

Steroid hormone profiles and relative body condition of calling and satellite toads: implications for proximate regulation of behavior in anurans

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Males of most anuran species (frogs and toads) vocalize to attract mates. However, individuals of many vocal species may also adopt alternative noncalling “satellite” tactics. Satellite males characteristically remain in close proximity to calling conspecifics and attempt to intercept incoming females attracted to advertising males. Emerson proposed that alternation between calling and noncalling behavior in anurans is mediated by a reciprocal interaction between circulating levels of corticosterone and androgens that is driven by depletion of energy reserves during vocalization. We tested this hypothesis by examining steroid hormone profiles and the relative body condition of calling and satellite Woodhouse’s toads (*Bufo woodhousii*) and Great Plains toads (*B. cognatus*). Consistent with Emerson’s hypothesis, callers had significantly higher circulating corticosterone levels and were in better condition than satellites. However, levels of testosterone and dihydrotestosterone did not differ significantly between satellites and callers, and we found no evidence that high levels of corticosterone had an inhibitory effect on androgen production in either species. These data thus support a relationship between corticosterone levels and depletion of energy reserves during bouts of vocalization but suggest that alternation between calling and satellite behavior may be associated with direct effects of corticosterone on brain vocal control centers. We propose a model that incorporates relationships among energy reserves, androgens, corticosterone, and arginine vasotocin-producing neurons in the telencephalon to explain transitions between calling and satellite tactics in toads. **Key words:** alternative mating tactics, anurans, body condition, hormones, satellite behavior, steroids. [*Behav Ecol* 15:313–320 (2004)]

Alternative mating behaviors occur in all major radiations of vertebrates (Gross, 1986; Rubenstein, 1980), but relatively few empirical data are available regarding underlying mechanisms associated with behavioral expression for most taxonomic groups. Proximate regulation of alternative reproductive tactics in fishes (Brantley et al., 1993; Oliveira et al., 2001; Uglem et al., 2002) and reptiles (Crews 1998; Moore, 1991; Moore et al., 1998; Rhen and Crews, 2002) have been investigated most extensively, and current models are largely based on these organisms. However, many taxa are likely to exhibit differences in proximate regulation of behavior at some level, given the disparate neural pathways associated with signal production. For instance, many fishes and squamate reptiles produce visual signals to attract mates, whereas most anuran amphibians (frogs and toads) and birds produce acoustic signals.

Alternative mating tactics are particularly pervasive in anurans (Waltz, 1982). Numerous investigations report the existence of nonvocalizing individuals (termed satellites) that attempt to intercept incoming females attracted to the vocalizations of calling males (for review, see Halliday and Tejedo, 1995). In most cases, individuals reportedly alternate between calling and satellite tactics within and/or between breeding bouts, indicating that the behaviors are condition-dependent strategies within genetically monomorphic males (sensu Gross, 1986).

Condition-dependent plastic adult phenotypes should be subject to activation effects of hormones that mediate behavioral expression (Moore, 1991; Moore et al., 1998). In anuran amphibians (and birds), production of vocalizations is usually mediated by androgens (Burmeister and Wilczynski, 2000; Chu and Wilczynski, 2001; Kelley, 1980; Moore, 1987; Wetzel and Kelley, 1983; Wingfield and Moore, 1987). Current understanding of the relationship between circulating steroid hormones and the expression of alternative mating tactics in anurans is largely based upon the work of Mendonça et al. (1985), who found that satellite male bullfrogs (*Rana catesbeiana*) possessed significantly higher androgens (testosterone and 5 α -dihydrotestosterone) relative to calling conspecifics. Mendonça et al. (1985) proposed that lower androgen levels in calling males resulted from stress responses (i.e., interactions between glucocorticoids and androgen-producing cells) associated with territorial defense (male bullfrogs aggressively defend territories and frequently engage in combat; Howard, 1978, 1984).

Vocal production in anurans is one of the most energetically expensive behaviors known for any ectotherm vertebrate, frequently elevating metabolic rates 20 times above basal rates (Wells, 2001). The energetic expenditure associated with vocal production combined with the common inhibition of androgen production associated with elevated glucocorticoid levels (Greenberg and Wingfield, 1987; Knapp and Moore, 1997; Licht et al., 1983; Orchinik et al., 1988; Wingfield and Ramenofsky, 1999) provided the impetus for the Energetics–Hormone Vocalization model (Emerson, 2001). This model is an extension of the Challenge Hypothesis (Wingfield et al., 1990) and proposes an interaction between corticosterone and androgens to explain transitions between calling and

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noncalling behavior in anurans. It predicts that the energetic demands imposed by vocal production will elevate circulating corticosterone, which, in turn, reaches a threshold level that inhibits androgen production. At this stage, a male ceases calling until energetic deficits are restored, circulating corticosterone is metabolized, and elevations in androgens mediate another bout of calling.

We tested predictions of the Energetics–Hormone Vocalization model in two anuran species with different satellite tactics. Noncalling satellite Woodhouse's toads (*Bufo woodhousii*) characteristically reside along the periphery of the chorus and actively amplex incoming individuals in an attempt to locate females (Sullivan, 1989). Individual *B. woodhousii* periodically alternate between calling and satellite tactics within and/or between breeding bouts (Sullivan, 1989). In contrast, Great Plains toads (*Bufo cognatus*) exhibit a satellite–host association whereby one or more noncalling satellites parasitize a single vocalizing individual male (Krupa, 1989; Sullivan, 1982a, 1983). Satellite males remain stationary, usually within 0.5 m of their “host” and also commonly alternate between calling and noncalling tactics (Krupa, 1989; Sullivan, 1982a, 1983).

Individuals of territorial and/or aggressive species may prevent conspecifics from exhibiting particular behaviors irrespective of their physiological condition. *B. woodhousii* and *B. cognatus* do not exhibit territoriality in the sense of long-term site fidelity, and neither species engages in combat or prolonged territorial bouts (Krupa, 1989; Sullivan, 1982a,b, 1983; Sullivan and Leek, 1986), as does *R. catesbeiana* (Howard, 1978). Although aggressive behaviors among males were reported for prolonged breeding populations of *B. woodhousii* in the desert southwest United States (Sullivan, 1982b), aggression has not been observed in explosive breeding populations in the central Great Plains (B.K. Sullivan, personal communication; C.J. Leary, personal observation). Hence, these nonterritorial, nonaggressive toad species were considered appropriate subjects to investigate proximate regulation of behavior because investigations are not confounded by social factors that potentially affect phenotypic expression. We focus on the relationship among circulating corticosterone, androgens, and relative body condition in an attempt to elucidate the factors regulating the expression of plastic adult phenotypes.

