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Physiological correlates of reproductive decisions: Relationships among body condition, reproductive status, and the hypothalamus-pituitary-adrenal axis in a reptile

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ABSTRACT

When opportunities to feed and reproduce are limited, females are often unable to recover sufficient energy stores to reproduce in consecutive years. Body condition has been used as a proxy for recent reproductive history in such species. We previously found that glucocorticoid responses to capture stress vary with body condition in female red-sided garter snakes (*Thamnophis sirtalis parietalis*), a species with limited seasonal breeding opportunities. Because variation in glucocorticoid receptor (GR) protein in the brain could explain these differences, we first assessed GR protein content in females in different body conditions. To investigate if body condition during the spring mating season accurately reflects recent reproductive history, we measured glucocorticoid responses to stress in females with different body conditions, assessed their mating behavior and brought mated females to our lab to determine which females would give birth during the summer (i.e., were parturient). Female red-sided garter snakes reproduce biennially, and therefore mated females that did not give birth were deemed non-parturient. In this study, glucocorticoid stress responses and mating behavior did not vary with body condition, nor was body condition related to brain GR or reproductive condition (parturient vs non-parturient). Only unreceptive females showed a significant stress-induced increase in glucocorticoids, suggesting that reduced stress responsiveness is associated with receptivity. Parturient females mated faster (were more proceptive) than non-parturient females. These data suggest that HPA axis activity modulates receptivity, while proceptivity is related primarily to reproductive condition.

1. Introduction

When opportunities for feeding and reproduction are limited, animals must make important “decisions” about whether or not to reproduce. Reproducing under unfavorable conditions jeopardizes both survival and overall reproductive success, but failure to reproduce under favorable conditions also jeopardizes fitness. The high stakes of navigating the trade-offs between reproduction and survival mean that the physiological mechanisms used by either sex to modulate reproductive investment are critically important, yet they remain poorly understood.

The hypothalamus-pituitary-adrenal (HPA) axis receives projections from many brain regions (Whitnall, 1993) and thus is ideally positioned to integrate information about internal status, such as energy balance and reproductive history, with external cues, such as day length, temperature, and the presence of conspecifics. All of these cues potentially influence reproduction. Hence, the HPA axis may integrate information from a variety of neural and neuroendocrine pathways and then contribute to reproductive decision-making via modulating the production of its major endocrine factor, glucocorticoids. In turn, glucocorticoids mobilize energy stores, fueling energetically intensive processes such as reproduction and migration (Landys et al., 2006; Schoech et al., 2009). As such, glucocorticoid concentrations are often elevated during the breeding season compared to other times of the year (Romero, 2002).

In addition to allostatic changes in baseline glucocorticoids, acute environmental perturbations can elicit a stress-induced increase in glucocorticoids that generally activates physiological processes and behaviors that support immediate survival while suppressing non-essential processes (Sapolsky et al., 2000). For example, activation of the HPA axis suppresses reproductive physiology and behavior in many animals (Crossin et al., 2016; Wingfield and Romero, 2001); these results can be mimicked by treatment with exogenous glucocorticoids.

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Taken together, these data suggest that while elevated glucocorticoids are often necessary to fuel the energetic demands of reproduction, if circulating glucocorticoid concentrations exceed some upper threshold, reproduction can be suppressed (Wingfield et al., 1998). In other words, there is a physiological range of glucocorticoid concentrations that facilitates and supports reproductive efforts.

Many studies examining the relationship between the HPA axis and reproduction hinge upon perturbing the HPA axis with standardized capture stress protocols. Such studies often report significant variation in both the magnitude of the stress-induced increase in glucocorticoids and how increased glucocorticoids affect reproduction (Wingfield and Romero, 2001; Wingfield and Sapolsky, 2003). Although there are fewer studies in females compared to males, the available data suggest that female reproductive condition is also an important factor. Indeed, glucocorticoid responses to capture stress vary with reproductive condition in species from all vertebrate groups (Anderson et al., 2014; Bauer et al., 2014; Jessop, 2001; Lutterschmidt et al., 2009; Narayan and Hero, 2013; Schreck et al., 2001; Vitousek et al., 2010; Wingfield et al., 1992; Woodley and Moore, 2002). In female tree lizards (*Urosaurus ornatus*), capture stress treatment elicits a significant increase in glucocorticoids unless the female is gravid, in which case no increase is observed (Woodley and Moore, 2002). These results suggest that the sensitivity of the HPA axis is modulated with the changing demands of the reproductive cycle, potentially reducing any negative impacts of glucocorticoids on female reproduction and/or embryonic development (Lupien et al., 2009).

