

## Respiratory Biology during Gravidity in *Crotaphytus collaris* and *Gambelia wislizenii*

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**ABSTRACT.**—During gravidity lizards experience a striking decrease in lung volume as a result of lung compression by eggs growing within the body cavity. In order to understand the effect of this decrease in lung volume on the respiratory biology of gravid egg-laying lizards, we measured changes in total lung volume, resting and postexercise expired volume, minute volume, respiratory frequency, and carbon dioxide production rate during reproduction in the Collared Lizard, *Crotaphytus collaris*, and the Leopard Lizard, *Gambelia wislizenii*. We found that compression of the lungs by shelled eggs resulted in an average 48% (range: 26–70%) decrease in total lung volume compared to the same postlaying *C. collaris* females, and an average 38% (range: 29–46%) decrease in *G. wislizenii*. CO<sub>2</sub> production rates were altered significantly during reproduction in female *C. collaris* and were 58% higher in females carrying late-stage follicles, compared to after laying. Despite the remarkable reduction in lung volume in both of these species and the increase in CO<sub>2</sub> production rates in *C. collaris*, no ventilation parameters changed over the course of reproduction. The highly distensible body cavities of *C. collaris* and *G. wislizenii* appear to be able to accommodate both growing eggs and adequate lung volumes for normal respiratory function during gravidity.

The respiratory system functions to match oxygen uptake and carbon dioxide release with the metabolic requirements of an organism. In ectotherms, changes in physical and physiological states, such as temperature, feeding and digestion, and exercise, alter metabolic requirements and result in dramatic compensatory changes in ventilation. This change in ventilation is achieved by altering the amount of air that is inhaled (tidal volume), breathing frequency, or both. For example, postprandial (after eating) increases in metabolic rate in the Savannah Monitor Lizard *Varanus exanthematicus* resulted in tidal volumes that were almost twice that of resting values, whereas breathing frequency remained about the same as resting (Hicks et al., 2000). In contrast, elevated metabolic rates due to exercise in the same lizard resulted in striking increases in breathing frequency and smaller than resting tidal volumes (Wang et al., 1997). Gravidity has also been shown to increase metabolic rates in lizards (Angilletta and Sears, 2000), but if and how ventilation changes to compensate for increased energetic costs during gravidity in egg-laying lizards is unknown. In fact, most of the literature on the respiratory biology of lizards has focused on males and nonreproductive females (Dawson and Templeton, 1963; Wood et al., 1995; Hicks et al., 2000; Butler et al., 2002), so we know little about the physiological changes that occur during a period that is crucial to the survival of individuals and their young.

Studies have shown that both performance and survivorship are affected by the physiological and load-bearing costs of reproduction (Miles et al., 2000; Olsson et al., 2000; Shine, 2003; Zani et al., 2008); therefore, the risk of mortality for both the mothers and offspring is elevated at this time. However, to our knowledge, the effect of clutch development on the respiratory physiology of lizards has only been examined in a single species, *Tiliqua rugosa* (Australian Sleepy Lizard) (Munns and Daniels, 2007).

Many lizards, such as the iguanids *Crotaphytus collaris* (the Collared Lizard, Say in James 1823) and *Gambelia wislizenii* (the Leopard Lizard, Baird and Girard 1852), and the scinid *T. rugosa*, possess simple, sac-like few- or single-chambered lungs

(Perry, 1989). These lungs are located within a continuous thoracic and abdominal cavity and are highly distensible (Mader, 1997). Because of their distensible nature and lack of a diaphragm, both naturally occurring and introduced materials within the body cavity (i.e., organs, food, eggs) may compress the lungs and reduce the volume available for gas exchange (Gilman, unpublished; Munns and Daniels, 2007). Munns and Daniels (2007) observed that lung compression due to fetus growth resulted in significantly decreased expired volume in females with no compensatory increase in breathing frequency. These results suggest that compression of the lungs may be partially responsible for the observed decrease in speed and endurance seen during gestation in this species (Munns and Daniels, 2007).

Although Munns and Daniels (2007) found a significant effect of lung compression on expired volume in *T. rugosa*, this may not be typical for many lizards. Physiological and morphological differences between this species and others may result in substantial differences in the degree to which their respiratory physiology is affected during reproduction. *Tiliqua rugosa* is a large, long-lived, slow-moving, herbivorous lizard and is typically pregnant for 6 to 7 months (Bull and Freaque, 1999; Munns and Daniels, 2007). This skink's lifestyle contrasts greatly with the active lifestyle of many other lizard species, e.g., it has a lower aerobic scope than more active lizards, and therefore might be expected to differ in other aspects of its respiratory physiology (Bennett and Dawson, 1976). Additionally, *T. rugosa* have markedly rigid bodies that resist expansion. In this species, it is difficult to determine the reproductive stage of females because they do not show identifiable changes in body shape (Munns and Daniels, 2007). In contrast, body shape is often used to determine reproductive stage in many lizards, particularly iguanids, and in many cases follicles and eggs can be easily palpated through the soft body cavity (Sloan and Baird, 1999; Zani et al., 2008).

