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Casey Gilman

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Casey Anderson Gilman

Candidate

Biology

Department

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RESPIRATORY PHYSIOLOGY DURING GRAVIDITY
IN
CROTAPHYTUS COLLARIS AND GAMBELIA WISLIZENII

BY

CASEY ANDERSON GILMAN

B.S., BIOLOGY, UNIVERSITY OF NEW MEXICO, 2006

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Science
in
Biology

The University of New Mexico
Albuquerque, New Mexico

May, 2010

DEDICATION

To my parents, whose love of nature inspired me as a child and an adult,

and whose encouragement gave me the strength to follow my heart;

and to Toni, my love, for being understanding during those times

when science got the best of me.

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ABSTRACT OF THESIS

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Bachelor of Science, Biology, University of New Mexico, 2006

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ABSTRACT

During gravidity lizards experience a significant decrease in lung volume as a result of compression by growing eggs within the body cavity. In order to understand the effect of this decrease in lung volume on respiratory physiology of gravid egg-laying lizards I measured changes in: total lung volume, resting and post-exercise expired volume, minute volume, respiratory frequency, and carbon dioxide production during reproduction in *Crotaphytus collaris* and *Gambelia wislizenii*. I found that compression of the lungs by eggs resulted in an average 48% (range: 26 to 70%) decrease in total lung volume in *C. collaris*, and an average 38% (range: 29 to 46%) decrease in *G. wislizenii*. Despite the significant reduction in lung volume in both of these species, only breathing frequency and rate of CO_2 production were significantly altered. Breathing frequency was greatest in both species when females were carrying shelled eggs, and CO_2 production rate was greatest in females of *C. collaris* when they were carrying late-stage follicles. The significant increases in breathing frequency in both species were large enough to compensate for the decrease in lung volume and maintain relatively constant minute ventilation in both species. *Crotaphytus collaris* and *G. wislizenii* appear to be able to withstand large changes in lung volume during reproduction with little respiratory compensation.

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INTRODUCTION

Although there have been a variety of studies published on the respiratory physiology of lizards, most of the literature focuses on males and non-reproductive females (Dawson and Templeton, 1963; Templeton and Dawson, 1963; Mcgregor et al., 1993; Wood et al., 1995; Wang et al., 1997; Hicks et al., 2000; Butler et al., 2002). These studies provide insight into the basic mechanisms that characterize the respiratory physiology of lizards and their physiological flexibility; they do not, however, offer insight into the physiological changes that occur during a period crucial to the survival of individuals and their offspring. Studies have shown that both performance and survivorship are affected by the physiological and load bearing costs of reproduction, and therefore the risk of mortality for both the mothers and offspring is elevated at this time (Miles et al., 2000; Olsson et al., 2000; Shine, 2003; Zani et al., 2008). To my knowledge, the affects of reproduction on respiratory physiology has only been examined previously in one species, *Tiliqua rugosa* (Munns and Daniels, 2007). This skink species (the Australian sleepy lizard), is a large, long-lived, slow moving, herbivorous lizard and is typically pregnant for 6 to 7 months (Bull and Freake, 1999; Munns and Daniels, 2007). Consequently, pregnancy potentially affects respiratory physiology for a considerable part of their annual cycle. However, *T. rugosa's* lifestyle contrasts greatly with the active lifestyle of many other lizard species', with a lower aerobic scope than more active, smaller lizards, and might be expected to differ in other aspects of its respiratory physiology (Bennett and Dawson, 1976). In this investigation I focus on changes in respiratory physiology during gravidity in two species of active, predatory lizards that inhabit hot deserts.

Iguanid lizards possess sac-like few-chambered (paucicameral) lungs, (Perry, 1989). These paired lungs are located within continuous thoracic and abdominal cavities and are highly distensible (Mader, 1997). Because of their distensible nature and lack of a diaphragm, both naturally occurring and introduced materials within the abdominal and thoracic cavities (i.e. organs, food, eggs) may compress the lungs and reduce the volume for gas exchange (Munns and Daniels, 2007; Gilman, unpublished). Munns and Daniels (2007) observed that the growing fetuses in *T. rugosa* caused unicameral lung compression, which decreased expired volume in females with no compensatory increase in breathing frequency. These results suggest that compression of the lungs by the young may be partially responsible for the observed decrease in speed and endurance seen during gestation in this species (Munns and Daniels, 2007).

