INTRINSIC AND EXTRINSIC FACTORS MEDIATING THE EXPRESSION OF

ALTERNATIVE MATING TACTICS IN MALE GREEN TREEFROGS,

HYLA CINEREA

by
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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the
requirements of the Sally McDonnell Barksdale Honors College.

Oxford
May 2016

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ACKNOWLEDGEMENTS

ABSTRACT

ALEAH R. LIDDELL: Intrinsic and Extrinsic Factors Mediating the Expression of Alternative
Mating Tactics in Male Green Treefrogs, *Hyla cinerea*
(Under the direction of Dr. Christopher Leary)

Males of many anuran species optimize their chances of reproductive success by vigorously calling to attract females or by adopting a non-calling "satellite" mating tactic. Satellite males perch silently near calling males and attempt to intercept females that the caller attracts. Models predict that tactic expression in male anurans is influenced by either extrinsic factors (i.e., the social-acoustic environment) or intrinsic factors (i.e., circulating hormone levels and body condition), but it is unclear how these factors potentially interact to influence tactic expression. For example, males often adopt a satellite mating tactic in response to the broadcast vocalizations of conspecific males, suggesting that males make mating tactic decisions based on the relative attractiveness of nearby calling males. However, the responses of males to broadcast vocalizations are often highly variable, suggesting that the physiological status of males may also be a primary factor influencing tactic decisions under varying social-acoustic contexts. For example, recent models propose that an increase in circulating glucocorticoids and a decrease in circulating androgens associated with the depletion of energy reserves during vocalization mediate transitions from calling to non-calling behavior in anurans. To examine how the social-acoustic environment and physiological status of males potentially interact to influence mating tactic decisions, I broadcast calls to calling male green treefrogs, *Hyla cinerea*, in natural choruses and observed whether males continued to call or adopted a non-calling satellite mating tactic. I then acquired blood samples and measured the size and weight of males to assess whether the probability that a male remained a caller or switched to a non-calling satellite mating tactic was influenced by circulating hormone levels and/or relative body condition. I found that

males that continued to call and males that adopted a non-calling satellite tactic in response to broadcast calls did not differ in circulating hormone levels. However, males that continued to call were generally larger, heavier, and in better condition than non-callers. Males that continued to call also produced longer, lower-frequency calls and invested more energy in calling. My results suggest that mating tactic expression in *H. cinerea* is determined by relative vocal attractiveness and that vocal quality is influenced by size, weight, and body condition.

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LIST OF ABBREVIATIONS

EHV   Energetics-Hormone Vocalization

T   Testosterone

DHT   Dihydrotestosterone

CORT   Corticosterone

INTRODUCTION

Competition among males is often intense, and in order to achieve reproductive success, some individuals deviate from behavioral norms and adopt alternative mating tactics. Alternative mating tactics are seen in a diverse array of animals, including insects, fish, amphibians, reptiles, mammals, and birds (Álvarez et al., 2013; Hume et al., 2013; Leary, 2014; Moore et al., 1998; Willisch & Neuhaus, 2009; Lank et al., 1995). In lampreys, for example, males may wrap their tails around females and vibrate rapidly as gametes are released or adopt a sneaking tactic. Sneaker males approach and circle spawning pairs during gamete release and swiftly release gametes of their own in an attempt to fertilize the female's eggs (Hume et al., 2013). The tactic which an individual adopts may depend on various factors, including stress level, body condition, and proximity to competitors (Mulrey et al., 2015). For example, giraffe weevils (*Lasiorhynchus barbicornis*) use fighting or sneaking tactics to gain access to females, and the tactic which a male adopts is influenced by body size (Painting & Holwell, 2014). Small males of this species adopt a sneaking tactic by either: 1) hiding under a mating pair and waiting on an opportunity to copulate, 2) copulating with or guarding a female while being guarded by a another male, or 3) tucking its rostrum (long snout) around the side of a female to avoid detection from approaching males. Individuals that hide usually do so until the copulating male is distracted by another male, and the sneaking male is able to intercept the female. Smaller males are more likely to employ a sneaking tactic than the fighting tactic. The fighting tactic involves violent tussling, with males using their elongated rostra and mandibles as weapons. Larger males rely solely on this aggressive behavior to secure females. Other factors aside from body size that may mediate tactic expression in weevils have not been studied, but factors that are likely to be influential are hormones, since they are known to promote sexual behavior in

males (Shuker & Simmons, 2014). Since hormones have been shown to underlie the variation in reproductive behavior of many animals (Oliveira et al., 2008), they are essential in the study of alternative reproductive tactics.

The Relative Plasticity Hypothesis provides a conceptual framework describing the hormonal basis for alternative reproductive tactics (Moore et al., 1998). This hypothesis makes the distinction between fixed and plastic alternative phenotypes. A fixed phenotype is permanently displayed throughout an individual's life and is predicted to arise because of organizational effects of hormones during early development. In contrast, activational effects of hormones may give rise to plastic phenotypes wherein individuals alternate between two or more mating tactics throughout life. Unlike fixed phenotypes, plastic phenotypes are controlled by variation in circulating hormone levels in adulthood. The Relative Plasticity Hypothesis thus predicts that males with fixed reproductive phenotypes should have similar hormonal profiles as adults, while those with plastic phenotypes should differ in terms of adult hormonal profiles (Moore, 1991).

One species that displays both fixed and plastic phenotypes is the tree lizard, *Urosaurus ornatus*. In this species, males vary in the color of the dewlap, a flap that extends below the neck that is used as a visual signal during social interactions (Moore et al., 1998). Males with solid orange dewlaps (orange males) and those with orange dewlaps with blue centers (orange-blue males) show distinct behavioral patterns, or fixed phenotypes; orange-blue males are consistently territorial, while orange males are nonterritorial. Variation in circulating progesterone levels during early development appear to be the hormonal trigger for this differentiation (Moore et al., 1998). Males with high progesterone levels during a critical developmental period, for example, become orange-blue males, whereas males with low progesterone levels become orange males.

