

Density-dependent mating tactic expression is linked to stress hormone in Woodhouse's toad

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The social environment can alter mating tactic expression through modulatory effects on the endocrine system. In anuran amphibians (frogs and toads), males often adopt noncalling mating tactics in response to increased chorus density, but the underlying physiological factors mediating this response are poorly understood. Recent models suggest that density-dependent expression of alternative mating tactics in anurans is associated with a cascade of hormonal events: high chorus density is expected to promote an increase in circulating androgen levels that elicits an increase in vocal effort, a reduction in energy reserves, and elevations in circulating corticosterone (CORT) levels that, in turn, suppress vocalization. Here, we show that chorus density was positively correlated with the proportion of males adopting a noncalling "satellite" mating tactic in explosive-breeding Woodhouse's toad (*Bufo woodhousii*). We also show that chorus density was positively correlated with circulating CORT levels in calling males, thereby linking density-dependent mating tactic expression to a hormone that has previously been shown to elicit noncalling mating tactics in this species. Chorus density, however, was not correlated with circulating androgen levels, vocal effort, or body condition. Our results suggest that satellite tactic expression may not be readily traceable to energetic constraints associated with calling behavior—a leading prediction of current models. *Key words*: androgens, body condition, chorus density, corticosterone, satellite behavior, vocal effort. [*Behav Ecol* 19:1103–1110 (2008)]

Males that facultatively alternate between mating tactics often respond to changes in the density of competing males (Greenfield and Shelly 1985; Brockmann 2001; Kokko and Rankin 2006). In particular, numerous studies on anuran amphibians (frogs and toads) describe a positive association between chorus density and the proportion of males adopting alternative noncalling mating tactics (reviewed in Gerhardt and Huber 2002, Table 9.2; see also Sullivan 1989; Ovaska and Hunte 1992). Although such context-dependent mating behaviors have been linked to various density-related social correlates in anurans, the hormonal factors that are potentially involved are poorly understood. Recent models suggest that density-dependent expression of alternative mating tactics in anurans is associated with a cascade of hormonal events; high chorus density is expected to promote an increase in circulating androgen levels that elicits an increase in vocal effort, a reduction in energy reserves, and elevations in circulating stress hormone (corticosterone [CORT]) levels that, in turn, suppress vocalization (Emerson 2001). Here, we examine density-dependent expression of alternative mating tactics in Woodhouse's toad, *Bufo woodhousii*, in the context of current endocrine models.

As with many anuran species, male *B. woodhousii* conditionally alternate between 2 alternative mating tactics: individuals may vocalize to attract mates or they may adopt a noncalling mating tactic (reviewed by Halliday and Tejedo 1995). Noncalling "satellite" males characteristically reside in close proximity to calling males and attempt to intercept females approaching vocalizing males. The majority of studies link satellite tactic expression in anurans to social factors that are expected to increase the occurrence of satellites under

dense chorus conditions (see reviews by Waltz 1982; Halliday and Tejedo 1995; Lucas and Howard 1995; Lucas et al. 1996; Gerhardt and Huber 2002). These mutually nonexclusive factors include the following: 1) increased interactions with particular males that are more attractive to females, 2) increased interactions with more dominant males, 3) increased vocal interference, and/or 4) a decrease in vacant calling sites. Empirical support for these hypotheses comes from several sources. For example, Arak (1988) found that the probability of satellite tactic expression in male natterjack toads (*Bufo calamita*) increased as the amplitude of broadcast calls increased (high-amplitude calls are often preferred by female anurans, Gerhardt and Huber 2002). These results are consistent with density-dependent patterns of satellite tactic expression due to a decrease in nearest neighbor distance and, hence, an expected increase in sound pressure level (SPL) under dense chorus conditions. Similarly, males in many anuran species time the production of their calls such that acoustic overlap with other calling males is minimized (reviewed by Halliday and Tejedo 1995; Schwartz 2001; Gerhardt and Huber 2002). These findings led to the hypothesis that competitively inferior males adopt noncalling mating tactics in response to an increase in vocal interference that is expected to occur under dense chorus conditions (Axtell 1958; Waltz 1982; Gerhardt and Klump 1988). Alternatively, in other highly territorial anuran species, males engage in aggressive vocal bouts or physical combat and the winners of such contests (usually larger/older males) subsequently defend calling sites against contest losers or other potential intruders that resort to a satellite mating tactic (i.e., *Rana catesbeiana*, Howard 1978, 1984). Hence, dominant males securing the limited number of calling sites is expected to increase the proportion of satellite males as chorus density increases. These findings do not preclude a potential role of hormones; the social environment has been shown to alter mating tactic expression through modulatory effects on the endocrine system in numerous taxa (Moore 1991; Moore et al. 1998; West-Eberhard 2003; Adkins-Regan 2005).

