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Vocal Behavior of the Explosively Breeding Neotropical Yellow Toad, *Incilius luetkenii*

NICK REHBERG-BESLER,[1,2] STEPHANIE M. DOUCET,[1] AND DANIEL J. MENNILL[1,2]

[1] *Department of Biological Sciences, University of Windsor, Ontario, Canada*

ABSTRACT.—**Frogs and toads exhibit complex vocal behavior, often in the context of mating and resource defense. Although over 6,500 species of frogs and toads have been described, detailed accounts of vocal behavior exist for very few species. In this study, we provide the first detailed investigation of the vocal behavior of Neotropical Yellow Toads, *Incilius luetkenii*, based on recordings collected from animals in northwestern Costa Rica. We provide quantitative measurements of two call types, we test the hypothesis that male body size is related to the structure of male calls, we investigate the context in which males produce calls, and we discuss the importance of their vocal behavior in the framework of an aggressive scramble competition mating system. We found that male *I. luetkenii* produce two vocalizations during their explosive breeding aggregations: advertisement calls and release calls. Advertisement calls are 2.5 sec-long vocalizations consisting of a series of pulses. Release calls are 0.6 sec-long vocalizations consisting of a small number of pulses and produced when other males attempt amplexus on them. As predicted, spectral properties of advertisement calls were inversely related to body size. Video analysis of male calling behavior revealed a call rate of 0.58 calls per minute and revealed that most advertisement calls occur within 2 sec following a contact with another male. Our findings suggest that advertisement calls and, by extension, chorusing behavior are associated with aggression between males during mating aggregations and that males may communicate acoustic information about their body size during male–male encounters.**

Frogs and toads produce complex vocalizations and provide us with a model system for studying acoustic communication (Gerhardt and Huber, 2002). The vocalizations of anurans vary in structure and function across species and they typically occur during the breeding period (Wells, 2007). Males are the more vocal of the two sexes and often have a repertoire of different types of vocalizations that they use in different contexts (Wells and Schwartz, 2007). The evolution of male advertisement calls appears to be driven by sexual selection for female mate choice and male–male competition (Ryan, 1988; Gerhardt, 1994). Specific properties of male advertisement calls can be assessed by females to choose a desirable mate (e.g., fundamental frequency; Ryan, 1980) or by rival males to assess location, body size, and fighting ability (Davies and Halliday, 1978; Gerhardt and Bee, 2007).

Across diverse animal taxa, vocal properties are related to body size, including in anurans (e.g., Gingras et al., 2013), fish (e.g., Ladich et al., 1992), mammals (e.g., Fitch, 1997), and birds (e.g., Martin et al., 2011). Information about the body size of calling males may be important to females interested in choosing a high quality breeding partner (e.g., Wilbur et al., 1978) or to other males engaged in aggressive interactions (e.g., Reby et al., 2005). In frogs specifically, many studies have provided empirical support for the hypothesis that call frequency varies allometrically with body size (e.g., Zweifel, 1968; Bee et al., 1999; Gingras et al., 2013). Therefore, this relationship appears to be a widespread component of communication in animals generally and anurans specifically.

In addition to their role in sexual selection, vocalizations can provide critical information for discriminating between closely related, cryptic, and elusive species (Padial et al., 2008; Vieites et al., 2009). Acoustic monitoring has helped to locate rare species, to quantify population densities, to monitor habitat restoration, and to provide a framework for wildlife management and conservation efforts (Blumstein et al., 2011; Marques et al., 2013; Lambert and McDonald, 2014). In spite of the importance of vocalizations for understanding the behavior, ecology, and conservation of anurans, relatively few species' vocalizations have been described in detail. A recent resurgence of rigorous quantifications of anuran vocal behavior has proven fruitful; for example, by helping describe elusive species in the Western Ghats of India (Bee et al., 2013a,b). With a 70% decline in overall population density of anurans in some Neotropical environments, understanding the behavior and ecology of anurans in these environments is increasingly important to facilitate conservation action (Whitfield et al., 2007).

Our goal in this study was to provide the first quantitative investigation of the vocalizations and vocal behavior of Neotropical Yellow Toads, *Incilius luetkenii* (formerly *Bufo luetkenii*, *Cranopsis luetkenii*, and *Ollotis luetkenii*). We sought to provide a rigorous quantification of the spectro-temporal features of their advertisement calls, to test the hypothesis that body size is related to acoustic features, and to determine the context of male vocal behavior. We predicted that, as in other anurans (e.g., Gingras et al., 2013) and nonanuran taxa (e.g., Martin et al., 2011), *I. luetkenii* would show a negative relationship between the spectral frequency of male advertisement calls and body size metrics.

METHODS

Study Site and Species.—We conducted this study in Sector Santa Rosa of the Guanacaste Conservation Area (10°40′N, 85°30′W), a United Nations Educational, Scientific, and Cultural Organization (UNESCO) World Heritage Site dominated by Neotropical dry forest habitat in northwestern Costa Rica (Fig. 1). The climate at this site is defined by little to no rain during the dry season from November to May and abundant rainfall during the rest of the year (Campos and Fedigan, 2013). The distribution range of *I. luetkenii* stretches from southern Mexico to central Costa Rica, and the animals are common in Sector Santa Rosa (Doucet and Mennill, 2010).