Females of live-bearing species in particular are subject to extraordinary energetic demands during reproduction, and some species cannot recover sufficient energy stores to reproduce in consecutive breeding seasons (Bonnet et al., 1998; Gregory, 2006; Whittier and Crews, 1990). A female's recent reproductive history could therefore influence whether or not she will reproduce in a given year. When this is the case, we might expect body condition to vary with reproductive condition such that females that have recently given birth have lower body condition compared to those that have not. In some species, the magnitude of the stress response varies with body condition (Dayger et al., 2013; Vitousek et al., 2010; Vitousek and Romero, 2013), but whether this variation is related specifically to reproductive history or condition, rather than body condition more generally, is unknown. For example, female red-sided garter snakes (*Thamnophis sirtalis parietalis*) in below-average body condition, but not above-average body condition, increased glucocorticoids in response to capture stress during the spring mating season (Dayger et al., 2013). Females in below-average body condition were also more sensitive to lower doses of exogenous corticosterone than those in above-average body condition, suggesting that the sensitivity of the HPA axis varies with body condition in this species (Dayger et al., 2013). Considering female red-sided garter snakes reproduce approximately every other year, and that reproductive condition and body condition are both related to variation in stress responsiveness in other species, we hypothesized that the association between body condition and stress responsiveness in females actually reflects variation in reproductive condition.

We tested this hypothesis in a well-studied population of red-sided garter snakes (*Thamnophis sirtalis parietalis*) by directly investigating the relationship between body condition, glucocorticoid and behavioral stress responses, and reproductive condition in females. Because variation in the quantity of glucocorticoid receptors (GR) could explain the relationship between stress responses and body condition, we first compared brain GR peptide content and glucocorticoid stress responses in females in different body conditions. Glucocorticoids can bind both GR and mineralocorticoid receptor (MR) (Landys et al., 2006; Whitnall, 1993), although GR is generally thought to mediate stress-induced signaling. Surprisingly, no published studies have investigated whether the quantity of GR varies with body condition in any taxa. Next, we tested whether behavioral stress responses vary with body condition using two measures of female reproductive behavior: receptivity and, among receptive females, proceptivity. Finally, we tested whether variation in body condition, glucocorticoid stress responses and/or behavioral stress responses are associated with variation in reproductive condition. We posited that body condition during spring emergence is related to recent reproductive history and thereby plays a role in the relationship between the HPA axis, reproductive behavior, and reproductive outcome.

1.1. Natural History of Study Organism

We conducted these studies in a population of red-sided garter snakes in Manitoba, Canada. During the winter, adult red-sided garter snakes brumate communally in underground dens for approximately 8 months before emerging in late April through May, upon which they engage in a short mating period lasting about 4 weeks (Gregory, 1973). Males begin emerging first, but remain at the den site searching for females to court. Females emerge during the course of the mating season and are courted vigorously by up to 100 males in a single mating ball (Garstka et al., 1982). If receptive to mating, the female will exhibit behaviors that are necessary and sufficient for copulation, including remaining relatively stationary during male courtship, rolling slightly to one side and raising the tail to expose the cloaca, and finally gaping the cloaca to permit intromission (e.g., Carpenter, 1977; Crews, 1976; Garstka et al., 1982). If a female is unreceptive, she will often completely evade male courtship by moving away suddenly and quickly (Crews, 1976; Mendonça and Crews, 1996) or she will flatten her body against the ground and not permit mails to engage in effective tail wrestling behavior that allows the male to align his cloaca with that of the female's (personal observations). Most females disperse from the den site to the surrounding aspen forest within 24 h of emergence, while males can remain at the den site for several weeks seeking further mating opportunities before eventually migrating to summer feeding grounds. Neither sex feeds during winter dormancy or the spring mating season (Gregory, 1973; O'Donnell et al., 2004; Whittier and Crews, 1990). Thus, energy stores acquired the previous year must sustain the snakes through the mating season and the initial migration to summer feeding grounds.

During the summer, females may become vitellogenic, ovulate, and fertilize eggs from sperm stored in the oviduct (Halpert et al., 1982). In late summer, pregnant females give birth to live young at the feeding grounds. Males and females migrate back to the den site in early fall before descending underground for the winter. The short, 3-month feeding season experienced by northern-latitude populations of red-sided garter snakes, coupled with the high energetic demands of viviparous reproduction, means that females are unable to reproduce in consecutive years and instead reproduce approximately every other year, making them biennial breeders (Gregory, 2006; Whittier and Crews, 1990). Red-sided garter snakes are also capital breeders that use stored energy to fuel reproduction (Bonnet et al., 1998; Drent and Daan, 1980). Thus, during the spring mating season, females that gave birth the previous summer are hypothesized to be in lower body condition than females that did not give birth and instead spent the summer feeding season acquiring and storing energy for future reproductive efforts.

2. Materials and methods

Experiments were conducted in the field with free-ranging red-sided garter snakes at a den site located in Inwood, Manitoba, Canada. The experiments described here were approved by Portland State University's Institutional Animal Care and Use Committee and conducted under the authority of Wildlife Scientific Permit WB14930 issued by the Manitoba Department of Sustainable Development (formerly the Department of Conservation).