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Received 5 November 2007; revised 16 June 2008; accepted 23 June 2008.

Circulating androgen and CORT levels are particularly important in regulating vocal behavior in male anurans (Emerson 2001). For example, whereas androgens promote vocalization by organizing and activating the neural substrates underlying vocal production (reviewed by Moore et al. 2005; Wilczynski et al. 2005), high CORT levels can inhibit androgen production and, hence, vocalization (Mendonça et al. 1985; Marler and Ryan 1996; Burmeister et al. 2001). Emerson (2001) proposed that reciprocal interactions between circulating androgens and CORT levels in anurans are driven by social interactions among males and the high energy requirements associated with vocal production. For example, the Energetics–Hormone Vocalization (EHV) model (Emerson 2001) proposes that increased social interactions among males should stimulate elevations in circulating androgen levels. This prediction was based largely on concepts of the Challenge Hypothesis (Wingfield et al. 1990), a model that has been rigorously tested and supported across a variety of vertebrate taxa (reviewed by Goymann et al. 2007; Moore 2007). Additionally, however, the EHV model posits that an increase in circulating androgen levels will mediate an increase in vocal effort. Androgen-mediated elevations in vocal effort were predicted to increase rates of energy depletion that, in turn, stimulate the production of CORT. Vocalization in anurans is known to be energetically demanding (reviewed by Wells 2001), and glucocorticoids play an established role in the mobilization of energy reserves (reviewed by McEwen and Wingfield 2003). The expected inverse relationship between energy reserves and CORT levels is of particular importance because it provides a potential link between the hormonal mechanisms mediating anuran mating tactic expression and satellite–caller dynamic game models that regard energetic state as a key variable in predicting tactic expression (i.e., Lucas and Howard 1995; Lucas et al. 1996; McCauley et al. 2000).

An alternative scenario, however, is that increased chorus density stimulates CORT production independently of changes in vocal effort or energy reserves. For example, crowding associated with high population density increased activity of the hypothalamic–pituitary–adrenal (HPA) axis in European starlings, and such effects may have occurred independently of food availability or metabolic state (Nephew and Romero 2003). To distinguish between these 2 possibilities, we measured circulating CORT levels, androgen levels, vocal effort, and body condition (the proxy for energy reserves) over 3 consecutive breeding seasons in *B. woodhousii*, and we examined these factors as they relate to chorus density and the proportion of males adopting satellite behavior. The first hypothesis, based on concepts of the EHV model, would be supported if density-dependent expression of alternative mating tactics is associated with elevations in circulating CORT levels that are driven by an increase in androgen levels and vocal effort and a decrease in body condition. The latter hypothesis would be supported if density-dependent expression of alternative mating tactics is associated with elevations in CORT levels only.

Bufo woodhousii is particularly well suited to such a study because we have previously established the role of androgens and CORT in satellite tactic expression. In this species, high CORT levels mediate changes from calling to satellite tactic expression, but these behavioral changes occur in the absence of any detectable negative effect of CORT on circulating androgen levels (Leary et al. 2004, 2006a). Additionally, circulating androgen levels are not positively correlated with vocal effort in this species but CORT levels are (Leary et al. 2008). Despite the apparent dissociation between androgen levels and vocal effort in this species (Leary et al. 2008), these findings do not exclude potential density-dependent effects

on androgen levels. For example, acoustic stimulation of the hypothalamic–pituitary–gonadal (HPG) axis and/or the gonads is well established in male anurans (Rabb 1973; Brzoska and Obert 1980; Wilczynski and Allison 1989; Burmeister and Wilczynski 2000, 2005; Chu and Wilczynski 2001), and increased social interactions among conspecifics should promote elevations in circulating androgen levels (concepts of the Challenge Hypothesis, Wingfield et al. 1990). Similarly, males of some anuran species respond to the vocalizations of nearby competitors by increasing the complexity of their calls (reviewed by Wells and Taigen 1986; Halliday and Tejedo 1995; Wells 2001), and in some cases, vocal effort is positively correlated with chorus density (Sullivan and Wagner 1988).

MATERIALS AND METHODS

Study site

Our study site was located on a privately owned horse pasture in central Oklahoma (Cleveland County) at the interface between the eastern deciduous forest and the central Great Plains grasslands. Vegetation at the site was composed primarily of grasses that were kept short via horse grazing and periodic mowing by the owner. Sparse overstory vegetation, combined with the large size of male *B. woodhousii* (mean \pm standard deviation [SD] snout–ischial length [SIL] = 79.8 ± 6.6 mm, $n = 543$) and their behavior (individuals can be approached without retreating or ceasing vocalization), allowed us to accurately assess chorus density and mating tactic expression periodically throughout the evening. The next nearest chorus was located approximately 3.2 km from the study site and consisted of no more than 12 individuals. Otherwise, the study site was isolated from any other choruses within a 10-km radius. There was no evidence that individuals emigrated from the study site to the other nearby chorus.