Incilius luetkenii emerge from underground hideaways at the onset of the rainy season and form breeding aggregations (Savage, 2002; Doucet and Mennill, 2010). Annually, we witness hundreds of individuals aggregating in seasonally dry creek

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[2] Corresponding authors. E-mail: rehbergbeslern@gmail.com, dmennill@uwindsor.ca

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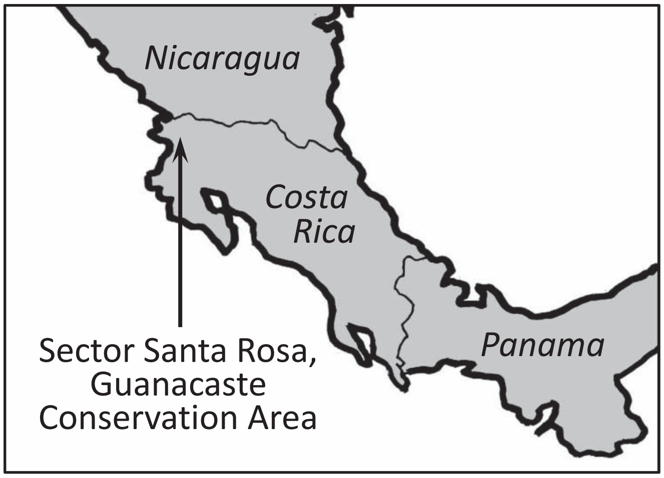

FIG. 1. Map of Costa Rica showing the study site in the Neotropical dry forests in Sector Santa Rosa of the Guanacaste Conservation Area.

beds that have been filled by the first significant rain of the year, which occurs at unpredictable times, usually in the month of May at our study site (range: 28 April to 6 June over the 9 yr between 2007 and 2015). At our site, aggregations form during the evening of the first rain, peak in number in the following morning, and may persist at lower numbers for an additional 1–2 days.

Within their breeding aggregations males spend their time calling, interacting with other males, and searching for gravid females as they arrive at the water's edge. Male *I. luetkenii* vocalize during the breeding event. Only one type of call—the advertisement call—has been described to date and only in rudimentary detail (Porter, 1966; Savage, 2002). When a male successfully enters amplexus with a female, the pair remains this way throughout the morning, or longer. We have observed egg laying only after most unmated males have left the pond, usually in the late morning or afternoon. The mating system of *I. luetkenii* can be best understood as scramble competition mating (Rehberg-Besler et al., 2015). Males do not hold resources but actively compete with rival males by engaging in male–male displacement behavior while searching for incoming females (Wells, 1977). Notably, males exhibit bright yellow coloration during their explosive breeding events and have been documented to rapidly shift to a cryptic, female-like brown coloration following amplexus (Doucet and Mennill, 2010).

Data Collection.—We recorded advertisement calls from 74 individual male toads during the 2013 breeding event (May 23–25) and then weighed and measured the mass and snout–vent length of each male (200 g spring scale accurate to 1 g and dial calipers accurate to 0.1 mm). We recorded males with AT815b (Audio-Technica, Inc., Tokyo, Japan) and ME66/K6 (Sennheiser, Inc., Wedemark, Germany) shotgun microphones and PMD-660 (Marantz, Inc., Kawasaki, Japan) solid-state digital recorders (recording format: WAVE; sampling frequency: 44.1 kHz; accuracy: 16 bits). We stood approximately 1 m from a male, pointing the shotgun microphone directly at him, and followed him until we had recorded at least 1 advertisement call (mean ± SE = 3.8 ± 0.3 advertisement calls recorded per toad; $n = 74$). We then captured the focal toad, measured his size, and released him 100 m away at a nearby pond to avoid the possibility of measuring the same animal twice. Males were readily distinguished from females based on their color and behavior (Doucet and Mennill, 2010; Rehberg-Besler et al., 2015). We did not measure the temperature of the toads at the time of capture; future research should explore how male vocalizations may vary with temperature (e.g., Zweifel, 1968).

We recorded release calls in the same way as advertisement calls. Release calls are uttered far less often than advertisement calls, and we were able to collect recordings of only eight release calls from five toads. Given our small sample size of release call recordings, we did not analyze release calls in the same depth as advertisement calls; we focused on basic measurements of length and dominant frequency. The animals we recorded producing release calls were different animals from those recorded producing advertisement calls.

Acoustic Quantification.—We measured nine spectro-temporal features of male advertisement calls: 1) call duration (the length from the start of the first pulse to the end of the last pulse); 2) call rise time (time from start of the first pulse to the start of the loudest pulse); 3) call fall time (time from the end of the loudest pulse to the end of the last pulse); 4) number of pulses per call; 5) pulse rate; 6) pulse duration (the average length of all pulses in the call); 7) pulse period (the average delay from the start of one pulse to the start of the next pulse); 8) frequency of the first spectral peak (see below); and 9) frequency of the second spectral peak (see below; Table 1). Each call was isolated and measured using Avisoft-SASLab Pro (V5.2, R. Sprecht, Berlin, Germany), relying on the automated measurement features of AviSoft to minimize human subjectivity in call measurement. For temporal properties, we used the pulse-train analysis feature of AviSoft (settings: rectification + exponential decay envelope; peak search

TABLE 1. Description of nine spectro-temporal features of male *Incilius luetkenii* advertisement calls that were measured in this study.

