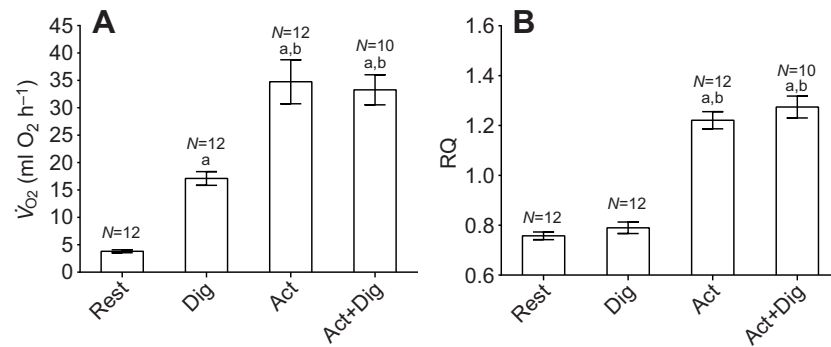
Fig. 4. Male *T. marcianus* whole-animal $\dot{V}_{O_2}$ and respiratory quotient values for each experimental treatment. (A) Whole-animal $\dot{V}_{O_2}$; (B) respiratory quotient (RQ). Mean±s.e.m. data were obtained at rest (resting metabolic rate, RMR), and during post-prandial digestion (Dig), fasting exhaustive activity (Act) and post-prandial exhaustive activity (Act+Dig). Lowercase letters above the values represent a statistically significant difference based on *post hoc* pairwise comparisons from a linear mixed model: a, significantly larger than Rest value; b, significantly larger than Dig value. Sample sizes are located above each value.

and embryonic development begins. After ovulation, the vitellogenic processes are rapidly down-regulated to pre-gestational levels (Dessauer and Fox, 1959; Garstka, et al., 1985; Bonnet et al., 1994). Thereafter, the oviduct continues to undergo extensive remodeling to provide the developing embryos with an appropriate environment for gas exchange and, in some species, to facilitate the maternal–fetal transport of varying quantities of trace nutrients (Masson and Guillette, 1987; Parker et al., 2010; Blackburn and Stewart, 2011; Van Dyke and Beaupre, 2012). Similar to patterns observed in the eggs of oviparous species, embryonic metabolism increases progressively to term, from negligible levels during early embryonic development, to substantially higher values prior to parturition (Packard et al., 1977; Robert and Thompson, 2000; Schultz et al., 2008). Recent work (Jackson, 2014) indicates that *T. marcianus* ovulates at least 6 weeks prior to parturition. Thus, the embryogenic measurements in the present study (3–4 weeks prior to parturition) were obtained approximately midway through development – when embryonic metabolism is still relatively low (Wang and Ji, 1997; Thompson and Russell, 1999). Therefore, we propose that much of the 1.6-fold metabolic increment during embryogenesis is associated with continued remodeling and maintenance costs of the oviduct, rather than embryonic metabolism, as would be expected during the later stages of development.

Digestion

In *T. marcianus*, $\dot{V}_{O_2}$ began to increase within hours of meal ingestion, and peak values occurred within 24 h, increasing to 4.4- to 5.2-fold above Rest values (Fig. 3A, Fig. 4A). There were no differences in RQ values between Rest and Dig (Fig. 3B, Fig. 4B).

The temporal patterns of increased $\dot{V}_{O_2}$ and the peak factorial increments exhibited by male and non-reproductive female *T. marcianus* are consistent with previously published values for snakes following a 10% body mass meal. More specifically, in *Thamnophis elegans*, *Lamprophis fuliginosus* and *Nerodia sipedon*, peak post-prandial $\dot{V}_{O_2}$ values are attained within 1 day of ingestion, and values range from ~3-fold in *T. elegans* and *N. sipedon* to ~5-fold in *Acanthophis praelongus* and *Python molurus* (Sievert and Andreadis, 1999; Roe et al., 2004; McCue et al., 2005; Britt et al., 2006; Christian et al., 2007).

During reproduction, females also reached peak levels of $\dot{V}_{O_2}$ that were ~4.5- to ~5.0-fold above Rest values within 24 h of ingestion. The apparently unchanged peak factorial levels were due to the 1.6- to 1.7-fold reproductive Rest increments, and absolute Dig $\dot{V}_{O_2}$ values were actually 30–50% larger during the vitellogenic and embryogenic phases of the reproductive cycle. The sum of the reproductive Rest increment and the non-reproductive Dig increment (i.e. ~54–55 ml O_2 h^{-1}) is substantially lower than the reproductive Dig increment (i.e. 64–74 ml O_2 h^{-1}). Therefore, our results indicate that an additional energetic cost (~10–20 ml O_2 h^{-1}) associated with digestion exists for reproductive females.

