

# Wet- and Dry-Season Steroid Hormone Profiles and Stress Reactivity of an Insular Dwarf Snake, the Hog Island Boa (*Boa constrictor imperator*)

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## ABSTRACT

Field endocrine studies providing new comparisons for inference into the evolutionary and ecological factors shaping organismal physiology are important, often yielding novel physiological insights. Here, we explored factors associated with the sex steroid hormone concentrations and adrenocortical response to capture stress in Hog Island boas (*Boa constrictor imperator*) in the Cayos Cochinos archipelago of Honduras to generate comparative field hormone data from a tropical reptile

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and test the island tameness hypothesis. Baseline concentrations of testosterone, corticosterone, estradiol, and progesterone were measured during the wet and dry seasons, and an acute stressor of 1 h in a cloth bag was used to assess the stress response. Plasma steroid concentrations in these snakes were generally low in comparison to other taxa. Higher testosterone concentrations in males and higher estradiol and corticosterone concentrations in females were observed during the wet season compared to the dry season, which may be indicative of mating activities and vitellogenesis during this period. Snakes displayed a 15-fold increase in corticosterone concentrations in response to capture stress, a rise that was not impacted by whether a snake had been captured during previous years. The adrenocortical stress response was greater in males and positively related to body temperature. We suggest that this system merits future inquiries into the physiology and behavior of *B. c. imperator*, particularly as a model for studying insular impacts on diverse life history characters.

## Introduction

Investigations into the hormonal regulation of reproduction in vertebrates are dominated by laboratory studies on model organisms that can elucidate the specific mechanisms by which hormones stimulate and inhibit reproductive behaviors and events in the reproductive cycle. However, field investigations of free-living animals are especially valuable for understanding the roles of hormones in seasonal reproductive events and behaviors, where reproductive endocrinology can be studied in a realistic, ecologically relevant setting. Among terrestrial vertebrates, field endocrine studies of small mammals and passerine birds have dominated the literature, with studies on amphibians, lizards, and snakes forming fewer yet still important contributions (see recent reviews in Norris and Lopez 2010). Snakes are particularly well suited for studying the relationship between hormones and reproduction because the group is quite speciose and diverse and many species are easy to study in the field. In addition, snakes exhibit a wide range of reproductive tactics, including asexual and sexual reproduction (Booth et al. 2012), oviparity and viviparity, capital and income breeding, and associated and dissociated relationships between hormonal peaks and reproductive events (reviewed in DeNardo and Taylor 2010; Taylor and DeNardo 2010). The vast majority of endocrine studies of snakes have been conducted on temperate, advanced

Caenophidian snakes (especially *Thamnophis* spp. and viperids), leading Taylor and DeNardo (2010) to call for studies on species representing a wider ecological and taxonomic grouping.

In particular, data on the reproductive endocrinology of tropical snakes would be of great interest in elucidating the relationships between hormones and reproduction in environments that are less seasonal than temperate zones, at least with respect to temperature. Some studies of tropical birds have shown that plasma androgen concentrations do not vary seasonally and are very low compared to temperate species (Wikelski et al. 2000; Goymann et al. 2004). Steroid hormone concentrations have been measured as correlates of reproductive and body condition in brown tree snakes (*Boiga irregularis*) on the tropical island of Guam (Waye and Mason 2003; Moore et al. 2005), but these snakes are an introduced species on the island and have been argued to be chronically stressed, possibly due to fluctuations in prey availability as a result of predation pressure. The few tropical snakes studied in their natural habitat are marine snakes; these show various degrees of association and dissociation of hormones with reproductive events (Gorman et al. 1981). Like many marine species, terrestrial tropical snake species experience little annual variation in temperature, but unlike marine species, their reproductive cycles may be entrained on seasonal patterns of rainfall (e.g., wet season vs. dry season; Shine and Brown 2008; Eikenaar et al. 2012). Alternatively, it is possible that terrestrial tropical snakes may reproduce at any time of year (continuous reproduction; Saint Girons 1982), although this pattern has yet to be documented.

While detailed studies of tropical snakes are therefore clearly desirable, these snakes can often be difficult to study because site accessibility and species detectability can be detriments to sample size in the tropics. However, the Hog Island boa (*Boa constrictor imperator*) bucks this trend. These populations of dwarf boa constrictors in the Central American *Boa* clade inhabit the Hog Island chain (Cayos Cochinos) of Honduras in the Caribbean Sea and reach population densities as high as 2,170/km<sup>2</sup> (Green 2010). These incredible densities allow collection of large amounts of data by researchers in a relatively short time period. Despite the high current population densities, island *B. c. imperator* are restricted to a total geographic range of only 2.2 km<sup>2</sup> (Wilson and Cruz Díaz 1993), have historically faced high poaching pressure from the pet trade (Reed et al. 2007; Green 2010), and are listed in appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora. Knowledge of their reproductive endocrinology therefore has the additional benefit of informing conservation efforts.