Type of acoustic feature	Description
Temporal properties	
Call duration (sec)	Time between onset of first pulse[a] and offset of the last pulse in a call
Call rise time (sec)	Time between onset of first pulse and the onset of the pulse with maximum amplitude
Call fall time (sec)	Time between onset of the pulse with maximum amplitude and offset of the last pulse
Pulses per call	Number of pulses in a call (k)
Pulse rate (pulses/sec)	Number of pulses minus 1 ($k - 1$) divided by the time between call onset and onset of last pulse
Pulse duration (sec)	Time between pulse onset and pulse offset
Pulse period (sec)	Time between pulse onset and onset of the next pulse in a call
Spectral properties	
Dominant frequency of first spectral peak (Hz)	Frequency at each pulse's first spectral peak, averaged across all pulses in a call
Dominant frequency of second spectral peak (Hz)	Frequency at each pulse's second spectral peak, averaged across all pulses in a call

[a] First pulse was determined as the first pulse that rose above background noise in a continuous bout of pulses within a call.

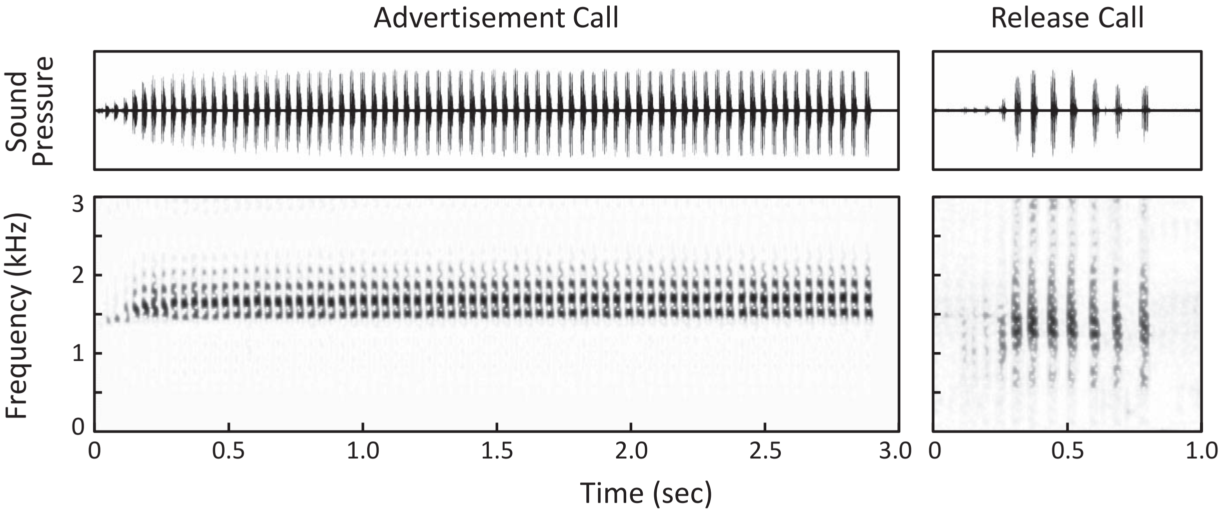

FIG. 2. Waveforms (top) and spectrograms (bottom) of a typical male Neotropical Yellow Toad *Incilius luetkenii* advertisement call (left) and release call (right).

with hysteresis; we adjusted the threshold to the minimum level that allowed us to separate the pulses as separate amplitude peaks). For spectral properties, we used the automatic parameter measurement feature of Avisoft (FFT length: 512 Hz; Hamming window; frame size 25%; temporal overlap 93.75%; calls were resampled to 6,000 Hz prior to measurement). We found that advertisement calls have two dominant spectral peaks (see Results) and substantial variation in regard to which of these two peaks was dominant (i.e., the peak with the highest relative amplitude). We therefore measured the first and second peaks separately, using the cutoff frequency function of AviSoft to isolate either the upper or lower frequency peak near the middle of a 200-Hz bandwidth frequency window (Fig. 2f,g). The frequency of each pulse was measured at the point of maximum amplitude, and pulses were automatically detected after adjusting the threshold above background but below pulse amplitude peaks. The 74 individuals used in statistical analysis contributed 279 advertisement calls, averaging 3.8 calls per individual (range: 1–11).

Video Data Collection.—To quantify the context of male calling behavior, we collected 10-min video recordings of 15 males during the 2015 breeding event that took place on 7 June 2015. We followed each focal male from a distance of approximately 1 m, recording his behavior with a DNV16HDZ video camera (Bell and Howell, Inc., Durham, North Carolina, USA), keeping the focal animal in the center of the frame. During these recordings we stated when the focal male vocalized, which was corroborated by observing vocal sac inflation during video analysis.