Chorus density and size estimates

The study site was monitored nightly throughout the early spring and summer months in 2002, 2003, and 2004 to assess chorus activity. Chorus density and chorus size were measured nightly, approximately every hour, beginning on the first night of chorus activity and on all subsequent nights of chorus activity. Initial nightly measurements began approximately 1 h after the onset of chorus activity and continued throughout the evening until calling activity waned or a large proportion of individuals were observed leaving the chorus. These times varied among nights but typically occurred around 2100 and 0300 h, respectively. It was necessary to begin data collection \sim 1 h after the onset of chorus activity and terminate data collection before complete cessation of all chorus activities because individuals were migrating to and from the pond and often called sporadically during these times. Hence, it was difficult to differentiate between calling and satellite males during these times. During periods in which measurements were taken, however, few or no individuals were observed entering or leaving the pond.

The total area of our study pond often changed dramatically over the course of the breeding period (range = $750\text{--}22\,000\text{ m}^2$), and calling males were not continuously or uniformly distributed across the pond. Thus, chorus density was not estimated from the total number of individuals present in the pond, as this approach would underestimate the local density where our response variables were measured. Chorus density estimates were based on local estimates of calling males, and all measured parameters (described below) were taken from areas where density estimates were obtained. Local chorus density estimates were made by counting all males (callers and

satellites) within three 1-m² plots; plots were arbitrarily selected among clusters of calling males. Females were not encountered in our plots during chorus density estimates; females rapidly paired with males on entering the chorus, and amplexed females typically resided in deeper regions of the pond, away from calling males. For each plot, the proportion of satellites was expressed as a fraction of the total number of males. These data were then averaged across the 3 plots to provide a mean chorus density for each sampling period. Estimates of chorus size (defined as the total number of males and females) were made by walking the perimeter of the pond while counting all individuals and plotting their locality on a map.

Behavioral and hormone data collection

Satellite male *B. woodhousii* typically remained in close proximity to aggregates of calling males and actively searched for and attempted to amplex females (Leary et al. 2004). Based on our previous work (Leary et al. 2004, 2005, 2006a, 2006b), behavioral phenotypes (callers vs. satellites) can be categorized in a few minutes of observation; satellites exhibited a low posture and did not show evidence of recent inflation of the vocal sac in contrast to calling males that exhibited an upright posture and partially or fully expanded vocal sac (see also Sullivan 1989). Behavioral observations and chorus density estimates were made using flashlights or low-powered LED headlamps and did not have any overt effects on behavior (see also Sullivan 1989).

We recorded a series of 6–10 consecutive vocalizations from individuals in aggregates of calling males (i.e., from areas in which density measures were taken) using Marantz PMD 222 recorders equipped with Sennheiser ME66 condenser microphones. Call amplitude was also measured from a subset of recorded individuals using RadioShack digital SPL meters (fast root mean square, C-weighting filter). A 30-cm heavy gauge wire was mounted on the SPL meters to control for changes in amplitude readings associated with variation in the distance between the subject and the SPL meter. Calling individuals were approached, and the wire mount on the SPL meter was oriented horizontal to the water substrate and placed directly in front of (almost touching) the calling male's expanded gular sac to obtain SPL readings (Leary et al. 2006b).

Body temperature typically affects various vocal parameters in anurans (Gerhardt and Huber 2002; for effects on *Bufo* calls, see Brown and Littlejohn 1972). Hence, we determined nearby water temperatures for each male subsequent to obtaining vocal recordings. All *B. woodhousii* in this population called while the hind legs, pelvis, and forelimbs were under water, and calling males maintained cloacal body temperatures that reflected water temperatures (Leary CJ, Garcia AM, personal observation).

Immediately after obtaining vocal recordings, we captured the males by hand and rapidly obtained a blood sample by cardiac puncture. Blood collection never took more than 30 s, and blood samples obtained in the field were kept on ice until they were returned to the laboratory (<8 h). Males were then measured from the tip of the snout to the distal end of the ischium and weighed to the nearest 0.1 g on a portable Ohaus digital scale. Lastly, all sampled individuals were numerically marked on the venter using a portable tattoo device (Tattoo-A-Pet, Fort Lauderdale, FL). Tattoos allowed identification throughout an entire season, but not between years due to fading. Males were not sampled more than once within a breeding season, but some males may have been sampled more than once across breeding seasons. However, resampling a subset of males across years is unlikely to affect the relationship between chorus density and the measured variables. The entire procedure (cardiac puncture, measurements, and marking) took ~1 min and did

not have a significant effect on circulating androgen or CORT levels (Leary et al. 2006a). Animals were then returned to the site of capture where males typically resumed vocal behavior within 1 min of release. On return to the laboratory, blood samples were centrifuged for 12 min at 3000 r.p.m., and plasma was subsequently stored at -20 °C until assayed for steroid hormones. Individuals were monitored by a veterinarian (A.M.G.) throughout the study. All methods and procedures were approved by the University of Oklahoma Institutional Animal Care and Use Committee (R01-005) and conformed to the ABS/ASAB ethical guidelines.

