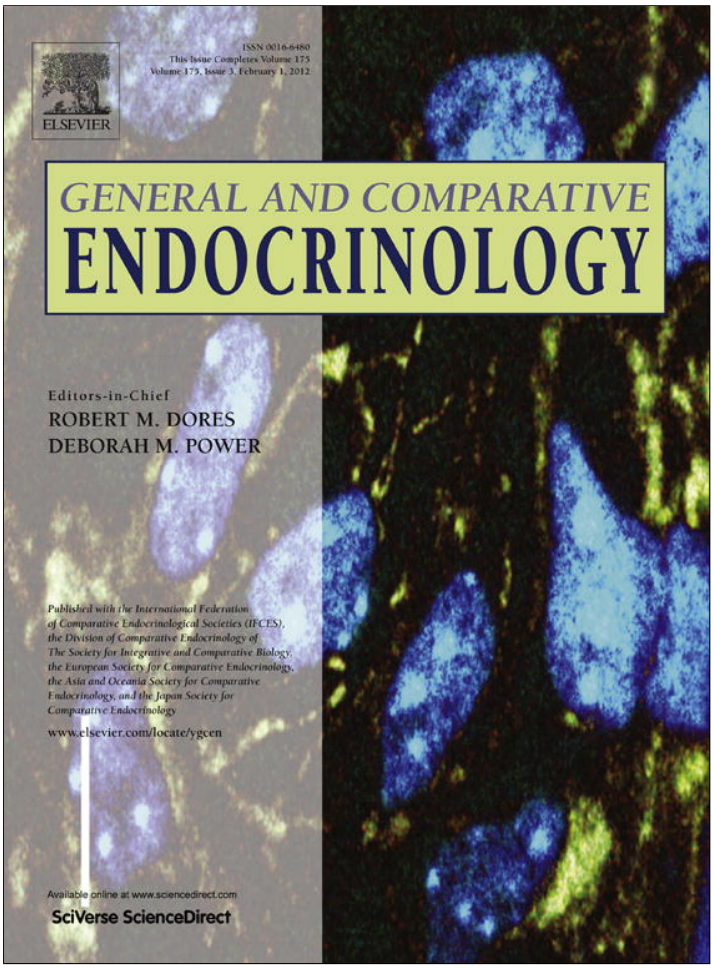

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Corticosterone and pace of life in two life-history ecotypes of the garter snake *Thamnophis elegans*

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ABSTRACT

Glucocorticoids are main candidates for mediating life-history trade-offs by regulating the balance between current reproduction and survival. It has been proposed that slow-living organisms should show higher stress-induced glucocorticoid levels that favor self-maintenance rather than current reproduction when compared to fast-living organisms. We tested this hypothesis in replicate populations of two ecotypes of the garter snake (*Thamnophis elegans*) that exhibit slow and fast pace of life strategies. We subjected free-ranging snakes to a capture-restraint protocol and compared the stress-induced corticosterone levels between slow- and fast-living snakes. We also used a five-year dataset to assess whether baseline corticosterone levels followed the same pattern as stress-induced levels in relation to pace of life. In accordance with the hypothesis, slow-living snakes showed higher stress-induced corticosterone levels than fast-living snakes. Baseline corticosterone levels showed a similar pattern with ecotype, although differences depended on the year of study. Overall, however, levels of glucocorticoids are higher in slow-living than fast-living snakes, which should favor self-maintenance and survival at the expense of current reproduction. The results of the present study are the first to relate glucocorticoid levels and pace of life in a reptilian system and contribute to our understanding of the physiological mechanisms involved in life-history evolution.

