Vocal and visual communication in an explosively breeding Neotropical toad

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University of Windsor

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VOCAL AND VISUAL COMMUNICATION IN AN EXPLOSIVELY
BREEDING NEOTROPICAL TOAD

By

NICOLAS REHBERG-BESLER

A Thesis
Submitted to the Faculty of Graduate Studies
through Biological Sciences
in Partial Fulfillment of the Requirements for
the Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada
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Vocal and visual communication in an explosively breeding Neotropical toad

by

Nicolas Rehberg-Besler

APPROVED BY:

______________________________________________
K. Drouillard, External Reader
Great Lakes Institute of Environmental Research

______________________________________________
D. Higgs, Internal Reader
Biological Sciences

______________________________________________
S. Doucet, Co-Advisor
Biological Sciences

______________________________________________
D. Mennill, Co-Advisor
Biological Sciences

August 7, 2014
Declaration of Co-Authorship

I hereby declare that this thesis incorporates material that is the result of joint research, as follows:

I am the sole author of chapter 1 and 5, and the principal author of chapters 2, 3, and 4. Chapters 2, 3, and 4 were conducted under the supervision of Dan Mennill and Stéphanie Doucet (University of Windsor)—both of whom helped guide the development of the studies, aided in the analyses and writing of the manuscripts, and provided financial and logistical support for all field research. Dan Mennill and Stéphanie Doucet both share authorship on all three data chapters.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis.

I certify that, with the above qualification, this thesis and the research to which it refers, is the product of my own work.

I certify that, to the best of my knowledge, my thesis does not infringe upon anyone’s copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the office of Graduate Studies, and that this thesis has not been submitted for a higher degree to any other University or Institution.
Abstract

Frogs and toads use both acoustic and visual signals to communicate within their noisy, competitive breeding aggregations. In this thesis, I studied acoustic and visual signals in the Neotropical yellow toad, *Incilius luetkenii*, in Costa Rica. I provide the first quantitative description of this species’ vocal behaviour, and I demonstrate that the spectral properties of male calls are correlated with body size; therefore, male calls may be used in size assessment. I experimentally demonstrate that chorus transmission distance is enhanced by the number of concurrently calling toads as well as the pitch of their calls; therefore choruses may attract more conspecific animals than a lone signaller. In terms of visual signals, I show that males respond more strongly to female-like brown models versus male-like yellow models; therefore colour facilitates sex recognition. My research expands our understanding of communication in a scramble competition mating system of an explosively breeding anuran.
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Chapter 1

General Introduction
Introduction

Animals communicate in multiple modalities including acoustic, visual, chemical, electrical, hydrodynamic, and tactile (Bradbury and Vehrencamp, 2011). Vocal and visual signals are two modalities of communication that are often shaped by sexual selection; these signals are used to attract mates and defend territories across diverse animal taxa (Andersson, 1994). Elaborate vocal and visual signals are widespread in frogs and toads (Order Anura), where breeding often takes place in large aggregations (Hödl and Amézquita, 2001; Gerhardt and Huber, 2002). Careful studies of communication within these aggregations have been applied to relatively few of the approximately 6500 species of frogs found on Earth (AmphibiaWeb, 2015). In this thesis, my goal was to conduct field studies of the vocal and visual communication in the Neotropical Yellow Toad, Incilius luetkenii. In this General Introduction I provide a brief summary of the literature to provide a general background for the three data chapters that follow.

Acoustic signalling and properties of sound

Acoustic signals can function in both short- and long-range communication. The physical properties of sound propagation determine how well acoustic signals transmit through the environment. Sounds attenuate through a phenomenon called “spherical spreading,” decreasing in intensity in a spherical pattern away from the sound source (Bradbury and Vehrencamp, 2011). Sounds experience degradation when they are modified by factors such as obstructions in the environment or atmospheric processes (Bradbury and Vehrencamp, 2011). High-frequency sounds are more subject to
atmospheric absorption and scattering due to environmental obstructions than are low-frequency sounds, thus high-frequency sounds attenuate and degrade more quickly than low-frequency sounds (Forrest, 1994).