The distensible nature of the body cavity may be advantageous during feeding in iguanids. The iguanids in the family Crotaphytidae, like *C. collaris* and *G. wislizenii*, often ingest prey up to 50% of their body length (other lizards, snakes, and rodents) (McAllister, 1985; Degenhardt et al., 1996), and having a flexible body wall may reduce the impact of large prey on movement and organ function. This flexibility may also be physiologically important during reproduction, as it would

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allow increases in egg volume without resulting in a one-to-one loss of space within the body cavity for organs and ingested prey. However, it is clear upon dissection that growing eggs within the abdomen do reduce some of the available body cavity space in these species, which appears to result in decreased lung volume (pers. obs.). Whether or not this apparent reduction in lung volume interferes with normal respiration in *C. collaris* and *G. wislizenii* is not clear.

In this study, we examine respiratory physiology during reproduction in two active, predatory, egg-laying lizard species: *C. collaris* and *G. wislizenii*. These lizards are moderately sized (~20–50 g), carnivorous, diurnal (Degenhardt et al., 1996), and are similarly shaped (they are neither dramatically elongate nor plate-like, which could affect the size, shape, and volume of the lungs). We address the question of whether, and to what degree, total lung volume, expired volume, breathing frequency, minute volume, and carbon dioxide production rate, change throughout the reproductive cycle in these species.

We test two hypotheses: 1) the growing clutch reduces total lung volume in gravid females, which results in decreased expired volume, as seen previously in *T. rugosa*, and conversely 2) the decrease in total lung volume is not great enough to affect expired volume significantly, potentially because of the highly distensible abdominal cavity of *C. collaris* and *G. wislizenii*. We also predict that if decreased lung volume during gravidity results in decreased tidal volume, breathing frequency will increase to maintain adequate minute ventilation to meet the energetic needs of these active species.

#### MATERIALS AND METHODS

We collected *C. collaris* (3 males and 10 females) and *G. wislizenii* (3 males and 11 females) from adjacent field sites in central New Mexico (Bernalillo County). We housed the animals until after all females laid their eggs and took respiratory measurements from the females at each reproductive stage following their capture until the end of the reproductive cycle (see below). We caught one female *C. collaris* that was carrying early-stage follicles, the others were carrying either late-stage follicles or eggs. One of the *C. collaris* females that was initially carrying late-stage follicles later developed a second clutch in captivity, and we were able to use that female for an additional set of early-stage follicle measurements. All *G. wislizenii* females were carrying late-stage follicles or eggs. We housed lizards outdoors in 1.2 × 0.6 × 0.6-m screen cages (LLL Reptile and Supply Co., Inc., Vista, CA) to allow access to natural light and climatic conditions. We outfitted cages with sand, wood, and rock refugia, and we covered one end of the cage with a 46 × 31-cm board to provide shade. We fed the lizards crickets every other day throughout the study and provided them with water daily. We housed two females to every male *C. collaris* and one male to every female *G. wislizenii*, when possible, to provide mating opportunities for both species to encourage normal reproduction. We placed females that we estimated to be close to oviposition in smaller cages filled with moist perlite to provide an appropriate substrate for laying eggs.

**Total Lung Volume.**—We used computed-tomography (Nano-SPECT/CT®) imaging to estimate lung capacity at two points during the reproductive cycle (while carrying shelled eggs and postlaying) in three females per species and at one point in two males of each species. Prior to measurements, we fasted the lizards for 48 h to limit the amount of food in their gut. To reduce activity, we placed each lizard in a cloth bag in a standard

laboratory refrigerator for ~20 min and/or freezer for ~5 min until cool (cooling the lizards should affect metabolic rate and therefore expired volume and breathing rate, but not total lung volume, which scales with mass and is a function of body size (Wood and Lenfant, 1976)). We then taped lizards to a cardboard restraint board with surgical tape (about the head, body, and limbs), to allow respiration but restrict other movement, and placed them in a cloth bag on an imaging bed. We acquired whole-body CT images of each lizard at 45kVp and 1 sec per projection. The total CT scan time was 6–7 min depending on the number of bed positions required to accommodate the length of the lizard. We estimated total lung volume with the use of the reconstructed CT data by fitting three-dimensional volumes to the interior of the lungs. We divided the lung volume into 3.2-mm axial regions consisting of eight reconstructed slices from the CT images. We determined the entire lung volume by summation of these regions. Because lizard breathing frequencies were low, tidal volumes were small, and images were averaged over the entire scanning period; respiration had a negligible effect on the estimation of lung volume.

Although we originally scanned three females (gravid and then postlaying) and three males of each species to determine total lung volume, one female of each species was carrying enlarged follicles postlaying. Therefore, we captured and scanned one additional postlaying female of each species to increase postlaying sample sizes.

We conducted the following procedures in the order presented below for each female, once a week. Male respiration was measured once during the study period.