METHODS

Field methods

Our study pond for *B. woodhousii* is located on a privately owned ranch in Norman, Oklahoma, where as many as 500 individuals may gather during the breeding season. *B. woodhousii* typically begin breeding after early spring rains (approximately April). We studied two *B. cognatus* breeding sites within 0.5 km of each other (also in Norman, Oklahoma) where a total of ~250 individuals engaged in breeding activity. Breeding activity for *B. cognatus* may or may not overlap with *B. woodhousii*, depending on weather conditions (heavy rainfall is required to initiate breeding activity in *B. cognatus*). Both species breed in ephemeral bodies of water in the same area, but *B. woodhousii* are largely allotopic to *B. cognatus*.

We recorded behavior of individuals under natural conditions for 10 to 15 min before collecting blood samples for hormone analyses. Observations were made with headlamps and/or flashlights and did not have any overt effects on behavior (see also Sullivan, 1983, 1989). In the time frame we used to assess behavioral expression, it was common to observe noncalling satellite males attempting to intercept incoming females and/or amplexing nearby males (Krupa, 1989;

Sullivan, 1983, 1989). Immediately after focal observations, blood was obtained in the field by cardiac puncture with a heparinized hypodermic needle. Bleeding procedures were completed in less than 3 min after hand capture. Blood samples (~200 µl) were collected from 112 *B. woodhousii* during breeding activity on 3–5, 8, 9, and 13 April and on 5 and 19 May 2001. Blood samples were collected from a total of 45 *B. cognatus* on 20 and 28–31 May and 1 June 2001. These dates largely encompassed the entire breeding season for both species.

Blood sampling took place during peak activity periods from approximately 2300 to 0100 h for *B. woodhousii* and from 2200 to 2400 h for *B. cognatus*. Both satellites and callers were sampled throughout observed activity periods. When satellites were sampled, a nearby caller was also sampled at the same time, but more blood samples were obtained for callers because they were more abundant than were satellites and, therefore, blood sampling was not always paired. Approximately 54% of all data points for *B. woodhousii* consisted of satellites and callers that had blood collected in a paired fashion; 84% of all *B. cognatus* data points consisted of caller/satellite associations that were sampled in a paired fashion. After blood collection, all individuals were weighed to the nearest 1.0 g with a portable OHAUS digital scale, measured from tip of snout to end of ischium to the nearest 1.0 mm, and numerically marked in sequence on the venter with a portable tattoo device (Tattoo-A-Pet, Inc., Fort Lauderdale, Florida) for future identification. Procedures had no overt effects on subsequent behavior; nonsatellite males typically resumed calling immediately after data collection and release. Blood samples obtained in the field were kept on ice until they were returned to the lab (less than 4 h) and centrifuged for 12 min at 3000 rpm. Plasma was collected and stored at –20°C until it was assayed for steroid hormones. All procedures were approved by the University of Oklahoma Animal Care and Use Committee (A3240-01).

Hormone assays

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 corticosterone (B). Hormone extraction, chromatography, and radioimmunoassay followed the methods of Harvey et al. (1997) and Knapp et al. (1999). Testosterone antibody was obtained from Research Diagnostics (Flanders, New Jersey) and used for both T and DHT assays. Corticosterone antibody was purchased from ICN Laboratories (Costa Mesa, California).

Plasma samples were analyzed for androgen and B levels in four assay runs for *B. woodhousii* and one assay run for *B. cognatus*. Mean intra-assay coefficients of variation for *B. woodhousii* for DHT, T, and B were 22.9, 8.6, and 5.4%, respectively, based on two to four standards run with each assay. Interassay coefficients of variation for *B. woodhousii* for DHT, T, and B were 26.1%, 5.5%, and 12.2%, respectively. Intra-assay coefficients of variation for *B. cognatus* for DHT, T, and B were 14.9, 9.2, and 11.5%, respectively, based on four standards run in the single assay. Although the extraction and chromatography procedures remove potential sources of nonspecific binding, we validated our assay procedures by verifying that curves generated by serial dilution of a plasma sample with known high hormone levels and from hormone-spiked charcoal-stripped plasma were parallel to the standard curves for each hormone (data not shown). Steroid hormone profiles for callers and satellites were analyzed separately for both species by using ANOVA.

Body condition

To determine if there was evidence of depleted energy reserves for satellites versus callers, the body mass of individuals was examined by using ANCOVA with snout-ischial length (SIL) as the covariate. Residual regression analyses were also performed to examine the relationship between body mass and body length for the two behavioral phenotypes. Lastly, we examined repeated measures of body mass for individuals that alternated between calling and satellite behavior (using two-tailed paired *t* tests) to determine if satellites were in significantly poorer condition than callers.

Significant differences are reported for $p < .05$ in all analyses. Data were analyzed by using Statview Statistical Software (SAS Institute Inc., Cary, North Carolina).

RESULTS

Hormone levels

Steroid hormone profiles of callers and satellites were similar for *B. woodhousii* and *B. cognatus* (Figure 1). There were no significant differences in DHT ($F_{1,110} = 0.41$, $p = .5$) or T levels ($F_{1,110} = 2.10$, $p = .1$) for satellite and calling *B. woodhousii*, but B levels were significantly higher for callers ($F_{1,110} = 3.90$, $p = .04$) (Figure 1A). There were also no significant differences in circulating DHT ($F_{1,43} = 0.05$, $p = .8$) or T levels ($F_{1,43} = 0.001$, $p = .9$) for satellite and calling *B. cognatus*. However, B concentrations were again significantly higher for callers ($F_{1,43} = 11.32$, $p = .001$) (Figure 1B). Although the intra- and interassay variation was greater than 20% for DHT, this variation does not mask potential differences in DHT levels between the tactics because (1) individual blood samples were randomized within each assay so that callers and satellites were represented in each assay, (2) T and DHT levels within an individual are tightly correlated in the current data set (data not shown), and (3) standard errors of the mean for DHT and T were similar or of the same magnitude between callers and satellites.

To compare our results with those reported for *R. catesbeiana* (Mendonça et al., 1985), we examined total androgen levels (T + DHT) for calling and satellite *B. woodhousii* and *B. cognatus*. Results remained similar in that there were no significant differences in total androgen concentrations for callers versus satellites in *B. woodhousii* ($F_{1,110} = 0.98$, $p = .3$) or *B. cognatus* ($F_{1,43} = 0.02$, $p = .8$).

