

# 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.

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## 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

each area) and subjected to a capture-stress protocol similar to Moore et al. (2000). Immediately upon capture a blood sample was obtained and snakes were then isolated individually in small, opaque cloth bags (20 × 20 cm) for either 1 or 4 h ( $n = 10$  for each capture stress duration). Immediately following the 1 or 4 h capture stress treatment, a second blood sample was collected to examine hormonal stress responses of snakes. All snakes were then weighed and measured to examine differences in body condition. Snakes were released at the point of capture at the conclusion of these experiments.

### 2.1. Blood sampling and radioimmunoassay

Blood samples (200  $\mu$ l) were obtained from the caudal vein as quickly as possible (mean  $\pm$  1 standard error: 73.0  $\pm$  3.8 s) using heparinized 1-cm<sup>3</sup> syringes and 25-g needles. Several studies have demonstrated that male red-sided garter snakes do not exhibit a significant response to either 1 or 4 h of capture stress during the spring mating season (Moore et al., 2001; Lutterschmidt and Mason, 2005). During the summer feeding period, male red-sided garter snakes exhibit a significant increase in corticosterone following 1 h of capture stress (Moore et al., 2001). Although a more complete timecourse of hormonal stress responses has not been investigated in red-sided garter snakes, our blood sampling times are well within the accepted range of less than 3 min for such studies of hormonal stress responses (e.g., Moore et al., 2000). Blood samples were stored on ice until return to the field station, where they were centrifuged and the plasma separated. Plasma samples were stored at  $-4^{\circ}\text{C}$  until return to Oregon State University, where they were stored at  $-70^{\circ}\text{C}$  until analyzed for corticosterone and testosterone concentrations following radioimmunoassay procedures modified from Moore et al. (2000).

Briefly, duplicate aliquots (70  $\mu$ l) of each plasma sample were incubated 12–24 h with 2000 cpm of tritiated steroid (Amersham Biosciences, Piscataway, NJ) to determine extraction efficiency. Steroids were extracted from each plasma sample twice with anhydrous ethyl ether. The ether phase was removed and dried under nitrogen gas in a warm water bath and the hormone extracts were then reconstituted in 600  $\mu$ l phosphate-buffered saline for direct assay. The methods used for direct radioimmunoassay of plasma samples for corticosterone and androgen concentrations in male red-sided garter snakes during the spring were described and validated in Lutterschmidt et al. (2004) and Lutterschmidt and Mason (2005). Because our testosterone antibody (Wein Laboratories, Inc., Succasunna, NJ) cross-reacts with 5- $\alpha$ -dihydrotestosterone (63.2% cross-reactivity), this direct radioimmunoassay measures both plasma testosterone and 5- $\alpha$ -dihydrotestosterone. Thus, we present data for total androgen concentrations.

Individual sample recoveries were determined from a 50- $\mu$ l aliquot of each extracted and reconstituted sample. For each steroid hormone being assayed, the remaining sample was allocated to two duplicate culture tubes for assay. Serial dilutions of the standard curve (performed in triplicate), 0% bound (or non-specific binding), 100% bound, and all samples were then incubated with 100  $\mu$ l tritiated steroid (1,2,6,7-<sup>3</sup>H testosterone or 1,2,6,7-<sup>3</sup>H corticosterone, Amersham Biosciences, Piscataway, NJ) and 100  $\mu$ l antiserum (testosterone antibody T3003 from Wein Laboratories, Inc., Succasunna, NJ; corticosterone antibody B3-163 from Esoterix Endocrinology, Calabasas Hills, CA) at  $4^{\circ}\text{C}$  for 12–24 h. Unbound steroid was separated from bound hormone using dextran-coated charcoal. The bound steroid was decanted into scintillation vials and incubated in toluene-based scintillation fluid for 12 h. The radioactivity of each sample was quantified in a Beckman LS 1800 scintillation counter.