**Carbon Dioxide Production Rate.**—We recorded CO<sub>2</sub> production rate, breathing frequency, and resting expired volume estimates between 2300 and 0400 during the lizards' rest phase to minimize activity. We fasted lizards for 48 h prior to all measurements to ensure they were postabsorptive (Hicks et al., 2000; Iglesias et al., 2003). We placed lizards in individual chambers in the dark within a temperature-controlled cabinet (33°C) for 2 h to acclimate before recording. We chose 33°C because this is a body temperature that is within the recorded range associated with activity in both species (Fitch, 1956; Parker and Pianka, 1976). We measured the ambient temperature within the chambers with the use of a 21-gauge Cu–Cn thermocouple and a TC-1000 thermocouple meter (Sable Systems, Las Vegas, NV) to ensure that incoming air did not vary the temperature in the chamber more than ±0.5°C. Chambers consisted of 473-ml glass jars fitted with airtight rubber stoppers with two 0.6-cm holes. Incoming and outgoing air flowed through rigid Bev-A-Line® running through the holes in each rubber stopper. Incoming dry, CO<sub>2</sub>-free air was produced by a FTIR Purge Gas Generator (Whatman, Newton, MA) and controlled by a flow meter (Omega FL-3402C and FL-3403G flow meters, Omega Engineering, Inc., Stamford, CT). Incoming air flowed through each chamber at 152 mL · min<sup>-1</sup> (STP). Outgoing air was sampled by a multiplexer (Systems Respiriometry Multiplexer V2.0, Sable Systems), water vapor was scrubbed with the use of a Drierite® column, and CO<sub>2</sub> was measured with the use of a CO<sub>2</sub> analyzer (LI-7000 CO<sub>2</sub>/H<sub>2</sub>O analyzer, LI-COR Biotechnology, Lincoln, NE). We calibrated the CO<sub>2</sub> daily with the use of dry, CO<sub>2</sub>-free air and a certified span gas containing 999 ppm CO<sub>2</sub> (Matheson Tri-Gas, Houston, TX). Output from the CO<sub>2</sub> analyzer was digitized with the use of a Universal Interface II (Sable Systems) and recorded on a personal computer with Datacan V data acquisition software (Sable Systems), with a sampling interval of 5 sec. We measured CO<sub>2</sub> production rate for a total of 20 min per animal a night (twice for

10 min). We used the lowest values of the two time periods to ensure that we were using the lowest resting metabolic rate for our analyses. We also measured baseline values for the incoming air before and after each individual's measurement. We calculated the rate of CO<sub>2</sub> production using the equation  $VCO_2 = FR(F_eCO_2 - F_iCO_2)/(1 - F_eCO_2(1 - [1/RQ]))$  (Lighton, 2008), where  $VCO_2$  is the rate of production,  $FR$  is the standard temperature and pressure flow rate of the incoming gas,  $F_eCO_2$  is the fractional concentration of CO<sub>2</sub> leaving the chamber,  $F_iCO_2$  is the fractional concentration of CO<sub>2</sub> entering the chamber, and  $RQ$  is the respiratory quotient. For our calculations we assumed an  $RQ$  of 0.7, reflective of oxidation of lipids, which has been shown to be typical of fasted lizards (Bennett and Dawson, 1976).

**Breathing Frequency, Resting Expired Volume, and Minute Volume Estimates.**—Following recording of the rate of CO<sub>2</sub> production, we replaced the two-hole rubber stoppers with one-hole stoppers fitted with a 15-cm lengths of 0.6 cm tubing and returned the lizards to the cabinet. We left the lizards in the dark for 1/2 h in the temperature chamber to allow them to return to a resting state. We connected the tubing from each chamber to a differential pressure transducer (PT100B, Sable Systems) to measure breathing frequency (number of breaths a minute) and resting expired (tidal) volume. We recorded the signal resulting from changes in air flow within each chamber with the use of ExpeData software (Sable Systems). We recorded resting expired volumes and breathing frequencies for 10 min at a time, twice per night, for each lizard and averaged all data for an individual per night for analysis. We calibrated expired volumes with the use of a 1-cc 1000 Series Hamilton Gastight syringe (model 1001) and injected known volumes of air at several injection frequencies into an empty chamber and recorded the resulting signal. We used volumes approximating the lizards' expected expired volume and breathing frequency range (Templeton and Dawson, 1963) for the calibration. Resulting regressions of injection volume on transducer signal at multiple injection rates showed a significant effect of injection rate on resulting transducer signal (ANCOVA test of intercept,  $P < 0.0001$ ), though all injection rates affected the resulting signal in the same way (ANCOVA test of slope,  $P = 0.95$ ). We used resulting relationships between these variables to estimate expired breath volumes (regressions of injection volume on transducer signal  $r^2 > 0.98$ ). Our procedure for estimating tidal volume is similar to the one recommended by Funk et al. (1986) for animals that are contained in a chamber. We calculated minute volumes, or the volume of air exchanged per minute, as a product of expired volume and breathing frequency. We also calculated the air convection requirement (minute ventilation [mL · min<sup>-1</sup>]/CO<sub>2</sub> production rate [mL · min<sup>-1</sup>]) for females and males of both species, to determine if there was evidence of hyper- or hypoventilation at any point during the study.

**Postexercise Expired Volume Estimates.**—Following our measurement of breathing frequency and resting expired volume, we placed lizards in cloth bags in the dark at room temperature (24.1 ± 0.12°C mean, SE) to rest until the following morning. Between 0900 and 1100 we brought the lizard bags into the light of the room for about an hour to allow the lizards to acclimate to the light. Because of the need to transfer the lizards into an open-air environment (not a closed chamber) to exercise them, we were not able to keep a consistent environmental temperature of 33°C while exercising them and recording postexercise expired volume (PEEV); instead this was accomplished at room temperature (24.1 ± 0.12°C mean, SEM). To obtain PEEV, we placed each lizard individually in a 0.9 × 0.6 × 0.9-m plastic tub. We encouraged

the lizard to run by tail tapping or simply following behind the lizard with our hand for 1 min and then immediately placed the lizard in a chamber to record expired volume for 1 min (as above). We placed lizards that were reluctant to run repeatedly on their backs to force them to right themselves. We used these activities to induce active, forced breathing, with expired volumes larger than resting expired volumes (potentially showing greater changes due to lung compression by the eggs than resting values). We repeated this procedure twice consecutively for each lizard. Once all respiratory parameters were measured, we weighed and scanned each lizard with ultrasound imaging to determine reproductive stage (Titan SonoSite Titan Portable Ultrasound system and a Titan L38 5–10-MHz broadband linear array transducer; SonoSite, Inc., Bothell, WA) (Gilman and Wolf, 2007). We then fed them and returned them to their respective cages.