The physiological mechanisms responsible for the large SDA response in snakes are understood to be related to increased organ mass and function (e.g. large increments in cardiopulmonary output, and intestinal brush-border protein transport capacity) and substantially increased rates of whole-animal protein synthesis (Houlihan, 1991; Hicks and Bennett, 2004; Secor, 2009). During reproduction, these processes are likely maintained, but to our knowledge this study represents one of the first examinations of the interaction effects of digestion and reproduction. Although our data
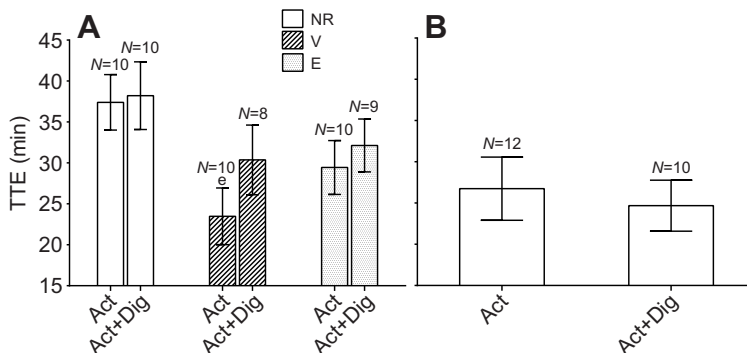
Fig. 5. Female and male *T. marcianus* time to exhaustion values associated with fasting exhaustive activity and post-prandial activity. Mean±s.e.m. time to exhaustion (TTE) for (A) females and (B) males during fasting exhaustive activity (Act) and post-prandial exhaustive activity (Act+Dig). Female reproductive condition is indicated in the key (NR, non-reproductive; V, vitellogenic; E, embryonic). Lowercase e, significantly lower than non-reproductive Act value, based on *post hoc* pairwise comparisons from a linear mixed model. Sample sizes are located above each value.

cannot elucidate the specific mechanisms associated with the 30–50% increase in $\dot{V}_{O_2}$, and only examined the first 24 h of the SDA response, previous work indicates that the presence of enlarged ovarian follicles within the posterior abdomen compresses the maternal organs (Munns and Daniels, 2007; Gilman et al., 2013), resulting in a substantially elevated cost of ventilation (e.g. 3-fold increase in *Tiliqua rugosa*) (Munns, 2013). It is possible that the mass/volume combination of the ovarian follicles with the 10% body mass meal was sufficient to produce a similar effect in *T. marcianus*. It is also possible that there are interaction effects in nutrient processing by the maternal liver between the vitellogenic/embryogenic processes (e.g. synthesis of vitellogenin and elevated rates of protein synthesis associated with oviduct remodeling) and those associated with digestion (e.g. liver remodeling, nutrient assimilation), resulting in substantially elevated rates of whole-animal protein synthesis.

Fasting exhaustive activity

Fasting exhaustive activity (Act) elicited ∼9.0-fold increment in $\dot{V}_{O_2}$ for non-reproductive females and males (Fig. 3A, Fig. 4A), led to exhaustion after 27 min (males) to 37 min (females; Fig. 5A,B), and gave RQ values of ∼1.2 (Fig. 3B, Fig. 4B). The aerobic scopes exhibited by male and female *T. marcianus* in the present study fall within the ∼2- to 12-fold range described for multiple species of snakes (e.g. Ruben, 1976; Ellis and Chappell, 1987; Andrade et al., 1997; Secor et al., 2000).

In the present study, both males and females (all reproductive conditions) exhibited RQ values of ∼1.2 during Act (Fig. 2B, Fig. 3B). Like all reptiles, snakes exhibit aerobic capacities that are approximately 1/10 the magnitude of those of a similarly sized endotherm (Bennett, 1994). Therefore, snakes employ anaerobic glycolysis to produce a large fraction (i.e. ∼50–90%) of the total ATP that is utilized during physical activity (Ruben, 1976; Gratz and Hutchison, 1977). Subsequently, byproducts from anaerobic glycolysis (i.e. lactate and hydrogen ions) accumulate in the bloodstream. The resulting activity-induced acid–base disturbance is buffered through ventilatory adjustments which serve to release bicarbonate-derived CO_2 from the lungs (Gleeson and Bennett, 1985). As a consequence, snakes and other reptiles routinely exhibit $\dot{V}_{CO_2}$ values that exceed $\dot{V}_{O_2}$ during physical activity, resulting in RQ values that surpass 1.0 (Gleeson and Bennett, 1982).