Another reason that endocrine studies on this population of island snakes may be desirable is that as a population free of natural and invasive predators, *B. c. imperator* may display traits associated with island tameness. The island tameness hypothesis postulates that in the absence of predation pressure, antipredator traits will become greatly attenuated or disappear altogether, resulting in a tame population of individuals (Darwin 1839; Blumstein and Daniel 2002). Famous among these are

the unfortunate cases of many island megafauna (e.g., giant tortoises, dodos) that became extinct due in large part to their tameness and therefore the ease with which they could be harvested by humans. In squamate reptiles, relaxed predation pressure on islands can result in the rapid loss of diverse defensive behaviors such as tail autotomy (Cooper and Pérez-Mellado 2010), the propensity to flee upon approach (Stone et al. 1994; Berger et al. 2007), and the avoidance of environmental space previously used by predators (Losos et al. 2004). For example, naïve marine iguanas (*Amblyrhynchus cristatus*) in the Galápagos archipelago flee from human approach later than mainland iguanid lizards (Berger et al. 2007), lack adrenocortical and cardiovascular stress responses to experimental chasing by a researcher, and can be easily captured during repeated attempts (Rödl et al. 2007; Vitousek et al. 2010). In particular, measuring stress reactivity—the adrenocortical response to a standardized stressor—is a fruitful way to evaluate the extent of the stress response in animals (Tokarz and Summers 2010). Animals exhibiting island tameness may lack or may have blunted stress reactivity (Rödl et al. 2007).

In this study, we report the results of a general assessment of the associations between season (wet vs. dry), sex, and body condition on the baseline plasma concentrations of testosterone (T) and corticosterone (CORT) in male and female *B. c. imperator*, as well as progesterone (P4) and estradiol (E2) in females. Additionally, we quantified the stress reactivity (increase in CORT from baseline) of the snakes when exposed to an acute stressor (handling followed by confinement in a cloth bag for 1 h) to test the hypothesis that these animals display island tameness. If this is true, then snakes subjected to capture stress should not display increases in plasma CORT concentrations. Since diverse factors have been shown to influence vertebrate stress reactivity, we include in our analysis and report estimated effect sizes for sex (Lutterschmidt et al. 2009), body condition (Moore and Jessop 2003), body temperature (Cree et al. 2003), and whether a snake had been previously captured (Rödl et al. 2007).

## Material and Methods

### Study Population

The study was carried out on Cayo Cochino Menor, the smallest island of the Cayos Cochinos archipelago. The 0.64-km<sup>2</sup> island is situated about 18 km off the Caribbean coast of Honduras (15°55'N, 86°30'W). It is a protected area managed by the Honduran Coral Reef Fund. While the island experiences rainfall throughout the year, marked seasonality exists in the form of distinct wet (October–January) and dry (February–September) seasons (Green 2010).

### Seasonal Steroid Concentrations

Visual searches conducted between 0700 and 1800 hours were used to locate *Boa constrictor imperator* during the periods of December 18, 2008–January 2, 2009 (wet season,  $n = 77$ ), and June 18–August 17, 2009 (dry season,  $n = 36$ ). The aims of

these visits during 2008–2009 were to ascertain circulating concentrations of CORT, T, E2, and P4 and to determine whether the concentrations of these hormones varied seasonally; hereafter, we refer to this study as the seasonal study.

When a snake was located, it was immediately captured and 0.5–1.0 mL of blood was obtained as quickly as possible by cardiac puncture using a 1-cc heparinized syringe with a 25-gauge needle. The time (min) between capture and obtaining the blood sample was recorded (mean = 5.5 min, range = 2–13 min), and baseline CORT concentrations were checked for an impact of time with nonlinear regression (no effect of time detected;  $P > 0.1$ ). Blood was then stored at ambient temperature in a 1.5-mL microcentrifuge tube until centrifugation later in the evening. Such transport of blood for several hours is unlikely to impact steroid hormone concentrations (Taylor and Schuett 2004). We determined the sex of snakes by observing the relative length of the cloacal spurs or by probing. Next, snout-to-vent length (SVL;  $\pm 0.5$  cm) was measured using a cloth measuring tape, and mass ( $\pm 0.5$  g) was determined using a digital scale. These measures were used to estimate a body condition index (BCI) as the residual of a linear regression of  $\log_{10}$  mass versus  $\log_{10}$  SVL. We scanned each snake for a passive integrated transponder (PIT) tag to determine whether the individual was a recapture, and if the snake was a new capture, we injected a PIT tag. Snakes were then released at their sites of capture. Blood was centrifuged for 2 min to separate cells from plasma, and plasma was removed and refrigerated prior to transport to California Polytechnic State University and then frozen at  $-80^{\circ}\text{C}$  until radioimmunoassay at Virginia Tech.