**Analyses.**—For analyses we placed females into one of five categories based on the size and stage of their eggs (early follicle, late-stage follicle, early egg, shelled egg, postlaying). We categorized females as containing early follicles if the follicles were spherical and echolucent (appearing dark) and smaller than 0.5 cm. Late-stage follicles were greater than 0.5 cm and spherical. Early eggs were elongated and echolucent, meaning they had been ovulated but were not shelled, and shelled eggs were easily identified because of their overall echogenic (appearing light) appearance, and in later stages by the separation of the echolucent albumin from the echogenic yolk (Gilman and Wolf, 2007). We used only one set of values for respiratory parameters per individual, per stage. If multiple sets of data were recorded for an individual of a particular stage (if, for example, an individual was measured for 2 weeks in a row and was carrying shelled eggs both weeks), we selected one set of measurements randomly for the analyses. We were unable to sample the same females consistently for each category, and so we present volume measurements as mass-specific to standardize the means, with the use of nongravid masses to remove the effect of the mass of the eggs on the values (Dawson and Templeton, 1963; Wang et al., 1997; Hicks et al., 2000; Secor et al., 2000). Because our data are unbalanced, we used mixed-model repeated-measures analyses to test for differences among stages in females and for post hoc between-stage analyses. We used reproductive stage as the fixed effect and individual as the random effect. When conducting post hoc analyses we corrected for multiple comparisons with the use of the sequential Bonferroni test for each set of tests (Rice, 1989). We tested for differences between postreproductive (postlaying) females and males with the use of Wilcoxon rank sum tests or two-sample  $t$ -tests. For all tests, we required a  $P < 0.05$  for the rejection of a null hypothesis.

## RESULTS

*Crotaphytus collaris* and *G. wislizenii* showed breathing patterns similar to those seen previously in *C. collaris*: active expiration, passive inspiration (back to resting lung position), active inspiration, passive expiration, respiratory pause (if present, lasting up to 4 min 20 sec in *C. collaris* and 2 min 38 sec in *G. wislizenii* during our study), followed again by active expiration (Templeton and Dawson, 1963; Wood and Lenfant, 1976). Individuals of both species showed considerable variability within this pattern in the parameters that constitute minute ventilation (i.e., tidal volume, breathing frequency and respiratory pause). For example, two females of *G. wislizenii* at the same reproductive stage had strikingly different breathing