Analyses

Calls were analyzed using Canary 1.1.1 Bioacoustics software, Cornell Bioacoustics Laboratory (Leary et al. 2006b). We examined call parameters known to be important in mate selection in bufonids and other anurans (Gerhardt and Huber 2002) or which were required for vocal effort estimates. Call parameters were averaged from 6 to 10 consecutive calls from each male and included call duration (seconds), pulse rate (number of pulses in a 1-s section in the middle portion of the call), and intercall duration (seconds). Vocal effort was calculated for each individual using the following equation (Leary et al. 2008):

$$\text{Vocal effort (pulses/s)} = \frac{[\text{call duration} / (\text{call duration} + \text{intercall duration})] \times \text{pulse rate}}{\quad} \quad (1)$$

SPL measures were found to be highly invariant across the subset of individuals that were measured (mean \pm SD = 93.7 \pm 1.3 dB SPL, range = 90–95, n = 85). Hence, SPL measurements were only taken periodically throughout the course of the study to verify that the measure exhibited minimal change. Because there was little variation in SPL, this measure was excluded from vocal effort estimates.

Vocal effort estimates were expected to reflect the values for the localized aggregate of calling males for a particular density but did not control for the total time a male spent calling (which could affect circulating hormone levels, Emerson 2001; Leary et al. 2008). However, males in this population typically arrived at the pond relatively rapidly after sunset, and peak chorus size was attained soon thereafter. Hence, most males were assumed to be calling for similar periods when they were sampled.

Body condition was calculated by obtaining the residual values from a linear regression of the cubed root body mass on SIL and dividing those values by the SIL, following the approach used by Baker (1992)M, Howard et al. (1997), and Howard and Young (1998). This was done for all individuals collectively across all years.

Hormone levels were determined using procedures that we have described previously (Leary et al. 2004). Briefly, plasma samples were extracted with diethyl ether, dried under nitrogen gas, and resuspended in 10% ethyl acetate in isooctane. Column chromatography was then used to separate dihydrotestosterone (DHT), testosterone (T), and CORT. T antibody was obtained from Research Diagnostics (Flanders, NJ) and used for both T and DHT radioimmunoassays. CORT antibody was purchased from ICN Laboratories (Costa Mesa, CA). Plasma samples were randomized across and within 6 assays with respect to time of night sampled and date and year sampled to prevent confounding of assay variability and temporal variation in hormone levels (Leary et al. 2008). Mean intra-assay coefficients of variation for DHT, T, and CORT were 12.9%, 11.5%, and 9.2%, respectively, based on 4 standards