#### *The Acute Stress Response*

We made a third visit to the island during the period of November 21–25, 2010, to characterize the stress reactivity of *B. c. imperator*; hereafter, we refer to this study as the stress study. Snakes ( $n = 100$ ) were located by visual search between 0800 and 1600 hours and captured, and body (cloacal) temperature ( $T_b$ ) was rapidly obtained using an Omega type T thermocouple reader and then immediately bled as described previously (mean = 3.3 min, range = 2–13 min). Following the initial bleeding, snakes were exposed to a standardized acute stressor: they were promptly placed into a white queen-sized pillowcase and carried as the search for additional animals continued. Exactly 1 h after initial capture, each snake was removed and bled again. We then measured SVL and mass, determined sex, scanned for a PIT tag to determine whether snakes had been captured on previous visits, PIT tagged if necessary, and released the snakes at their sites of initial capture. Blood samples were treated and stored as previously described. Contrary to the results from the seasonal data set, a significant quadratic effect of latency to initial blood draw on baseline CORT concentration was detected for the stress study ( $T = 2.55$ ,  $P = 0.012$ ,  $R^2 = 0.21$ ). Therefore, we excluded data on snakes ( $n = 13$ ) bled six or more minutes after initial capture to remove researcher-induced impacts on baseline CORT and the magnitude of the

CORT stress response; this exclusion was the minimum exclusion necessary to remove the relationship between latency to blood draw and baseline CORT ( $T = 0.87$ ,  $P = 0.388$ ,  $R^2 = 0.009$ ) and is an acceptable estimate of baseline CORT in reptiles (Romero and Reed 2005).

#### *Radioimmunoassay*

Concentrations of steroids were measured by standard radioimmunoassay techniques following extraction and chromatographic separation (Lind et al. 2010). In the stress study, CORT levels were determined by direct radioimmunoassay with no chromatography. We used 20  $\mu\text{L}$  of plasma when steroid concentrations were predicted to be high (such as CORT following acute stress) and 50  $\mu\text{L}$  of plasma when steroid concentrations were predicted to be low (such as T in females). For individual extraction efficiency determination, we equilibrated each sample overnight with 2,000 cpm of tritiated steroid for each hormone measured. Each sample was extracted with 5 mL of distilled dichloromethane with the dichloromethane phase removed and dried in a warm-water bath, under a stream of nitrogen gas, and resuspended in 10% ethyl acetate in isooctane. For the removal of neutral lipids and isolation of individual steroids, samples were transferred to diatomaceous earth (Celite, Sigma) columns for chromatographic separation. For separation, steroids were eluted through the columns with increasing concentrations of ethyl acetate in isooctane. After this, samples were dried in a  $40^{\circ}\text{C}$  water bath under nitrogen gas, resuspended in 600  $\mu\text{L}$  phosphate-buffered saline, and maintained overnight at  $4^{\circ}\text{C}$ .

Individual extraction efficiency for each steroid was determined from 100  $\mu\text{L}$  of the sample while 200  $\mu\text{L}$  of the sample was allocated to each of two duplicates for the assay. For the seasonal study, recoveries were CORT, 63%; E2, 74%; P4, 33%; T, 81%. Mean recovery for CORT in the stress study was 96.2%. Serial dilutions for the standard curves were performed in triplicate (CORT curve range = 4–2,000 pg, P4 curve range 2–1,000 pg, E2 curve range 1–500 pg, T curve range = 1–500 pg). All samples were incubated overnight with 100  $\mu\text{L}$  of antiserum (CORT: Esoterix Endocrinology, Calabasas Hills, CA; P4: Fitzgerald Industries, Acton, MA; E2: Biogenesis, Poole, Dorset, UK; T: T-3003, Wien Laboratories, Succasunna, NJ) and 100  $\mu\text{L}$  of tritiated steroid. Unbound steroid was separated using dextran-coated charcoal and the bound steroid decanted into scintillation vials. Samples were counted on a liquid scintillation counter and final concentrations corrected for individual extraction efficiency. Average intra-assay coefficients of variation were CORT, 12.7%; E2, 19.7%; P4, 11.1%; T, 6.3%, during the seasonal study, and 19.9% for CORT during the stress study. Limits of detection during the seasonal study were as follows: CORT,  $\sim 0.7$  ng/mL; P4,  $\sim 0.21$  ng/mL; E2,  $\sim 0.09$  ng/mL; male T,  $\sim 0.4$  ng/mL; female T,  $\sim 0.06$  ng/mL.

### Statistical Analysis

Nondetectable hormone concentrations were given a value at the limit of detection of the assay to prevent biasing estimates toward higher concentrations. This level varied by individual sample depending on the plasma volume used in the assay and the individual extraction efficiency. All hormone concentrations were unexpectedly low, resulting in a high number of samples below the limit of detection during the seasonal study. More than half the plasma samples had nondetectable hormone concentrations for male dry season T (16/23 samples), female wet season T (30/37), female dry season T (all 13 samples), dry season E2 (all 13 samples), dry season P4 (all 13 samples), and wet season P4 (36/37). As a result, female T and P4 hormone concentrations were not subjected to statistical analysis, and the reported values may reflect slightly positively biased estimates. Male T concentrations and female E2 concentrations were analyzed with the following modeling procedures because wet-season concentrations of both hormones were mostly in the detectable range and generally higher than dry-season concentrations, meaning statistical differences would still be appropriate and informative, given that our estimates of the difference between seasonal means are actually conservative.