We analyzed the video recordings using JWatcher (v.1.0; Blumstein and Daniel, 2007) and noted the time of each of the following six behaviors: 1) focal male produced an advertisement call ($n = 76$; numbers represent total number of instances of the behavior across all of our video recordings); 2) nonfocal male produced an advertisement call ($n = 81$); 3) focal male initiated contact with another male ($n = 171$); 4) nonfocal male initiated contact with focal male ($n = 116$); 5) focal male approached within one body length of nonfocal male without contacting him ($n = 62$); and 6) nonfocal male approached within one body length of focal male without contacting him ($n = 54$). Although we watched for cases of males approaching a female, none occurred in our sample. We did not detect release calls in our video recordings. In two of the 15 videos, the focal male did not call during the 10-min trial, and these trials were excluded from our analyses.

Statistical Analysis.—Our two body size measurements (snout–vent length and mass) were highly correlated ($r = 0.82$, $n = 74$) and we therefore chose to use snout–vent length as our measurement of body size (hereafter, we refer to this measure simply as "body size"). We used Pearson correlations to describe the association between body size and nine spectro-temporal features of male advertisement calls. To maintain an overall significance threshold of 0.05 across these nine correlations (i.e., body size vs. the nine spectro-temporal features), we applied a Bonferroni correction and considered correlations with associated P-values lower than 0.0056 to be significant in these analyses.

We analyzed our time-stamped behavioral video data by quantifying how many times each of the above six behaviors occurred, how many times each behavior directly preceded a male advertisement call, and the average length of time between the focal male call and the behavior that directly preceded it.

We calculated coefficients of variation (CV = 100% × standard deviation / mean) to quantify the degree of variation in different call features (as in, for example, Gerhardt, 1991; Gerhardt and Huber, 2002). We were interested in quantifying sources of within-individual variation and between-individual variation, as in previous studies (e.g., Bee et al., 2013a,b; Thomas et al., 2014). We computed within-individual variation (CV_w) from all males that produced more than one advertisement call ($n = 59$, range: 2–11 calls). We calculated CV_w using the average and standard deviation for each male and then averaged to get a single mean CV_w for each call feature. We calculated between-individual CV (CV_b) by using the overall average and standard deviation from all males to create a single CV_b value for each call feature.

All statistical values are presented as mean ± SE, all tests are two-tailed, and all analyses were conducted in SPSS 20 (IBM, Chicago, IL, USA).

RESULTS

Call Properties.—Male *I. luetkenii* produced advertisement calls (Fig. 2) with a length of 2.5 ± 0.7 sec. These calls consisted of a series of rapid pulses (pulse duration: 22.5 ± 0.3 msec) with a pulse rate of 22.1 ± 0.3 pulses per second. The call generally

TABLE 2. Mean and standard errors of nine spectro-temporal features of the advertisement calls of male *Incilius luetkenii* ($n = 74$). Coefficients of variation (CV) are presented for nine spectro-temporal features for between individuals (CV_b) and within individuals (CV_w) ($n = 59$).

Type of acoustic feature	Feature	Mean	SE	CV_b	CV_w
Temporal properties	Call duration (sec)	2.52	0.1	26.3	26.1
	Call rise time (sec)	1.76	0.1	33.8	41.0
	Call fall time (sec)	0.76	0.1	62.5	69.9
	Pulses per call	55.7	1.5	24.5	25.6
	Pulse rate (pulses/sec)	22.1	0.3	11.3	1.8
	Pulse duration (sec)	0.023	0.0003	12.9	5.4
	Pulse period (sec)	0.046	0.005	11.2	2.3
Spectral properties	Frequency of first spectral peak (kHz)	1.42	0.01	6.1	3.9
	Frequency of second spectral peak (kHz)	1.56	0.01	5.5	3.4

reached its peak amplitude after 1.75 ± 0.07 sec and persisted for 0.75 ± 0.06 sec after this peak. Power spectra of advertisement calls revealed two dominant spectral peaks. These two peaks varied in terms of which one had a higher amplitude, sometimes from pulse-to-pulse within a male's call. The lower peak had an average frequency of 1.42 ± 0.11 kHz whereas the higher peak had an average frequency of 1.56 ± 0.10 kHz (Table 2).

Male advertisement calls contained some properties that showed high coefficients of variation and some with low coefficients of variation. Call duration, call rise time, call fall time, and number of pulses per call were all highly variable both within and between individuals (i.e., CVs > 24%; Table 2). Pulse rate, pulse duration, pulse period, and both spectral frequency measures showed low coefficients of variation both within and between individuals (i.e., CVs < 13%). When looking solely at within-individual variation, each of these five properties exhibited little variation (i.e., $CV_w < 6\%$; Table 2).

Males also produced release calls (Fig. 2). These calls were produced much less frequently; we recorded this call in eight instances from five individuals. Males produced release calls when another male fully clasped the focal male in an amplexus-like position, letting go after the release call. We found an average release call duration of 0.58 ± 0.05 sec, with 8.2 ± 0.5 pulses per call, and an average dominant frequency of 1.2 ± 0.04 kHz.