Following exhaustion, *T. marcianus* required 5–10 h to return to resting $\dot{V}_{O_2}$ levels, a time frame considerably longer than the approximately 2–3 h recovery period reported for the snake *Nerodia rhombifera*, multiple species of lizards, and crocodilians (Gratz and Hutchison, 1977; Gleeson, 1982; Gleeson and Dalessio, 1989; Hartzler, et al., 2006). However, the trial duration required to elicit exhaustion for *T. marcianus* was substantially longer than the 5–15 min protocols used in previous studies (Ruben, 1976; Ellis and Chappell, 1987; Andrade et al., 1997; Secor et al., 2000). Previous work has demonstrated that recovery time is directly related to the duration of the activity protocol, and this discrepancy may explain the long recovery times in *T. marcianus* (Gleeson and Hancock, 2002). Based on previous work in reptiles, it is likely that much of the excess post-exercise oxygen consumption (EPOC) is attributable to lactate oxidation and glycogenesis, phosphocreatine repletion and ATP repletion (Gratz and Hutchison, 1977; Gleeson, 1991; Gleeson and Hancock, 2002).

Contrary to our predictions, reproductive females exhibited aerobic scopes and RQ values that were not different from values obtained in the non-reproductive state, and reached exhaustion up

to ∼14 min sooner (i.e. reduced performance), indicating a prioritization pattern of oxygen delivery (Fig. 3A and Fig. 5A). The decrement in activity performance during vitellogenesis reported in the present study is consistent with previous work in *T. marcianus* (Seigel et al., 1987), and a number of species of lizards (Shine, 1980, 2003; Cooper et al., 1990; Sinervo et al., 1991). We are not aware of previous work that has examined how the competing oxygen demands from reproduction and activity are resolved, but it is possible that the metabolic processes associated with reproduction are temporarily compromised during physical activity, or that the small signal-to-noise ratio of the reproductive metabolic increments (1.6- to 1.7-fold) relative to activity (∼9-fold) made it difficult to detect an interaction effect.

In the present study, males exhibited endurance times (TTE) that were shorter than those of non-reproductive females (by ∼10 min); however, their aerobic scopes (∼9-fold above Rest values), RQ values (∼1.2) and recovery times were roughly equivalent (i.e. between 5 and 10 h). Female *T. marcianus* have been reported to undergo more than one reproductive cycle per annum (Ford and Karges, 1987), and therefore may be required to locomote more frequently throughout the season than males to capture appropriate prey items to match caloric intake with elevated demand. Thus, it is possible that females may have a greater selection pressure for elevated endurance capacity than males; however, future work is required to carry out additional tests and elucidate the specific causal mechanisms responsible for this apparent discrepancy in endurance time.

Post-prandial exhaustive activity

Contrary to our predictions, males and females (all reproductive conditions) exhibited a prioritization pattern of oxygen delivery during Act+Dig; that is, aerobic scopes were similar to values attained during Act (Fig. 3A, Fig. 4A). The prioritization pattern of $\dot{V}_{O_2}$ exhibited by *T. marcianus* in the present study contrasts with previous work in *Varanus exanthematicus* and *P. molurus* (Secor et al., 2000; Bennett and Hicks, 2001), but is consistent with work in rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*Oncorhynchus tshawytscha*), sablefish (*Anoplopoma fimbria*) and cane toads (*Bufo marinus*) (Andersen and Wang, 2003; Furnell, 1987; Aslop and Wood, 1997; Thorarensen and Farrell, 2006).

During Act+Dig, males and non-reproductive females must locomote with the 10% body mass meal, and reproductive females are further encumbered by a 20–30% body mass increment associated with the enlarged ovarian follicles. In vertebrates, additional weight during locomotion results in a linear increase in the force required for muscular contraction, and therefore increased metabolic cost (i.e. a ∼10% increase in $\dot{V}_{O_2}$ for each additional 10% in body mass) (Taylor et al., 1980). For reproductive females, the combined mass and volume constraints associated with the ingested meal and enlarged ovarian follicles may further result in elevated costs of ventilation (Munns, 2013). Therefore, if the cardiopulmonary system has sufficient capacity for delivering O_2 to all metabolically active organ beds simultaneously (hence additivity; Bennett and Hicks, 2001), the individual $\dot{V}_{O_2}$ increments from digestion and activity would sum together, and the increased costs of performing locomotion and ventilation would result in a still larger increase in $\dot{V}_{O_2}$ during Act+Dig (i.e. ∼15-fold increase in $\dot{V}_{O_2}$ over Rest values). This was not the case in the present study. Instead, the similar aerobic scopes exhibited by *T. marcianus* during Act and Act+Dig may indicate that this

species' cardiopulmonary system has a limited capacity for oxygen transport (peaks at ~9- to 10-fold above Rest).