Nonterritorial males of this species also show phenotypic flexibility. For example, they switch between nomadic and sedentary behaviors based on environmental conditions. In dry, environmentally harsh years, they adopt nomadic behavior; but during wetter years, they are sedentary. This variation is prompted by changes in stress and sex steroid levels in adulthood (Goodenough et al., 2010). Under stressful conditions, for example, corticosterone (CORT) levels rise and induce a drop in circulating testosterone levels in nonterritorial males, triggering nomadic behavior. When stress levels are not as high, such reciprocal interactions between CORT levels and circulating androgens do not occur, and these individuals remain sedentary.

Although the Relative Plasticity Hypothesis has been incredibly influential in directing research on alternative mating tactics, its fundamental underpinnings have recently been called into question. For example, plastic adult phenotypes are predicted to have different hormonal profiles, but whether these differences are causally associated with mating tactic expression or are a result of tactic expression is often not clear (Oliveira et al., 2001). In the house finch (*Haemorhous mexicanus*), for example, males possess either dull-colored feathers or red feathers. Individuals with red feathers provide higher levels of parental care than do dull males, but dull males are socially dominant in competition for access to food and mates (Oliveira et al., 2008). Testosterone elevation in this species increases investment in mating behavior through increased song rate but decreases investment in parental care. Since high levels of testosterone are generally associated with greater male dominance, a study was conducted to assess whether dominance in duller males reflected a testosterone-dependent mating tactic (Duckworth et al., 2004). Results showed that although testosterone injection in captive males increased dominance rank, free-living redder males with high testosterone levels were not dominant. This implies that testosterone level is unrelated to dominance among free-living house finches and that the social

environment and access to food play a greater role in influencing behavior of these animals than the effects of testosterone. How the social environment and physiology interact to influence mating tactic expression, however, remains largely unexplored.

Anuran amphibians are an ideal system in which to examine influences of the social environment on mating tactic expression. Anurans produce androgen-dependent vocal signals that are used to communicate aggressively with rival males and to attract mates (Leary, 2009). Females use male vocal signals to select males and prefer certain vocal characteristics. Females generally prefer males that have longer calls and shorter intercall durations (Gerhardt & Huber, 2002; Davis & Leary, 2015), and these parameters reflect the energy invested in vocalization (McGregor, 2005). Moreover, spectral characteristics of calls seem to be important in mate selection by females (Gerhardt & Huber, 2002). Some anurans produce calls with spectra that consist of low-frequency and high-frequency components (Gerhardt & Huber, 2002), and female preferences for frequencies vary among species. For example, female *Hyla chrysoselis* prefer low-frequency calls to high-frequency calls, and *Hyla cinerea* females typically prefer calls of average frequency to those of low or high frequency (Gerhardt & Huber, 2002). These vocal characteristics contribute to vocal quality, which may play a role in mating tactic expression among anurans. For example, males of many anuran species alternate between a calling tactic and an alternative non-calling "satellite" mating tactic (Gerhardt & Huber, 2002; Leary, 2009). Non-calling satellite males remain near calling "host" males and attempt to intercept females attracted to the vocalizations of calling males (Perrill et al., 1982). One prevailing hypothesis is that males assess the vocal quality of male competitors and adopt a non-calling satellite mating tactic when the attractiveness of male competitors exceeds their own attractiveness. To address this hypothesis, researchers often use vocal playback studies in which vocalizations are broadcast

to conspecific calling males, and the probability of adopting the satellite tactic or calling behavior is observed. In one such study, green treefrogs (*H. cinerea*) were exposed to a synthetic mating call that reflected the physical properties of a 'typical' conspecific male call. Eleven of the fourteen males that were exposed to this broadcast stimulus adopted the satellite tactic (Perrill et al., 1982). In another playback experiment on rhacophorid frogs, *Philautus variabilis*, 3 of 12 males stopped calling in response to a multi-note advertisement call stimulus, 1 did not change its calling behavior, and 8 switched from a single note to multi-note advertisement call (Srivastava & Rawat, 2001), presumably increasing their localizability (Narins et al., 2000). All 12 individuals changed their orientation to face the speaker and made at least 1 move toward the speaker during the playback. Results from these experiments illustrate the behavioral variation that exists among anurans during social interactions with conspecific males. This behavioral variation, which may be influenced by differences in vocal characteristics between males, makes anurans ideal subjects to study the effects of the social environment on mating tactic expression.

In addition to the social environment, circulating hormone levels are thought to influence mating tactic expression in anurans. For example, the Energetics-Hormone Vocalization (EHV) model predicts that calling behavior is accompanied by increased levels of the hormones testosterone (T) and CORT (Emerson, 2001). Testosterone is responsible for the development of male secondary characteristics and stimulates call production in anurans (Wilczynski et al., 2005). CORT is a glucocorticoid, which is elevated in response to stressors; it promotes mobilization of peripheral energy stores and delays processes in the body that are not needed during stressful events. According to the EHV model, CORT rises during calling because of the energetic demands of calling, and at a certain threshold, it elicits a stress response, which causes levels of circulating T to decrease. This results in a reciprocal relationship between the two

hormones (Nelson, 2005) and is predicted to explain shifts from calling to non-calling behavior. The model predicts that individuals with lower androgen levels and higher CORT will produce less attractive signals and be more likely to become satellites during social interactions. This idea was supported in a study that showed how male-male social interactions can influence hormone levels and behavior in *H. cinerea* (Leary, 2014). In this study, males were exposed to broadcast advertisement calls and aggressive calls. Both types of vocalizations caused androgen levels to drop in males that heard the signals, and aggressive calls caused CORT levels to rise. These results reflect differences in circulating hormone levels for males adopting calling and satellite behavior in this species (Leary & Harris, 2013). For example, calling males have higher circulating androgens and lower CORT levels than satellite males, suggesting that vocal interactions between competing males can drive changes in circulating hormone levels that mediate shifts in mating tactic expression.