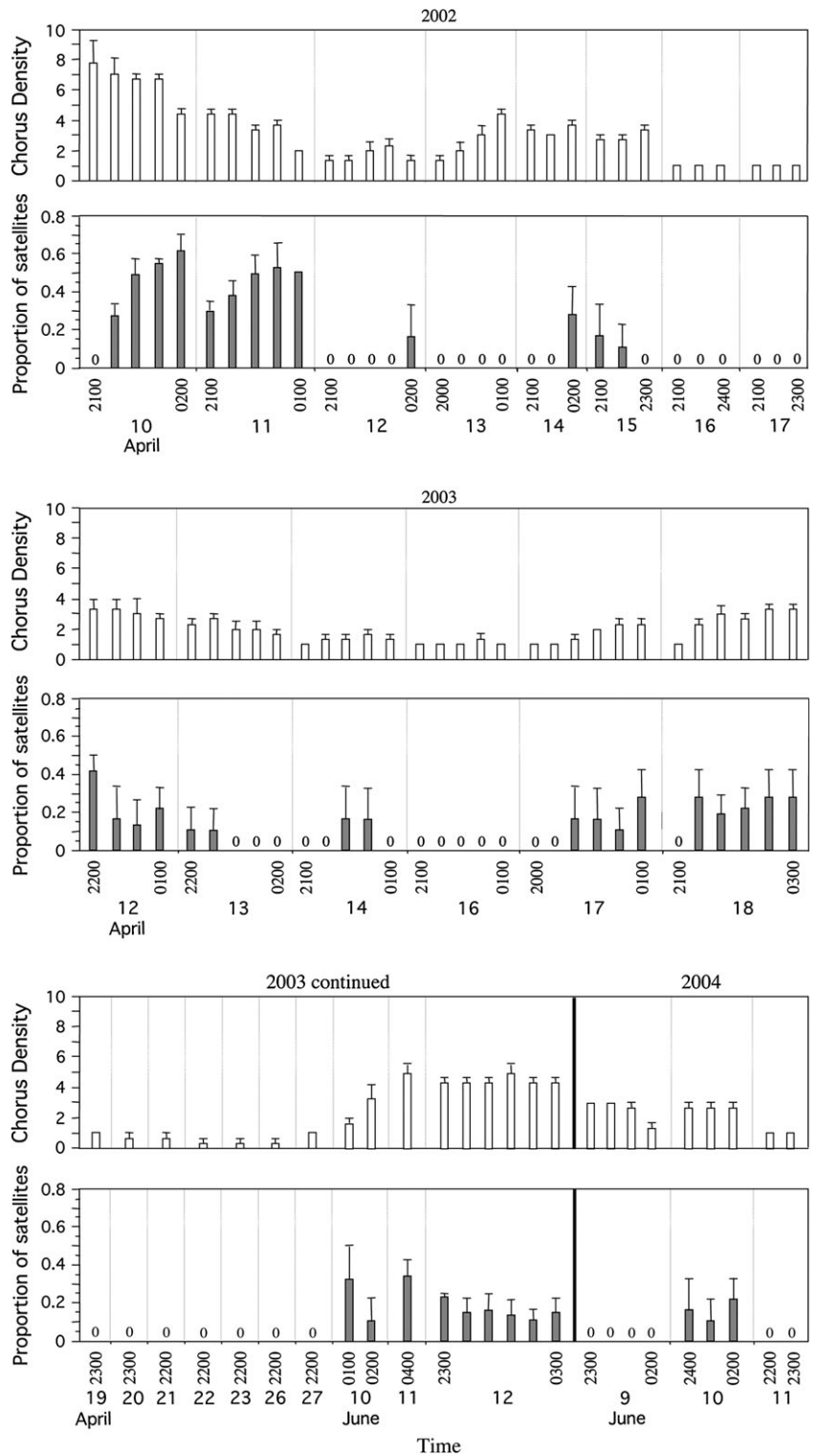


Figure 1
Temporal changes in chorus density (# males per m²) and the proportion of satellite males within nights and across the 2002–2004 breeding seasons. Data are presented as means ± standard errors from three 1-m² plots for each sampling period.

run with each assay. Interassay coefficients of variation for DHT, T, and CORT were 19%, 16.3%, and 10.8%, respectively.

Statistics

We first examined whether chorus density was positively correlated with the frequency of male *B. woodhousii* adopting the satellite tactic. We then examined how CORT levels, vocal

effort, body condition, and androgen levels were related to chorus density using simple linear regressions. Relationships among hormone levels, vocal effort, and body condition are provided in Leary et al. (2008). Many of these variables are highly correlated, and hence, independent contrasts between chorus density and hormone levels, vocal effort, and body condition were conducted. To remove the effects of temperature on vocal effort, residual values were obtained from

a linear regression of the 2 variables and used in regression analyses.

RESULTS

Chorus activity patterns varied considerably across the 3 years of study, spanning a total of 8, 16, and 3 nights (2002, 2003, and 2004, respectively) between the months of April and June (Figure 1). Chorus activity varied across these 27 nights of chorus activity as well, resulting in a total of 87 1-h intervals for which chorus density estimates were obtained. Satellite males were present for 42 of these intervals (Figure 1). Mean local chorus density estimates ranged from 1 to 7.66 males per m² (mean ± SD = 2.79 ± 1.21 males per m²), and the mean proportion of satellite males (the fraction of satellite males to total males) ranged from 0 to 0.62 (0.12 ± 0.13) (Figure 1). Across all years combined, chorus density was positively correlated with the proportion of males that adopted a satellite tactic ($F_{1,85} = 40.38, P < 0.0001, r^2 = 0.32$; Figure 2). Chorus density was also positively related to the size of the chorus (i.e., the number of individuals present at the pond) ($F_{1,85} = 157.43, P < 0.0001, r^2 = 0.65$) despite the fact that the pond always contained large areas where no males were present.

Blood samples were collected from a total of 281 males in the 3 years of study and across the range of chorus densities for determination of plasma hormone levels. Chorus density was found to be significantly and positively correlated with circulating CORT level ($F_{1,279} = 4.91, P = 0.02, r^2 = 0.017$), but there was no correlation with androgen level ($F_{1,278} = 1.33, P = 0.20, r^2 = 0.005$) (Figure 3).