Alternatively, sufficient capacity for oxygen delivery may exist, but the presence of a large meal within the stomach, and ovarian follicles in reproductive females, may alter ventilation patterns (i.e. constraining maximal levels of O_2 and CO_2 exchange in the lungs). More specifically, regions of the lung may be compressed or collapse as a result of the expansion of the ovarian follicles during reproduction and the distention of the stomach following ingestion, thereby limiting tidal volume and hence the respiratory fraction of fresh, oxygen-rich air in contact with the respiratory surfaces (Munns and Daniels, 2007). In addition to altered ventilation patterns, the combination of intense physical activity, enlarged ovarian follicles and the distention of the stomach may increase intra-abdominal pressure in *T. marcianus* sufficiently to reduce blood flow through the inferior vena cava, thereby limiting venous return and constraining maximal levels of cardiac output (Farmer and Hicks, 2000; Munns et al., 2004).

During Act+Dig in the present study, all groups were capable of maintaining activity performance (TTE) in spite of the additional 10–40% body mass load (Fig. 5A,B). Our data cannot discern why *T. marcianus* is capable of overcoming this burden without any noticeable decrement in activity performance, but it is possible that most of the metabolic processes associated with digestion and reproduction are temporarily compromised during exhaustive activity, and blood supply to the gastrointestinal tract and oviduct are preferentially redistributed to the active skeletal muscles, thus prioritizing activity performance (Furnell, 1987; Thorarensen and Farrell, 2006). Moreover, a decrease in right–left shunt fraction in reptiles may result in elevated O_2 extraction from ventilated air and thus increased O_2 diffusion gradient at the tissues (Secor et al., 2000; Bennett and Hicks, 2001). A similar effect may occur if this species decreases ventilation/perfusion heterogeneity at the lungs and/or decreases intrapulmonary shunting (Bennett and Hicks, 2001). Such cardiopulmonary adjustments may have provided *T. marcianus* with sufficient O_2 delivery capacity to simply maintain $\dot{V}_{O_2}$ levels and activity performance.

In the present study, both male and female (all reproductive conditions) *T. marcianus* exhibited a shift in RQ from ~1.2 during Act to ~1.3–1.5 during Act+Dig. This increase in RQ may reflect the additional muscular work required to perform locomotion with the additional body mass burden that cannot be met by aerobic metabolism, thus resulting in a more pronounced utilization of anaerobic pathways to fuel activity performance (Gleeson and Bennett, 1982). Moreover, because $\dot{V}_{O_2}$ remains unchanged between Act and Act+Dig, and activity performance (i.e. TTE) is maintained, it is plausible that a sizable portion of ATP that was previously synthesized through aerobic pathways during Act was synthesized anaerobically during Act+Dig. Ultimately, the RQ values presented in this study indicate a pattern of increased reliance on anaerobic metabolism during Act+Dig in male and female *T. marcianus* (Gleeson and Bennett, 1982). However, additional measurements of total body lactate production are required in order to elucidate the precise extent to which anaerobic metabolic pathways contribute to total ATP production during Act and Act+Dig.

Conclusions
Under natural conditions, animals may perform more than one metabolically demanding function simultaneously. Laboratory

studies on the patterns of physiological prioritization that occur under these conditions represent an important area of investigation if conclusions are to be extrapolated to predicting performance outcomes in the natural environment (Jackson, 1987; Andersen and Wang, 2003). In the present study, we examined how the simultaneous oxygen demands associated with reproduction, digestion and activity are resolved, and our results indicate that prioritization is the primary pattern of oxygen delivery. During Act+Dig, activity performance was likely prioritized at the expense of digestive and perhaps reproductive functions. We hope that future work examines the specific cardiopulmonary mechanisms associated with the prioritization response described in the present study. More specifically, measurements of the components of cardiac output and minute ventilation, in addition to measurements of P_{O_2} and P_{CO_2} within the systemic and venous circulation, would be useful for identifying possible limitations in oxygen transport (Secor et al., 2000; Bennett and Hicks, 2001). Furthermore, measurements of mesenteric artery and hepatic portal blood flow would be helpful for identifying possible shifts in blood flow distribution during post-prandial activity (Secor and White, 2010).

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