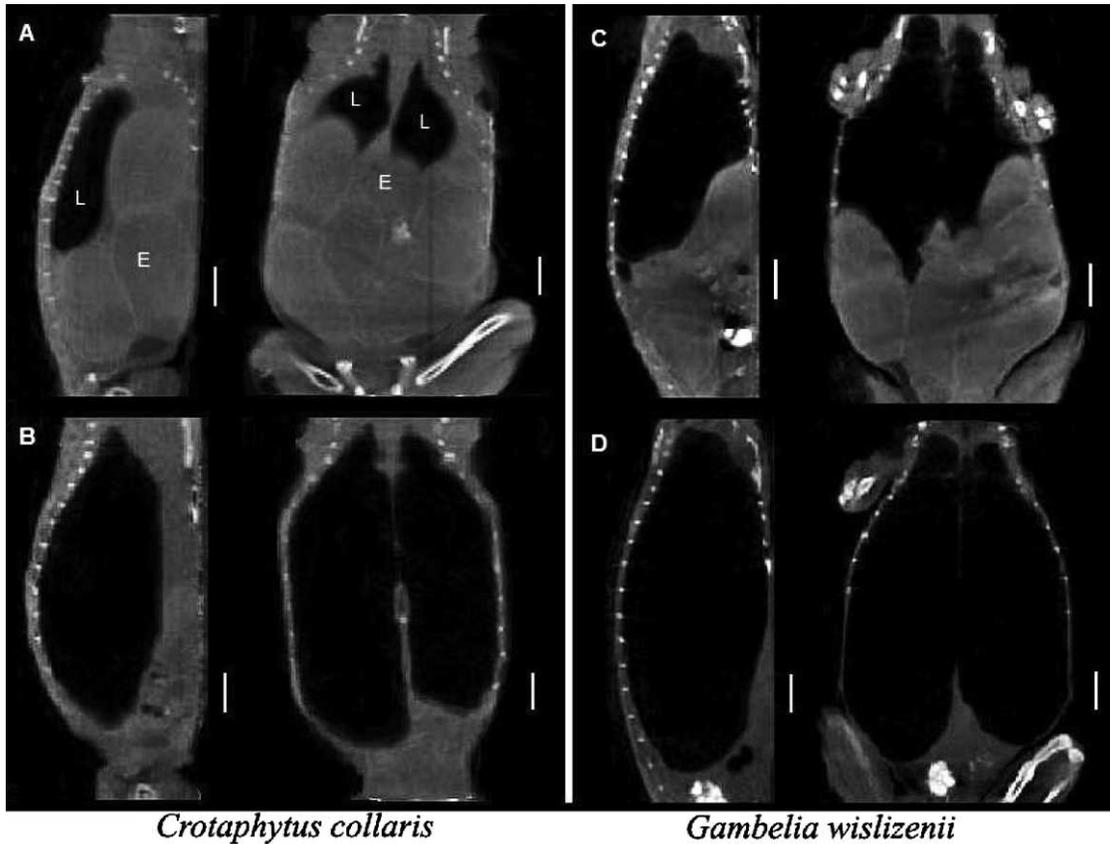


FIG. 1. Images from computed-tomography (CT) scans of females of *Crotaphytus collaris* and *Gambelia wislizenii*. The four groups of images are lateral (left image) and dorsal-ventral (right image) views. Black areas within the body cavity are lungs (L) and gray areas at the mid to lower end of the body are eggs (E) in A and C. (A) Gravid female of *C. collaris* (relative clutch mass 28% with associated 70% reduction in lung volume). (B) The same female of *C. collaris* after laying. (C) Gravid female of *G. wislizenii* (relative clutch mass 44% with associated 46% reduction in lung volume). (D) The same female of *G. wislizenii* after laying. White 5-mm scale bars are located in the lower right area of each scan. Image depth is 0.2 mm.

patterns. Although one female breathed infrequently (3 breaths  $\cdot$  min $^{-1}$ ) and had large expired volumes (0.02 cc  $\cdot$  g $^{-1}$ ), the other showed the reverse pattern: many frequent (31 breaths  $\cdot$  min $^{-1}$ ), smaller (0.006 cc  $\cdot$  g $^{-1}$ ) breaths.

Compression of the lungs by eggs in gravid females resulted in greatly reduced lung volumes in both species (Fig. 1). Females of *C. collaris* had 26 and 70% reductions in lung volume when gravid compared to postlaying, and females of *G. wislizenii* had 29 and 46% reductions. Males and postreproductive females of a species did not differ in their mass-specific lung volumes (*C. collaris*:  $t[2] = -1.20$ ,  $P = 0.35$ ; *G. wislizenii*:  $t[2] = -0.79$ ,  $P = 0.51$ ) (Table 1).

There were significant changes in CO<sub>2</sub> production rates during reproduction in females of *C. collaris* ( $F_{4,8} = 4.76$ ,  $P = 0.01$ ) (Fig. 2A). Females carrying late-stage follicles had 58% higher CO<sub>2</sub> production rates than postlaying ( $F_{1,6} = 15.38$ ,  $P = 0.03$ , nonsignificant with sequential Bonferroni correction). In contrast, there were no significant changes in CO<sub>2</sub> production rate during reproduction in females of *G. wislizenii* ( $F_{3,7} = 1.05$ ,  $P = 0.44$ ) (Fig. 2B). CO<sub>2</sub> production rate in postreproductive females was not different from that of the males, in either species (*C. collaris*:  $n_{\text{males}} = 3$ ,  $n_{\text{females}} = 5$ ,  $W = 21$ ,  $P = 0.77$ ; *G. wislizenii*:  $n_{\text{males}} = 3$ ,  $n_{\text{females}} = 6$ ,  $W = 29$ ,  $P = 0.90$ ).

Although gravidity resulted in a striking reduction in lung volume in both species, this reduction was not compensated for

TABLE 1. Sizes and estimated lung volumes of female and male *Crotaphytus collaris* and *Gambelia wislizenii*.  $n = 3$  for all categories. Values shown as mean  $\pm$  SE.

	Nongravid mass (g)	Snout-vent length (mm)	Postlaying lung volume (cc)	Gravid lung volume (cc)	Postlaying lung volume (cc $\cdot$ g $^{-1}$ )	Gravid lung volume (cc $\cdot$ g $^{-1}$ )
<i>Crotaphytus collaris</i>						
Female	27.74 $\pm$ 2.09	91.00 $\pm$ 0.91	11.97 $\pm$ 2.27	6.71 $\pm$ 2.76	0.45 $\pm$ 0.12	0.27 $\pm$ 0.12
Male	41.92 $\pm$ 2.50	99.00 $\pm$ 2.65	12.98 $\pm$ 1.18		0.31 $\pm$ 0.01	
<i>Gambelia wislizenii</i>						
Female	26.79 $\pm$ 0.56	105.25 $\pm$ 3.07	14.15 $\pm$ 4.36	7.97 $\pm$ 2.67	0.53 $\pm$ 0.16	0.29 $\pm$ 0.10
Male	17.13 $\pm$ 1.29	89.33 $\pm$ 0.67	6.46 $\pm$ 1.19		0.38 $\pm$ 0.06	