1. Introduction

Life-history strategies vary profoundly among organisms, but this diversity is not random; it falls mainly along a slow-to-fast pace of life continuum that suggests constraints in the evolutionary diversification of life histories [30,33]. At one extreme of the continuum, 'fast-living' organisms are characterized by rapid growth, early maturation, and high reproductive rate, but a short life span. 'Slow-living' organisms at the other end of the continuum, display a long lifespan, but slow growth, delayed maturation, and a lower reproductive rate [28,30]. Life-history theory proposes that this pattern arises because constraints in the allocation of limited resources between different functions result in trade-offs among life-history traits such that maximal investment in both current reproduction and survival (and future reproduction) is not possible [29,33]. Understanding the proximate physiological mechanisms mediating such trade-offs is critical to understanding the evolution of life-histories and the existence of a pace of life continuum of strategies [16,30,46].

The endocrine system is a candidate for such physiological mechanisms, and hormones have been hypothesized to be mediators of life-history trade-offs (e.g., follicle-stimulating hormone [25,36], testosterone [14,16], juvenile hormone [12,45], insulin-like growth factor [24,38], prolactin [2], and glucocorticoids [30,43]. Among these different hormones, glucocorticoids, such as cortisol and corticosterone, are considered major candidates for mediating the costs of reproduction (i.e., the trade-off between current and future reproduction) [30,43,44]. Glucocorticoids are secreted by the adrenals and usually circulate in the bloodstream at baseline levels, regulating critical metabolic processes such as energy acquisition, storage, and utilization [20,35]. Upon exposure to unpredictable adverse events such as food dearth, severe climatic conditions, or predation risk, however, the activity of the hypothalamic–pituitary–adrenal (HPA) axis – or hypothalamic–pituitary–interrenal axis (HPI) in reptiles [13] – is up-regulated and secretion of glucocorticoids is increased. The resulting stress-induced levels cause a shift in physiology and behavior such that self-maintenance and survival processes are prioritized, many times at the expense of less immediately vital functions such as reproduction and other non-survival related activities [3,43]. In this way, by redirecting resources and behaviors, glucocorticoids are thought to regulate the balance between current reproductive effort versus survival (and future reproduction) in the face of environmental challenges [3].

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The value of this glucocorticoid-mediated allocation decision, however, is likely to be shaped by the relative importance of current reproduction versus survival in maximizing fitness of the organisms [30,43]. In particular, it has been hypothesized that glucocorticoid levels should differ among organisms along the fast-slow continuum of life-history strategies [4,15,30]. This hypothesis predicts that slow-living organisms – whose fitness depends more on adult survival than on annual fecundity – should show higher stress-induced glucocorticoid levels that favor self-maintenance in the face of a stressor, while fast-living organisms – whose fitness depends heavily on current reproduction – should show lower levels to be able to maintain the current reproductive effort even at the expense of future survival and reproduction. Two recent comparative studies have provided support for this association between glucocorticoid levels and pace of life in birds. The first found that among 64 bird species, those making a lower investment in current reproduction (i.e., have a lower brood value) show higher stress-induced corticosterone responses when subjected to the acute stress of capture and restraint [4]. Likewise, the second study found higher maximum stress-induced corticosterone levels in response to capture-restraint in passerine species with higher annual adult survival [15]. To our knowledge, tests of this hypothesis in other vertebrate taxa have not been performed to date, and therefore the generality of this association between glucocorticoid levels and pace-of life remains to be determined.

In this study, we use a five-year dataset to test for an association between glucocorticoid levels and pace of life in replicate populations of the western terrestrial garter snake, *Thamnophis elegans* [39], which exhibit distinct fast-living and slow-living life-history ecotypes (Table 1). A recent study in this system [32] found that free-ranging gravid females of the fast-living ecotype have lower baseline corticosterone levels – the major glucocorticoid in reptiles [13] – than those of the slow-living ecotype, suggesting that the ecotypes may differ more generally (i.e., in all stages and both sexes) in the function of their HPI axes. Sex and reproductive status can affect corticosterone levels in wild vertebrates, although no consistent patterns have so far emerged in reptiles [27,40]. Thus, the present study expands on those early findings by including males and non-gravid females in addition to gravid females and by testing the following prediction of the hypothesis linking pace-of life with glucocorticoid levels: fast-living snakes, who invest more in current reproduction, should show lower stress-induced corticosterone levels than M-slow snakes, who prioritize investment in self-maintenance and survival. We tested this prediction by subjecting free-ranging snakes of both ecotypes to a standard capture-restraint protocol and compared the resultant stress-induced levels of corticosterone. In addition, we used a five-year dataset to assess whether baseline corticosterone levels followed the same pattern as stress-induced levels in relation to pace of life in this system.