In addition to the individuals from which blood samples were obtained, vocal recordings were acquired from 66 other individuals (a total of 347 males). Chorus density was not significantly correlated with the residual values for vocal effort from a regression of vocal effort versus temperature ($F_{1,345} = 1.70, P = 0.2, r^2 = 0.005$; Figure 4A). Results were similar when analyses were restricted to males for which measurements for all variables were available ($F_{1,147} = 0.395, P = 0.5, r^2 = 0.003$). Results were also nonsignificant when the raw vocal effort values were used ($F_{1,345} = 1.56, P = 0.2, r^2 = 0.004$; $F_{1,147} = 0.05, P = 0.8, r^2 = 0.003$). The means, SDs, and ranges for vocal attributes (uncorrected for temperature) are provided in Table 1.

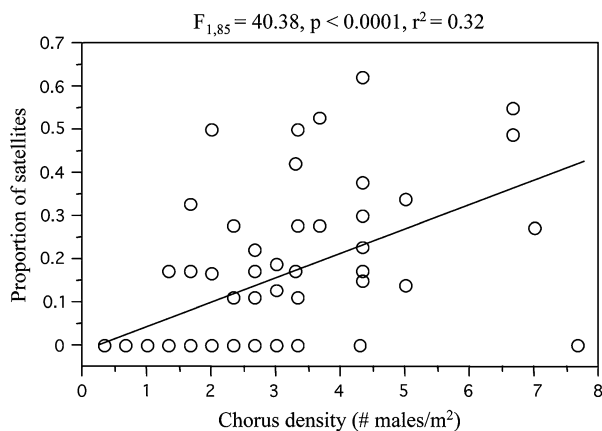


Figure 2
Simple linear regression between chorus density and the proportion of satellite males for 2002–2004 data combined. Estimates of chorus density and proportion of satellites are based on the mean values obtained from three 1-m² plots for each sampling period shown in Figure 1.

Chorus density also was not significantly correlated with body condition for all males sampled across years ($F_{1,531} = 0.98, P = 0.32, r^2 = 0.002$; Figure 4B).

DISCUSSION

Our results suggest that high chorus density stimulates the production of CORT in *B. woodhousii*, and this effect appears to be unrelated to an increase in vocal effort or a decrease in body condition. High CORT levels have previously been found to elicit satellite behavior in this species (Leary et al. 2006a). Hence, density-related elevations in circulating CORT levels probably contribute to the increased proportion of satellite males observed under dense chorus conditions.

Negative energy balance is known to stimulate CORT production (McEwen and Wingfield 2003) and is central to predictions of the EHV model (Emerson 2001). The HPA axis, however, can also be activated by various other intrinsic and extrinsic factors (reviewed by Wingfield and Sapolsky 2003). The inhibitory effect of CORT on vocal production in anurans, combined with the multiple physical, social, and internal factors that may trigger activity of the hypothalamic–pituitary–interrenal (HPI) axis, suggests that satellite behavior in

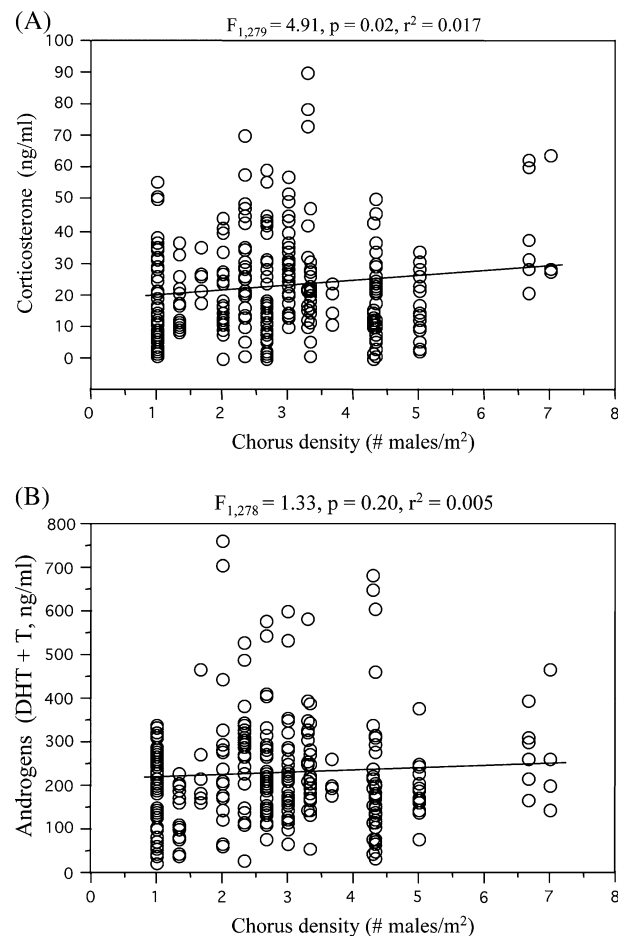


Figure 3
Simple linear regression between chorus density and plasma (A) CORT level (nanograms per milliliter) and (B) androgen levels (DHT + T, nanograms per milliliter). Blood samples for hormone analyses were obtained from males within approximately 1 h of making chorus density estimates. Chorus density is based on the mean value from the three 1-m² plots during each sampling period shown in Figure 1.