In this study, I examine respiratory changes associated with reproduction in two egg-laying lizard species: *Crotaphytus collaris* and *Gambelia wislizenii* (Family Crotaphytidae). These lizards are moderately-sized (~20-50g), carnivorous, diurnal (Degenhardt, 1996), and have similar body forms (they are neither dramatically elongate nor plate-like, as can be seen in other desert species, which could affect the size, shape and volume of the lungs); both are considered classic sit-and-wait predators (Pianka, 1986). Here, I address the question of whether and to what degree total lung volume, expired volume, breathing frequency, minute volume, and carbon dioxide production change throughout the reproductive cycle in two egg-laying lizard species.

METHODS

I collected *Crotaphytus collaris* (Say in James 1823) (3 males and 10 females) and *Gambelia wislizenii* (Baird and Girard 1852) (3 males and 11 females) from adjacent field sites in central New Mexico (Bernalillo County) (University of New Mexico Animal Care Protocol Approval # A4023-01). Lizards were housed outdoors in 4′ x 2′ x 2′ screen cages (LLL Reptile and Supply Co., Inc, CA, USA) to allow access to natural light and climatic conditions. Cages were outfitted with sand, wood and rock refugia, and one end of the cage was covered with an 18″ x 12″ board to provide shade. Lizards were fed crickets every other day throughout the study. *Crotaphytus collaris* were housed with two females to every male and *G. wislizenii* as one male to every female, when possible, to provide mating opportunities for both species. Females estimated to be close to oviposition were placed in smaller cages filled with moist perlite to provide an appropriate substrate for laying.

Sample Sizes

Although I was able to catch at least 10 females of each species, all females except one *C. collaris* were carrying either late-stage follicles or eggs at the time of capture. One female of *C. collaris* had a second clutch in captivity and I was able to use that female for an additional set of early-stage follicle measurements. I was unable to collect any early-follicle stage females of *G. wislizenii*, so my analyses began with late-follicle stage females for that species. Post-exercise expired volumes were estimated only during the second year of the study, so sample sizes for those estimates are smaller than for other parameters. Additionally, due to equipment failure and human error, I was unable to

obtain measurements for every female during every stage of reproduction. Sample sizes for each parameter measured are shown in the figures.

Carbon Dioxide Production

The following procedures were conducted in the order presented for each female once a week in order to get measurements for every female at every reproductive stage, when possible. Male respiration was measured once for each male during the study period. Lizards were fasted for 48 hours prior to all measurements to ensure they were post-absorptive. CO_2 production, breathing frequencies and resting expired volume estimates were recorded between 2300 and 0400 during the rest phase of their day to minimize activity. Lizards were placed in individual chambers in the dark within a temperature-controlled cabinet (33ºC) for two hours to acclimate before recording began. The T_a within the cabinet was regulated using a temperature-controlled circulator (Model 1187, VWR Scientific Products, West Chester, PA, USA), which pumped fluid through copper tubing. Air within the cabinet was mixed using a small electric fan. The T_a within the chambers was measured using a 21-gauge Cu–Cn thermocouple and a TC-1000 thermocouple meter (Sable Systems, Las Vegas, NV, USA) to ensure that incoming air did not vary the chamber temperature (no more than ± 0.5ºC). Lizard chambers consisted of 473 ml glass jars fitted with air-tight rubber stoppers with two ¼″ holes through which tubing was run. Incoming dry, CO_2-free air produced by a FTIR Purge Gas Generator (Whatman, Newton, MA, USA) and controlled by a flowmeter (Omega FL-3402C and FL-3403G flowmeters, Omega Engineering, Inc., Stamford, CT, USA) flowed through each chamber at 152 ml*min^{-1} (STP). Outgoing air was sampled by a multiplexer

(Systems Respirometry Multiplexer V2.0, Sable Systems) and measured using a CO_2

analyzer (LI-7000 CO_2/H_2O analyzer, LI-COR Biotechnology, Lincoln, NE, USA),

which was calibrated daily using dry, CO_2-free air and a certified span gas containing 999

p.p.m. CO_2 (Matheson Tri-Gas, Houston, TX, USA). Output from the CO_2 analyzer was

digitized using a Universal Interface II (Sable Systems) and recorded on a personal

computer using Datacan V data acquisition software (Sable Systems), with a sampling

interval of 5 seconds. CO_2 production was measured for a total of twenty minutes per

animal a night (twice for ten minutes). Baseline values for the incoming air were

measured before and after each individual's measurement. The rate of CO_2 production

was calculated using the equation $V\mathrm{CO}_2 = FR(F_e\mathrm{CO}_2 - F_i\mathrm{CO}_2)/(1-F_e\mathrm{CO}_2(1-(1/RQ)))$