2. Methods

2.1. Study populations

The study system of garter snakes comprises populations of fast- and slow-paced life history ecotypes in close proximity (1–25 km) [22] in the vicinity of Eagle Lake, California, USA. Replicate populations of the fast pace of life ecotype inhabit the shoreline of lake and are characterized by rapid growth to large body sizes, early maturation, high annual reproduction and short median life span. Within a few kilometers of the lake, replicate populations of the slow pace of life ecotype reside in montane meadows, and exhibit the opposite combination of life-history traits (Table 1). Population genetic studies of these populations, that utilized neutral molecular markers [21,22], M. Manes and A. Bronikowski unpublished data) support the interpretation of this system as an interconnected metapopulation, with both small but significant genetic divergence (Average Fst = 0.02, range = 0.016–0.086) primarily between populations of opposing ecotypes, as well as lower levels of gene flow between ecotypes than among replicate populations of the same ecotype [21]. These fast-living lakeshore (hereafter L-fast) and slow-living meadow (hereafter M-slow) snakes have been the focus of study for over 30 years and show marked differences in a variety of physiological parameters such as mass-specific metabolic rate, cellular metabolism, DNA-repair efficiency, and immune function [5,6,26,31,32,37,38]. In addition, these ecotypes show significant genetic divergence in growth rates as established by a common-garden experiment [7], as well as heritable variation in coloration, scalation and vertebral number [22], suggesting the possibility that genetic divergence also underlies glucocorticoid physiology in this system.

Table 1
Life-history differences between replicate populations of fast-living lakeshore (L-fast) and slow-living meadow (M-slow) ecotypes of the western terrestrial garter snake *Thamnophis elegans* (Bronikowski and Arnold [8]).

Life-history trait	Fast-living	Slow-living
Maximum body size	700 mm	550 mm
Size at maturity	450 mm	400 mm
Age at maturity	3 years	5–7 years
Reproductive rate	Annual	Resource-dependent
Mean litter size	8 neonates	5 neonates
Median lifespan	4 years	8 years

2.2. Field work

Blood samples for corticosterone (CORT) measurements were obtained from free-ranging snakes captured by hand while basking or foraging, or from under rocks or in grasses. We sampled animals from 2 to 4 replicate L-fast and 2 to 4 replicate M-slow populations in 2006 through 2009 (baseline) and 2010 (baseline and stress-induced). Samples for baseline CORT were collected in the five study years between mid-May and early-July, with some variation in sampling periods among years: 2006 (May 13–July 10, $n = 85$ snakes), 2007 (May 17–July 8, $n = 66$), 2008 (May 11–21, $n = 68$), 2009 (July 2–8, $n = 78$), and 2010 (June 11–22, $n = 103$). Individuals were bled from their caudal vein using heparin-rinsed syringes. Plasma was separated by centrifugation, snap-frozen in liquid nitrogen in the field, and stored at −80 °C in the laboratory until analysis. All blood samples included in our analyses were collected within 10 min of capture of the animals. Data for 2010, for which we had records of exact handling times (i.e., time from capture to end of blood collection), indicate that CORT does not show an increase with handling time within this time frame (Fig. 1). We therefore consider the measures of CORT obtained within 10 min of capture as representative of baseline (or background) levels in this system, as is the case in other reptile species (e.g., [10]. Samples for stress-induced CORT were obtained from 28 free-ranging snakes in 2010 (7 L-fast females, 4 L-fast males, 10 M-slow females, 7 M-slow males) following a standard capture-restrain protocol. The initial blood sample (~20–30 μl) was collected within 10 min of capture (range: 1–10 min, mean ± SE: 3.28 ± 0.47 min, $n = 28$), hereafter time 0. Snakes were then placed in cloth bags and additional blood samples collected 15, 45, 90, and 180 min after time 0. Once blood collection was completed, all snakes were weighed (g), measured for snout-vent length (SVL, mm), and sexed via hemipene eversion. In addition, females were palpated to determine gravidity. All snakes were then released at their site of capture.