To further investigate the potential for a reciprocal interaction between B and circulating androgens, we examined linear regressions for B versus T + DHT for calling *B. woodhousii* and *B. cognatus*. There was a weak positive relationship between circulating B and total androgens for *B. woodhousii* ($r^2_{1,81} = 0.04$, $p = .06$) and *B. cognatus* ($r^2_{1,25} = 0.1$, $p = .02$; Figure 2).

Body condition

Males practicing a satellite tactic were significantly smaller than callers for *B. woodhousii* ($F_{1,148} = 6.9$, $p = .009$; mean \pm SE, satellites: 74 ± 1.1 mm, callers: 78 ± 0.8 mm) and *B. cognatus* ($F_{1,57} = 14.5$, $p = .0003$; satellites: 81 ± 1.5 mm, callers: 87 ± 0.8 mm). Sample sizes differ from those reported for hormone analyses because statistical analyses of SIL included individuals that were observed and measured but not sampled for hormone levels. Results were similar when analyses included only individuals from which blood was collected.

Differences in SIL for the two behavioral phenotypes precluded the use of condition indices (i.e., calculating individual scores based on the proportion of body mass to

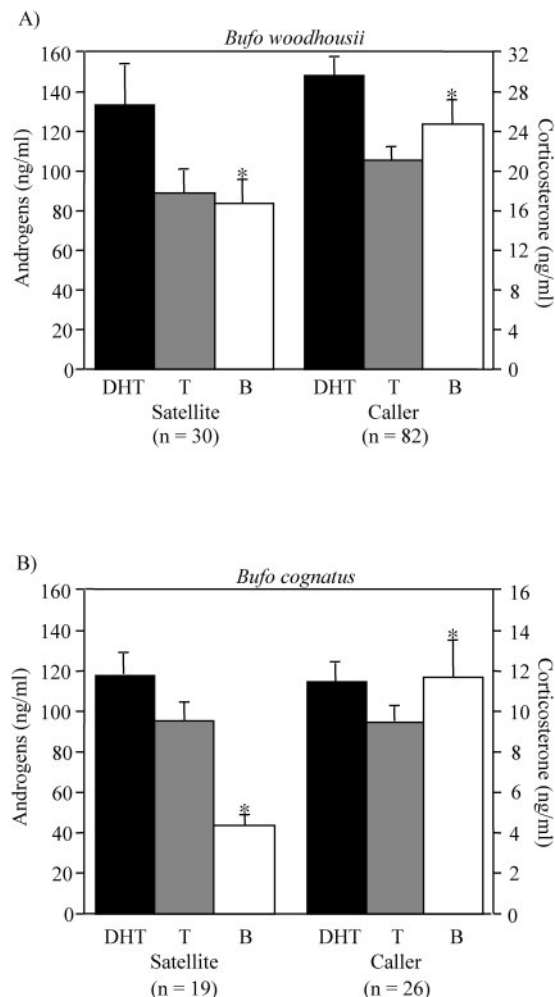


Figure 1
Mean (\pm SE) steroid hormone levels for satellite and calling male *Bufo woodhousii* (A) and *B. cognatus* (B). Dihydrotestosterone (DHT) and testosterone (T) were not significantly different for the two behavioral categories, but corticosterone (B) was significantly higher in callers (indicated with an asterisk).

body length) because ratios do not control for SIL (Tomkins and Simmons, 2002). We therefore followed the statistical procedures outlined by Tomkins and Simmons (2002) to determine if satellites differed from callers in relative body condition. ANCOVA was initially performed (on log-transformed data) with body mass as the dependent variable, behavior (satellite versus caller) as the factor, and SIL as the covariate. Analysis for *B. woodhousii* revealed a significant interaction between mass and SIL ($F_{1,121} = 11.07$, $p = .001$) (Figure 3). There was no significant interaction between mass and SIL for satellite versus calling *B. cognatus* ($F_{1,41} = 0.56$, $p = .4$), and satellite males were not in significantly poorer condition than callers ($F_{1,41} = 0.50$, $p = .4$).

Because heterogeneity of slopes violates the assumptions of ANCOVA (Sokal and Rohlf, 1981), log body mass for satellite and calling *B. woodhousii* was adjusted to the common mean log SIL (1.88 mm) by using the equation calculated from the pooled slope (see Tomkins and Simmons, 2002). ANOVA was then used to determine if satellites and callers differed significantly in body mass corrected to the mean SIL. Satellite *B. woodhousii* were in significantly poorer condition than callers ($F_{1,123} = 10.41$, $p = .001$).

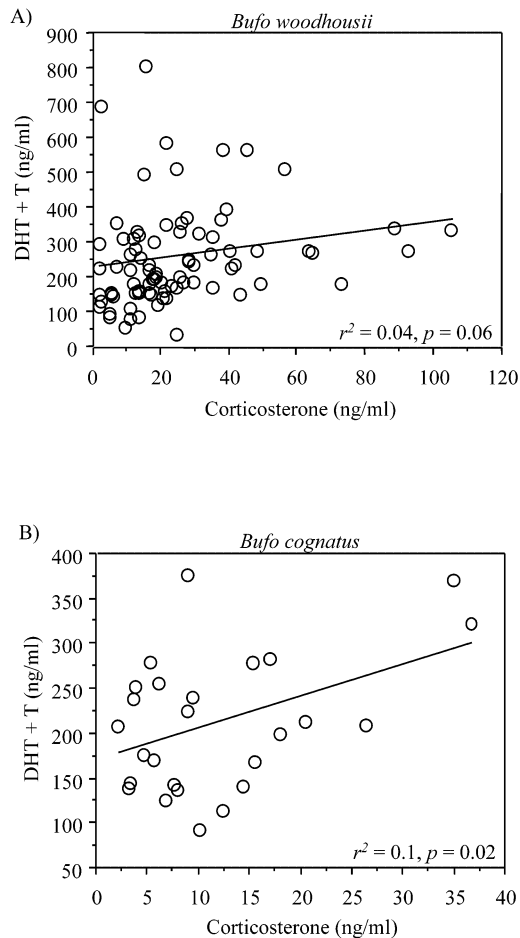


Figure 2

Linear regressions showing the relationship between corticosterone and total androgens (testosterone [T] + dihydrotestosterone [DHT]) in *Bufo woodhousii* (A) and *Bufo cognatus* (B).