(Lighton, 2008), where $V\mathrm{CO}_2$ is the rate of production, FR is the standard temperature

and pressure flow rate of the incoming gas, $F_e\mathrm{CO}_2$ is the fractional concentration of CO_2

leaving the chamber, $F_i\mathrm{CO}_2$ is the fractional concentration of CO_2 entering the chamber,

and RQ is the respiratory quotient. For my calculations I assumed an RQ of .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 the recording of CO_2 production, the two-hole rubber stoppers were

replaced with one-hole stoppers fitted with one 15cm length of ¼″ tubing and the lizards

were returned to the cabinet. Lizards were left in the dark for one half hour within the

temperature chamber to be allowed to return to a resting state. I connected the tubing

from each chamber to a differential pressure transducer (Sable Systems) just prior to

recording breathing frequency (number of breaths a minute) and resting expired (tidal) volume. Resting expired volumes and breathing frequencies were recorded for 10 minutes at a time, twice per night. Expired volumes were calibrated using a 1cc 1000 Series Hamilton Gastight syringe (model 1001) to inject known volumes of air at several injection frequencies into an empty chamber and recording the resulting signal. Volumes approximating the lizards' expected expired volume and breathing frequency range (Templeton and Dawson, 1963) were used 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 < .0001), though all injection rates affected the resulting signal in the same way (ANCOVA test of slope, P =.95). I used resulting relationships between these variables to estimate expired breath volumes (regressions of injection volume on transducer signal r^2>.98). My procedure is similar to the one recommended by Funk et al. (1986) to estimate tidal volumes when animals are contained in a chamber. Minute volumes, or the volume of air exchanged per minute, were calculated as a product of expired volume and breathing frequency.

Post-Exercise Expired Volume Estimates

Following the measurement of breathing frequency and resting expired volume, lizards were placed in cloth bags in the dark at room temperature to rest until the morning. Between 0900 and 1100 the lizard bags were brought 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, I was not

able to keep a consistent environmental temperature of 33°C while exercising them and recording post-exercise expired volume (PEEV). So, this was all done at room temperature (24.1 ± .12°C mean, s.e.m.). To obtain PEEV, each lizard was placed individually in a 3′ x 2′ x 3′ plastic tub. I encouraged the lizard to run by tail tapping or simply following behind the lizard with my hand for one minute and then immediately placed them in a chamber to record expired volume for one minute (as above). Lizards that were reluctant to run were placed repeatedly on their back to force them to right themselves. These activities were used 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). This procedure was repeated twice for each lizard. Once all respiratory parameters were measured, lizards were weighed, scanned with ultrasound imaging to determine reproductive stage (Gilman and Wolf, 2007), fed, and then returned to their respective cages.

Total Lung Volume

Computed-Tomography (NanoSPECT/CT®) imaging was used to estimate lung capacity at two points during the reproductive cycle (gravid, post-laying) in females and at one point (arbitrarily determined) in the males. Individuals were fasted for 48 hours to limit the amount of food in the digestive tract. In order to reduce activity, each lizard placed within a cloth bag in a standard lab refrigerator for ~20 minutes and/or freezer for ~5 minutes until cool (note: 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 simply a function of body size (Wood and Lenfant, 1976)). Lizards were then taped to

a cardboard restraint board with surgical tape (about the head, body and limbs), to allow respiration but restrict other movement, and placed within a cloth bag on an imaging bed. Whole body CT images of each lizard were acquired at 45kVp and one second per projection. The total CT scan time was six to seven minutes depending upon the number of bed positions required to accommodate the length. Lung volume was estimated using the reconstructed CT data by fitting 3-dimensional volumes to the interior of the lungs. The lung volume was divided into 3.2 mm axial regions consisting of 8 reconstructed slices from the CT images. The entire lung volume was determined by summation of these regions. Because lizard breathing frequencies were low, tidal volumes were small, and images were essentially averaged over the entire scanning period, respiration had a negligible effect on the estimation of lung volume. Three females and three males of each species were originally scanned to determine total lung volume. However, due to the presence of follicles in one post-gravid female of each species I was only able to obtain gravid and post-gravid volumes in two females of each species. One additional non-gravid female of each species was captured and scanned to increase non-gravid sample sizes.

