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Corticosterone and the transition from courtship behavior to dispersal in male red-sided garter snakes (*Thamnophis sirtalis parietalis*)

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Abstract

Seasonal modulation of baseline glucocorticoid concentrations as well as the sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis plays an important role in supporting critical life-history events such as seasonal reproduction and migration. Despite numerous studies on adrenocortical modulation, little is known about the exact timing of this seasonal modulation with respect to critical life-history stages. We tested the hypothesis that seasonal modulation of the HPA axis during the spring mating season in male red-sided garter snakes (*Thamnophis sirtalis parietalis*) is temporally linked to the mechanisms regulating dispersal. We compared hormonal responses to capture stress in courting male red-sided garter snakes collected from the den site and den perimeter to those of dispersing snakes collected 0.6 km from the den. We also investigated possible changes in steroid hormones during the spring mating season. These studies support previous findings that plasma androgen and corticosterone concentrations significantly decline over the mating season. Our results demonstrate that males 0.6 km into a 15–20 km route to the feeding grounds have lower baseline corticosterone concentrations than male snakes actively courting at the den. Dispersing males also exhibit a typical stress response marked by a significant increase in corticosterone while actively courting males do not. Capture stress did not significantly influence androgen concentrations of either courting or dispersing male red-sided garter snakes. There were no significant differences in body composition indices among male snakes collected from the den, den perimeter, or 0.6 km away from the den. However, we did observe a significant negative correlation between baseline corticosterone levels and body composition indices. These data suggest that breeding is a distinct stage accompanied by specific physiological parameters that differ from those during dispersal to the feeding grounds. Our results indicate that declining baseline corticosterone concentrations may play a role in the behavioral switch between actively courting and dispersing (i.e., feeding) in the late spring.

Keywords: Corticosterone; Androgen; Stress; Dispersal; Feeding; Garter snake; *Thamnophis sirtalis parietalis*

1. Introduction

In vertebrates, the response to virtually any stressor includes activation of the hypothalamic-pituitary-adrenal (HPA) axis resulting in an increased secretion of glucocorticoids (Selye, 1950; reviewed in Sapolsky, 1992). Glucocorticoids modulate a variety of physiological and behavioral processes that promote survival while suppressing behaviors, such as reproduction, that are not crucial to immediate survival (Wingfield and Silverin, 1986; Wingfield et al., 1998). The physiological actions of glucocorticoids include promotion of gluconeogenesis and a subsequent increase in available glucose (Hadley, 1996).

Although this class of steroid hormones is frequently associated with acute stress, a seasonal elevation in glucocorticoid levels can be a very useful tool during periods of high activity. A correlation between activity levels and elevated baseline glucocorticoid concentrations has been well documented in many vertebrate species. For example, avian migration is particularly energetically demanding, and some studies have indicated an elevation in baseline corticosterone concentrations specifically in association with migratory flight (e.g., Holberton, 1999; Landys-Ciannelli et al., 2002; O'Reilly and Wingfield, 1995; Piersma et al., 2000; Reneerkens et al., 2002). Vocalization in male

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anurans is also very energetically demanding; several species of male frogs show elevated corticosterone levels in association with calling (Emerson and Hess, 2001). Glucocorticoids are also elevated during the breeding season in several amphibians and reptiles (Moore et al., 2001; Schramm et al., 1999; Wilson and Wingfield, 1992, 1994; Zerani and Gobbetti, 1993; reviewed in Moore and Jessop, 2003).

In addition to the modulation of baseline glucocorticoid levels to aid in critical life-history events, the sensitivity of the adrenocortical response to acute stress can also be modulated (Romero, 2001; Wingfield, 1994). For example, animals that live in extreme environments with limited reproductive opportunities often suppress the typical response to stressors during the breeding season (Moore et al., 2001; Silverin et al., 1997; Wingfield et al., 1992, 1995). This minimizes the negative effects of stress on reproductive behavior (primarily from negative interactions between the HPA axis and the hypothalamic-pituitary-gonadal (HPG) axis (Greenberg and Wingfield, 1987)), thereby maximizing reproductive potential. Despite numerous studies on adrenocortical modulation, little is known about the exact timing of this seasonal modulation with respect to critical life-history stages. In addition, there is a lack of information regarding the mechanisms regulating such modulation, especially in reptiles.