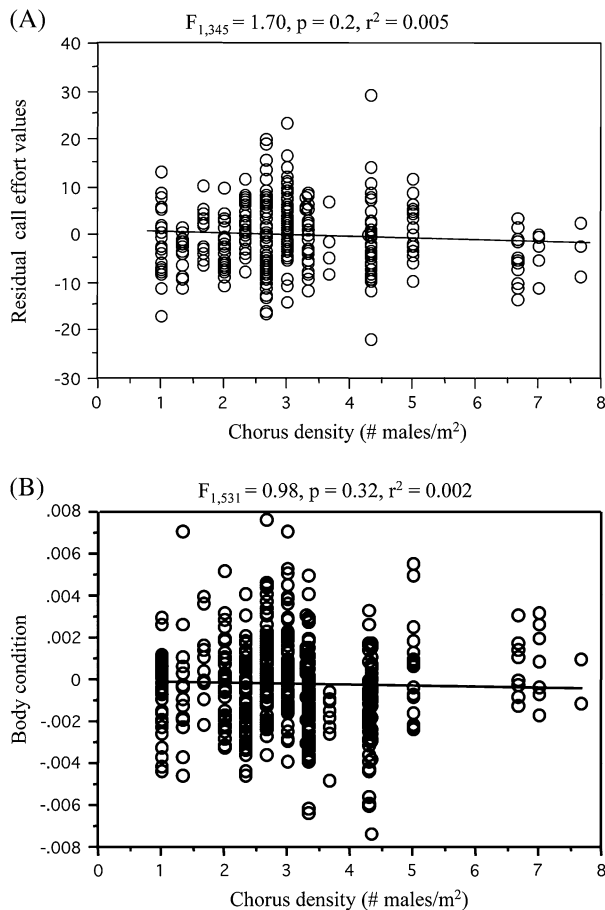


Figure 4

Simple linear regression between chorus density and (A) residual vocal effort values and (B) body condition of calling males. Residual vocal effort values were obtained from a linear regression of body temperature against vocal effort. Chorus density is based on the mean from the three 1-m² plots during each sampling period shown in Figure 1.

anurans may not be readily traceable to energetic constraints on calling behavior—a leading hypothesis for the adoption of satellite/noncalling behavior (Lucas and Howard 1995; Lucas et al. 1996; McCauley et al. 2000; Emerson 2001; Leary et al. 2004). Although there is some evidence that satellite male anurans are in poorer condition than calling males (Robertson 1986; Leary et al. 2004), our current results suggest that potential modulatory effects of chorus density on CORT levels may also be operating to activate the HPI axis independently of energetic state.

One might predict that the various intrinsic and extrinsic factors that potentially influence circulating CORT levels would result in a relatively weak correlation between chorus density and CORT. For instance, heavy rainfall appears to be a major contributor to temporal changes in CORT levels in *B. woodhousii* (Leary et al. 2008). Accordingly, chorus density explained only a small proportion of the total variance in circulating CORT levels in the present study. In addition, however, the involvement of CORT in the transition from calling to satellite behavior (Leary et al. 2004, 2006a) is also likely to be an important explanation for why the correlation between CORT levels of calling males and chorus density was not stronger; as CORT levels increase with increasing density, males that switched tactics were not included in our analyses. Satellites were excluded because these males appear to rapidly

Table 1

Means, SDs, and ranges for vocal attributes measured from 347 *Bufo woodhousii*

Vocal parameter	Mean	SD	Range
Call duration (s)	2.50	0.61	0.96–4.40
Intercall duration (s)	2.69	2.69	2.77–28.67
Pulses/s	127.8	15.4	89.8–165.0
Vocal effort (pulses/s)	31.2	7.3	8.4–64.2

Recordings were made across temperatures ranging from 17 °C to 25 °C (mean = 20, SD = 2.6). Vocal effort was positively correlated with temperature ($F_{1,345} = 41.5$, $P < 0.001$, $r^2 = 0.11$).

clear circulating CORT after threshold levels (that elicit the satellite tactic) are reached. For instance, although high CORT levels increase the probability that males adopt a satellite tactic (Leary et al. 2006a), satellite males have significantly lower levels of circulating CORT than callers (Leary et al. 2004). These findings may be explained by the relatively long (~15 min) focal observational periods used in our previous study (Leary et al. 2004) and the fact that males stopped calling and adopted the satellite tactic for some (unknown) amount of time before they were sampled. During this time, evidence suggests that males metabolize a fraction of the CORT that accumulated while calling. For example, prolonged focal observations of a small number of calling individuals ($n = 5$) that were observed to change to the satellite tactic and sampled soon thereafter had CORT levels that were generally higher than the average levels of other callers (Leary CJ, unpublished data). Hence, a detailed account of the amount of time passed since satellite behavior was initially adopted would be required to control for the effects of time on the clearance of circulating CORT. We did not have adequate data to address this issue in the present study.