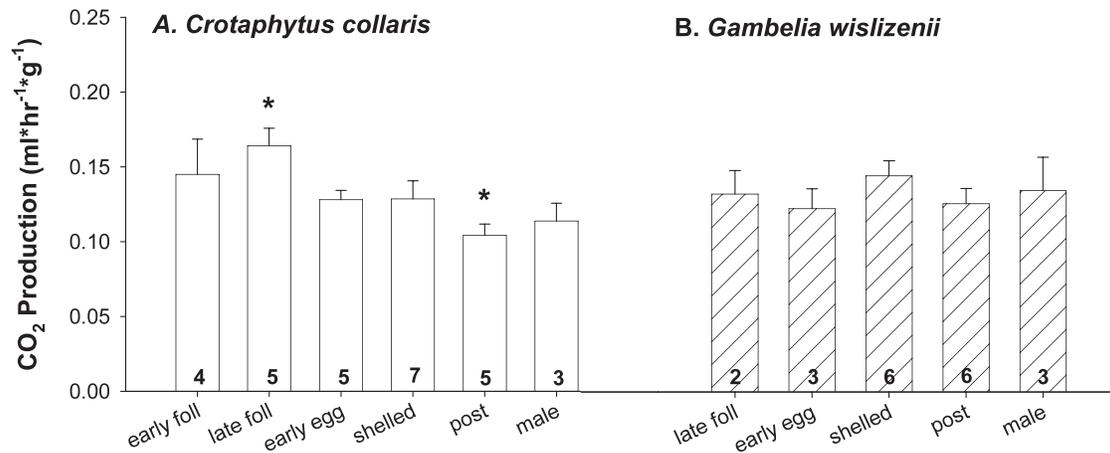


FIG. 2. CO<sub>2</sub> production rate throughout the reproductive cycle in females, and males of *Crotaphytus collaris* and *Gambelia wislizenii*. Values are shown as mean and SE. The sample size for each category is shown at the base of each bar. There were significant changes in CO<sub>2</sub> production rates during reproduction in females of *C. collaris* ( $P = 0.01$ ). Stars denote the largest difference between groups (58%).

by changes in the ventilation parameters we measured. Breathing frequency, resting expired volume, minute volume, and postexercise expired volume did not differ across stages in females of either species (breathing frequency: *C. collaris*:  $F_{4,9} = 2.11$ ,  $P = 0.13$ ; *G. wislizenii*:  $F_{3,10} = 1.52$ ,  $P = 0.28$  [Fig. 3A,B]; resting expired volume: *C. collaris*:  $F_{4,8} = 2.08$ ,  $P = 0.14$ ; *G. wislizenii*:  $F_{3,7} = 0.87$ ,  $P = 0.50$  [Fig. 3C,D]; minute volume: *C. collaris*:  $F_{4,8} = 0.61$ ,  $P = 0.66$ ; *G. wislizenii*:  $F_{3,7} = 1.53$ ,  $P = 0.29$  [Fig. 3E,F]; postexercise expired volume: *C. collaris*:  $F_{4,4} = 0.31$ ,  $P = 0.86$ ; *G. wislizenii*:  $F_{3,3} = 1.25$ ,  $P = 0.40$  [Fig. 4]). There were also no significant differences between postreproductive females and males of the same species in most of the ventilation parameters we measured, except for breathing frequency in *C. collaris* (breathing frequency, resting expired volume, minute volume, postexercise expired volume: *C. collaris*:  $n_{\text{males}} = 3$ ,  $n_{\text{females}} = 5$ ,  $W = 30$ ,  $P = 0.04$ ,  $W = 19$ ,  $P = 0.37$ ,  $W = 27$ ,  $P = 0.23$ , postexercise  $n_{\text{males}} = 3$ ,  $n_{\text{females}} = 3$ ,  $W = 11$ ,  $P = 1.00$ ; *G. wislizenii*:  $n_{\text{males}} = 3$ ,  $n_{\text{females}} = 7$ ,  $W = 36$ ,  $P = 0.65$ ,  $W = 44$ ,  $P = 0.25$ ,  $W = 41$ ,  $P = 0.65$ , postexercise  $n_{\text{males}} = 3$ ,  $n_{\text{females}} = 3$ ,  $W = 10$ ,  $P = 1.00$ ).

In addition to tests of the effect of lung compression on tidal volume across all females in our study, we were interested in whether or not females with the largest relative clutch masses (ratio of clutch mass to nonreproductive female body mass) might be affected to a greater degree than those with smaller relative clutch masses. A reduction in resting expired volume during gravidity was evident when we examined four *G. wislizenii* with the largest relative clutch masses ( $38.3 \pm 2.2\%$ , compared to the mean of  $31.4 \pm 3.6\%$ ). These females had significantly smaller resting expired volumes when gravid ( $0.007 \pm 0.002 \text{ cc} \cdot \text{g}^{-1}$ ), compared to after laying ( $0.011 \pm 0.002 \text{ cc} \cdot \text{g}^{-1}$ ) (paired  $t$ -test,  $n = 4$ ,  $t = -3.68$ ,  $P = 0.04$ ). However, females of *C. collaris* did not show the same trend (relative clutch mass  $36.3 \pm 3.53\%$ , compared to the mean of  $30.6 \pm 4.3\%$ ; volumes  $0.005 \pm 0.002$  shelled,  $0.007 \pm 0.003 \text{ cc} \cdot \text{g}^{-1}$  post,  $n = 3$ ,  $P = 0.42$ ).

Although there was an increase in CO<sub>2</sub> production rate in *C. collaris* late-stage follicles and no change in minute ventilation across stages, air convection requirement values did not differ across reproductive stage for either species (*C. collaris*:  $F_{4,4} = 0.31$ ,  $P = 0.86$ ; *G. wislizenii*:  $F_{3,3} = 1.25$ ,  $P = 0.40$ ). Air convection requirement values ranged from  $28.06 \pm 8.63$  to  $51.94 \pm 11.75$  in *C. collaris* and from  $26.49 \pm 4.14$  to  $48.08 \pm 12.72$  in *G. wislizenii*

(reproductive stage mean  $\pm$  SE). Individuals in our study appeared to be able to match ventilation with CO<sub>2</sub> output, regardless of reproductive stage.

#### DISCUSSION

Carbon dioxide production rate, tidal volume, and breathing rate at 33°C for all individuals of *C. collaris* in this study were comparable to males of *C. collaris* as reported by Dawson and Templeton (1963) and Templeton and Dawson (1963) at the same temperature. Respiratory parameters for individuals of *G. wislizenii* also fell within the same ranges.