For the seasonal study, we examined how season, body condition, and, in the case of CORT, sex influenced steroid concentrations by considering a set of a priori models designed to test the importance of these major life history factors on steroid hormones. We deemed a model selection approach to be most appropriate here, given the exploratory nature of our data and the substantial number of possible models (Burnham and Anderson 2002; Mazerolle 2006). Season and sex effects were included in our full models due to their well-established impacts on steroid hormone concentrations (see Taylor and Denardo 2010 for review). Additionally, body condition and T concentrations are often negatively associated as part of a trade-off in energy allocation in times of stress (Moore and Jessop 2003). Due to the high prevalence of cases where T is higher in males than in females (Taylor and DeNardo 2010), we chose to model T in each sex separately to home in on the potential impacts of season and body condition on the hormone levels. Thus, we could evaluate the four factorial combinations of season, body condition, and their interactions, in addition to an intercept-only (null) model for male T and female T, E2, and P4. We present results from and rank all five models for each response, as this is the recommended approach given a small model set (Arnold 2010). Effects sizes (with 95% confidence interval [CI]) presented in the text for main effects were obtained with a multimodel approach using model averaging, which creates a more robust estimate of effect size given model uncertainty (Burnham and Anderson 2002; Mazerolle 2006). Models of plasma CORT included all possible combinations of sex, season, and body condition main effects. Additionally, we included interactions between sex and season and between sex and body condition, as well as a single model with a sex by season by body condition three-way interaction, because season and body condition often confer different biological impor-

tance—related to reproductive state—between the sexes (Lutterschmidt et al. 2009; Taylor and DeNardo 2010). We produced 14 models in all to evaluate CORT. We present the top model and all close competitors (models  $\leq 2$   $\Delta$ AIC [Akaike Information Criterion] from the top model). We ranked models of all hormones based on an information-theoretic approach (Burnham and Anderson 2002), specifically using AIC corrected for small sample size ( $AIC_c$ ) to evaluate the relative likelihood of each model (hypothesis) given the data set. The  $AIC_c$  values, likelihood estimates, and effect size estimates presented here were obtained using the generalized linear model with maximum likelihood estimation in SPSS Statistics, version 20 (IBM, New York). Plasma CORT and male plasma T concentrations required  $\log_{10}$  transformations to alleviate concerns of overdispersion during modeling, and model-based effect size estimates for these variables should be interpreted in terms of the transformed variables.

We evaluated whether *B. c. imperator* mounted an adrenocortical response to acute handling and capture stress using the repeated-measures general linear model function in SPSS to model plasma CORT concentrations, which conformed to the model assumptions of equality of the covariance matrices and normality of the multivariate residuals without the need for transformation. We specified time (baseline bleed or stressed bleed) as the within-subjects factor. Sex and whether the snake had been previously captured were between-subjects factors, and body temperature at initial capture and snake body condition were included as covariates in the model. Additionally, exploratory analyses to ensure linear fit of each predictor and CORT revealed that baseline CORT is better predicted by a quadratic effect of body condition, so a quadratic main effect of body condition was also entered as a covariate. Thus, our model included main effects of time, sex, previous capture experience, body temperature, body condition, body condition squared, and interactions between time and each of the other predictors. Time of day (known to influence CORT concentrations; Tarlow et al. 2003; Lutterschmidt and Mason 2009) was not included in this model, as exploratory analyses indicated that it was highly confounded with snake body temperature (increasing temperatures to midday, after which they drop). Temperature and time of day are therefore potentially confounded in our model.

We evaluated the statistical significance of each model predictor at an  $\alpha$  level of 0.05 by subjecting each  $P$  value to the two-stage sharpened adjustment for false discovery rate (FDR; Benjamini et al. 2006). The FDR-adjusted  $P$  values are reported in Results. Two significant interactions between the within-subjects factor of time and another model variable (sex and  $T_b$ ) were detected, meriting post hoc analyses to determine the nature of the significant effects. In the case of sex, we compared the magnitude of the change in plasma CORT concentration (stressed minus baseline) between males and females, so a single two-sample  $t$ -test was most appropriate. As  $T_b$  is a continuous variable, further exploration of the relationship between  $T_b$  and time was accomplished through simple linear regression. This approach to post hoc testing after repeated-measures analysis

Table 1: Concentrations (ng/mL; means  $\pm$  1 SEM) of testosterone, corticosterone, estradiol, and progesterone in *Boa constrictor imperator* during December–January (wet) and June–August (dry) visits to the Cayo Cochino Menor

Sex and season	<i>n</i>	Testosterone	Corticosterone	Estradiol	Progesterone
Male:					
Dry	23	1.50 $\pm$ .474 <sup>a</sup>	6.23 $\pm$ 1.76	...	...
Wet	40	7.36 $\pm$ 1.31	3.84 $\pm$ .416	...	...
Female:					
Dry	13	.059 $\pm$ .002 <sup>a</sup>	6.83 $\pm$ 1.35	.078 $\pm$ .002 <sup>a</sup>	.226 $\pm$ .015 <sup>a</sup>
Wet	37	.070 $\pm$ .003 <sup>a</sup>	3.85 $\pm$ .364	.119 $\pm$ .006	.237 $\pm$ .015 <sup>a</sup>

<sup>a</sup>Values are composed of >50% nondetectable samples.

of CORT concentrations is analogous to that of French et al. (2010).