Body Size and Advertisement Calls.—Male *I. luetkenii* had a snout–vent length of 82.1 ± 0.6 mm (range: 71.1–93.5 mm) and weighed 47.5 ± 1.0 g (range: 31–72 g; $n = 74$). The spectral features of male *I. luetkenii* advertisement calls showed a significant inverse relationship with body size (i.e. snout–vent length; Table 3, Fig. 3), with larger males producing lower-frequency calls. We found no significant correlations between temporal measurements and body size (all $r < 0.15$, $P > 0.11$; $n = 74$).

TABLE 3. Pearson correlations between body size (snout–vent length) and nine spectro-temporal features of advertisement calls of male *Incilius luetkenii* ($n = 74$).

Type of acoustic feature	Feature	Body size	
		r	P
Temporal properties	Call duration (sec)	0.03	0.82
	Call rise time (sec)	0.13	0.28
	Call fall time (sec)	−0.11	0.33
	Pulses per call	0.01	0.95
	Pulse rate (pulses/sec)	−0.06	0.64
	Pulse duration (sec)	0.06	0.60
	Pulse period (sec)	0.06	0.59
Spectral properties	Frequency of first spectral peak (kHz)	−0.55	0.001
	Frequency of second spectral peak (kHz)	−0.51	0.001

Video Analysis of Calling Behavior.—Video analysis revealed that male advertisement calls were always associated with inflation of the male vocal sac (Fig. 4). Our observations of release calls, in contrast, suggest this call does not require full inflation of the vocal sac. Males produced advertisement calls at a rate of 0.58 ± 0.16 calls per minute ($n = 76$ calls from 13 males). In 66% of cases where we recorded a focal male calling, the focal male called immediately after being contacted by another male (average time interval: 1.04 ± 0.12 sec between contact and vocalization); in 12% of cases, the focal male called immediately after making contact with another male (average time interval: 1.67 ± 0.26 sec between contact and vocalization); in another 12% of cases, the focal male called immediately after another male within frame produced an advertisement call (average time interval: 1.79 ± 0.11 sec between call 1 and call 2); in 9% of cases, the focal male's call was preceded only by his previous call (average time interval: 3.28 ± 0.44 sec between calls); and in the final 1% of cases, the focal male called after approaching a nonfocal male within one body length (time interval: 3.42 sec).

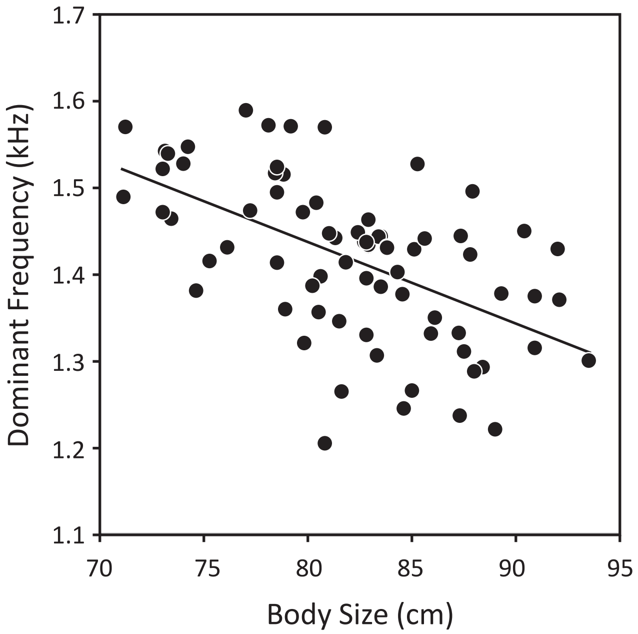

FIG. 3. The dominant frequency of the advertisement calls produced by male Neotropical Yellow Toads *Incilius luetkenii* shows an inverse relationship with male body size (measured as snout–vent length).