While it is clear that hormones often play a key role in influencing mating tactic expression, no previous studies have assessed whether hormone levels explain the behavioral variation observed during vocal playback studies in anurans. The purpose of my study was to assess how the social-acoustic environment and circulating hormone levels potentially interact to influence mating tactic expression in *H. cinerea*. In order to do this, I recorded the calls of males in natural choruses and subsequently exposed these males to broadcast conspecific advertisement calls. I observed their subsequent behavior, collected blood samples, and measured body size and weight to determine if males that called and males that adopted a satellite tactic in response to broadcast calls differed in circulating levels of androgens, glucocorticoids, or estimates of body condition. Based on the predictions of the EHV model, I predicted that males that became satellites in response to the broadcast call would possess higher levels of CORT and lower levels

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of androgens than males that continued to call. I also predicted that males that continued to call would produce calls that were more attractive than those of males that stopped calling in response to broadcast calls.

METHODS

I studied *H. cinerea* at the University of Mississippi Field Station in May and June of 2015. I performed vocal playback experiments and collected data between 2000 and 2300 hours, during peaks of reproductive activity. I used a headlamp with a red light for observations to avoid disturbing the frogs and affecting their behavior.

Vocal Playback Experiment

I used a Marantz PMD 222 recorder with a Sennheiser directional condenser microphone (Model ME-66) to record the calls of males and to broadcast calls to them. First, I located calling males in natural choruses and subsequently recorded a series of 30 calls from each male. I then broadcast two stimuli to the male for about five minutes per stimulus while observing and recording the male's behavioral reaction. The first broadcast stimulus consisted of a white noise control stimulus, which reflected the amplitude envelope of a normal *H. cinerea* advertisement call. The purpose of using this stimulus was to assess whether these frogs react specifically to conspecific male advertisement calls. The second stimulus consisted of an exemplar conspecific male advertisement call that reflected the average values for the study population (dominant carrier frequencies = 800 and 2600 Hz, call duration = 189 ms, intercall duration = 612 ms, vocal effort = 0.23). The recorder was held steadily about one foot away from each calling male as the

stimulus was broadcast to calling males, and during each trial, the focal male's calls were recorded. In response to the stimulus, males either continued to call or stopped calling and adopted a satellite mating tactic. Immediately after presenting both stimuli and recording each male's behavior, I obtained the temperature of calling males using an infrared thermometer and acquired about 100 µl of blood from each male via cardiac puncture with a 28 gauge heparinized needle. Blood samples took up to seven minutes to obtain. After blood collection, weight and snout-vent length (SVL) were measured with a digital OHAUS scale and ruler, and the subjects were tattooed with a "Tatoo-a-Pet" portable tattooing device for future identification. Afterwards, they were immediately released at the site of capture. Blood samples were stored on ice for about 2-5 hours until it was brought to the lab, where it was centrifuged for 12 minutes at 2200 rpm in order to separate the plasma and blood cells. Subsequently, the plasma was frozen at -20ºC until androgen and glucocorticoid levels were measured via radioimmunoassay.

Vocal Analysis

Calls that were recorded before and during the presentation of the broadcast stimulus were imported into Raven Pro acoustics software (Cornell Bioacoustics Laboratory) for analysis of acoustic parameters. About 10 calls from the middle of each vocal bout were selected, and waveforms and audiospectrographs were generated. Vocal parameters known to be important in mate selection were measured for each call. These parameters included call duration (ms), intercall duration (ms), and low and high peak carrier frequencies (Hz). Call duration and intercall duration were incorporated into the following formula to generate a measurement of vocal effort:

$$\text{Vocal Effort} = \frac{\text{Call Duration (ms)}}{\text{Call Duration (ms)} + \text{Intercall Duration (ms)}}$$

Column Chromatography and Radioimmunoassay Procedures

The separation of hormones and quantification of hormone concentrations followed the methodology of Leary et al. (2004). Plasma samples were incubated with radiolabeled hormone overnight in order to obtain recoveries from the samples. Diethyl ether was used to extract steroids from the samples and was subsequently dried at 40ºC under nitrogen gas. Dried samples were then resuspended in 10% ethyl acetate in iso-octane and loaded onto a column of diatomaceous earth, containing 3:1 diatomaceous earth:distilled water "glycol trap" and a 1:1 propanediol:ethylene glycol mixture. Mixtures of 10%, 20%, and 52% ethyl acetate in iso-octane were then used to collect DHT, T, and CORT, respectively. Eluates were dried under nitrogen gas and then resuspended in phosphate-buffered saline, which comprised 0.3% gelatin (PBSg) for use in radioimmunoassay. All samples were assayed in duplicate. The CORT antibody was purchased from MP Biomedicals, LLC (Solon, Ohio). The T antibody used to measure DHT and T was purchased from Fitzgerald Industries International, Inc. (Acton, Massachusetts).

Statistical analysis

All analyses were performed using Statview (SAS Institute, Inc.) and SPSS (IBM Software). First, I determined body condition by taking the residual value from a linear regression of the cubed root body mass on SVL and dividing that value by SVL. This allowed me to take into account the scaling relationship between size and weight, which served as a proxy for energy reserves. Next, effects of ambient temperature on vocal behavior were

accounted for through the correlation of acoustic parameters and body temperature. Temperatures varied between 18° C and 28° C with a mean of 24ºC ($SD = 2$ºC). Linear regressions of temperature on each parameter were produced, and residual values were added to the regression line and adjusted to the mean temperature of 24ºC. Vocal effort was recalculated from the adjusted acoustic parameters. A one-way analysis of variance (ANOVA) was used to compare body condition, body sizes, weights, hormone concentrations, and vocal characteristics of males that continued to call and those that stopped calling and adopted a satellite tactic in response to the broadcast call. Chi-square contingency tests were performed to determine the probability of a male becoming a satellite, based on vocal attractiveness. Acoustic variation in response to the stimuli within groups of frogs that reacted similarly was measured through a paired t-test that incorporated pre-broadcast vocal quality and vocal quality during presentation of the broadcast stimulus. A logistic regression was performed to determine the most likely predictor of reproductive behavior in response to the stimuli. Lastly, vocal parameters of calls produced when males were unchallenged (before the playback) and challenged (during exposure to the playback) were compared using simple linear regression.

RESULTS

Data were obtained from 68 calling male *H. cinerea*. Fifty-four males (79%) continued to call, and fourteen males (21%) stopped calling and became satellites in response to the broadcast advertisement call.