## Results

### Seasonal Steroid Concentrations

Mean concentrations of all measured hormones are shown in table 1. The best-supported model for male plasma T concentrations included season, body condition, and a season by body condition interaction; this received a moderately high model weight ( $w_i = 0.50$ ; fig. 1; table 2). Our model-averaged estimate for the effect of season indicates that male T is higher during the wet season ( $\tilde{\beta} = -0.55 \pm 0.3$ ; effect size here based on  $\log_{10}$ -transformed T), and T was positively related to male body condition ( $\tilde{\beta} = 2.57 \pm 1.62$ ). The 95% CI around the single estimate available for the effect of the season by body condition interaction includes 0 (table 2), and the variability surrounding this estimate is to blame for the proximity of the next-best model, which includes only main effects of season and body condition. Taken in concert with the number of males where dry season T was nondetectable, these results are inconclusive concerning a potential interaction between season and body condition on male T concentrations.

Model selection for female plasma E2 concentrations in females consisted of only a season main effect ( $w_i = 0.40$ ), and the competing model with a main effect of body condition was simply more complex by one parameter and should therefore be interpreted with additional caution (Burnham and Anderson 2002; Arnold 2010). Model averaging produced an estimate ( $\tilde{\beta}$ ) for the season effect of  $-0.036 \pm 0.02$  ng/mL, with higher plasma E2 during the wet season.

Out of 14 models, plasma CORT concentrations were best approximated by a model including only a main effect of season, with higher CORT in the dry season ( $w_i = 0.23$ ; table 3). Competing models ( $<2 \Delta AIC_c$  from top model) included the null (intercept-only) model and a model including sex, season, and a sex by season interaction. Model averaging produced an estimate for the effect size of season on  $\log_{10}$  CORT concentrations that included 0 ( $\tilde{\beta} = 0.08 \pm 0.12$ ), and indeed the season-only model was estimated to be only 1.7 times more likely than the null model; season appears to have a weak effect that is obscured by high variability among individual snakes. Estimates for the other main effects considered in relation to

CORT concentrations also included 0 ( $\tilde{\beta}_{\text{sex}} = 0.04 \pm 0.11$ ;  $\tilde{\beta}_{\text{BCI}} = -0.24 \pm 0.58$ ).

### The Acute Stress Response

Our repeated-measures general linear model for plasma CORT concentrations at baseline and following acute stress revealed a significant effect of time (baseline vs. stressed;  $F_{1,79} = 7.23$ ,  $P = 0.009$ ), where all animals displayed an increase in plasma CORT in response to the stressor. There was also a significant main effect of sex ( $F_{1,79} = 7.21$ ,  $P = 0.009$ ), such that males had higher CORT than females. Additionally, CORT concentration showed a positive association with body temperature ( $F_{1,79} = 15.63$ ,  $P < 0.001$ ). The main effect of body condition was highly nonsignificant ( $F_{1,79} = 0.44$ ,  $P = 0.51$ ), while the quadratic effect was marginally nonsignificant ( $F_{1,79} = 3.56$ ,  $P = 0.06$ ). Last, we detected time by sex ( $F_{1,79} = 5.31$ ,  $P = 0.020$ ) and time by body temperature ( $F_{1,79} = 12.3$ ,  $P = 0.002$ ) interactions, while the tests for the interactions between time and body condition were nonsignificant ( $P > 0.3$ ). We did not detect a main effect of whether a snake was a new capture

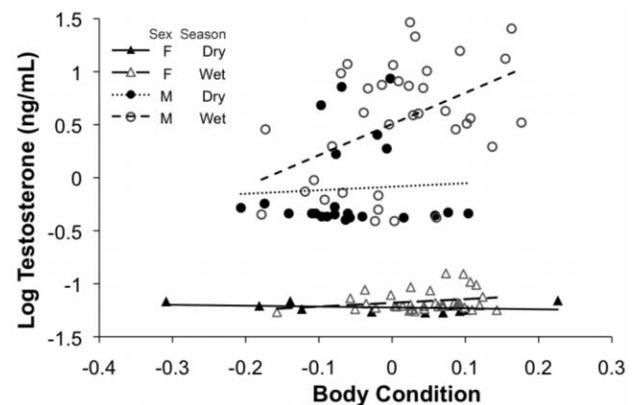


Figure 1. Relationship between  $\log_{10}$ -transformed testosterone concentration and body condition index values for male and female *Boa constrictor imperator* on Cayo Cochino Menor, Honduras. Testosterone is positively associated with snake body condition index in males and is higher during the wet season. Female testosterone was, for the most part, nondetectable in our assays.