In our study, females of both species showed dramatic decreases in total lung volume during gravidity as a result of compression by the eggs, with no associated decrease in either resting or postexercise expired volume, with the exception of the female *G. wislizenii*, with the largest relative clutch masses. The lack of an effect of decreased lung volume on expired volume in most individuals may be, in part, related to body cavity distensibility. Relative clutch masses of *T. rugosa* in the study by Munns and Daniels (2007) were  $21.6 \pm 2.6\%$ , whereas *C. collaris* and *G. wislizenii* in our study had relative clutch masses of  $33.7 \pm 2.7\%$  and  $31.4 \pm 3.6\%$ , respectively. Given the larger relative clutch masses in our study species and the need of body cavity to accommodate the clutch, we might expect the effect of lung compression to be greater in our species than in *T. rugosa*. However, although it is difficult to tell the reproductive stage of females of *T. rugosa* because they do not show obvious changes in body shape and their abdomens are covered in large ossified scales (Munns and Daniels, 2007), the abdomens of *C. collaris* and *G. wislizenii* are quite pliant, and reproductive stage can be determined relatively easily by palpation (Husak, 2006). The relative flexibility of the abdomens in our study species is also demonstrated in that the size of the clutch is not reflected directly in the degree of lung compression in *C. collaris* and *G. wislizenii*. The two females of *C. collaris* we scanned with CT had relative clutch masses of 28.2 and 25.9% with reductions in lung volume during gravidity of 70.1 and 26.5%, respectively, and the two females of *G. wislizenii* had relative clutch masses of 43.5 and 26.9% and reductions in lung volume of 46.2 and 28.7%, respectively. The abdominal flexibility in *C. collaris* and *G. wislizenii* may allow for greater relative clutch masses than in *T. rugosa*, with overall no effect on expired volume.

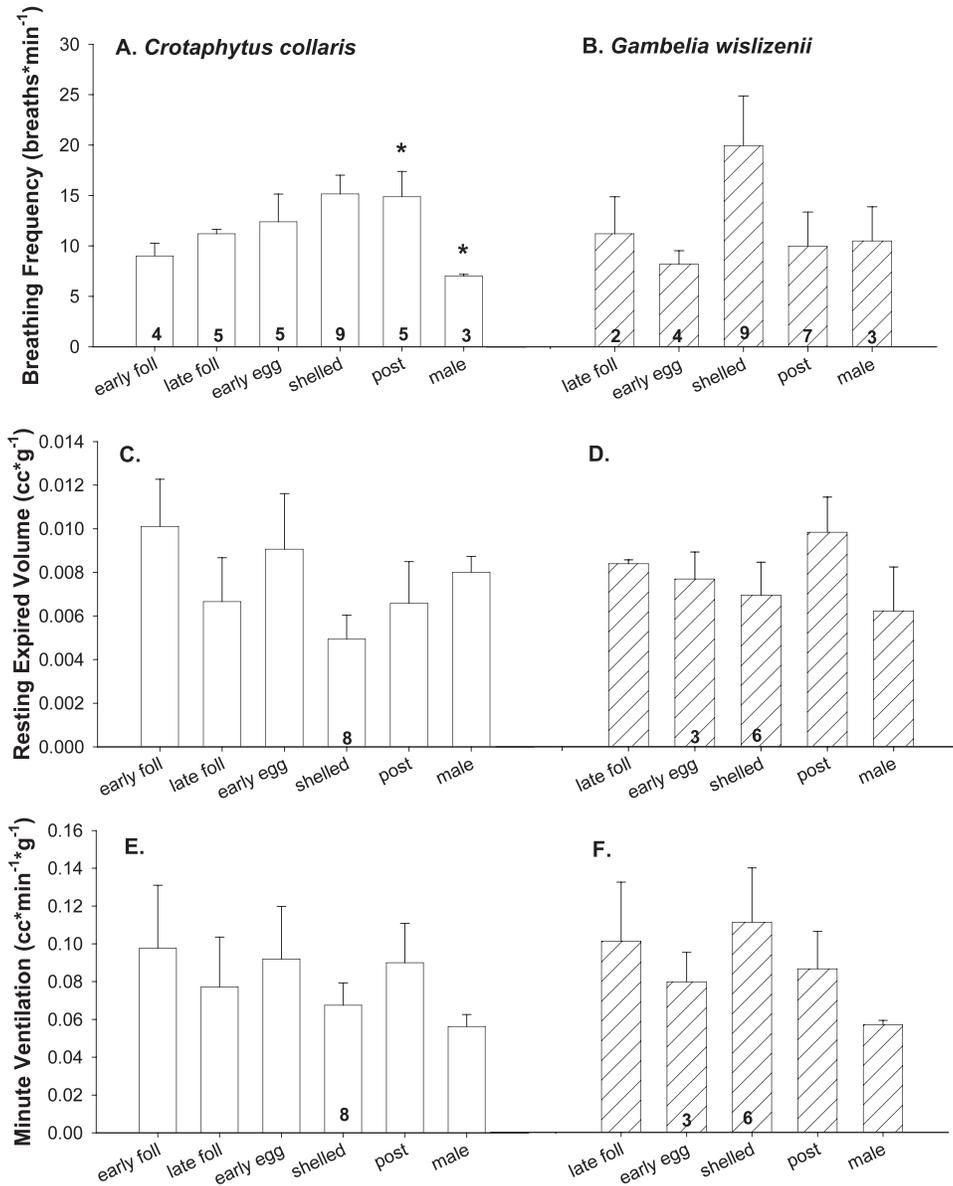


FIG. 3. Breathing frequency, resting expired volume, and minute ventilation of individuals of *Crotaphytus collaris* and *Gambelia wislizenii*. Values are shown as mean and SE. Sample sizes for each category are shown at the base of each bar and are the same within each category for all respiratory parameters unless noted otherwise. There were no significant differences among different stages in females in either species, or between postreproductive female and male *G. wislizenii*. Postreproductive females had significantly higher breathing frequencies than males of *C. collaris* ( $P = 0.04$ ).