2.3. Corticosterone radioimmunoassay

Levels of plasma CORT were determined in 17 assays using double-antibody radioimmunoassay kits (Catalog # 07–102103, MP

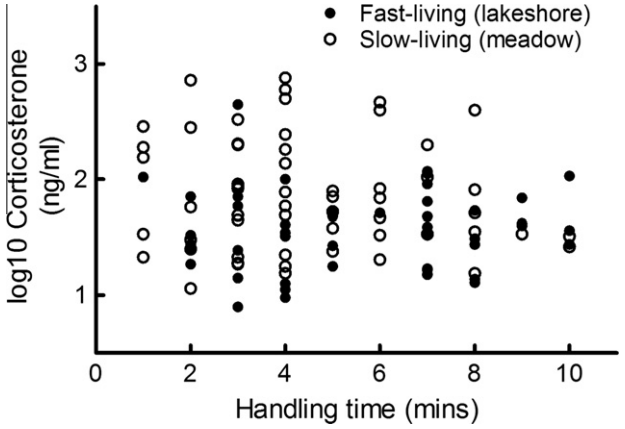

Fig. 1. Scatterplot of plasma corticosterone level (log_{10} transformed) as a function of handling time (i.e., time from capture to end of bleeding) in free-ranging individuals of the two life-history ecotypes of the garter snake *Thamnophis elegans*.

Biomedical, Orangeburg, NY, USA) that had already been validated for use in our study system [32]. We followed the protocol by Robert et al. [32], except that plasma samples were diluted 1:80 (instead of 1:40) because this dilution proved optimal for the range of samples in the present study. Samples from different years were assayed on separate kits on the year of sample collection. All samples for a given year were assayed within a 24–48 h time period using reagents from a single kit (n = 3–6 assays), with samples from L-fast and M-slow snakes included in each assay. In 2010, we analyzed the repeated samples from a given individual (i.e., from the capture-restraint protocol) in the same assay to minimize within-individual variation, while samples from different individuals within each ecotype were randomly assigned to assays. We used the kit-provided low control to calculate intra-and inter-assay coefficients of variation of CORT concentrations. Mean intra-assay variation was 14.2% whereas mean inter-assay variation was 19.4%. All samples were run in duplicate.

2.4. Statistical analyses

Statistical analyses were conducted using the mixed-model procedure (Proc Mixed) in SAS software (SAS 9.2, SAS Institute Inc., Cary, NC, USA). Corticosterone levels were log_{10} transformed before analyses to normalize the data.

Stress-induced CORT was analyzed using the following repeated measures general linear model:

$$Y = \mu + \text{time} + \text{ecotype} + \text{time} \times \text{ecotype} + \varepsilon$$

Time is the effect of the five repeated measures of CORT from the capture-restraint protocol and ecotype is the fixed effect of the L-fast versus M-slow ecotypes. Initial models also included SVL, body condition, day, time of day, sex/gravidity group, and all two-way interactions between effects. SVL (i.e., body size) is a covariate used as a proxy for age of the snakes [37,41]. Body condition is a covariate representing the size-corrected weight of the individuals, calculated as the residuals of the regression of the log of body weight on the log of SVL [42], using separate regression lines for gravid females versus non-gravid females and males. Day is a covariate representing the day of the year when each individual was sampled (January 1st = day 1), while time of day is the time each individual was sampled on a given day. Sex/gravidity group is the effect of sex/gravidity with three categories: male, non-gravid female, or gravid female. Non-significant terms were removed from the final model described above, except for the interaction between time and ecotype. Significance was assessed with an α-level of 0.05. Effects and interactions excluded from the final model exceeded an α-level of 0.1.