To visually examine the body mass distribution of the two behavioral phenotypes, residual body mass values obtained from a pooled regression of satellite and calling *B. woodhousii* and *B. cognatus* were plotted against SIL. The majority of *B. woodhousii* satellites (76%) fell below the zero y-axis value, whereas callers were approximately equally distributed above (52%) and below (48%) the line (Figure 4A), indicating that satellites were generally in poorer condition than were callers. In contrast, only 57% of satellite *B. cognatus* fell below the zero y-axis, whereas 46% of callers were distributed above the line (Figure 4B).

We further examined the relationship between relative condition and behavior by comparing repeated measures of body mass from individuals that were observed to alternate between calling and satellite behavior. Repeated measures were taken from only a few individuals because handling and measuring procedures potentially have adverse effects on subsequent hormone levels and behavior (Moore et al., 1991). In the present study, we were interested in determining natural changes in behavioral expression, and therefore, we attempted to minimize handling and did not collect multiple blood samples from individual toads. Because of the small number of individuals repeatedly sampled ($n = 4$ for *B. cognatus* and $n = 14$ for *B. woodhousii*), we combined the data for both species to examine differences in body mass for callers versus satellites. A paired t test (two-tailed) indicated that individuals were

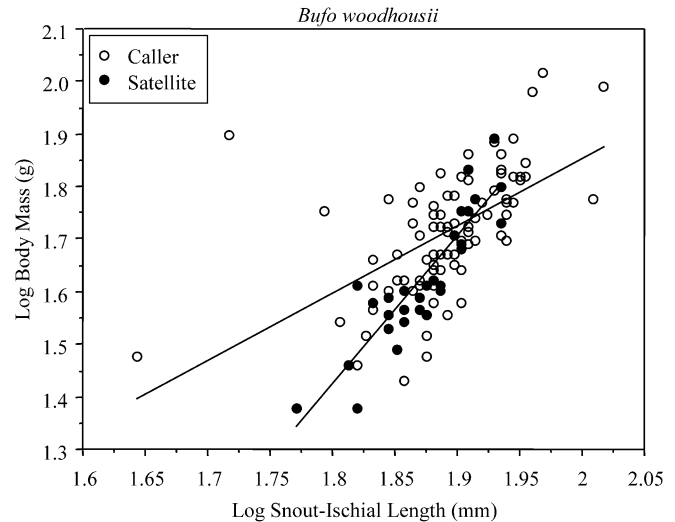


Figure 3

Relationship between log snout-ischial length and log body mass for satellite (solid circles) and calling (open circles) *Bufo woodhousii*. There was a significant interaction between the two variables across behavioral phenotypes (see text).

significantly heavier when calling compared with when they were exhibiting satellite behavior ($t_{1,17} = 3.91$; $p = .001$) (Figure 5). The duration between repeated measures of body mass associated with transitions in behavior ranged from 47–195 h for *B. woodhousii* (mean \pm SE = 120 ± 14 h) and 23–72 h for *B. cognatus* (36 ± 12 h). Individuals practicing a satellite tactic were, on average, 4.0 g lighter than when they were calling. Based on the mean body mass for each species (*B. cognatus* = 67.0 g; *B. woodhousii* = 49.0 g), this difference represents a 6–8% reduction in body mass.

DISCUSSION

Circulating levels of androgens (DHT + T) did not differ for calling and satellite *B. woodhousii* or *B. cognatus* despite significantly higher circulating B levels for calling males. Further investigation revealed no evidence of a significant interaction between B and androgens in either species. Given that individual *B. woodhousii* and *B. cognatus* were observed alternating between behaviors, a significant decrease in relative body condition while exhibiting satellite tactics suggests that high B levels in calling males are associated with depletion of energetic reserves during vocal production.

Differences in body condition are not likely to be the result of differential hydration states for the two behavioral categories because all individuals remained partially submerged while calling or exhibiting satellite behavior. Our results suggest that satellites are physiologically constrained from sustained calling activity and must forage before initiating subsequent calling bouts (Marler and Ryan, 1996). Sullivan (1982a) reported that tactic expression in *B. cognatus* is also influenced by social context, because satellite males typically adopted calling behavior after removal of callers from the chorus. In Sullivan's (1982a) investigation, focal observations were terminated when satellites were observed to call (Sullivan BK, personal communication). By using similar procedures, we have found that satellites sometimes adopt vocal behavior after removal of the associated host as reported by Sullivan (1982a), but only for a very brief period of time. Individuals subsequently resume satellite behavior after ephemeral bouts of calling (C.J. Leary, personal observation).

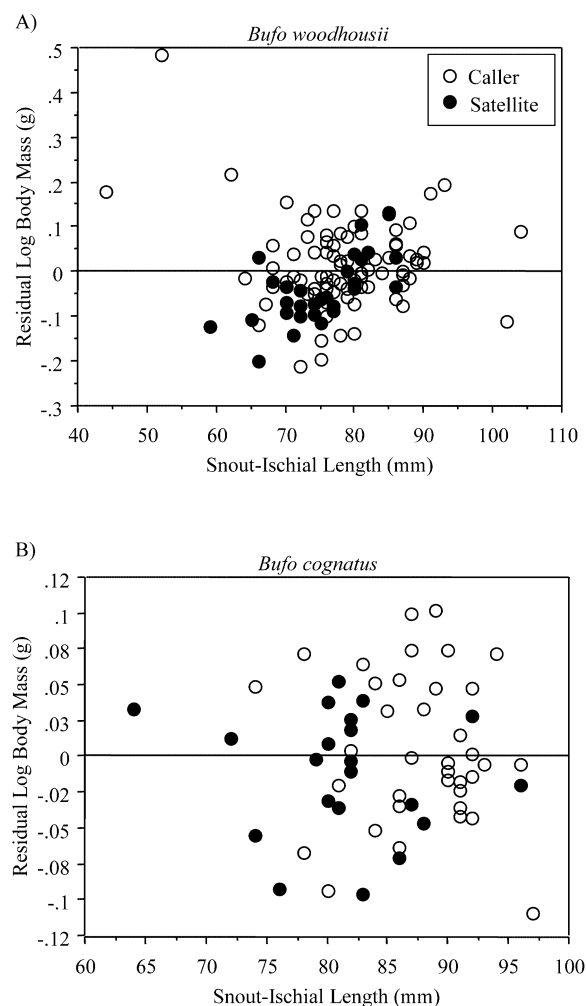


Figure 4
Regression of residual values of log body mass plotted against snout-ischial length for satellite and calling *Bufo woodhousii* (A) and *B. cognatus* (B). The majority of satellites fell below the zero y-axis value for both species (see text).

