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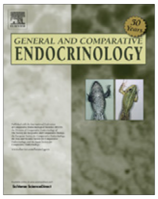

# Corticosterone and pace of life in two life-history ecotypes of the garter snake *Thamnophis elegans*

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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 ($\sim$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