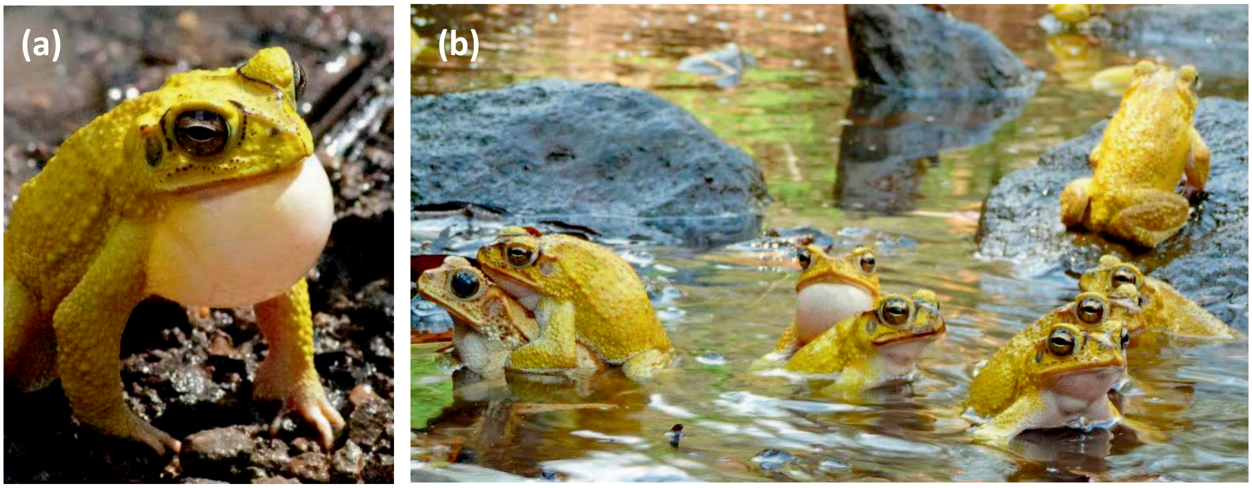

FIG. 4. An adult male *Incilius luetkenii* calling with an inflated vocal sac (a). Male *I. luetkenii* aggregate in large numbers at breeding ponds that form at the start of the rainy season (b); this photograph shows a male in amplexus with a female (at lower left) and other males interacting.

DISCUSSION

In this study, we describe the vocalizations and the vocal behavior of male Neotropical Yellow Toads, *I. luetkenii*. We provide quantitative measurements of the spectro-temporal properties of *I. luetkenii* advertisement calls as well as basic measurements of their release calls. We also report a significant relationship between the spectral properties of male advertisement calls and body size, consistent with the widely supported hypothesis that body size and vocal frequency have an allometric relationship. Finally, we quantify the context of male advertisement calls using video analysis of vocal behavior and find an association between vocalizations and male–male physical interactions, suggesting that chorusing might arise as a by-product of male–male interactions. Together, this work provides the first comprehensive account of the vocal behavior of this little-studied Neotropical toad species.

Our rigorous quantification of the advertisement calls of *I. luetkenii* expands our understanding of the calls of this species beyond previous anecdotal descriptions. To date, the advertisement calls of *I. luetkenii* have been described as being “about 4 seconds” in duration (Savage, 2002), with a dominant frequency of 1.6–1.95 kHz and a pulse rate of 21 pulses per second (Porter, 1966; Savage, 2002). Our data reveal that typical male advertisement calls are 2.5-sec long with two spectral peaks at 1.4 kHz and 1.6 kHz and, similar to the previous anecdotal description, a pulse rate of 22 pulses per second. The difference in call length and spectral frequency between our study and the previous description (Porter, 1966; Savage, 2002) likely arises from the low sample size of the previous study (4 toads) compared to our study (74 toads), although they may also reflect as-yet unstudied population-level differences.

We quantified coefficients of variation within and between individuals, as in other studies of anuran communication (e.g., Gerhardt, 1991; Gerhardt and Huber, 2002; Bee et al., 2013a,b; Thomas et al., 2014). Gerhardt (1991) distinguished between “static” and “dynamic” properties of anuran vocalizations based on the extent of within-individual variation; static properties exhibit low coefficients of variation (i.e., $CV_w < 5\%$) and are generally constrained by vocal morphology and used in species recognition whereas dynamic properties exhibit high coefficients of variation (i.e., $CV_w > 12\%$) and often are under more-active control. Our results reveal that dominant frequency and pulse rate are static traits with low within-individual variation, suggesting that these features are constrained by vocal morphology. Our results reveal that call duration and associated temporal features are dynamic traits with high within-individual variation (Gerhardt and Huber, 2002).

The body size of our study animals correlated to the frequency components of their vocalizations; larger males produced lower frequency advertisement calls. This is consistent with patterns observed in many animals (Ladich et al., 1992; Fitch, 1997; Bee et al., 1999; Martin et al., 2011) including many anurans (Gingras et al., 2013). Indeed, a recent comparative study determined widespread inverse correlation between male body size and spectral properties of vocalizations across 136 species in four clades of anurans (*Bufo*, Hylinae, *Leptodactylus*, and *Rana*; Gingras et al., 2013). Spectral features of anuran advertisement calls are known to have low within-individual variation (Gerhardt, 1991). This relationship between body size and frequency, therefore, may be an important aspect in mate choice; females prefer larger males in many species (e.g., Robertson, 1986; Morris and Yoon, 1989), and therefore males may enhance mate attraction by advertising their size. Males also may use call dominant frequency as a signal to assess the fighting ability of rival males. Male Common Toads (*Bufo bufo*), for example, can settle mating contests using vocalizations that honestly reflect body size (Davies and Halliday, 1978). Judging male size based on vocalizations in highly competitive aggregations may provide fitness benefits to males in the form of reduced energy expenditure and reduced risk of injury if they can accurately assess the size of a competitor. Our observation that calls are associated with aggressive interactions suggests that this type of communication may be important for *I. luetkenii*.