Response to Control Stimulus vs. Advertisement Call Stimulus

Of the 68 calling males used, 20 were presented with the white noise control stimulus. One hundred percent of these males continued to call during the control playback experiments and did not move from their original calling sites. However, 11 of these 20 males reacted differently in response to the advertisement call. Three called normally before becoming aggressive; two called and abandoned their calling sites; three called, became aggressive, and then abandoned their calling sites; and three became aggressive and stopped calling. These results suggest that males do not simply respond to any sound but rather only respond to specific attributes of conspecific male advertisement calls.

Hormone Levels

Blood acquisition time was significantly correlated with an increase in CORT ($r^2_{1,64} = 0.25$, $p = < 0.001$) and a decrease in total androgens ($r^2_{1,69} = 0.10$, $p = 0.01$), so I excluded all blood samples that took more than four minutes to acquire. Using the remaining 63 samples, I regressed body condition against levels of circulating DHT, T, and CORT and found that body condition was positively correlated with levels of DHT ($r^2_{1,61} = 0.10$, $p = 0.01$) (Figure 1A) and T ($r^2_{1,61} = 0.07$, $p = 0.04$) (Figure 1B) and negatively correlated with CORT ($r^2_{1,61} = 0.10$, $p = 0.01$) (Figure 1C). In other words, males in better condition had higher levels of DHT and T and lower levels of CORT. However, there was no detectable relationship between size (i.e., snout-ischial length) and levels of DHT ($r^2_{1,61} = 0.005$, $p = 0.57$), T ($r^2_{1,61} = 0.18$, $p = 0.30$), and CORT ($r^2_{1,61} = 0.007$, $p = 0.17$). Lastly, ANOVA results revealed that there was no significant difference in levels of DHT ($F_{1,61} = 1.15$, $p = 0.29$), T ($F_{1,61} = 0.94$, $p = 0.34$), and CORT
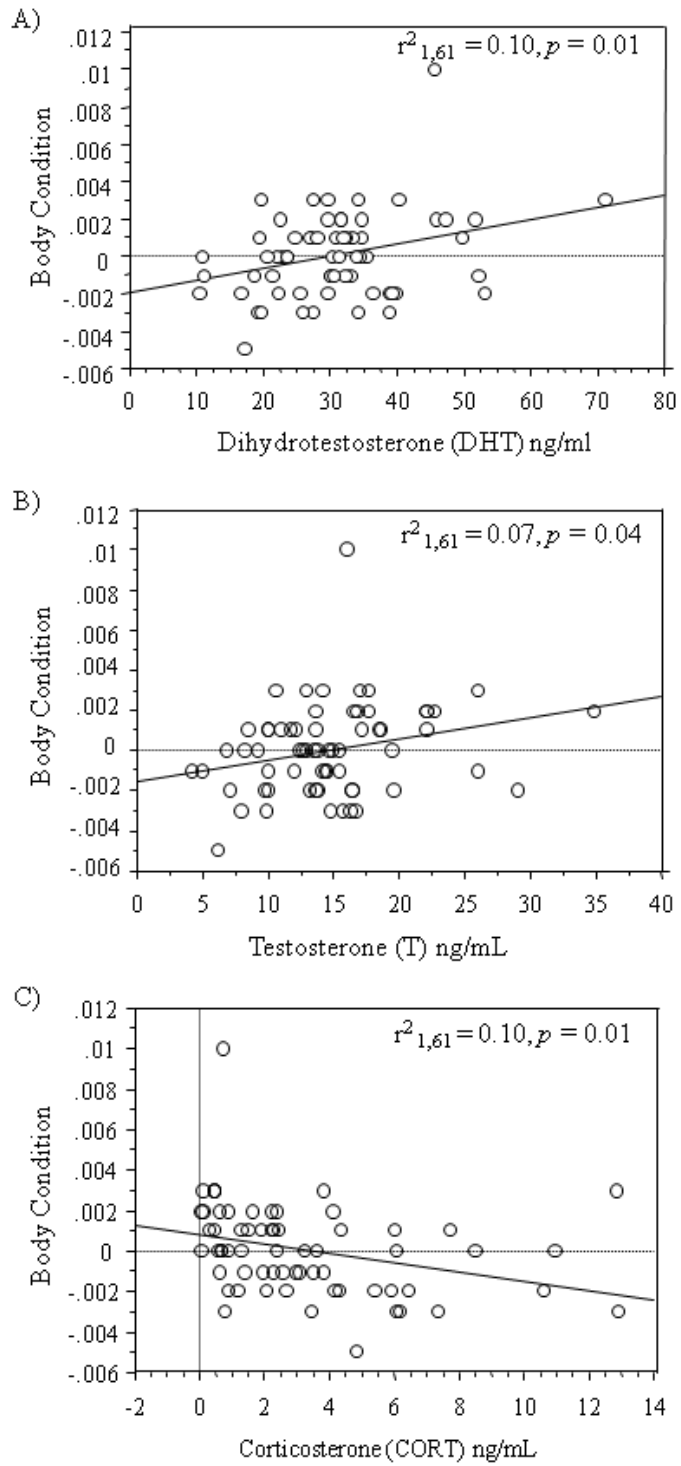
Figure 1. Relationships between body condition and levels of (A) DHT, (B) T, and (C) CORT.

($F_{1,61} = 2.43$, $p = 0.12$) between males that stopped calling and those that continued to call in response to the broadcast stimulus.

Body Condition, Size, and Weight

Males that continued to call in response to the broadcast advertisement call were significantly larger ($F_{1,66} = 12.82$, $p < 0.001$) (Figure 2A), weighed more ($F_{1,66} = 18.78$, $p < 0.001$) (Figure 2B), and were in better body condition ($F_{1,66} = 4.23$, $p = 0.04$) (Figure 2C) than males that became satellites.

Call Attributes

Low Frequency

Males that stopped calling in response to the playback produced calls with higher average carrier frequencies in the low frequency peak of the advertisement call than males that continued to call ($F_{1,65} = 4.06$, $p = 0.05$). Furthermore, larger males produced lower-frequency calls ($r^2_{1,65} = 0.14$, $p = 0.002$) (Figure 3A) as did heavier males ($r^2_{1,65} = 0.14$, $p = 0.002$) (Figure 3B). There was no detectable relationship between low frequency and body condition ($r^2_{1,65} = 0.01$, $p = 0.47$) (Figure 3C) or low frequency and circulating levels of DHT ($r^2_{1,60} = 0.001$, $p = 0.86$), T ($r^2_{1,60} = 0.01$, $p = 0.41$), and CORT ($r^2_{1,60} = 0.0002$, $p = 0.92$).