All samples were randomly distributed across 5 steroid assays; hormone concentrations were corrected for individual recovery variation. Mean intra-assay variation was 15.9% and 19.1% for androgen and corticosterone, respectively. Inter-assay variation was 12.1% for androgen and 24.4% for corticosterone.

### 2.2. Statistical analyses

To investigate whether androgen and corticosterone concentrations decrease during the late spring, we used a one-way analysis of variance (ANOVA) on ranks followed by a Student-Newman-Keuls multiple com-

parisons procedure. To investigate possible differences in baseline (i.e., pre-stress) corticosterone concentrations among snakes collected from different locations, we used a one-way ANOVA followed by a Tukey's multiple comparisons procedure. Likewise, we used a one-way ANOVA followed by a Tukey's multiple comparisons procedure to investigate possible differences in baseline (i.e., pre-stress) androgen concentrations among snakes collected from different locations. As expected, there were no significant differences between the pre-stress hormone levels of the 1 h and 4 h capture stress groups. Thus, we collapsed the 1 h and 4 h treatment groups prior to examining differences in baseline hormone concentrations among snakes collected from different locations. Prior to analysis, both corticosterone and androgen concentrations were square root-transformed to correct for non-normality.

We used a one-way repeated measures ANOVA followed by a Tukey's multiple comparisons procedure to investigate possible differences in corticosterone responses to capture stress (i.e., pre- versus post-stress corticosterone levels) among snakes collected from different locations. Likewise, we used a one-way repeated measures ANOVA followed by a Tukey's multiple comparisons procedure to investigate possible differences in androgen responses to capture stress (i.e., pre- versus post-stress androgen levels) among snakes collected from different locations. For these repeated measures analyses, both the location of capture and duration of capture stress were included in the analyses as between-subjects factors. Prior to analysis, corticosterone concentrations were natural log-transformed to correct for non-normality; androgen concentrations were square root-transformed to correct for non-normality.

Lastly, we investigated possible differences in the body composition index of courting versus dispersing snakes. Body composition index is defined as each individual's residual from a regression of body mass on snout-vent length for all courting and dispersing snakes used in this experiment. We used a one-way ANOVA to investigate differences in the body composition index among courting and dispersing snakes collected from the den, den perimeter, and 0.6 km from the den. A correlation analysis was used to determine if there was a significant association between baseline (i.e. pre-stress) corticosterone levels and body composition index in courting and dispersing snakes. We used SigmaStat<sup>®</sup> 2.03 (SPSS, 1997) and SPSS<sup>®</sup> 13.0 (SPSS, 2004) for all statistical analyses. All statistical comparisons were considered significant at  $P \leq 0.05$ .

## 3. Results

We found that androgen ( $H = 27.619$ ,  $df = 4$ ,  $P < 0.001$ ) and corticosterone ( $H = 12.35$ ,  $df = 4$ ,  $P = 0.015$ ) plasma concentrations decreased over the second half of the mating season (Fig. 1; from a one-way ANOVA on ranks for androgen and a one-way ANOVA on ranks for corticosterone). Both androgen and corticosterone concentrations were significantly higher on the first sampling date (May 14) than on all following sampling dates (17–26 May; from 2 separate one-way ANOVAs on ranks followed by Student-Newman-Keuls multiple comparisons procedures for androgen and corticosterone).

We examined differences in baseline and stress-induced hormone levels of male snakes from three different areas: (1) den, (2) den perimeter, and (3) 0.6 km from the den on the migratory route. Results from a one-way ANOVA indicate no statistically significant differences in baseline (i.e., pre-stress) androgen concentrations among snakes collected from different locations. Results from a repeated measures ANOVA indicate no statistically significant differences in androgen responses to capture stress among snakes. Neither the location of capture nor the duration of capture stress had a statistically significant