In addition to the repeated measures analysis for stress-induced CORT, we also conducted an analysis using the maximum CORT level achieved by each individual during the capture-restraint protocol (i.e., max CORT) as the dependent variable, following the recent study in birds by Hau et al. [15]. As in the latter study, max CORT in the snakes was strongly positively correlated with two other indices of the stress-response: the difference between maximum and baseline CORT (Spearman's ρ = 0.929, $p < 0.0001$) and the area under the curve or integrated CORT response (Spearman's ρ = 0.968, $p < 0.0001$).

Baseline CORT was analyzed using the following general linear model:

$$Y = \mu + \text{year} + \text{ecotype} + \text{year} \times \text{ecotype} + \text{body condition} + \text{day} + \varepsilon$$

Year is the fixed effect of year of study (2006–2010) while the remaining terms are as defined above. Initial models included also SVL, time of day, sex/gravidity group, population nested within ecotype, and all two-way interactions between effects. Population nested within ecotype represents the effect of the different replicate lakeshore and meadow populations (n = 4 populations per ecotype), treated as a fixed rather than a random effect to reflect complex microhabitat variation among populations within ecotypes. Non-significant effects were dropped from the model. Because the sampling periods differed among years (see Section 2.2 above) we also conducted analyses including only the subset of snakes that were sampled over similar periods each year. Limiting our dataset in this way did not change our main results (data not shown); we thus present the analyses including the complete dataset. The final models presented (i.e., for stress-induced and baseline CORT) were supported by the use of Akaike's information criterion for small sample sizes (AICc values) (data not shown), which provide a measure of the parsimony of the models, combining their likelihood with a penalty for extra parameters [9].

3. Results

3.1. Stress-induced corticosterone levels

The repeated measures analysis indicated a significant effect of time and ecotype on the levels of CORT measured during the capture-restraint protocol, but no significant interaction between those terms (Table 2). That is, M-slow snakes showed overall higher CORT at all time points including baseline than L-fast snakes (Fig. 2). Similarly, max CORT was on average higher in M-slow (315.9 ± 50.2 ng/ml) than L-fast snakes (173.7 ± 34.4 ng/ml) ($F_{1,26}$ = 5.62, p = 0.026).

3.2. Baseline corticosterone levels

The effect of ecotype on baseline CORT was dependent on the year of study, as indicated by the significant ecotype × year interaction (Table 3). In three out of the five study years (2006, 2007

Table 2
Repeated measures general linear model for corticosterone levels during the capture-restraint protocol (stress-induced CORT) in the two life-history ecotypes of garter snake.

Source	F	df_1,df_2	p
Time	38.79	4100	<0.0001
Ecotype	5.02	126	0.034
Time × ecotype	1.20	4100	0.314

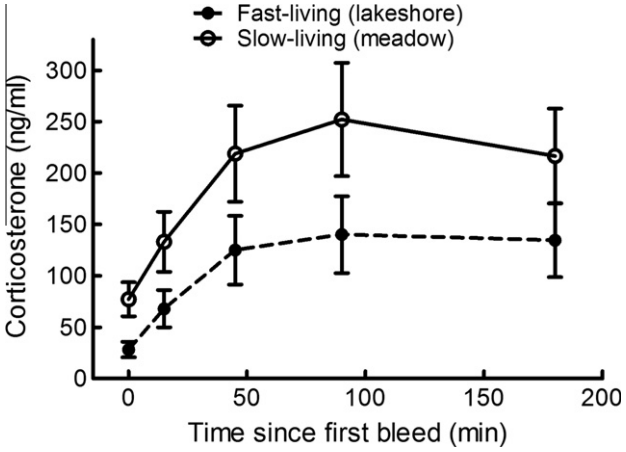

Fig. 2. Plasma corticosterone levels in response to a capture-restraint challenge (stress-induced CORT) in free-ranging individuals of two life-history ecotypes of the garter snake *Thamnophis elegans*. Time = 0 min indicates baseline corticosterone level (See Section 2 for details). Data are backtransformed least square means ± SE from the statistical model in Table 2. Sample sizes: M-slow, $n = 11$; L-fast, $n = 17$.