High Frequency

Males that stopped calling and those that continued to call in response to the broadcast stimulus did not differ in the carrier frequency of the high frequency peak of the advertisement
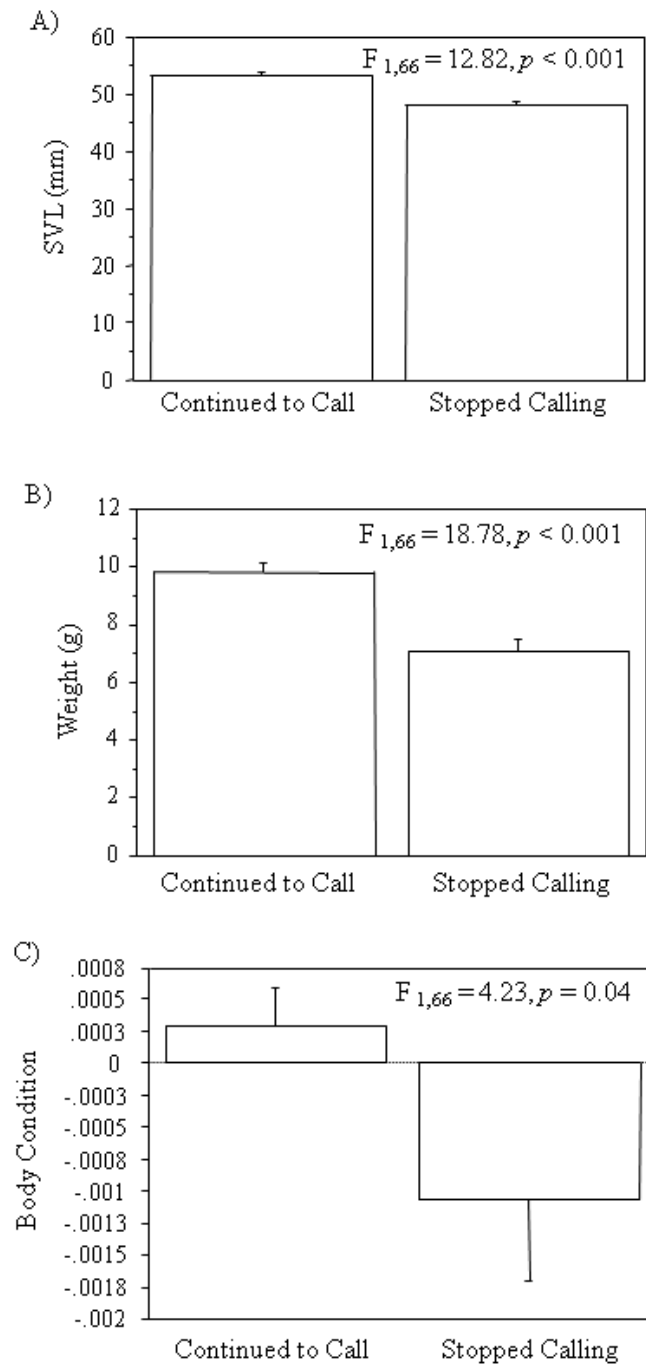
Figure 2. Comparisons of (A) size, (B) weight, and (C) body condition for males that continued to call and those that stopped calling in response to the advertisement call.
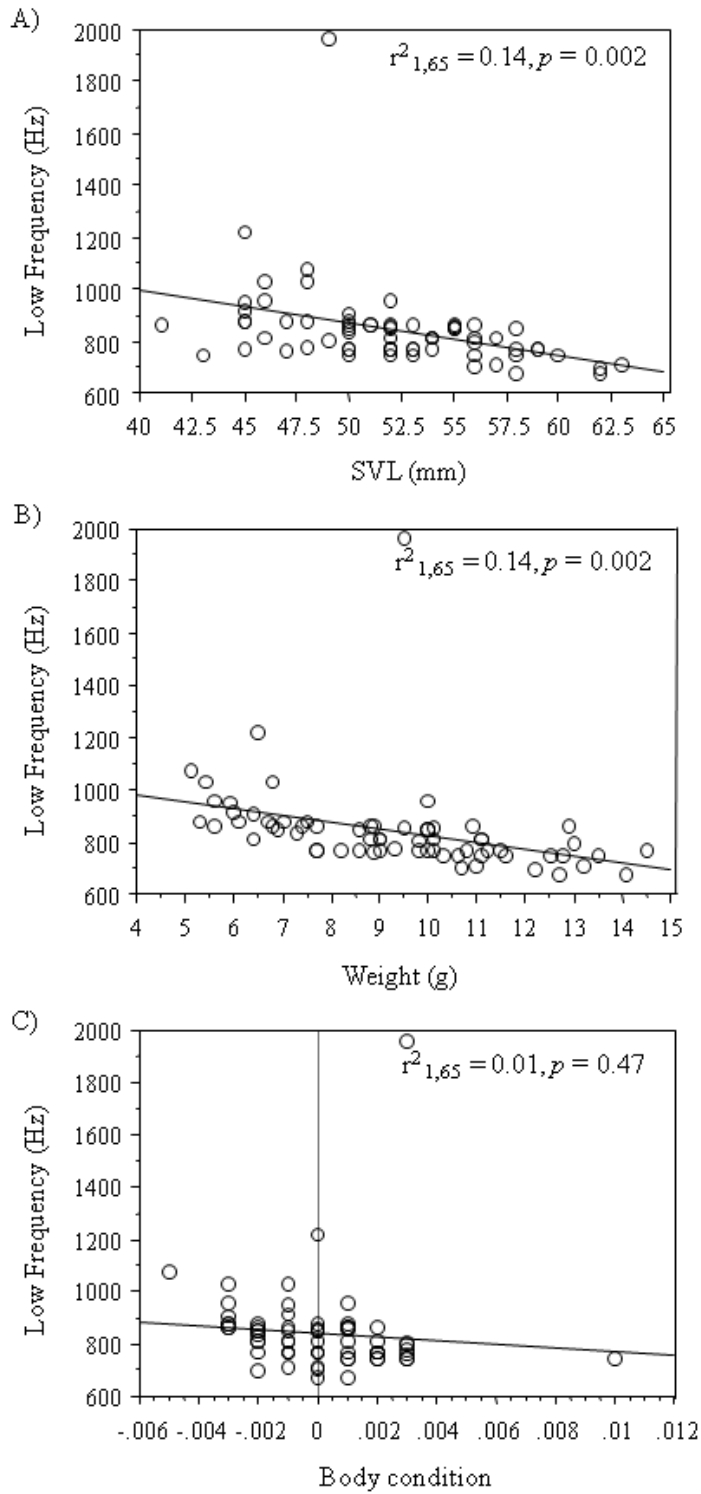
Figure 3. Relationships between low frequency and (A) size, (B) weight, and (C) body condition.