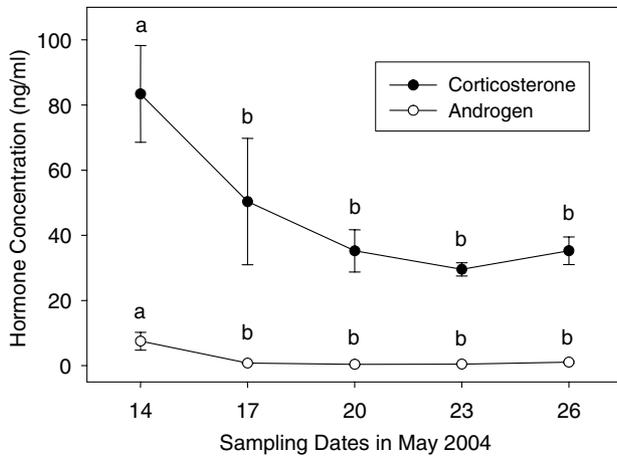


Fig. 1. Change in basal corticosterone and androgen concentrations during the latter portion of the mating season in male red-sided garter snakes, *Thamnophis sirtalis parietalis* ( $n = 14$  for each sampling date). Standard errors ( $\pm 1$ ) are shown by the vertical lines. Statistically significant differences in hormone concentrations among sampling dates are indicated by letters above each standard error bar.

effect on androgen concentrations (Fig. 2A; results from a repeated measures ANOVA). There were no statistically significant interactions between androgen responses to capture stress, the location of capture, and the duration of capture stress treatment (from a repeated measures ANOVA).

Snakes collected 0.6 km from the den had statistically significantly lower baseline (pre-stress) corticosterone concentrations than snakes collected from both the den site and the den perimeter (Fig. 2B;  $F = 5.606$ ;  $df = 2$ ;  $P = 0.006$ , from a one-way ANOVA followed by a Tukey's multiple comparisons procedure). Corticosterone responses to capture stress differed significantly among courting and dispersing snakes collected from the den, den perimeter, and 0.6 km from the den ( $F = 18.296$ ;  $df = 1$ ;  $P < 0.001$ ; from a repeated measures ANOVA). The location of capture had a statistically significant effect on corticosterone responses to capture stress (Fig. 2B;  $F = 3.482$ ;  $df = 2$ ;  $P = 0.038$ , from a repeated measures ANOVA), and there was a statistically significant interaction between corticosterone responses to capture stress and the location of capture ( $F = 7.730$ ;  $df = 2$ ;  $P = 0.001$ , from a repeated measures ANOVA). Results from a Tukey's multiple comparisons procedure indicate that only snakes collected 0.6 km from the den showed a significant increase in corticosterone in response to capture stress (Fig. 2B). The duration of capture stress treatment had no significant influence on corticosterone responses to capture stress. There were no statistically significant interactions between the duration of capture stress treatment, location of capture, and corticosterone responses to capture stress (from a repeated measures ANOVA).

There were no significant differences in body composition index among courting and dispersing snakes collected from the den, den perimeter, and 0.6 km from the den (Fig. 3; from a one-way ANOVA). Baseline (pre-stress) cor-