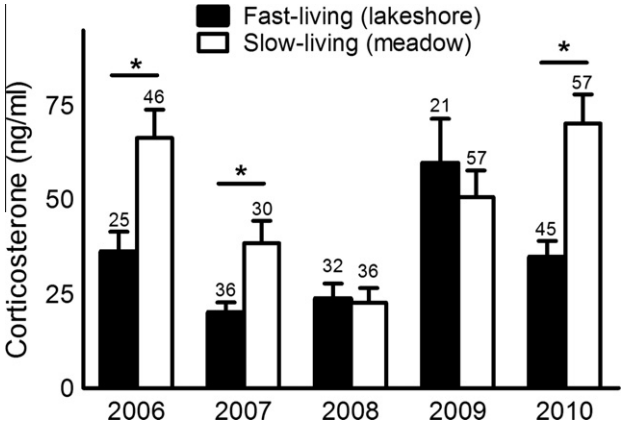

Fig. 3. Plasma baseline corticosterone levels (baseline CORT) in free-ranging snakes of two life-history ecotypes of *Thamnophis elegans* sampled in five consecutive years. Data are backtransformed least square means ± SE from the statistical model in Table 3. Sample sizes are provided above the error bars. Asterisks denote years when significant differences between the ecotypes were detected.

and 2010), M-slow snakes showed higher baseline CORT than L-fast snakes, while in the remaining 2 years (2008 and 2009) there was no ecotype difference (Fig. 3). Independently of ecotype and year, baseline CORT was weakly negatively related to body condition ($r_p = -0.186$, $p = 0.0002$, $n = 398$) and to day of the year ($r_p = -0.147$, $p = 0.0032$, $n = 398$) snakes were sampled (Table 3).

4. Discussion

In the present study we found support for the hypothesis linking glucocorticoid levels with pace of life in two life-history ecotypes of the western terrestrial garter snake, *T. elegans*. Slow-living (M-slow) snakes showed overall higher corticosterone levels during a capture-restraint challenge than fast-living (L-fast) snakes. In accordance with the hypothesis, the higher corticosterone levels should favor behaviors and physiological adjustments promoting self-maintenance and immediate survival in these snakes with a slow-pace life-history strategy. In contrast, the lower corticosterone levels should facilitate current reproduction, even at the expense of survival and future reproduction, in snakes with the fast pace of life strategy. Similarly, our five-year dataset on baseline corticosterone levels indicated that when differences between the ecotypes were present (i.e., in 3 of the 5 years of study), it was always M-slow snakes that showed higher levels than L-fast snakes, a pattern resembling that for stress-induced levels. Therefore, from our data it is apparent that garter snakes of the slow-living ecotype are exposed to overall higher levels of circulating glucocorticoids – both baseline and stressed-induced – than garter snakes of the fast-living ecotype. Our study, thus, shows an association between glucocorticoid levels and pace of life in a reptilian system, as has been recently documented for birds [4,15], supporting the possible role of glucocorticoids as mediators of life-history trade-offs in this vertebrate group. It remains to be determined whether the ecotypic difference in stress-induced corticosterone levels is only observed in certain years – as is the case for baseline levels – and whether the two measures show parallel patterns or vary independently across years.

Table 3
General linear model for baseline corticosterone level (baseline CORT) in the two life-history ecotypes of garter snake across the 5 years of study.