call ($F_{1,65} = 0.20$, $p = 0.07$). However, size ($r^2_{1,65} = 0.11$, $p = 0.01$) (Figure 4A), weight ($r^2_{1,65} = 0.19$, $p < 0.001$) (Figure 4B), and body condition ($r^2_{1,65} = 0.06$, $p = 0.05$) (Figure 4C) were negatively associated with high frequency. There was no significant relationship between high frequency and levels of DHT ($r^2_{1,60} = 0.001$, $p = 0.79$), T ($r^2_{1,60} = 0.01$, $p = 0.35$), and CORT ($r^2_{1,60} = 0.04$, $p = 0.14$).

Call Duration

Males that continued to call in response to the broadcast stimulus produced calls that were longer in duration compared to males that stopped calling ($F_{1,65} = 6.92$, $p = 0.01$) (Figure 5A). Also, call duration of calls produced before the playback was positively correlated with size ($r^2_{1,65} = 0.10$, $p = 0.01$) and weight ($r^2_{1,65} = 0.09$, $p = 0.01$) but was not correlated with body condition ($r^2_{1,65} = 0.01$, $p = 0.49$). There was no relationship between call duration and levels of DHT ($r^2_{1,60} = 0.01$, $p = 0.57$), T ($r^2_{1,60} = 0.03$, $p = 0.18$), and CORT ($r^2_{1,60} = 0.003$, $p = 0.67$).

Intercall Duration

There was no difference between intercall duration for males that continued to call and those that stopped calling in response to the broadcast stimulus ($F_{1,65} = 0.07$, $p = 0.80$). Intercall duration was unrelated to size ($r^2_{1,65} = 0.02$, $p = 0.29$), weight ($r^2_{1,65} = 0.01$, $p = 0.47$), body condition ($r^2_{1,65} = 0.03$, $p = 0.69$), and levels of DHT ($r^2_{1,60} = 0.004$, $p = 0.63$), T ($r^2_{1,60} = 0.002$, $p = 0.70$), and CORT ($r^2_{1,60} = 0.003$, $p = 0.67$).

Vocal Effort

Vocal effort before the stimulus was broadcast was significantly higher for males that continued to call in response to the playback than for those that stopped calling ($F_{1,65} = 6.38$, $p = 0.01$) (Figure 5B). Vocal effort was not significantly related to size ($r^2_{1,65} = 0.04$, $p = 0.12$),
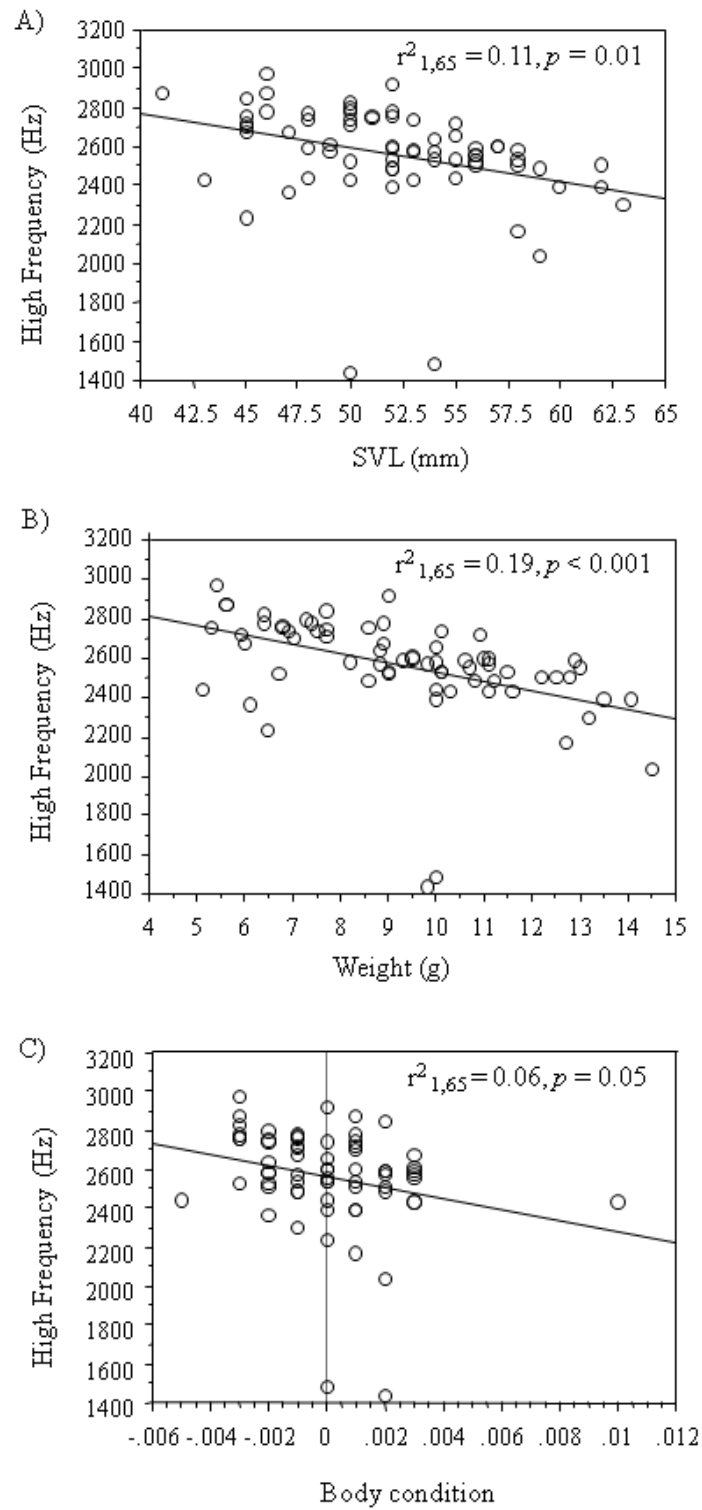
Figure 4. Relationships between high frequency and (A) size, (B) weight, and (C) body condition.