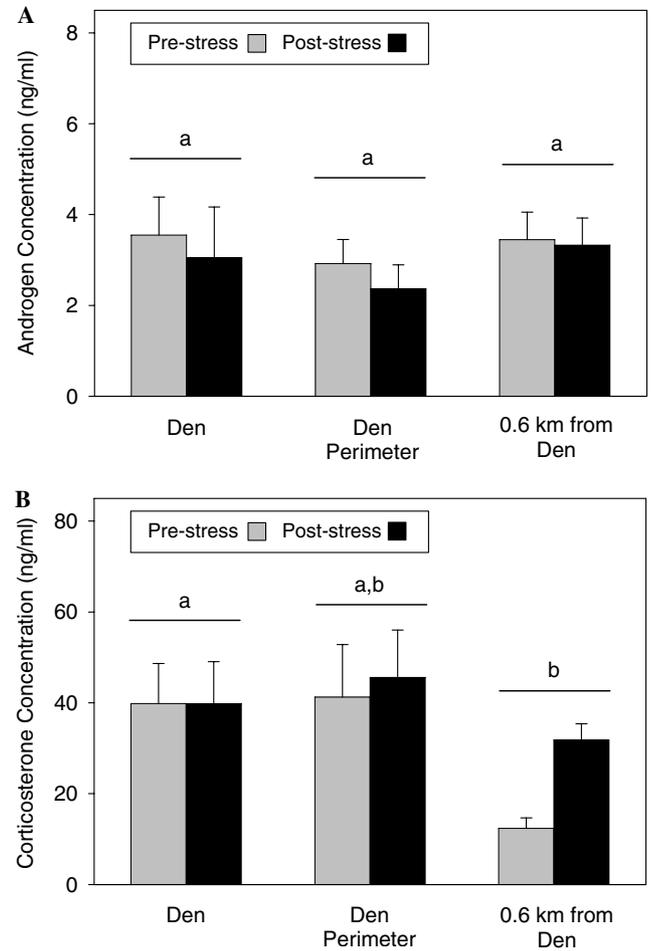


Fig. 2. Hormonal responses of male red-sided garter snakes (*Thamnophis sirtalis parietalis*) to capture stress during the spring mating season. This study was conducted during the last quarter of the mating season (20–25 May 2004), a time when snakes can be found transitioning from active courtship behavior to dispersal to the feeding grounds. Courting male garter snakes were collected from the den and den perimeter; snakes collected approximately 0.6 km from the den were dispersing to the feeding grounds. For ease of visual interpretation, and because the duration of capture stress did not significantly influence hormonal stress responses, we collapsed the 1 h and 4 h capture stress treatment groups as shown ( $n = 20$  for each location). (A) Mean plasma androgen concentrations of snakes; (B) mean plasma corticosterone concentrations. Standard errors ( $\pm 1$ ) are shown by the vertical lines. Statistically significant differences in hormonal responses to capture stress among males collected from different sites are indicated by letters above each group.

ticosterone concentrations of courting and dispersing snakes were significantly negatively correlated with body composition index (Fig. 4;  $r = -0.281$ ;  $P = 0.029$ ).

#### 4. Discussion

We tested the hypothesis that seasonal modulation of the HPA axis during the spring mating season in male red-sided garter snakes (*T. sirtalis parietalis*) is temporally linked to the mechanisms regulating dispersal to the feeding grounds. Our results provide evidence that males 0.6 km into a 15–20 km route to the feeding grounds have different baseline hormone

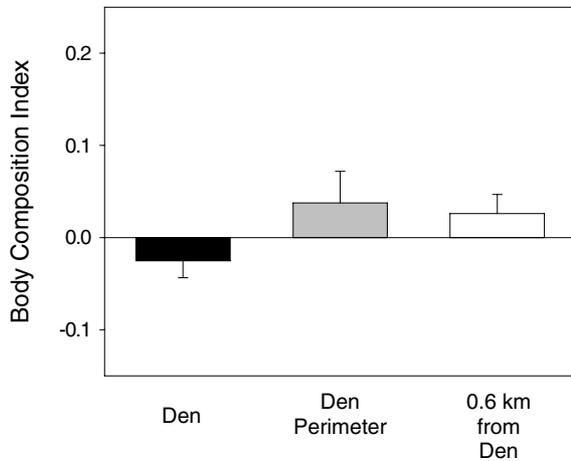


Fig. 3. Body composition index of male red-sided garter snakes (*Thamnophis sirtalis parietalis*) during the spring mating season. Courting male garter snakes were collected from the den and den perimeter; snakes collected approximately 0.6 km from the den were dispersing to the feeding grounds. Standard errors (+1) are shown by the vertical lines.