Analyses

For analyses, females were placed into one of five categories, based on the size and stage off the eggs (early follicles, late-stage follicles, early egg, shelled egg, post-laying). Because I was unable to sample the same females consistently for each category, I present volume measurements as mass-specific to standardize the means, as is standard for these types of studies (Dawson and Templeton 1963, Templeton and Dawson 1963,

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Hopkins et al. 1995, Wang et al. 1997, Hicks et al. 2000, Secor et al. 2000, Munns and Daniels 2007). Mass-specific values were calculated using non-gravid masses, to remove the effect of the mass of the eggs on the values. Only one set of values for respiratory parameters were used 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 two weeks in a row and was carrying shelled eggs both weeks), one set of measurements was randomly selected for the analyses. Statistical analyses of resting and active respiratory parameters were performed using Kruskal-Wallis and Mann-Whitney non-parametric tests and significance was determined as $P<0.05$. Tests of significance for CT estimates of lung volumes were performed using 2-sample and paired t-tests ($P<0.05$).

Eggs caused considerable lung compression in gravid females compared to after these females layed (Fig. 1). The two females of each species that were CT scanned while gravid and non-gravid had significantly smaller total lung volumes when gravid compared to after-laying (paired t-test, $P =.04$; *C. collaris*: 26 and 70% difference, *G. wislizenii*: 29 and 46% difference). Total and mass-specific lung volumes of all individuals scanned are shown in Table 1. There were no significant differences in mass-specific lung volumes between the species of females at the same reproductive stage. Non-reproductive females did not have significantly different mass-specific lung volumes than males of the same species.

Females of *C. collaris* increased their breathing frequencies throughout the reproductive cycle, with the highest values occurring when carrying shelled eggs ($P=.04$) (Fig. 2A). Compared to males, females took significantly more breaths when carrying late-stage follicles, shelled eggs, and after laying ($P=.04, .03, .04$, respectively). Females' mass-specific resting expired volumes did not differ significantly throughout the reproductive cycle (Fig 2B). Females and males of *C. collaris* did not have significantly different mass-specific resting expired volumes at any point in the females' cycle. Mass-specific minute volumes did not change significantly throughout the reproductive cycle in *C. collaris* (Fig 2C). Mass-specific minute volumes of males were not significantly different than females' at any reproductive stage. There was no significant difference in mass-specific post-exercise expired volumes (PEEVs) among reproductive stages in females (Fig. 3A). PEEVs of males of *C. collaris* were not significantly different from those of females.

Females of *G. wislizenii* had significantly greater breathing frequencies when carrying shelled eggs, compared to early-egg and post-laying stages (P=.03, .01) (Fig. 2D). There were no significant differences between breathing frequencies of females and males at any stage. Females' resting expired volumes did not change significantly throughout the reproductive cycle (Fig 2E). Males' resting expired volumes were similar to volumes of post-ovipositional females. There was no significant change in minute volumes of females throughout the reproductive cycle (Fig 2F). Minute volumes of males were similar to those of females. There was little difference in PEEVs among stages in females and between males and females (Fig. 3B). There were no significant differences species in breathing frequencies, resting expired volumes, minute volumes, or post-exercise expired volumes between females of *C. collaris* and *G. wislizenii* of any stage, or between males of these species.

Females of *C. collaris* had significantly greater CO_2 production while carrying late-stage follicles compared to after-laying and compared to males (P=.0122, .0369) (Fig. 4A). Females of *G. wislizenii* showed no changes in CO_2 production throughout the reproductive cycle, and their CO_2 production was similar to that of the males (Fig. 4B). There were no significant differences between females of *C. collaris* and *G. wislizenii* of any stage, or between males of these species.

Air convection requirement (minute ventilation ($ml*min^{-1}$) / CO_2 production ($ml*min^{-1}$)) ranged from 28.06 ± 8.63 to 51.94 ± 11.75 in *C. collaris* and 26.49 ± 4.14 to 48.08 ± 12.72 (reproductive stage mean ± s.e.m.). There were no significant differences among the stages in each species, and there were no significant differences between the species.