A)

Call Duration (ms)

$F_{1,65}=6.92, p=0.01$

Continued to Call   Stopped Calling

B)

Vocal Effort

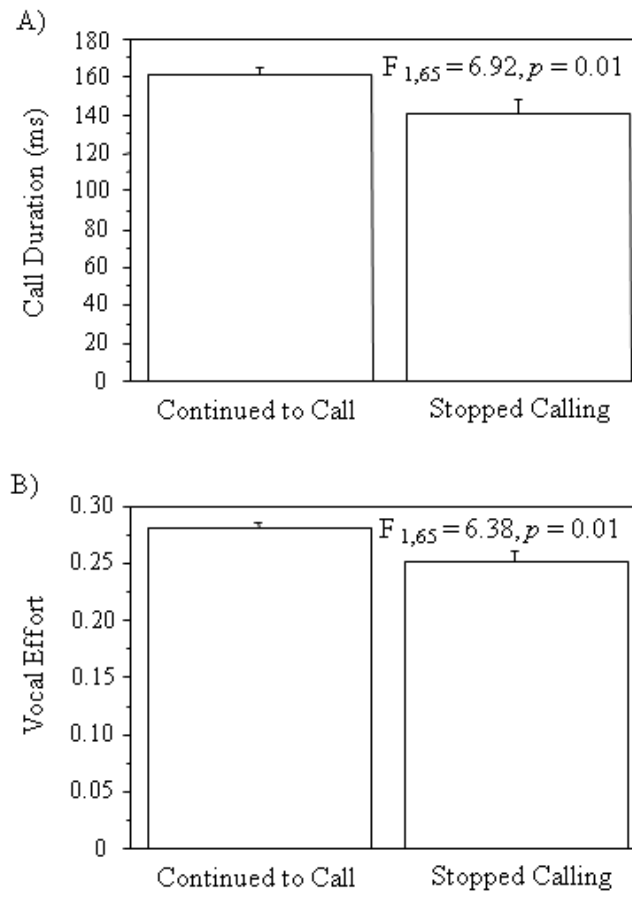$F_{1,65}=6.38, p=0.01$

Continued to Call   Stopped Calling

Figure 5. Differences in (A) call duration and (B) vocal effort between males that continued to call and those that stopped calling in response to the playback.

weight ($r^2_{1,65} = 0.03$, $p = 0.15$), or body condition ($r^2_{1,65} = 0.003$, $p = 0.65$). There was no relationship between vocal effort and circulating levels of DHT ($r^2_{1,60} = 0.0001$, $p = 0.93$), T ($r^2_{1,60} = 0.02$, $p = 0.31$), or CORT ($r^2_{1,60} = 0.0004$, $p = 0.88$).

Probability of Calling

I performed chi-square tests to determine whether the vocal characteristics of males predicted their likeliness to call in response to the broadcast call. Male behavior during exposure to the playback could not be predicted by their vocal characteristics before the broadcast stimulus was presented (Table 1). However, behavior could be predicted by low frequency $\chi^2 (1, N = 67) = 4.62$, $p = 0.03$, high frequency $\chi^2 (1, N = 67) = 7.40$, $p = 0.01$, and vocal effort $\chi^2 (1, N = 67) = 3.95$, $p = 0.05$ of calls produced during exposure to the broadcast call (Table 1). In terms of low frequency, 15% of males that eventually stopped calling had low frequencies that were lower than that of the broadcast call, while 48% of males that continued to call had lower low frequencies (Table 1). A greater percentage of calling males (97%) than males that stopped calling (77%) had greater calling effort than that of the broadcast call during the playback (Table 1). Lastly, 84% of males that stopped calling had calls of higher high frequencies than the broadcast call, whereas the majority of males that continued to call (57%) had calls of lower high frequencies than the broadcast call (Table 1).

Within-group Variation between Changes in Vocal Quality

In order to determine the extent to which vocal characteristics changed within the 2 behavioral groups upon exposure to the advertisement call, I performed paired t-tests. I did not use values that were adjusted for temperature, since paired t-tests involve within-group variation. Males that continued to call significantly increased their call duration ($t_{51} = -7.56$, $p < 0.001$) and

Chi-Square Values for Vocal Parameters

	Vocal Parameter	Broadcast Quality	Continued to Call		Stopped Calling		Chi Square	*p* Value
			Percentage Higher	Percentage Lower	Percentage Higher	Percentage Lower		
Pre-Broadcast	High Carrier Frequency (Hz)	2600 Hz	42.59%	57.41%	46.15%	53.85%	0.05	0.82
	Low Carrier Frequency (Hz)	800 Hz	53.70%	46.29%	76.92%	23.07%	2.32	0.13
	Call Duration (ms)	189 ms	11.11%	88.89%	7.69%	92.31%	0.13	0.72
	Intercall Duration (ms)	612 ms	3.70%	96.29%	0.00%	100.00%	0.49	0.48
	Vocal Effort	0.23	90.74%	9.26%	76.92%	23.08%	1.90	0.17
During Broadcast	High Carrier Frequency (Hz)	2600 Hz	42.59%	57.41%	84.62%	15.39%	7.40	**0.01**
	Low Carrier Frequency (Hz)	800 Hz	51.85%	48.15%	84.62%	15.39%	4.62	**0.03**
	Call Duration (ms)	189 ms	20.37%	79.63%	0.00%	100.00%	3.17	0.07
	Intercall Duration (ms)	612 ms	7.41%	92.59%	15.39%	84.62%	0.82	0.37
	Vocal Effort	0.23	97.44%	5.56%	76.92%	23.08%	3.95	**0.05**

Table 1. Chi-square contingency test results that show the probability that a male will continue to call or stop calling in response to the broadcast call. Significant *p* values are bolded. Percentages represent the proportion of males that produced calls of either higher or lower value for each parameter relative to the broadcast call.

intercall duration (t_{51} = -3.35, p = 0.002), which resulted in no net change of vocal effort. However, these males did not change the carrier frequencies of their calls. Males that stopped calling did not significantly change either call parameter.