The positive correlation between chorus density and circulating CORT levels does not appear to be associated with aggression or territoriality (Sullivan 1989; Leary et al. 2004). Male *B. woodhousii* also do not seem to be awaiting vacancy of calling sites; calling males typically aggregated in various areas of the pond, resulting in large areas that were uninhabited by males despite no obvious structural differences from sites used by callers. No studies have assessed how vocal interactions potentially modulate CORT levels in anurans and, thus, it remains unknown whether variation in chorus density modulates CORT levels through changes in the acoustic environment. Density-dependent effects on CORT levels, however, have been documented in several larval amphibians where vocalizations are absent (see reviews by Cooperman et al. 2004; Belden et al. 2007).

One possible explanation for the positive correlation between chorus density and circulating CORT levels in *B. woodhousii* is that the increase in the proportion of satellite males increases their detection by calling “host” males, thereby creating social circumstances that activate the HPI axis in calling males. If true, the positive correlation between chorus density and CORT levels in calling males may be a consequence of an increase in the proportion of satellite males and not attributable to chorus density per se. However, in the Great Plains toad (*B. cognatus*), males with satellites had significantly lower CORT levels than nearby males without satellites (Leary et al. 2006b). Whether these findings are applicable to *B. woodhousii* is unknown but, nonetheless, suggest that the presence of satellite males does not elicit elevations in CORT.

An alternative explanation for the positive correlation between chorus density and circulating CORT levels is that environmental cues that promote large, dense choruses (i.e., heavy

rainfall) also stimulate the HPI axis (for potential relationships between environmental variables and circulating CORT levels in *B. woodhousii*, see Leary et al. 2008). Currently, without experimental manipulation of chorus density, we cannot separate the potential effects of the abiotic and biotic environments on circulating CORT levels. To our knowledge, no study has experimentally manipulated chorus density to determine the potential effects on alternative mating tactics in anurans. Thus, it is unknown whether correlations between chorus density and mating tactic expression are attributable to environmental conditions that stimulate physiological changes and elicit changes in behavior. Nevertheless, our results suggest that there are physiological factors that could contribute to density-dependent patterns of mating tactic expression in *B. woodhousii*. Our results warrant further investigation to ascertain the factors that mediate density-related endocrine and mating tactic responses in this and other anuran species.

The lack of a significant positive correlation between chorus density and circulating androgen levels in *B. woodhousii* was unexpected given the results of various other studies. For example, conspecific calls have been shown to stimulate the HPG axis and/or hormone production by the gonads in several anuran species (Rabb 1973; Brzoska and Obert 1980; Wilczynski and Allison 1989; Burmeister and Wilczynski 2000, 2005; Chu and Wilczynski 2001). One possible explanation is that in explosive-breeding *B. woodhousii*, the HPG is rapidly activated by certain environmental conditions (i.e., rainfall), creating a ceiling effect with respect to circulating androgen levels (Leary et al. 2008). This hypothesis is consistent with implicit predictions of the Challenge Hypothesis for explosive-breeding species (Wingfield et al. 1990) and with recent studies indicating that environmental as well as social factors can play major roles in modulating androgen levels (Goymann et al. 2007). There is no evidence that high CORT levels suppress androgen levels in *B. woodhousii* (Leary et al. 2004, 2006a), and hence, the lack of a significant positive correlation between chorus density and androgen levels is not attributable to reciprocal interactions between circulating CORT and androgen levels.

Vocal effort is positively correlated with circulating CORT levels in *B. woodhousii* (Leary et al. 2008), and therefore, any density-dependent increase in vocal effort is expected to affect circulating CORT levels. Density-related changes in CORT level in *B. woodhousii*, however, were not related to changes in vocal effort, suggesting that the positive correlation between chorus density and CORT levels is not associated with increased energy expenditure. Clearly, further comparative studies are needed to assess how hormone levels, vocal effort, and energetic state potentially contribute to variation to density-dependent patterns of mating tactic expression in anurans.

FUNDING

M. Blanche Adams and M. Frances Adams Memorial Research Scholarships (University of Oklahoma, Department of Zoology); The University of Oklahoma Graduate Student Senate; The American Society of Ichthyologists and Herpetologists; Sigma Xi; The Animal Behavior Society; The Society of Integrative and Comparative Biology; The Society for the Study of Amphibians and Reptiles; National Science Foundation (IBN 0308958) to C.J.L.

We thank M. Guffey, D. Fox, and S. Reynolds for assistance in the field.

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