DISCUSSION

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 my study), followed again by active expiration (Templeton and Dawson, 1963; Wood and Lenfant, 1976). Individuals of both species showed considerable variability within this pattern, particularly in the combination of tidal volume, breathing frequency and respiratory pause used to estimate individual minute ventilation (Fig. 5). Carbon dioxide production, tidal volume, and breathing rate at 33ºC 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). Respiratory parameters for individuals of *G. wislizenii* also fell within the same ranges.

In my study, females of both species showed significant decreases in total lung volume during gravidity as a result of compression by the eggs, with no associated decrease in either resting or post-exercise expired volume. The lack of reduction of expired volume throughout the reproductive cycle in *C. collaris* and *G. wislizenii* may, in part, be due to the large total lung volume to expired lung volume ratio. Resting expired lung volumes were a small fraction of total lung volumes (resting: 1.31% of total lung volumes post-reproduction and 0.8 to 2.31% while gravid; post-exercise: 3.94% post-reproduction and 7.54 to 20.42% while gravid). My observations suggest that even a 70% reduction in total lung volume still allows for maintaining breath volumes during gravidity as large as those in non-reproductive females of *C. collaris* and *G. wislizenii*.

In contrast to the lack of change in expired volume observed, I did find significant increases in breathing frequency in *C. collaris* and *G. wislizenii* during gravidity. Somewhat surprisingly, these increases in breathing frequency did not result in significantly increased minute ventilation, even though the breathing rate increased as much as 144% in *G. wislizenii* (shelled eggs versus early-stage eggs). Increases in breathing frequency during gravidity allowed for the maintenance of consistent minute volumes throughout the reproductive cycle. Increases in expired volumes were not observed although expired volumes were such a small fraction of total lung volumes, and it would appear that increasing expired volume would be a feasible means of increasing minute volumes. Other researchers (Perry 1989) have shown, however, that increasing expired volumes is energetically more costly than increasing breathing frequency during periods of increased oxygen demands.

Carbon dioxide production was greatest in females of *C. collaris* when they were carrying late-stage follicles. In my 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_2 production that were nearly two times those of observed post laying and 44% higher than values observed in males. These observations suggest a high metabolic cost for *C. collaris* females during vitellogenesis (yolk-deposition). I did not observe the same increase in rates of CO_2 production in *G. wislizennii*. Increased energetic costs during reproduction up to 122% (Angilletta and Sears, 2000) have, however, been observed in another egg-laying lizards and other reptiles (Stewart, 1989; DeMarco and Guillette, 1992; Bonnett et al., 1994). Because both Angilletta and Sears (2000), and my study measured only resting

metabolism, these increases reflect the increased energetic cost of producing the eggs, but do not reflect the load-carrying costs also associated with reproduction. Additionally, because I did not examine changes in other energetic parameters (i.e. growth) and therefore potential energetic trade-offs during reproduction, the extent of the cost is not clear.

Interestingly, the increase in CO_2 production observed in my 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_2 production and minute ventilation was apparent when these parameters were evaluated separately, but the air convection requirements ((minute ventilation (ml*min^{-1}) / CO_2 production (ml*min^{-1})) did not change significantly throughout the cycle. The air convection requirements of the animals in my study were consistent with the literature, and did not provide evidence of hyper- or hypoventilation at any point in their reproductive cycle (Wang et al. 1997, Hicks et al. 2000) Again, it appears that the parameters in respiratory physiology that changed significantly during reproduction were not great enough to produce significant changes in total respiratory output.

Unlike my study, Munns and Daniels (2007) found a significant decrease in expired volume due to the compression of the lungs by the growing fetuses in the live-bearing lizard, *T. rugosa*. Given that all these lizards have large total lung volume to expired (tidal) volume ratios (Wood and Lenfant 1976), I believe the differences in response to compression of the lungs during reproduction among these species may be attributed to differences in body cavity distensibility. Relative clutch masses (ratio of clutch mass to non-reproductive female body mass) of females in their study were 21.6 ± 2.6%, while *C.*