Female *I. luetkenii* face a barrage of aggressive males when they approach a mating aggregation. In scramble competition mating systems, male mating success is closely related to being the first male to find a female and also to dominating male–male interactions (Schwagmeyer and Woontner, 1986). Our anecdotal observations of female *I. luetkenii* do not suggest that they assess multiple males and then facilitate amplexus; instead, females appear to approach an aggregation of males and become the target of amplexus attempts by many males simultaneously. Although males of some anuran species do not engage in intrasexual struggles (Cherry, 1992), *I. luetkenii* males are aggressive, which appears to limit the opportunities for female mate choice. Of course, females may still shape the outcome of successful amplexus in other ways, such as delaying the time of oviposition (Eberhard, 1996). Given the apparently limited context for female choice in this species, the aggressive nature of males during their brief breeding season, and the fact that male vocalizations usually follow physical contact with other males, we suggest that male *I. luetkenii* advertisement calls play an important role within aggressive male–male contexts. In other explosively breeding Bufonids that engage in scramble competition for mates, breeding aggregations exhibit significant male–male aggression and, in some, acoustic chorusing behavior is absent (Höglund and Robertson, 1988). Male *I. luetkenii*, in contrast, seem to chorus loudly and often at high densities. Interestingly, our findings suggest that chorusing behavior may directly result from the high level of intrasexual aggression among males. This difference is important for future work with *I. luetkenii*, which could further examine the function of choruses in scramble competition systems.

Acoustic monitoring tools for remote animal surveys rely on rigorous quantification of animal vocal behavior. Acoustic monitoring has the potential to guide conservation efforts and locate elusive and rare species, among many other benefits (reviewed in Blumstein et al., 2011). Further, some species that share similar appearances may be accurately identified only by their vocalizations. Savage (2002) reports three congeners that are visually similar to *I. luetkenii* and have overlapping distributions: *Incilius melanochlorus*, *Incilius valliceps*, and *Incilius coccifer*. Original maps (Porter, 1966) sometimes misidentified *I. luetkenii* as *I. valliceps*, underscoring the importance of acoustic quantification (Savage, 2002). Although subtle visual differences are present between the four species, acoustic analysis may be the quickest and most reliable way to distinguish species that are similar in appearance. Future analyses describing the vocalizations of these congeners, and other anuran taxa, will facilitate acoustic surveys.

Conclusion.—In this study, we provide the first rigorous, quantitative description of male *I. luetkenii* vocalizations. We present detailed information on the acoustic properties of male advertisement calls and confirm a significant inverse relationship between body size and dominant frequencies. We suggest that males produce advertisement calls during interactions with other males in an aggressive context, which may lead to chorusing behavior because of the high rate of aggressive interactions. Understanding the ecology and behavior of anurans is important in light of the rapid decline of amphibian species worldwide (Stuart et al., 2004). Acoustic monitoring has become an increasingly popular method for assessing the presence and density of species and thus guiding conservation efforts. With an increase in the use of this methodology, it is critical to increase the rigorousness of vocal descriptions such that species are not misidentified. This is especially important in Neotropical areas that are facing precipitous declines in amphibian populations.

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Literature Cited

Bee, M. A., S. A. Perrill, and P. C. Owen. 1999. Size assessment in simulated territorial encounters between male green frogs (*Rana clamitans*). Behavioral Ecology and Sociobiology 45:177–184.

Bee, M. A., R. Suyesh, and S. D. Biju. 2013a. Vocal behavior of the ponmudi bush frog (*Raorchestes graminirupes*): repertoire and individual variation. Herpetologica 69:22–35.

———. 2013b. The vocal repertoire of *Pseudophilautus kani*, a shrub frog (Anura: Rhacophoridae) from the Western Ghats of India. Bioacoustics 22:67–85.

Blumstein, D. T., and J. C. Daniel. 2007. Quantifying behaviour the JWatcher way. Sinauer, USA.

Blumstein, D. T., D. J. Mennill, P. Clemins, L. Girod, K. Yao, G. Patricelli, J. L. Deppe, A. H. Krakauer, C. Clark, K. A. Cortopassi, et al. 2011. Acoustic monitoring in terrestrial environments using microphone arrays: applications, technological considerations and prospectus. Journal of Applied Ecology 48:758–767.

Campos, F. A., and L. M. Fedigan. 2013. Urine-washing in white-faced capuchins: a new look at an old puzzle. Behaviour 150:763–798.

Cherry, M. I., 1992. Sexual selection in the leopard toad, *Bufo pardalis*. Behaviour 120:164–176.

Davies, N. B., and T. R. Halliday. 1978. Deep croaks and fighting assessment in toads *Bufo bufo*. Nature 274:683–685.

Doucet, S. M., and D. J. Mennill. 2010. Dynamic sexual dichromatism in an explosively breeding Neotropical toad. Biology Letters 6:63–66.

Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press, USA.

Fitch, W. T. 1997. Vocal tract length and formant frequency dispersion correlate with body size in rhesus macaques. Journal of the Acoustical Society of America 102:1213–1222.

Gerhardt, H. C. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. Animal Behaviour 42:615–635.

———. 1994. The evolution of vocalizations in frogs and toads. Annual Review of Ecology and Systematics 25:293–324.

Gerhardt, H. C., and M. A. Bee. 2007. Recognition and localization of acoustic signals. Pp. 113–146 in P. M. Narins, A. S. Feng, R. R. Fay, and A. N. Popper (eds.). Hearing and Sound Communication in Amphibians: Springer Handbook of Auditory Research. Volume 28. Springer Publishing, USA.