Although satellites had lower B levels than did callers in both toad species, B concentrations showed a 1.5-fold difference for satellite and calling *B. woodhousii* but a 3-fold difference for satellite and calling *B. cognatus*. These differences may be related to species-specific differences in B sensitivities and/or production, but we also suspect that these results are attributable, in part, to striking behavioral differences between the two species. Satellite *B. cognatus* parasitize a single vocalizing individual by remaining stationary and in close proximity of their host, often for prolonged periods of time (i.e., in excess of 2 h; C.J. Leary, personal observation). In contrast, satellite *B. woodhousii* actively patrol the periphery of chorusing males and persistently engage in amplexus with other males in an attempt to locate incoming females (nondiscriminating male-male amplexus is common in *Bufo*; Leary, 2001a,b). This satellite tactic in *B. woodhousii* was also reported by Sullivan (1989) and is similar to that reported by Forester and Thompson (1998) for *B. americanus* (referred to as gauntlet behavior). Satellite *B. woodhousii* in our study population, however, are not terrestrial (as reported for *B. americanus*) but are restricted to deeper water surrounding the calling males located in shallow water. Hence, noncalling satellite *B. woodhousii* may exhibit higher B levels relative to callers than do *B. cognatus* satellites because

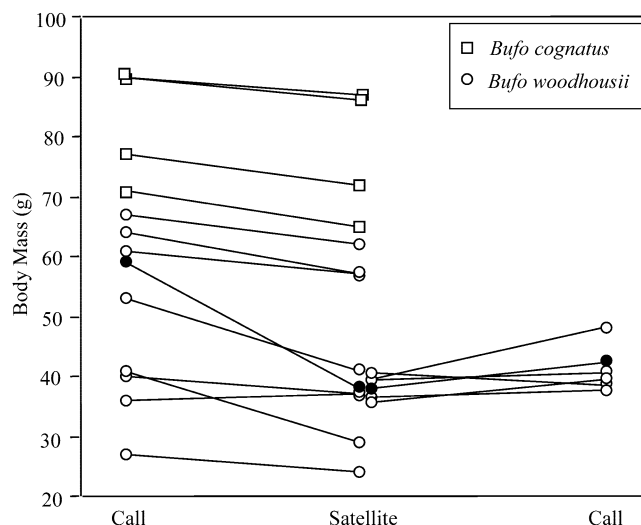


Figure 5
Changes in body mass associated with transitions in behavior for individual *Bufo woodhousii* and *B. cognatus*. (Left) Data from individuals first observed calling and subsequently observed exhibiting satellite behavior. (Right) Data from individuals first observed exhibiting satellite behavior and then subsequently observed calling. Solid circles represent an individual *B. woodhousii* that was observed alternating between behaviors on two occasions.

satellite *B. woodhousii* continue to exhibit energetically demanding activities (i.e., swimming/frequent amplexus), whereas satellite *B. cognatus* characteristically remain inactive.

Territorial and aggressive interactions have been shown to elevate circulating B and suppress androgen production in numerous vertebrates (Greenberg and Crews, 1990; Greenberg and Wingfield, 1987; Knapp and Moore, 1995, 1996). However, Emerson and Hess (2001) have questioned whether a reciprocal relationship exists between B and androgens for territorial *R. catesbeiana* (as reported by Mendonça et al., 1985). Emerson and Hess (2001) pointed out that satellite individuals in many species have a larger testes-to-body size ratio, which may provide an alternative explanation for observed elevations in circulating androgens in satellite *R. catesbeiana*. Our data do not reveal a reciprocal relationship for these hormones in nonterritorial *B. woodhousii* and *B. cognatus*, but we do not have sufficient data to adequately address Emerson and Hess's (2001) hypothesis. Differences in testes size may still exist in satellite and calling *B. woodhousii* and *B. cognatus* even though behavioral phenotypes are not "fixed" in these species because for the majority of anurans studied thus far (including *Bufo* and *Rana*) satellites are significantly smaller and presumably younger. Therefore, differences in gonosomatic "indices" may be a function of allometric growth rates. Currently, comparative analyses are not sufficient to determine whether general differences in hormonal patterns exist for territorial and nonterritorial anuran species. Behavioral differences among *R. catesbeiana*, *B. woodhousii*, and *B. cognatus* may thus contribute to interspecific differences in steroid hormone profiles for satellites versus callers.

Ecological factors may also play a role in interspecific differences in steroid hormone profiles among anurans. For instance, in the population studied by Mendonça et al. (1985), *R. catesbeiana* bred over a prolonged period (several months), whereas *B. woodhousii* and *B. cognatus* are explosive breeders, often completing the breeding season in less than 1 week (Krupa, 1989; Sullivan, 1982a, 1983, 1989; Wells, 1977; C.J. Leary, personal observation). Explosively breeding arctic bird

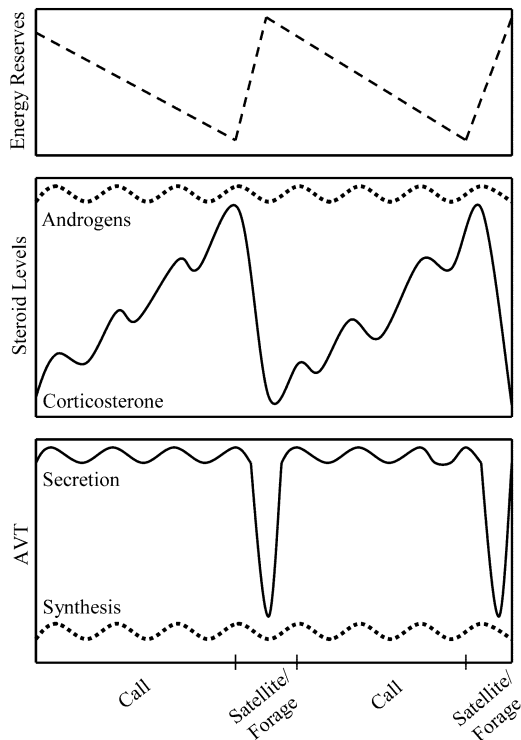


Figure 6

Proposed mechanism controlling alternation between calling and satellite behavior in *Bufo woodhousii* and *B. cognatus*. The model proposes a reciprocal interaction between corticosterone (B) and arginine vasotocin (AVT) secretion. Elevated B levels are driven by depletion of energetic reserves over time (between successive calling bouts) and probably fluctuate during inactive daytime periods and active night time periods. The slopes for B concentration and energetic reserves is dependent upon condition and prey availability. Androgens exhibit temporal fluctuations that result in fluctuations in AVT synthesis, but androgens do not exhibit a reciprocal relationship with B.

species modulate adrenocortical responses to stressors (see Wingfield et al., 1995, 1998) by altering sensitivity to elevated B (e.g., by varying B binding protein and/or receptor levels; for review, see Breuner and Orchinik, 2002). Physiological and/or behavioral regulation of B (i.e., alternating between vocal and nonvocal behavior) would potentially be advantageous in anuran species with a narrow window of opportunity for reproduction. For example, assuming that elevated androgens are responsible for the production and/or maintenance of sexual behavior (Harvey et al., 1997), a reciprocal interaction between B and androgens in an explosive breeding species would potentially remove individuals from the pool of conspecifics engaged in breeding activity for a significant portion of the reproductive period. In addition, elevated androgens are necessary to initiate spermatogenesis (Harvey et al., 1997). Hence, strong selective pressures may maintain high androgens in explosive breeding species to maximize individual reproductive potential.