Table 2: Model selection results for evaluating the impacts of season (wet or dry) and body condition index (BCI) on the baseline concentrations of male testosterone (T) and female estradiol (E2) in *Boa constrictor imperator* found on Cayo Cochino Menor

Response and model	Effect size estimates			AIC <sub>c</sub>	ΔAIC <sub>c</sub>	w <sub>i</sub>
	Season	BCI	Season × BCI			
Male T:						
<b>Season + BCI + season × BCI</b>	<b>−.592 ± .292</b>	<b>2.941 ± 1.733</b>	<b>−2.612 ± 3.178<sup>a</sup></b>	<b>92.67</b>	<b>0</b>	<b>.5</b>
Season + BCI	−.496 ± .274	2.164 ± 1.485		92.83	.16	.46
Season	−.646 ± .270			98.20	5.52	.03
BCI		3.174 ± 1.512		101.97	9.30	<.001
Intercept only (null)				114.66	21.98	<.001
Female E2:						
Season + BCI + season × BCI	−.033 ± .021	.159 ± .152	−.143 ± .190	−184.66	.85	.26
Season + BCI	−.036 ± .021	.067 ± .093		−185.07	.45	.32
<b>Season</b>	<b>−.040 ± .020</b>			<b>−185.51</b>	<b>0</b>	<b>.40</b>
BCI		.111 ± .101		−176.83	8.69	.01
Intercept only (null)				−174.71	10.81	<.001

Note. Effect size estimates included 95% confidence interval. All models included an intercept. The best-supported model is shown in bold. AIC<sub>c</sub> = Akaike Information Criterion adjusted for small sample size. w<sub>i</sub> = model weights, indicating likelihood that the model is best given the data.

<sup>a</sup>Note that this effect size estimate includes 0.

(mean rise in CORT and 95% CI = 46.84 ± 5.8 ng/mL) or had been previously captured (43.45 ± 6.5 ng/mL;  $F_{1,79} = 0.43$ ,  $P = 0.51$ ), nor was there evidence of an interaction between this variable and time ( $F_{1,79} = 0.2$ ,  $P = 0.66$ ). Post hoc tests performed to elucidate the nature of the significant interactions with time showed that the CORT stress response was of greater magnitude in male snakes (50.85 ± 6.3 ng/mL rise) than in females (40.03 ± 5.8 ng/mL rise;  $T = -2.47$ ,  $df = 84$ ,  $P = 0.016$ ) and was positively correlated with body temperature ( $\beta = 6.56$ ,  $P < 0.001$ ,  $R^2 = 0.16$ ; fig. 2).

## Discussion

This is the first study to report steroid hormone concentrations in a basal Alethinophidian snake and the first to describe seasonal variation (wet vs. dry) in steroid hormone concentrations in a terrestrial, tropical snake in its native habitat. The steroid hormone concentrations of *Boa constrictor imperator* are very low compared to most other snakes studied. For example, baseline CORT concentrations of *B. c. imperator* tended to be in the range of 5–10 ng/mL, about an order of magnitude lower than most other snake species studied to date (Moore et al. 2001; Tokarz and Summers 2010). Testosterone, E2, and P4 concentrations also appeared unusually low, with female T and P4 concentrations generally lower than the limits of detection, such that potential impacts of season will require further study. Whether the low circulating concentrations are characteristic of basal Alethinophidians or of this family of snakes (Boidae) or are unique to island or tropical populations remains to be answered. This topic has been most thoroughly studied in tropical birds, where circulating concentrations of androgens tend

to be lower than in temperate species. Goymann et al. (2004) refined this concept by noting that androgens are lowest in tropical bird species with long mating seasons. The introduced *Boiga irregularis* on Guam had relatively low CORT, E2, P4, and T, but chronic stress and interannual variation in food availability could potentially play a role in the observed differences between free-ranging and captive animals (Moore et al. 2005). Eikenaar et al. (2012) found no evidence for a relationship between latitude and T concentrations among reptile species but demonstrated a negative relationship between T concentrations and length of the breeding period (i.e., species with longer breeding seasons have lower T concentrations). In

Table 3: The four best-supported models approximating baseline plasma corticosterone (CORT) concentrations in *Boa constrictor imperator* on Cayo Cochino Menor, including the top model (i.e., lowest sample size–corrected Akaike Information Criterion [AIC<sub>c</sub>] value) and models within two AIC<sub>c</sub> units of the top model

Model	K	Log (L)	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	w <sub>i</sub>
Season	4	−5.813	17.86	0	.23
Season + sex	6	−5.209	18.82	.96	.14
Intercept only (null)	2	−7.418	18.95	1.09	.13
Season + sex + season × sex	10	−4.369	19.34	1.48	.11

Note. All models included intercepts, and those with interactions included main effects. K = number of parameters in model including the intercept and deviance estimate. Log (L) = maximized log-likelihood estimate. AIC<sub>c</sub> = Akaike Information Criterion adjusted for small sample size. w<sub>i</sub> = model weights, indicating likelihood that the model is best given the data.