Gerhardt, H. C., and F. Huber. 2002. Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions. University of Chicago Press, USA.

Gingras, B., M. Boeckle, C. T. Herbst, and W. T. Fitch. 2013. Call acoustics reflect body size across four clades of anurans. Journal of Zoology 289:143–150.

Höglund, J., and J. G. Robertson. 1988. Chorusing behaviour, a density-dependent alternative mating strategy in male common toads (*Bufo bufo*). Ethology 79:324–332.

Ladich, F., C. Bischof, G. Schleinzer, and A. Fuchs. 1992. Intra- and interspecific differences in agonistic vocalization in croaking gouramis (genus: *Trichopsis*, Anabantoidei, Teleostei). Bioacoustics 4:131–141.

Lambert, K. T., and P. G. McDonald. 2014. A low-cost, yet simple and highly repeatable system for acoustically surveying cryptic species. Austral Ecology 39:779–785.

Marques, T. A., L. Thomas, S. W. Martin, D. K. Mellinger, J. A. Ward, D. J. Moretti, D. Harris, and P. L. Tyack. 2013. Estimating animal population density using passive acoustics. Biological Reviews 88: 287–309.

Martin, J. P., S. M. Doucet, R. C. Knox, and D. J. Mennill. 2011. Body size correlates negatively with the frequency of distress calls and songs of Neotropical birds. Journal of Field Ornithology 82:259–268.

Morris, M. R., and S. L. Yoon. 1989. A mechanism for female choice of large males in the treefrog *Hyla chrysoscelis*. Behavioral Ecology and Sociobiology 25:65–71.

Padial, J. M., J. Koehler, A. Munoz, and I. De la Riva. 2008. Assessing the taxonomic status of tropical frogs through bioacoustics: geographical variation in the advertisement calls in the *Eleutherodactylus discoidalis* species group (Anura). Zoological Journal of the Linnean Society 152: 353–365.

Porter, K. R. 1966. Mating calls of six Mexican and Central American toads (Genus *Bufo*). Herpetologica 22:60–67.

Reby, D., K. McComb, B. Cargnelutti, C. Darwin, W. T. Fitch, and T. Clutton-Brock. 2005. Red deer stags use formants as assessment cues during intrasexual agonistic interactions. Proceedings of the Royal Society of London B: Biological Sciences 272:941–947.

Rehberg-Besler, N., D. J. Mennill, and S. M. Doucet. 2015. Dynamic sexual dichromatism produces a sex signal in an explosively breeding Neotropical toad: a model presentation experiment. Behavioural Processes 121:74–79.

Robertson, J. G. 1986. Female choice, male strategies and the role of vocalizations in the Australian frog *Uperoleia rugosa*. Animal Behaviour 34:773–784.

Ryan, M. J. 1980. Female mate choice in a Neotropical frog. Science 209: 523–525.

———. 1988. Constraints and patterns in the evolution of anuran acoustic communication. Pp. 637–677 in B. Frizsch (ed.), The Evolution of the Amphibian Auditory System. John Wiley & Sons Inc., USA.

Savage, J. M. 2002. The Amphibians and Reptiles of Costa Rica. University of Chicago Press, USA.

Schwagmeyer, P. L., and S. J. Woontner. 1986. Scramble competition polygyny in thirteen-lined ground squirrels: the relative contributions of overt conflict and competitive mate searching. Behavioral Ecology and Sociobiology 19:359–364.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. Rodrigues, D. J. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Thomas, A., R. Suyesh, S. D. Biju, M. A. Bee. 2014. Vocal behavior of the elusive purple frog of India (*Nasikabatrachus sahyadrensis*), a fossorial species endemic to the western Ghats. PLoS ONE 9:e94121.

Vieites, D. R., K. C. Wollenberg, F. Andreone, J. Köhler, F. Glaw, and M. Vences, 2009. Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proceedings of the National Academy of Sciences USA 106:8267–8272.

Wells, K. D., and J. J. Schwartz. 2007. The behavioural ecology of anuran communication. Pp. 44–86 in P. M. Narins, A. S. Feng, R. R. Fay, and A. N. Popper (eds.), Hearing and Sound Communication in Amphibians: Springer Handbook of Auditory Research. Volume 28. Springer, USA.

Wells, K. G. 1977. The social behaviour of anuran amphibians. Animal Behavior 25:666–693.

———. 2007. The ecology and behavior of amphibians. University of Chicago Press, USA.

Whitfield, S. M., K. E. Bell, T. Philippi, M. Sasa, F. Bolaños, G. Chaves, J. M. Savage, and M. A. Donnelly. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. Proceedings of the National Academy of Sciences USA 104:8352–8356.

Wilbur, H. M., D. I. Rubenstein, and L. Fairchild. 1978. Sexual selection in toads: the roles of female choice and male body size. Evolution 32: 264–270.

Zweifel, R. G. 1968. Effects of temperature, body size, and hybridization on mating calls of toads, *Bufo a. americanus* and *Bufo woodhousii fowleri*. Copeia 1968:269–285.

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