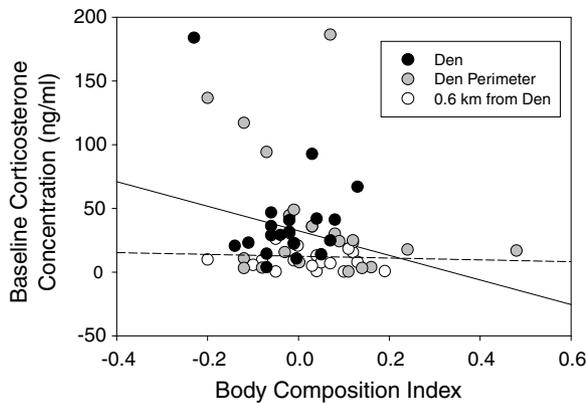


Fig. 4. Correlation between baseline corticosterone concentrations and body composition index of male red-sided garter snakes (*Thamnophis sirtalis parietalis*) during the spring mating season. Shaded symbols indicate whether snakes were collected from the den, den perimeter, or approximately 0.6 km from the den. The solid line represents the association between baseline corticosterone concentrations and body composition index for all courting and dispersing snakes ( $r = -0.281$ ;  $P = 0.029$ ;  $n = 60$ ). The dashed line represents the association between baseline corticosterone concentrations and body composition index for dispersing male snakes only ( $r = -0.067$ ;  $P = 0.779$ ;  $n = 20$ ).

concentrations and a different hormonal response to capture stress. 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. 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.

#### 4.1. Baseline androgen and corticosterone concentrations

As predicted, our study indicates that plasma androgen concentrations are quite low during the spring breeding

season (Fig. 1). Our results also support the decline in plasma testosterone concentrations reported by Krohmer et al. (1987) in male red-sided garter snakes during the spring. Red-sided garter snakes have a dissociated reproductive pattern with peak gametogenesis occurring in late summer, at a time when snakes are not actively mating (Crews, 1984). Indeed, several studies have indicated that sex steroid hormones do not play a role in regulating reproductive behavior of male snakes (Crews, 1991; Crews et al., 1984). Thus, changes in already basal androgen levels likely do not play a role in initiating the transition from reproductive behavior to dispersal in male red-sided garter snakes. However, it should be noted that the decline in testosterone concentrations reported by Krohmer et al. (1987) paralleled a significant decline in courtship intensity in male red-sided garter snakes. Whether this reported decline in courtship intensity results from a decline in testosterone concentrations or some other factor requires further investigation.

Our data support the relationship between seasonal activity levels and seasonal baseline corticosterone concentrations (e.g., Wilson and Wingfield, 1994; Holberton, 1999). Male snakes 0.6 km away from the den had significantly lower baseline (i.e., pre-stress) concentrations of corticosterone as compared to both actively courting males at the den and males captured in the vicinity of the den (Fig. 2B). Our results suggest that elevated baseline corticosterone concentrations facilitate reproductive behavior in this species (e.g., Moore and Jessop, 2003). These data also suggest that male red-sided garter snakes utilize seasonally elevated baseline corticosterone concentrations predominantly during the breeding season and not during dispersal. Once male snakes cease courting females and disperse from the den site, they will begin to feed if prey items are available (e.g., Gregory and Stewart, 1975; O'Donnell et al., 2004). Indeed, we observed earthworms in the stomach contents of a few dispersing male snakes during this study. Thus, dispersal behavior of male red-sided garter snakes is likely supported energetically by active foraging, whereas sustained courtship behavior is likely facilitated by elevated baseline corticosterone concentrations.