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collaris in my study had relative clutch masses of 33.7 ± 2.7% and *G. wislizenii* had relative clutch masses of 31.4 ± 3.6%. Given the larger relative clutch masses of my study species and the fact that the body cavity must accommodate the clutch, one might expect the effect of lung compression to be greater in my species than in *T. rugosa*. However, *T. rugosa* has a body cavity that does not appear to distend and accommodate the growing clutch with as much flexibility as the species in my study. Using body shape as an indicator of reproductive stage is possible in both my species, however it is difficult to identify the reproductive stage of females of *T. rugosa* because they do not show identifiable changes in body shape (Munns and Daniels, 2007). Inter-individual variability in body distensibility can even be seen across and within the species I studied. The two females of *C. collaris* had relative clutch masses of 39.4 and 34.9% with a reduction in lung volume during gravidity of 70.1 and 26.5%, respectively, while the two females of *G. wislizenii* had relative clutch masses of 38.9 and 36.8% and reductions in lung volume of 46.2 and 28.7%, respectively. Due to variability in the positioning of the eggs and flexibility of the abdomen, differences in relative clutch mass are not directly reflected in the degree of lung compression (Fig. 6). This highly distensible nature of the body wall in Crotaphytids may also provide an advantage during feeding for this group. These species often ingest relatively large prey (other lizards, snakes and rodents) (Degenhardt, 1996), and having a flexible body wall may reduce the impact of large prey on movement and organ function. In this study, it appears that this flexibility and may also allow for only small changes to be necessary in respiratory function during reproduction.
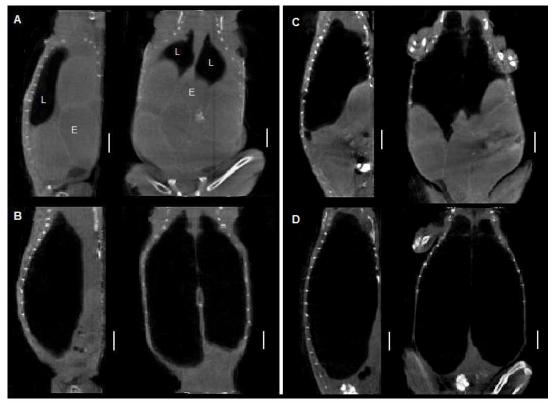
In conclusion, I found that gravidity and resulting compression of the lungs by growing follicles and eggs does result in compensatory changes in respiratory physiology, with the most significant changes seen in carbon dioxide production and breathing frequency for both species. Surprisingly, many component variables showed no significant changes. This appears to be due to the highly distensible nature of the body wall, the flexibility of the respiratory system in reptiles, and the evolution of large lung volumes in reptiles, all of which allow for reduction in lung volume from ingestion of large meals and reproduction with little change in respiratory parameters. It is important to note, however, that this study focused on inactive animals and therefore further research is needed to elucidate how lung compression affects respiratory physiology and performance during activity.

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Figure 1. Images from Computed-Tomography (CT) scans of females and males of *C. collaris* and *G. wislizenii*. 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 39% 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 39% with associated 46% reduction in lung volume). D) The same female of *G. wislizenii* after laying. White 5mm scale bars are located in the lower right area of each scan. Image depth is 0.2mm.
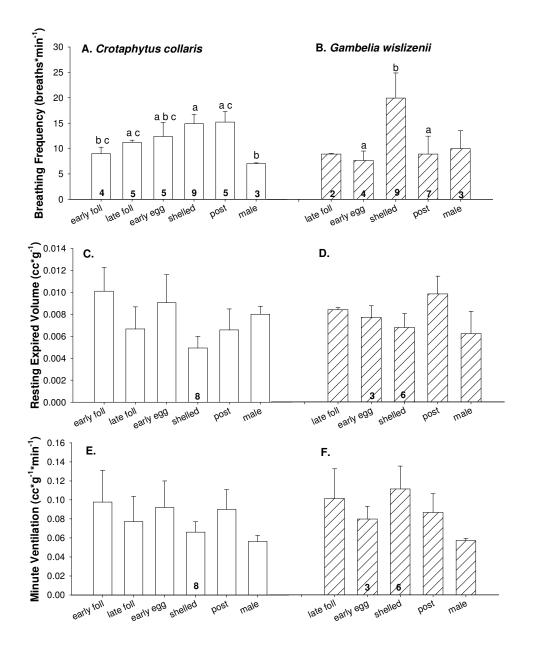

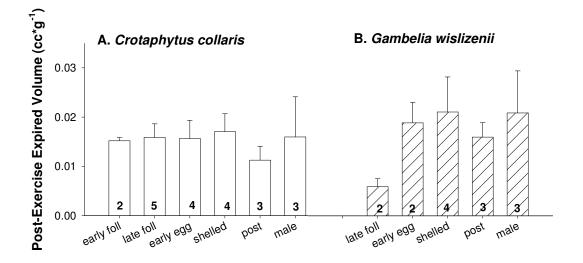Crotaphytus collaris   Gambelia wislizenii

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 ± s.e.m.