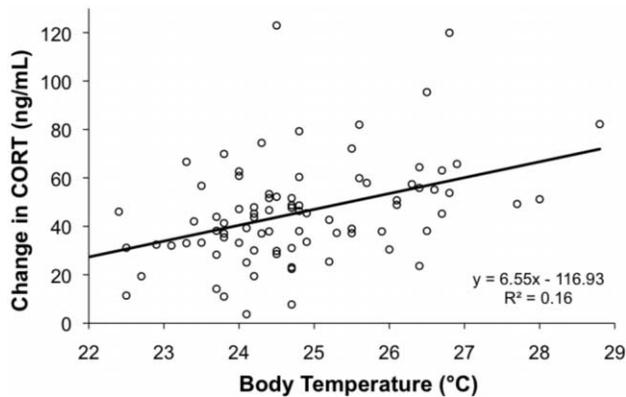


Figure 2. Magnitude of the rise in corticosterone (CORT) concentration induced by acute stress versus body temperature ( $^{\circ}\text{C}$ ) of *Boa constrictor imperator* on Cayo Cochino Menor, Honduras. The magnitude of the corticosterone stress response is positively related to snake body temperature.

concordance with this idea, marine snakes also displayed extremely low concentrations of androgens (Gorman et al. 1981), with the lowest levels in a species that produces sperm and mates most of the year (*Laticauda colubrina*), higher levels in a species with a slightly more restricted mating season (*Cerberus rhynchops*), and still higher levels in a species with an even shorter mating season (*Acrochordus granulatus*). Unfortunately, the exact timing of spermatogenesis and mating behaviors in free-ranging *B. c. imperator* is unknown. The only data available on *B. constrictor* are from a temperate population of *B. c. occidentalis* in Argentina, which undergo vitellogenesis, spermatogenesis, and mating during the period of April–September (Bertona and Chiaraviglio 2003).

Because precipitation varies more than temperature on an annual basis in many tropical environments including the Cayos Cochinos, any seasonality observed in the reproductive cycles of animals may be more entrained to wet and dry seasons than to temperature (Shine and Brown 2008). The elevated T observed during the wet season suggests that androgen-dependent processes, such as mating, spermatogenesis, or both, may occur at this time of year in *B. c. imperator*. The exact timing of spermatogenesis remains to be tested via histological examination of gonads. Furthermore, although mating activity in *B. c. imperator* on the Cayos Cochinos is seldom observed, it occurs during the wet season on other Caribbean island populations (S. Boback, personal communication). Testosterone concentrations are elevated during the mating season(s) of most snakes studied to date (reviewed in DeNardo and Taylor 2010). Studies of rattlesnakes (*Crotalus* sp.) with bimodal mating seasons (one in late summer associated with spermatogenesis and the next in the following spring, dissociated from spermatogenesis) have shown that T concentrations are elevated during both mating seasons (Schuett et al. 1997, 2002; Taylor et al. 2004; Lind et al. 2010); this strongly suggests that T plays a

role in stimulating mate-searching and/or courtship behaviors in snakes (but see Camazine et al. 1980; Lutterschmidt 2012).

The elevation of E2 in females during the wet season likely reflects seasonal events in the reproductive cycle in *B. c. imperator*. Island boas in Belize mate during the wet months of January–March, at which time females are vitellogenic and males are spermatogenic (S. Boback, personal communication); it is probable that these events also occur during the wet season in the population we studied. Estradiol is the major hormone that stimulates vitellogenesis in female reptiles (Ho et al. 1982), so we would expect it to be elevated during the wet season in *B. c. imperator*. It is impossible to speculate on the function of T in female *B. c. imperator* because levels were so low (most nondetectable). The fact that almost all female P4 concentrations were below the detection limits may be because we failed to collect pregnant females during our study. Progesterone is typically elevated in females during pregnancy, as it acts generally in the maintenance of pregnancy (Custodia-Lora and Callard 2002). Females are pregnant during the dry season on Cayos Cochinos and often hide under large logs and rocks, which likely biased our samples toward nonreproductive females with low P4.

While we certainly expected to observe sex differences in circulating androgens in *B. c. imperator*, most studies have found no differences in CORT concentrations between male and female free-ranging snakes (Taylor et al. 2004; Lutterschmidt et al. 2009; Sperry et al. 2009; Lind et al. 2010; Palacios et al. 2012). In this study, our best-supported model similarly lacked an effect of sex on baseline CORT concentrations during our first two visits to the island (December–January and June–August), but during our third visit in November we found that males had higher CORT than females. The significance of this pattern is difficult to understand without a more complete understanding of seasonal patterns of reproductive events in this species. It is possible that males were mate searching in November, which may mark the beginning of mating activity. CORT levels could be elevated in order to facilitate energy mobilization for increased activity at the onset of mating behaviors. In both sexes, CORT was higher during the dry season than during the wet season, which could arise from regular seasonal patterns of food availability. When prey availability is limited, CORT concentrations increase as a mechanism of mobilizing energy from storage (Wilson and Wingfield 1992).

*Boa constrictor imperator* exhibited a hormonal response to acute stress in the form of an average 15-fold increase in plasma CORT concentrations. Such a response is comparatively large (Tyrrell and Cree 1998; Tokarz and Summers 2010). In terms of the island tameness hypothesis, this result is informative in showing that fewer than a few thousand years of isolation from mainland *B. constrictor* populations and predators (Green 2010) have not resulted in the loss of a hormonal response to acute capture stress. Island-living song sparrows (*Melospiza melodia*; Clinchy et al. 2004) and blue tits (*Parus caeruleus*; Müller et al. 2007) both display lower baseline and stressed CORT concentrations on islands, but these studies did not address whether the magnitude of the acute stress response is different among

island and mainland populations. Previous studies conducted on marine iguanas isolated for 5–15 million years on the Galápagos Islands have revealed complex patterns in stress reactivity, where plasma CORT concentrations increase in response to capture (Berger et al. 2007; Rödl et al. 2007; French et al. 2010) but not in response to experimental chasing alone unless the animal had been previously captured (Rödl et al. 2007). Vitousek et al. (2010) demonstrated that even prior capture experience does not cause marine iguanas to mount a cardiovascular stress response to human approach, although they do mount such a response to the approach of native avian predators. In this study, the stress response of *B. c. imperator* was not affected by whether an animal had been captured during previous years of study, suggesting that previous capture by humans does not modulate adrenocortical stress reactivity in these snakes. However, the time period between captures of these snakes varied dramatically among individuals and was much longer than that of the marine iguanas studied by Rodl et al. (2007).