DISCUSSION

Circulating levels of DHT, T, and CORT did not differ between male *H. cinerea* that stopped calling and those that continued to call in response to the broadcast vocalizations. This is interesting, because in natural choruses, satellites typically have higher levels of CORT and lower levels of androgens (Leary & Harris, 2013). I did find that androgen levels were positively linked with body condition and that males in better body condition were more likely to continue to call in response to the broadcast stimulus. These results suggest that hormones are linked to characteristics that make males more or less likely to call, but hormone levels do not determine mating tactic expression. What I found to be the best predictor of mating tactic expression was relative vocal attractiveness; males that were more attractive were more likely to continue to call in response to the broadcast stimulus.

Compared to males that continued to call in response to the broadcast stimulus, males that eventually stopped calling were more likely to produce calls of higher frequency than the broadcast stimulus, and they invested less energy in their calls. This suggests that males stopped calling and adopted the satellite tactic because of their relative unattractiveness. The influence of size and body condition on attractiveness may explain this finding. Research on *H. cinerea* has shown that body condition can influence tactic expression and acoustic properties (Humfeld, 2013). Males of poor body condition produce higher-frequency calls because of energy depletion

from calling. This depletion leads to vocal fatigue, which is characterized by increased airflow rates through the respiratory system. When muscle glycogen is depleted, performance in the trunk muscles that generate pulmonary pressure is reduced; and this leads to forceful expulsions of air that form the short-duration, high-frequency calls often produced by males in poor body condition (Humfeld, 2013). Thus, males in poor condition may be physically constrained to produce unattractive calls. Like poor body condition, small size may also place an energetic constraint on males because if its association with decreased energy stores (Tejedo, 1992). Though body condition is expected to influence call duration, I did not observe this effect. However, body condition was positively correlated with weight and size. Furthermore, males that stopped calling and adopted the satellite tactic produced calls that were shorter in duration, were smaller, and were in poorer body condition than males that continued to call. My results suggest that males adopted the satellite tactic because their calls were less attractive than the broadcast call.

Anuran males that become satellites usually parasitize males with lower dominant frequencies (Berec & Bajgar, 2011), perhaps because they are larger and more likely to be reproductively successful. It is advantageous to parasitize larger males, because these larger males are the most attractive to females and tend to have loud calls and defend large territories, sometimes making localization by females easier. This may help explain relative vocal attractiveness as a predictor of reproductive behavior and the overall difference in size between callers and satellites.

I found that males that continued to call exerted more vocal effort than those that stopped calling, but vocal effort was not related to size, weight, body condition, or hormone levels. This supports the idea that males adopted the satellite tactic because of their relative attractiveness,

rather than their physiology. Interestingly, there was no correlation between any other vocal parameter and hormone levels, further suggesting that vocal quality varied among individuals independently of hormones. These results do not support the EHV model, which predicts that there will be a graded relationship between vocal quality and circulating androgen and CORT levels.

Furthermore, I found a negative correlation between size and call carrier frequencies as well as weight and call frequencies. Thus, larger, heavier males had overall lower-frequency calls. These results are consistent with previous findings and support the idea that larger males produce more attractive calls (Berec & Bajgar, 2011). This idea was further supported by my finding that larger males produced calls that were longer in duration. All of these traits that were characteristic of larger, heavier males were also characteristic of males that continued to call in response to the playback; they had lower frequencies and longer call durations than males that became satellites. Surprisingly, intercall duration was unlinked to all other variables in the study.

Within-group variation between vocal characteristics of males before and after exposure to the broadcast call was different for satellites and callers. Callers did not significantly change in terms of low frequency or high frequency, but they did significantly increase their intercall duration and call duration, resulting in no net change of vocal effort. In contrast, males that stopped calling did not significantly alter any vocal parameter.

In summary, my research shows that mating tactic expression in *H. cinerea* is influenced by weight, body condition, size, and vocal quality. Hormone levels were related to body condition, which is known to affect the propensity to call, but it is clear that elevated glucocorticoids and low androgens are not required to elicit changes from calling to non-calling satellite tactic expression in this species. In a previous study on *H. cinerea*, satellite males had

higher levels of CORT and lower levels of T and DHT than callers (Leary & Harris, 2013).

However, I observed variation in reproductive behavior without these hormonal differences.

Thus, hormone levels were not predictors of mating tactic expression. My results imply that

males choose to adopt a certain tactic based on their relative vocal attractiveness to other males.

Further research should be conducted on other possible effects of the social environment

and physiology on *H. cinerea* tactic expression. This could be done by observing the extent to

which tactic expression is dependent on population density and possible advantages of choosing

to adopt one tactic over another in areas of high and low density. Other factors that should be

assessed are possible age-dependent differences in behavior. In bullfrogs (*Rana catesbeiana*),

larger males are typically older than smaller males and are less likely to adopt the satellite tactic.

Older males of this species are presumably at an advantage, because they demonstrate the ability

to survive through various environmental conditions (Howard, 1978, 1983, 1984). However, this

age-size-behavior relationship is not seen in Woodhouse's toads (*Bufo woodhousii*) and Great

Plains toads (*Bufo cognatus*) (Leary et al., 2005). These results suggest that reproductive

behavior reflects age-dependent differences in some anurans but not others. Further research

should be conducted on *H. cinerea* to discover whether there is a relationship between age and

behavior within this species. This may lead to a better understanding of the factors that give rise

to variation in mating tactic expression in *H. cinerea*.

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