Regardless of behavioral and/or ecological differences and potential physiological mechanisms that may modulate stress responses, our results suggest that decision rules governing the expression of satellite tactics in *B. woodhousii* and *B. cognatus* are independent of androgens. Although other studies have examined the relationship between steroid hormone levels and calling/noncalling behavior in anurans (for review see Burmeister et al., 2001), comparisons may not be appropriate because noncalling behavior is set within very different social

and ecological contexts. For instance, Townsend and Moger (1987) examined noncalling male *Eleutherodactylus coqui* exhibiting parental care of terrestrial eggs, Marler and Ryan (1996) examined noncalling male *Physalaemus pustulosus* foraging away from breeding ponds, and Harvey et al. (1997) studied noncalling “prebreeding” and “postbreeding” male *Scaphiopus couchii*. Mendonça et al. (1985) and the present study focus on noncalling satellite males that attempt to intercept females for mating. One commonality among the three anuran species for which there is information specifically addressing alternative noncalling satellite tactics is that callers have elevated levels of B. It may be that glucocorticoids mediate the expression of alternative mating tactics in anurans without necessarily having a negative effect on androgen production. One such mechanism could involve direct B-mediated regulation of arginine vasotocin (AVT).

AVT is produced in sexually dimorphic vocal control centers of the anuran brain and induces calling behavior when administered to males of various frog and toad species (Wilczynski and Chu, 2001), including *B. cognatus* (Propper and Dixon, 1997). A role of AVT in mediating reproductive behaviors has also been demonstrated in other vertebrates, including salamanders (Moore et al., 2000), fishes (Bastian et al., 2001; Semsar et al., 2001), mammals (vasopressin is the mammalian homologue; Bester-Meredith and Marler, 2001), and birds (Voorhuis et al., 1991). AVT production, in turn, can be regulated by androgens (Boyd, 1994a,b). When this process is incorporated into the Energetics–Hormone Vocalization model proposed by Emerson (2001), it suggests that cyclic fluctuations in androgen levels are accompanied by fluctuations in AVT production. Such a mechanism may explain cyclic patterns of behavioral expression in bullfrogs but does not appear to be occurring in *B. woodhousii* or *B. cognatus*. Therefore, we need to look elsewhere for a potential mechanism mediating behavioral tactics in these species.

Corticosterone acts directly on AVT neurons in the rough-skinned newt, *Taricha granulosa*, via a nongenomic mechanism (i.e., does not require gene transcription; Moore and Evans, 1999; Orchinik et al. 1991). In this species, AVT also mediates courtship behavior (i.e., clasping responses associated with amplexus; Moore et al., 2000; Rose et al., 1995), which can be directly and rapidly inhibited by exogenous application of corticosterone (Moore and Miller, 1984; Rose et al., 1993, 1998). If the results obtained in *T. granulosa* are applicable to anurans, then direct effects of B on AVT neurons may regulate cyclic patterns of calling and noncalling activity. Burmeister et al. (2001) addressed this possibility in green treefrogs (*Hyla cinerea*), but the results were equivocal. In túngara frogs, B implants suppressed calling activity, but the treatment caused a significant decrease in circulating androgens (Marler and Ryan, 1996). Hence, it was unclear if effects resulted from a decrease in androgens or were a direct result of B.

We propose a model (Figure 6) that incorporates AVT to explain cyclic patterns of calling and satellite behavior in *B. woodhousii* and *B. cognatus*. This model is based on our data for relative body condition and steroid hormone profiles for these species, models interrelating energetics and corticosterone action (i.e., concepts of the Emergency Life History Stage theory) proposed by Wingfield and Ramenofsky (1999), and the Energetics–Hormone Vocalization model proposed by Emerson (2001). Our model differs from previous models in that it proposes a B-mediated response that is independent of the hypothalamic–pituitary–gonadal axis (i.e., there is no reciprocal interaction between B and androgens). The model proposes that fluctuations in androgen concentrations coincide with AVT synthesis/release (Figure 6). Periodic elevations in androgens are based on findings reported for other anuran species (for review, see Emerson and Hess, 2001), and

the association between androgens and AVT is based on Boyd (1994a,b). Corticosterone concentration, in turn, parallels calling duration (Emerson and Hess, 2001), but prolonged calling (over several consecutive nights) potentially elevates circulating B (depending on condition and prey availability) and initiates interactions between B and AVT-producing neurons when threshold levels are reached (Figure 6). Our model does not incorporate call effort, which is expected to coincide with androgen and B levels (Emerson, 2001), nor does it consider the entire breeding season (i.e., androgens are expected to diminish toward the end of the breeding season; Itoh et al., 1990).

To date, only one study in anurans bears on our model, and these data may be consistent with a nongenomic effect of B on AVT neurons. Satellite male cricket frogs (*Acris crepitans*) had higher concentrations of forebrain AVT than did callers, suggesting that AVT synthesis is not inhibited in satellites (Marler et al. 1999). However, potential depletion of AVT reserves during vocal production complicates determining whether or not AVT synthesis is inhibited, and steroid hormone profiles for callers and satellites are not available for this species.

Further investigations and comparative studies are necessary to elucidate the proximate mechanisms regulating the expression of noncalling satellite tactics in anurans. Future investigations should establish natural steroid hormone levels for satellites and callers given that numerous ecological factors (i.e., duration of breeding season) and behavioral characteristics (i.e., aggression) vary from species to species and potentially contribute to interspecific variation in physiology. The differences in steroid hormone profiles for satellites and callers in the species studied thus far suggest two different pathways that may regulate expression of alternative mating tactics in anurans: a direct B/AVT-mediated response versus a B/androgen/AVT-mediated response. We are currently working to distinguish between these two potential mechanisms in *B. woodhousii* and *B. cognatus*.