Further research is necessary to determine the temporal relationship between changes in corticosterone concentrations, dispersal, and feeding behavior. For example, if corticosterone inhibits feeding behavior, then a decline in corticosterone concentrations would remove this inhibition and initiate dispersal to the feeding grounds. Alternatively, it is possible that feeding causes a decline in corticosterone concentrations. We do not think this is a likely mechanism, however, as only a few dispersing snakes collected during this study were observed to be feeding. Thus, most dispersing snakes had lower baseline corticosterone levels despite a lack of feeding behavior. Furthermore, courting male snakes choose female pheromone trails over worm trails during the mating season (O'Donnell et al., 2004), indicating that courting male snakes refuse food even when available. The transition from courtship behavior to feeding/

dispersal behavior is therefore likely regulated by changes in corticosterone concentrations (or other factor), rather than feeding behavior inducing changes in corticosterone concentrations. Future research examining the temporal relationship between feeding and dispersal would be particularly informative regarding the importance of corticosterone in initiating the transition between these life-history events.

To determine the time course of the decline in corticosterone levels among courting and dispersing snakes, we collected males from the den and housed them in an outdoor arena. Male snakes were monitored for steroid hormone concentrations during the late breeding season. Males showed a significant decrease in baseline corticosterone concentrations over the two-week time period (Fig. 1). The lowest concentrations of corticosterone observed during late spring (i.e., 20–26 May; Fig. 1) in this part of the study are higher than those baseline hormone concentrations measured in dispersing male snakes from 20 to 25 May (Fig. 2B). It is possible that corticosterone concentrations of snakes housed in the arena were still declining at the end of our observation period, especially if these snakes had not “transitioned” to dispersal behavior by the end of this study. It is also possible that housing snakes in the arenas increased corticosterone concentrations. However, 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.

The lowest concentrations of corticosterone observed in males kept in nylon cloth arenas and monitored for hormone levels (20–26 May; Fig. 1) are similar to the baseline corticosterone concentrations of actively courting males collected at the den and den perimeter from 20 to 25 May (Fig. 2B; approximately  $40 \text{ ng ml}^{-1}$ ). These results suggest that corticosterone concentrations decrease over the breeding season while male snakes are still actively courting. Thus, elevated corticosterone levels facilitate reproduction during the breeding season, but corticosterone concentrations gradually decline during the spring. We suggest that male snakes reach a certain threshold of baseline corticosterone concentrations, below which triggers the physiological switch from courtship behavior to dispersal to the feeding grounds.

#### 4.2. Hormonal stress responses

Our results demonstrate that males traveling to the feeding grounds in the spring exhibit a hormonal stress response marked by a significant increase in corticosterone while males at the den and den perimeter do not (Fig. 2B). These results support our hypothesis that male red-sided garter snakes undergo a change in physiological state toward the end of the breeding season and during the start of dispersal to the feeding grounds. Neither courting snakes at the den and den perimeter nor dispersing snakes exhibited a change in plasma androgen concentrations in

response to capture stress (Fig. 2A). This study and others (e.g., Crews et al., 1984; Krohmer et al., 1987; Moore et al., 2001; Whittier et al., 1987) indicate that baseline plasma androgen concentrations are minimal during the late spring. These low androgen concentrations during the spring breeding season most likely preclude significant changes in plasma androgen in response to capture stress. These already basal androgen concentrations likely also contribute to the lack of a significant difference among baseline androgen concentrations in snakes collected from the den, den perimeter, and 0.6 km from the den (Fig. 2B).

The absence of a hormonal stress response, marked by increased corticosterone concentrations, in courting male snakes collected from the den and den perimeter may result from seasonal modulation of the adrenocortical response to stress. For example, during the summer feeding season, male red-sided garter snakes respond to capture stress with a significant increase in corticosterone concentrations (Moore et al., 2001). In contrast, Moore et al. (2001) and Lutterschmidt and Mason (2005) demonstrated that male red-sided garter snakes do not show significant hormonal responses to capture stress during the spring mating season (but see Lutterschmidt and Mason (2005) for discussion of seasonal and annual variation in hormonal stress responses of red-sided garter snakes). Alternatively, the lack of a hormonal stress response in male snakes during the spring mating season has been hypothesized to result from the already elevated baseline corticosterone levels observed in these animals (e.g., Lutterschmidt and Mason, 2005; Moore et al., 2001). However, we demonstrate a lack of a significant hormonal stress response in courting male snakes even when basal (pre-stress) corticosterone concentrations are not maximally elevated (i.e.,  $40 \text{ ng ml}^{-1}$ ; Fig. 2B). Thus, seasonal modulation of the adrenocortical response to stress is supported.