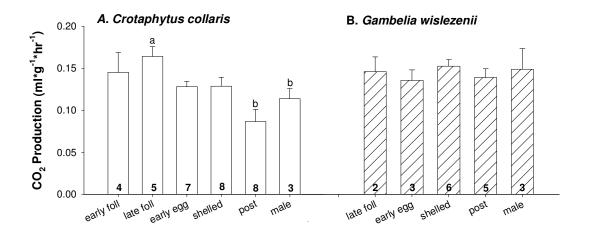|	Non-Gravid Mass (g)	Snout-Vent Length (mm)	Non-Gravid Lung Volume (cc)	Gravid Lung Volume (cc)	Non-Gravid Lung Volume $(cc \cdot g^{-1})$	Gravid Lung Volume $(cc \cdot g^{-1})$
Crotaphytus collaris						
female	27.7 ± 2.1	91.0 ± 0.9	12.0 ± 2.3	6.7 ± 2.8	0.5 ± 0.1	0.3 ±0.1
male	41.9 ± 2.5	99.0 ± 2.7	13.0 ± 1.2		0.3 ± 0.0	
Gambelia wislizenii						
female	26.8 ± 0.6	105.3 ± 3.1	14.2 ± 4.4	8.0 ± 2.7	0.5 ± 0.2	0.3 ± 0.1
male	17.1 ± 1.3	89.3 ± 0.7	6.5 ± 1.2		0.4 ± 0.1	

Figure 2. Breathing frequency, resting expired volume, and minute ventilation of individuals of *C. collaris* and *G. wislizenii*. Values are shown as mean and s.e.m. 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.
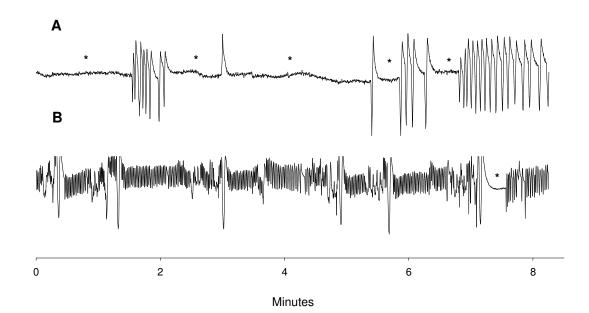
Figure 3. Post-exercise expired volume throughout the reproductive cycle in females, and males of *C. collaris* and *G. wislizenii*. Values are shown as mean and s.e.m. The sample size for each category is shown at the base of each bar.

Figure 4. CO_2 production throughout the reproductive cycle in females, and males of *C. collaris* and *G. wislizenii*. Values are shown as mean and s.e.m. The sample size for each category is shown at the base of each bar. Letters above the bars denote significant differences between values within a species ($P<0.05$).

Figure 5. Breathing traces from two females of *Gambelia wislizenii* within 48 hours of laying their eggs. Inspiration is shown by a downward deflection of the breath trace (negative pressure) and exhalation by the upward deflection of the trace (positive pressure). Periods of respiratory pause can be seen before inhalation (in most cases). The respiratory pattern of female A shows considerably more and longer periods of respiratory pause (*). In contrast, female B has more smaller-volume breaths and only a single period of respiratory pause.

Figure 6. Images from Computed-Tomography (CT) scans of two females of *C. collaris*. 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 (relative clutch mass 39% with associated 70% reduction in lung volume). B) Second gravid (relative clutch mass 35% with associated 27% reduction in lung volume). White 5mm scale bars are located in the lower right area of each scan. Both females have similar clutch volumes, but due to the different positioning of the eggs in the females, lung volumes were not similarly decreased during gravidity. Image depth is 0.2mm.