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REFERENCES

- Bastian J, Schniederjan S, Nguyenkim J, 2001. Arginine vasotocin modulates a sexually dimorphic communication behavior in the weakly electric fish *Apteronotus leptorhynchus*. *J Exp Biol* 204:1909–1923.
- Bester-Meredith JK, Marler CA, 2001. Vasopressin and aggression in cross fostered California mice (*Peromyscus californicus*) and white-footed mice (*Peromyscus leucopus*). *Horm Behav* 40:51–64.
- Boyd SK, 1994a. Arginine vasotocin facilitation of advertisement calling and call phonotaxis in bullfrogs. *Horm Behav* 28:232–240.
- Boyd SK, 1994b. Gonadal steroid modulation of vasotocin concentrations in the bullfrog brain. *Neuroendocrinology* 60:150–156.
- Brantley RK, Wingfield JC, Bass AH, 1993. Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal bases for male dimorphism among teleost fishes. *Horm Behav* 27:332–347.
- Breuner CW, Orchinik, M, 2002. Beyond carrier proteins: plasma binding proteins as mediators of corticosteroid action in vertebrates. *J Endocrinol* 175:99–112.
- Burmeister S, Somes C, Wilczynski W, 2001. Behavioral and hormonal effects of exogenous vasotocin and corticosterone in the green treefrog. *Gen Comp Endocrinol* 122:189–197.
- Burmeister S, Wilczynski W, 2000. Social signals influence hormones independently of calling behavior in the treefrog (*Hyla cinerea*). *Horm Behav* 38:201–209.
- Chu J, Wilczynski W, 2001. Social influences on androgen levels in the southern leopard frog, *Rana sphenoccephala*. *Gen Comp Endocrinol* 121:66–73.
- Crews D, 1998. On the organization of individual differences in sexual behavior. *Am Zool* 38:118–132.
- Emerson SB, 2001. Male advertisement calls: Behavioral variation and physiological processes. In: *Anuran communication* (Ryan MJ, ed). Washington: Smithsonian Institution Press; 36–44.
- Emerson SB, Hess DL, 2001. Glucocorticoids, androgens, testis mass, and the energetics of vocalization in breeding male frogs. *Horm Behav* 39:59–69.
- Forester DC, Thompson KJ, 1998. Gauntlet behavior as a male sexual tactic in the American toad (Amphibia: Bufonidae). *Behaviour* 135:99–119.
- Greenberg N, Crews D, 1990. Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinensis*. *Gen Comp Endocrinol* 77:246–255.
- Greenberg N, Wingfield J, 1987. Stress and reproduction: reciprocal relationships. In: *Hormones and reproduction in fishes, amphibians and reptiles* (Norris DO, Jones RE, eds). New York: Plenum Press; 461–489.
- Gross MR, 1986. Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol Evol* 11:92–98.
- Halliday TR, Tejedo, M, 1995. Intrasexual selection and alternative mating behaviour. In: *Amphibian biology: social behaviour*, vol. 2 (Heatwole H, Sullivan BK, eds). Chipping Norton, NSW: Surrey Beatty and Sons; 419–468.
- Harvey LA, Propper CR, Woodley SK, Moore MC, 1997. Reproductive endocrinology of the explosively breeding desert spadefoot toad, *Scaphiopus couchii*. *Gen Comp Endocrinol* 105:102–113.
- Howard RD, 1978. The evolution of mating strategies in bullfrogs, *Rana catesbeiana*. *Evolution* 32:850–871.
- Howard RD, 1984. Alternative mating behaviors of young male bullfrogs. *Am Zool* 24:397–406.
- Itoh M, Inoue M, Ishii S, 1990. Annual cycle of pituitary and plasma gonadotropins and plasma sex steroids in a wild population of the toad, *Bufo japonicus*. *Gen Comp Endocrinol* 78:242–253.
- Kelley DB, 1980. Auditory and vocal nuclei in the frog brain concentrate sex hormones. *Science* 207:553–555.
- Knapp R, Moore MC, 1995. Hormonal responses to aggression vary in different types of agonistic encounters in male tree lizards, *Urosaurus ornatus*. *Horm Behav* 29:85–105.
- Knapp R, Moore MC, 1996. Male morphs in tree lizards, *Urosaurus ornatus*, have different delayed hormonal responses to aggressive encounters. *Anim Behav* 52:1045–1055.
- Knapp R, Moore MC, 1997. Male morphs in tree lizards have different testosterone responses to elevated levels of corticosterone. *Gen Comp Endocrinol* 107:273–279.
- Knapp R, Wingfield JC, Bass AH, 1999. Steroid hormones and parental care in the plainfin midshipman fish (*Porichthys notatus*). *Horm Behav* 35:81–89.
- Krupa JJ, 1989. Alternative mating tactics in the Great Plains toad. *Anim Behav* 37:1035–1043.
- Leary CJ, 2001a. Evidence of convergent character displacement in release vocalizations of *Bufo fowleri* and *B. terrestris* (Anura; Bufonidae). *Anim Behav* 61:431–438.
- Leary CJ, 2001b. Investigating opposing patterns of character displacement in release and advertisement vocalizations of *Bufo americanus* and *B. fowleri* (Anura; Bufonidae). *Can J Zool* 79:1577–1585.
- Licht P, McCreery BR, Barnes R, Pang R, 1983. Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog, *Rana catesbeiana*. *Gen Comp Endocrinol* 50:124–145.
- Marler CA, Boyd SK, Wilczynski W, 1999. Forebrain arginine vasotocin correlates of alternative mating strategies in cricket frogs. *Horm Behav* 36:53–61.
- Marler CA, Ryan MJ, 1996. Energetic constraints and steroid hormone correlates of male calling behavior in the Tungara frog. *J Zool Lond* 240:397–409.