Our data suggest that seasonal modulation of the adrenocortical stress response and baseline corticosterone levels occurs only during courtship activity, as snakes that have initiated dispersal show a more typical hormonal response to capture stress. Such a suppression of the hormonal stress response during the breeding season would reduce the negative impacts of stress on reproduction. This would be especially critical to the northern-latitude red-sided garter snake, as reproductive opportunities are quite limited. Although little is known regarding the mechanisms of adrenocortical modulation, our results suggest that such modulation in red-sided garter snakes is linked to the mechanisms regulating the physiological transition between courtship behavior and dispersal. Factors likely involved in this physiological transition, and hence adrenocortical modulation, include changing baseline corticosterone levels and body condition indices.

#### 4.3. Body condition

Mean body composition index (as defined by the residuals from a regression of mass on snout-vent length) of male

snakes at the den, den perimeter and 0.6 km from the den did not differ significantly. These results suggest that body condition does not play a direct role in initiating dispersal of male red-sided garter snakes. We found that baseline corticosterone concentrations of male red-sided garter snakes were negatively correlated with body composition index during the late spring. These results are similar to a previous study on this population indicating a negative relationship between body condition and corticosterone levels across seasons (Moore et al., 2001).

Because glucocorticoids are involved in energy homeostasis, it is not surprising that baseline glucocorticoid concentrations are often correlated with energetic condition. We investigated hormonal stress responses and body condition during late spring, when actively courting males have expended up to 1% of their body mass per day courting (e.g., Shine et al., 2001). Dispersing snakes are no longer maintaining courtship behavior, and this change in behavior is likely related to a decrease in corticosterone concentrations. However, no significant differences were observed among the body composition indices of males collected from the den, den perimeter, or 0.6 km from the den (Fig. 3). Thus, the observed correlation between baseline corticosterone concentrations and body condition may not be physiologically relevant, especially with regard to the transition from courtship behavior to dispersal from the den site.

It is interesting to note that dispersing male snakes do not show the same strong negative correlation between baseline corticosterone concentrations and body composition index as do courting males at the den and den perimeter (Fig. 4). Indeed, a correlation analysis of baseline corticosterone concentrations and body composition index for only dispersing males yields no significant association ( $r = -0.067$ ,  $P = 0.779$ ). This provides further evidence that elevated corticosterone concentrations may facilitate courtship behavior in male snakes with low energetic condition at the den and den perimeter, but elevated corticosterone levels are not utilized for dispersal behavior. In contrast, dispersal behavior is apparently supported energetically by active foraging, as snakes begin to feed soon after dispersal from the den site (e.g., Gregory and Stewart, 1975; O'Donnell et al., 2004).

Collectively, these experiments demonstrate that seasonal modulation of baseline corticosterone concentrations and hormonal stress responses may occur within a specific but brief portion of the mating season. Such seasonal modulation of the HPA axis appears to be tightly linked to the transition between reproductive and non-reproductive states in red-sided garter snakes. Our results also provide evidence that declining baseline corticosterone concentrations likely play a role in initiating dispersal (and feeding) behavior of male snakes. We hypothesize that the transition between reproductive and non-reproductive behaviors in male red-sided garter snakes occurs when baseline corticosterone levels fall below a certain threshold level. Future research examining the influence of corticosterone antagonists on the duration of courtship activity and dispersal

behavior would help elucidate corticosterone's role in regulating the transition between mating and dispersal in this northern-latitude ectothermic model.

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