Although flexibility of the body cavity wall may be at least partly responsible for the lack of change in mean expired volumes across reproductive stage in both species, some individuals were affected by the resulting decrease in total lung volume. This suggests that there may be a limit to the advantage afforded by body cavity flexibility in *G. wislizenii*, and potentially *C. collaris*, with extremely high clutch volumes. Experimental increases in clutch volume and reproduction of our study would be useful. In addition, it is important to note that our measurements of postexercise expired tidal volume were conducted at room temperature. It is possible that repeating these tests at higher temperatures, when individual metabolic rates and oxygen requirements are increased, would reveal limitations in body wall distensibility.

Carbon dioxide production rate was greatest in females of *C. collaris* when they were carrying late-stage follicles. In our study, females who were carrying medium to large follicles (meaning they were still investing a large amount of yolk to the follicles) were included in this category. These females had rates of CO<sub>2</sub> production that were 58% higher than postlaying females (Fig. 2). This suggests an increased metabolic cost when females are mobilizing nutrients for yolk. Carbon dioxide production rate was not as elevated dramatically while females were carrying shelled eggs (23% greater than postlaying), suggesting that the cost of mobilizing nutrients for yolk was greater than the cost incurred by the embryos, though we did not measure egg CO<sub>2</sub> production rate after laying. Increased energetic costs during reproduction have been observed in other egg-laying lizards and other reptiles (Stewart, 1989; DeMarco and Guillette, 1992;

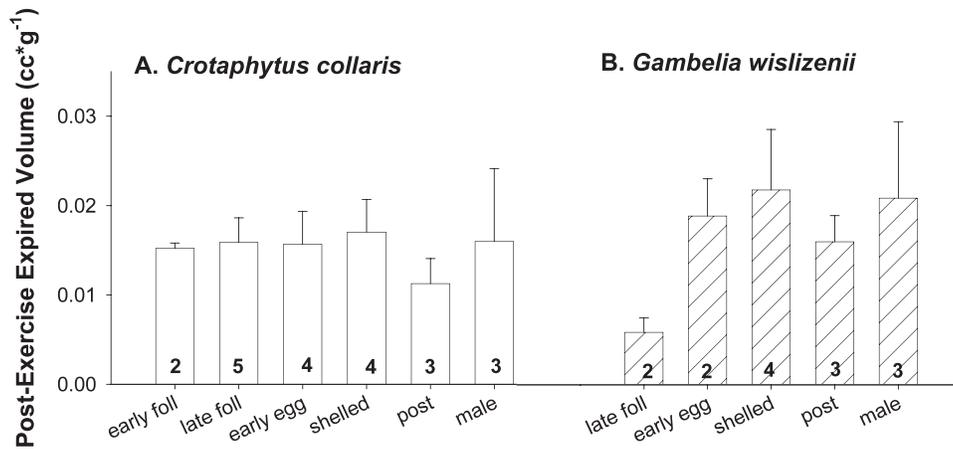


FIG. 4. Postexercise expired volume throughout the reproductive cycle in females, and males of *Crotaphytus collaris* and *Gambelia wislizenii*. Values are shown as mean and SE. The sample size for each category is shown at the base of each bar. There were no significant differences in postexercise expired volume among different stages in females and between postreproductive females and males, within a species.

Bonnett et al., 1994; Angilletta and Sears, 2000). However, our study is the first to measure the metabolic cost of both vitellogenesis and gravidity in oviparous lizards.

The increase in CO<sub>2</sub> production rate observed in our study was not reflected in an increase in the amount of gas exchanged on a minute-to-minute basis (minute ventilation). The observed disconnect between CO<sub>2</sub> production rate and minute ventilation was apparent when these parameters were evaluated separately, but the air convection requirements (minute ventilation [mL · min<sup>-1</sup>]/CO<sub>2</sub> production rate [mL · min<sup>-1</sup>]) did not change significantly throughout the reproductive cycle. Air convection requirements of the animals in our study were consistent with the literature, and did not provide evidence of hyper- or hypoventilation at any point in their cycle (Wang et al., 1997; Hicks et al., 2000). However, the high interindividual variability in expired volume, which resulted in high variability in minute ventilation, may have masked any significant disconnect between CO<sub>2</sub> production rate and minute ventilation.

In conclusion, we found that gravidity resulted in substantial reductions in total lung volume with no changes in expired tidal volumes, breathing rates or minute volumes in *C. collaris* and *G. wislizenii*. Distention of the abdominal wall may have reduced the effect of clutch volume on lung compression, seen in the variable ratio between relative clutch mass and lung volume reduction. Our results suggest that the highly distensible nature of the body wall may allow for reduction in lung volume from ingestion of large meals and reproduction with little change in most respiratory parameters.

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