- Mendonça MT, Licht P, Ryan MJ, Barnes R, 1985. Changes in hormone levels in relation to breeding behavior in male bullfrogs (*Rana catesbeiana*) at the individual and population levels. *Gen Comp Endocrinol* 58:270–279.
- Moore FL, 1987. Regulation of reproductive behaviors. In: *Hormones and reproduction in fishes, amphibians and reptiles* (Norris DO, Jones RE, eds). New York: Plenum Press; 505–522.
- Moore FL, Evans SJ, 1999. Steroid hormones use non-genomic mechanisms to control brain functions and behaviors: a review of evidence. *Brain Behav Evol* 54:41–50.
- Moore FL, Miller LJ, 1984. Stress-induced inhibition of sexual behavior: corticosterone inhibits courtship behaviors of a male amphibian (*Taricha granulosa*). *Horm Behav* 18:400–410.
- Moore FL, Richardson C, Lowry CA, 2000. Sexual dimorphism in numbers of vasotocin-immunoreactive neurons in brain areas with reproductive behaviors in the roughskin newt. *Gen Comp Endocrinol* 117:281–298.
- Moore MC, 1991. Application of organization-activation theory to alternative male reproductive strategies: a review. *Horm Behav* 25: 154–179.
- Moore MC, Hews DK, Knapp R, 1998. Hormonal control and evolution of alternative male phenotypes: generalizations of models for sexual differentiation. *Am Zool* 38:133–151.
- Moore MC, Thompson CW, Marler CA, 1991. Reciprocal changes in corticosterone and testosterone levels following acute and chronic handling stress in the tree lizard, *Urosaurus ornatus*. *Gen Comp Endocrinol* 81:217–226.
- Oliveira RF, Canario AVM, Grober MS, 2001. Male sexual polymorphism, alternative reproductive tactics, and androgens in combtooth blennies (Pisces: Blenniidae). *Horm Behav* 40:266–275.
- Orchinik M., Licht P, Crews D, 1988. Plasma steroid concentrations change in response to sexual behavior in *Bufo marinus*. *Horm Behav* 22:338–350.
- Orchinik M, Murray TF, Moore FL, 1991. A corticosteroid receptor in neuronal membranes. *Science* 252:1848–1851.
- Propper CR, Dixon TB, 1997. Differential effects of arginine vasotocin and gonadotropin-releasing hormone on sexual behaviors in an anuran amphibian. *Horm Behav* 32:99–104.
- Rhen T, Crews D, 2002. Variation in reproductive behavior within a sex: neural systems and endocrine activation. *J Neuroendocrinol* 14:517–531.
- Rose JD, Kinnaird JR, Moore FL, 1995. Neurophysiological effects of vasotocin and corticosterone on medullary neurons: Implications for hormonal control of amphibian courtship behavior. *Neuroendocrinology* 62:406–417.
- Rose JD, Marrs GS, Moore FL, 1998. Rapid, corticosterone-induced disruption of medullary sensorimotor integration related to suppression of amplexic clasping in behaving roughskin newts (*Taricha granulosa*). *Horm Behav* 34:268–282.
- Rose JD, Moore FL, Orchinik M, 1993. Rapid neurophysiological effects of corticosterone on medullary neurons: Relationship to stress-induced suppression of courtship clasping in an amphibian. *Neuroendocrinology* 57:815–824.
- Rubenstein DI, 1980. On the evolution of alternative mating strategies. In: *Limits to action: the allocation of individual behavior* (Staddon JER, ed). New York: Academic Press; 65–100.
- Semsar K, Kandel FLM, Godwin J, 2001. Manipulations of the AVT system shift social status and related courtship and aggressive behavior in the bluehead wrasse. *Horm Behav* 40:21–31.
- Sokal RR, Rohlf FJ, 1981. *Biometry*, 2nd ed. San Francisco: W.H. Freeman and Company.
- Sullivan BK, 1982a. Male mating behavior in the Great Plains toad (*Bufo cognatus*). *Anim Behav* 30:939–940.
- Sullivan BK, 1982b. Sexual selection in Woodhouse's toad (*Bufo woodhousei*), I: chorus organization. *Anim Behav* 30:680–686.
- Sullivan BK, 1983. Sexual selection in the Great Plains toad (*Bufo cognatus*). *Behaviour* 84:258–264.
- Sullivan BK, 1989. Mating system variation in Woodhouse's toad (*Bufo woodhousei*). *Ethology* 83:60–68.
- Sullivan BK, Leek MR, 1986. Acoustic communication in Woodhouse's toad (*Bufo woodhousei*). *Behaviour* 98:305–319.
- Tomkins JL, Simmons LW, 2002. Measuring relative investment: a case study of testes investment in species with alternative male reproductive tactics. *Anim Behav* 63:1009–1016.
- Townsend DS, Moger WH, 1987. Plasma androgen levels during male parental care in a tropical frog (*Eleutherodactylus*). *Horm Behav* 21: 93–99.
- Uglen I, Mayer I, Rosenqvist G, 2002. Variation in plasma steroids and reproductive traits in dimorphic males of corksing wrasse (*Symphodus melops* L.). *Horm Behav* 41:396–404.
- Voorhuis TAM, De Kloet ER, De Wied D, 1991. Effect of a vasotocin analog in singing behavior in the canary. *Horm Behav* 25:549–559.
- Waltz EC, 1982. Alternative mating tactics and the law of diminishing returns: the satellite threshold model. *Behav Ecol Sociobiol* 10:75–83.
- Wells KD, 1977. The social behaviour of anuran amphibians. *Anim Behav* 25:666–693.
- Wells KD, 2001. The energetics of calling in frogs. In: *Anuran communication* (Ryan MJ, ed). Washington: Smithsonian Institution; 45–60.
- Wetzel DM, Kelley DB, 1983. Androgen and gonadotropin effects on male mate calls in South African clawed frogs, *Xenopus laevis*. *Horm Behav* 17:388–404.
- Wilczynski W, Chu J, 2001. Acoustic communication, endocrine control, and neurochemical systems of the brain. In: *Anuran communication* (Ryan MJ, ed). Washington: Smithsonian Institution; 23–35.
- Wingfield JC, Hegner R, Dufty AM Jr, Ball GF, 1990. The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am Nat* 136: 829–846.
- Wingfield JC, Maney DL, Breuner CW, Jacobs JD, Lynn S, Ramenofsky M, Richardson RD, 1998. Ecological bases of hormone-behavior interactions: the “emergency life history stage.” *Am Zool* 38:191–206.
- Wingfield JC, Moore MC, 1987. Hormonal, social and environmental factors in the reproductive biology of free-living male birds. In: *Psychobiology of reproductive behavior: an evolutionary perspective* (Crews D, ed). Englewood Cliffs, New Jersey: Prentice Hall; 149–175.
- Wingfield JC, O'Reilly KM, Astheimer LB, 1995. Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. *Amer Zool* 35:285–294.
- Wingfield JC, Ramenofsky M, 1999. Hormones and the behavioral ecology of stress. In: *Stress physiology in animals* (Balm PHM, ed). Sheffield: Sheffield Academic Press; 1–51.