Source	F	df_1, df_2	p
Ecotype	16.90	1386	<0.0001
Year	7.95	4386	<0.0001
Ecotype × year	4.70	4386	0.0010
Body condition	13.92	1386	0.0002
Day	8.59	1386	0.0036

Given that comparative studies do not allow inferring causation – or disentangling genetic, environmental, and maternal effects – alternative interpretations of the association between glucocorticoid levels and pace of life among species or populations cannot be ruled out without the use of an experimental approach [15]. In our study system, for instance, the higher corticosterone levels (especially baseline concentrations) exhibited by snakes from M-slow populations in comparison to those form L-fast populations could be a reflection of the lower and less predictable levels of food that characterize meadow habitats in comparison to lakeshore habitats [8]. Low food availability has been linked to elevated baseline corticosterone levels in vertebrates, including reptiles (e.g., [11,17]). In addition to food availability, other environmental factors that could potentially impact glucocorticoid levels also differ between the two habitat types in our study system, including ambient temperature (higher in lakeshore), presence of a trematode parasite (only found in meadows), and risk of predation (higher in lakeshore). Common-garden experiments can be used to tease apart the relative contribution of life-history strategy (or other genetic differences) versus current environmental factors in explaining physiological variation documented in the field (e.g., [1,23,26]). Potential effects of chronic stress in captivity (e.g., [32]), however, could make inferences about the function of physiological systems of free-living animals complicated in some systems (see [18]). Alternatively, hormonal manipulations in the field (e.g., [19]), might provide a more relevant way to determine the role of glucocorticoids in mediating life-history trade-offs and their associations with disparate life-history strategies in free-living animals.

An intriguing finding of our study is that the ecotypic variation in baseline corticosterone levels was not observed in two of the five study years, 2008 and 2009. The reason for this pattern is not clear at present. Neither data on climatic variables (e.g., snowpack depth, precipitation, temperature), nor field notes on food availability (e.g., presence or absence of breeding anurans in the meadow habitats) explained the observed inter-annual pattern (data not shown). One difference between the study years is that in 2008 and 2009 samples were collected during a restricted sampling period in early May and early July respectively (see Section 2). In contrast, in the remaining three years collection occurred during a longer period that included days during June, the peak foraging month for the snakes. Seasonal patterns of variation in glucocorticoid levels are

common in vertebrates including reptiles (reviewed by [34]). Thus, it is possible that differences in baseline corticosterone levels between the two ecotypes become obvious only at certain times of the year; for instance, during periods when snakes are most actively seeking food. In accordance with this suggestion, baseline corticosterone levels are thought to have primarily metabolic functions, allowing the adjustment of foraging behaviors, locomotor activity, and energy metabolism to varying seasonal demands associated with the life-cycle (reviewed by [20]).

Our study also contributes basic data on glucocorticoid responses in a free-living reptile for which life-history data are also available. This kind of information is essential for building a dataset that will allow testing the hypothesis linking glucocorticod levels and life history in a comparative study across reptile species, as has been recently accomplished for birds [4,15]. Furthermore, as suggested for other physiological systems [47], the unique set of characteristics (e.g., only ectothermic amniotes) and key phylogenetic position of reptiles among vertebrates, make a better understanding of their endocrine physiology paramount for understanding the evolutionary history of this physiological system. Finally, a more complete characterization of the variation in glucocorticoid physiology– and its association to the slow-fast continuum of life-history strategies in this and other groups of animals – will also require the measurement of downstream regulatory factors such as corticosterone binding globulins and glucocorticoid receptors [4,15], which can enhance or attenuate the biological effects of circulating hormone levels.

5. Conclusions

The results of the present study constitute the first test of the hypothesis linking plasma glucocorticoid levels to life-history strategies in a reptile species and contribute to the understanding of the physiological mechanisms involved in life-history evolution. Similar to patterns recently described for birds, our data support the prediction that in reptiles, slow-living animals – whose fitness depends more on adult survival than on annual fecundity – should display higher glucocorticoid levels that favor self-maintenance and immediate survival, while fast-living animals – whose fitness depends more heavily on current reproduction – should show lower glucocorticoid levels that would not interfere with current reproduction. Future studies in this and other systems should evaluate the relative roles of life-history strategy *per se* versus current environmental factors in explaining the observed association between glucocorticoid levels and pace of life, for which experimental approaches will be important.

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