Stress reactivity in these snakes appears to be modulated by sex and body temperature. Stress reactivity was higher in male boas, and males had higher baseline CORT concentrations during this portion of the study. Sex differences in adrenocortical secretion during stress have been previously documented in reptiles. Sykes and Klukowski (2009) showed that female water snakes (*Nerodia sipedon*) had a nearly threefold higher rise in CORT concentration during capture and confinement than did males, a result opposite to our findings in *B. c. imperator*. Gravid female tree lizards (*Urosaurus ornatus*) do not increase CORT secretion during capture stress, whereas plasma CORT concentrations of vitellogenic females and males do increase; this effect may protect developing offspring from high CORT concentrations (Woodley and Moore 2002). In the marine iguana, no sex difference in CORT secretion during stress was detected (Romero and Wikelski 2001). Seasonal modulation of the CORT stress response is common among squamate reptiles, including snakes (Moore et al. 2001; Cease et al. 2007; Lutterschmidt et al. 2009), and these changes are often associated with reproduction and dispersal. Our stress study was conducted during the month of November, which is likely the beginning of the mating season, as T concentrations are elevated in the following month (they may have been elevated in November, but we did not measure them). The larger stress responses of males may result from a seasonally sensitized hypothalamo-pituitary-adrenal axis that is adaptive for dealing with the energetically expensive stressors of mate searching, warding off other males, and courting females in this high-density population (Moore and Jessop 2003).

The magnitude of the CORT stress response in *B. c. imperator* was also positively related to body temperature. This is not surprising, as the rates of diverse physiological processes are temperature dependent in ectotherms. While baseline CORT concentrations are related to temperature in lizards (Cree et al. 2003), Sykes and Klukowski (2009) found no impact of experimentally imposed temperature treatments on the adrenocortical response to capture and confinement in free-ranging

water snakes. It may be that body temperature at the onset of an acute stressor may be crucial in determining the absolute magnitude and/or rapidity of the stress response (the water snakes did not differ in body temperature at the time of capture and likely took a number of minutes to change temperature). As we mentioned previously, temperature and time of day were confounded during the stress study, so a controlled study would be necessary to parse out the different effects of diel and temperature-associated variation in CORT physiology. Possibly the most important point of discussion regarding the impact of temperature on the hormonal stress response is an applied one: researchers should always take temperature (and time of day) into account when conducting field studies of physiological responses, especially in ectotherms.

This field endocrinology study of *B. c. imperator* adds important knowledge on hormone–life history interactions in squamate reptiles, especially snakes, by expanding the geographic and taxonomic scope of the current literature. Almost all of the work on hormonal physiology in snakes has been conducted within the advanced Caenophidian lineage, and the majority of those studies were on two genera, *Crotalus* (rattlesnakes) and *Thamnophis* (garter snakes; Taylor and DeNardo 2010). Our characterization of the circulating concentrations of steroids in a boid snake has broadened the comparative framework for assimilating knowledge of squamate endocrinology and generating additional questions. We have shown that circulating baseline concentrations of all sex steroids and CORT are low in *B. c. imperator* relative to other snakes. Determining whether this trait is characteristic of basal or boid snakes or of tropical or island snakes will require further comparative hormonal studies. Both baseline CORT concentrations and the stress response of the island boas in our study may actually be low compared to mainland *B. constrictor*, which could be explained as island tameness. We can consider this only a possibility without additional work. A comparison of our results with hormonal stress response data from *B. constrictor* populations on other islands and from mainland Central and South America would be an important next step in determining whether island tameness has arisen in Hog Island boas. After all, complete absence of a physiological or behavioral response may not be expected to result from isolation from predators; varying degrees of attenuation of such responses may indeed be the norm for island endemics (Cooper and Pérez-Mellado 2010), and relaxed selection may not result in the loss of a response if it serves other functions (Blumstein and Daniel 2002). Populations of *B. constrictor* exist in many parts of mainland Central and South America, as well as on 43 offshore islands other than the Cayos Cochinos archipelago (Boback 2005). These island populations of *B. constrictor* likely differ greatly in the time since isolation from mainland populations occurred, presence or absence of predators, prey types, density, and a myriad of other factors. The broad geographic range (68° of latitude) of *B. constrictor* (Boback 2005), combined with the great number and diversity of insular populations, makes it an ideal model for examining the way in which

organismal physiology evolves in island populations and shapes those organisms' life histories.

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