We investigated changes in baseline corticosterone (the primary glucocorticoid in reptiles (Idler, 1972)) and testosterone concentrations as well as modulation of the adrenocortical stress response during the highly active spring breeding season in a well-studied population of red-sided garter snakes (*Thamnophis sirtalis parietalis*). The red-sided garter snake is the most northerly living reptile in North America. To survive harsh winters, these northern-latitude populations enter a winter dormancy period in underground dens for up to eight months. Snakes emerge in the spring and immediately enter an intense mating season for approximately 4–5 weeks. During this time, gonads are regressed, sex steroid levels are decreasing and glucocorticoid levels are elevated (Crews et al., 1984; Krohmer et al., 1987; Moore et al., 2001; Whittier et al., 1987). Female red-sided garter snakes generally exit the den post-copulation, or within a day or two of emergence (Shine et al., 2001). In contrast, males typically stay in the vicinity of the den, exhibiting courtship behavior for several weeks (Shine et al., 2001). Once snakes leave the den, they travel as far as 20 km to summer feeding grounds (Gregory, 1977). Male red-sided garter snakes are of particular interest because they enter this costly courtship period subsequent to an eight-month dormancy period. In addition, snakes remain aphagic while actively courting (O'Donnell et al., 2004). Thus, elevated baseline corticosterone levels may facilitate reproduction in male red-sided garter snakes by mobilizing much-needed energy stores to sustain energetically costly courtship activity (Moore and Jessop, 2003).

Previous studies in red-sided garter snakes have shown that male snakes have a reduced response to capture stress in the spring (Moore et al., 2001; Lutterschmidt and Mason, 2005; but see Moore et al., 2000). Because corticosterone inhibits courtship behavior of male snakes (Moore and Mason, 2001; Lutterschmidt et al., 2004), seasonal modulation of the adrenocortical response to stress during the spring mating season is adaptive in that it increases reproductive opportunities. In contrast, male snakes have pronounced hormonal stress responses during the summer feeding season (Moore et al., 2001). We hypothesized that male snakes undergo a physiological and behavioral switch between mating at the dens in the early spring and foraging at the feeding grounds in the summer. We investigated whether this change in physiological state is related to the mechanisms regulating dispersal. Specifically, we addressed the following questions: (1) Do baseline plasma corticosterone and testosterone concentrations change during the mating season? (2) Do baseline corticosterone and androgen concentrations differ between courting and dispersing snakes? (3) How do stress responses of actively courting snakes compare to those of dispersing snakes? and (4) How does body condition of actively courting snakes compare to that of dispersing snakes?

2. Materials and methods

Male red-sided garter snakes, *T. sirtalis parietalis*, were captured in and around a winter hibernaculum in Inwood, Manitoba, Canada (50°31.58′ N, 97°29.71′ W), during May 2004. To describe patterns in baseline steroid hormone concentrations of male snakes during the latter half of the spring mating season, we captured 70 actively courting males from the den on the morning of 14 May 2004. Male snakes were randomly assigned to one of five sampling groups and numbered via unique clips on the ventral scales. Animals were housed together in an outdoor nylon cloth arena (1 m × 1 m × 1 m) near the den site where they were exposed to similar ambient temperatures and photoperiod as snakes at the den. Moore and Mason (2001) demonstrated that housing male red-sided garter snakes in these outdoor arenas does not influence either corticosterone or androgen concentrations of snakes during the spring mating season. Hide boxes placed inside the arena provided cover; water was provided *ad libitum*. Following acclimatization to the arenas, a randomly selected group of snakes ($n = 14$) was bled during the afternoon of 14 May and every three days thereafter (17, 20, 23, 26 May). No snake was bled more than once during the experiment. All blood samples were obtained between 1100 and 1400 h.

To investigate differences in baseline and stress-induced levels of corticosterone and testosterone between actively courting and dispersing snakes, we examined hormonal stress responses in male red-sided garter snakes collected from three areas around the den: den, den perimeter, and 0.6 km from the den. The den lies in an open rocky area in a limestone quarry, while the den perimeter is predominately aspen woodland. During the spring mating season, courting snakes can be found both within the den area as well as up to 200 m away from the den in the surrounding aspen woodland (Shine et al., 2001, 2003). We collected snakes in the aspen woodland approximately 50–70 m from the den (hereon referred to as the den perimeter) to investigate possible differences in baseline and stress-induced hormone levels in snakes that are still courting but are beginning to disperse from the den site (e.g., Shine et al., 2006). Thus, snakes collected from the den perimeter are an intermediate group between those actively courting at the den and those that are dispersing to the feeding grounds. In contrast, we are confident that snakes collected 0.6 km away from the den are dispersing to the feeding grounds and not returning to the den for further courtship opportunities (e.g., Gregory and Stewart, 1975; O'Donnell et al., 2004; Shine et al., 2001, 2003).

This study was conducted during the last quarter of the mating season (20–25 May 2004), a time when snakes can be found transitioning from active courtship behavior to dispersal to the feeding grounds. Equal numbers of snakes were collected from each area on any given day ($n = 20$ for