The frequency at which animals vocalize is often closely linked to morphology. Across many animal taxa, body size is inversely linked to the spectral frequency of their vocalizations (e.g., Fitch, 1997; Reby et al., 2005; Martin et al., 2011; Gingras et al., 2013). Larger animals produce lower-frequency signals and their signals consequently retain more energy at greater distances than smaller animals (Penna and Solís, 1998). This link to body size has been well demonstrated in anurans and can be explained largely by the dimensions of the larynx; larger animals tend to have larger larynges, and a larger sound production organ can produce sounds with longer wavelengths that are lower in frequency (Gingras et al., 2013).

Most frogs produce sounds by forcing pressurized air through the larynx, which vibrates the vocal cords and inflates the vocal sac (Gerhardt and Huber, 2002). Anuran vocal signals vary in structure and function across species and contexts and are typically produced by males during the breeding period to attract mates (Gerhardt and Huber, 2002; Wells, 2007). Males often have a vocal repertoire, including a subset of advertisement calls, encounter calls, aggressive calls, release calls, distress calls, and courtship calls (Gerhardt and Huber, 2002). Some authors use the term “advertisement call” as a catchall phrase when referring to anuran vocalizations, especially when the intended receiver is unknown, or the function of the call is unclear (Wells, 1977). The evolution of male advertisement calls is widely understood to be driven by sexual
selection through female choice and male-male competition (Ryan, 1988). Females are able to assess specific properties of male advertisement calls to choose desirable mates (Gerhardt and Huber, 2002), whereas males can use the calls to assess location, body size, and fighting ability of males (Davies and Halliday, 1978; Gerhardt and Bee, 2007).

**Chorus dynamics and prolonged breeding**

Frogs and toads often breed in large aggregations that can involve tens to tens-of-thousands of individuals (Wells, 1977). The timing of these aggregations usually coincides with seasonal changes in the environment that support breeding, such as the onset of the rainy season in the tropics or the arrival of warm weather in the temperate zone, and at locations with abundant sites for oviposition or foraging (Gerhardt and Bee, 2007). In most species, males produce advertisement calls intensely and concurrently during these aggregations, creating a loud chorus (Gerhardt and Huber, 2002). Each male broadcasts his advertisement call, while females attempt to localize and choose the best mate (Wells, 1977; Gerhardt, 1991; Gerhardt, 1994; Gerhardt and Huber, 2002; Gerhardt and Bee, 2007). Frog choruses exhibit both broad-scale and fine-scale patterns of variation. At a broad scale, frogs most often chorus at night, a time when light levels are too low for diurnal predators to visually locate individuals (Gerhardt and Huber, 2002). The large number of males in choruses creates a competitive mating environment, sometimes selecting for males that are better able to broadcast their signals (Narins and Zelick, 1988; Gerhardt and Huber, 2002). This is especially true in prolonged breeders (i.e., species that have a long breeding season) where the primary
mating strategy used by males is to attract gravid females using vocalizations rather than to actively search for gravid females (Wells, 1977). Males of some species adjust the timing of their signals to stand out above background noise and neighbouring competitors by avoiding call overlap (e.g., Schwartz, 1993). As a result, some frog and insect species are well known for timing the production of calls during the very short gaps in background noise (e.g., Narins and Zelick, 1988). Extensive work on female mate choice in frogs has relied on phonotaxis experiments; females are given a choice between the playback of different male calls, and the female’s approach responses are used to make inferences about female preferences (Gerhardt and Huber, 2002). Results from these experiments reveal that some anurans have developed strategies to increase the opportunity for females to distinguish between individuals in the cacophony of the chorus (e.g., sound source segregation; Bee, 2007a).

Vocal and visual signals in explosive breeders

Not all anurans have prolonged breeding seasons; some have an explosive breeding season wherein individuals aggregate at high densities and breed for a short amount of time (Wells, 1977). Explosively breeding anurans face the challenge of locating a breeding aggregation during a short time window, sometimes only a few hours (Wells, 1977). The chorus signal produced by males may serve to attract conspecifics that are within the broadcast range of the chorus (Gerhardt and Klump, 1988). This broadcast range can be thought of in terms of the active space of the signal. Active space is the “maximum distance from the source at which the signal is still above
the threshold of detectability” (Bradbury 1981, p. 141). Chorusing behaviour likely has a benefit over individual calling in terms of active space; the calls of multiple males in immediate proximity are expected to interfere constructively, increasing the active space of the chorus and possibly leading to enhanced female mate attraction (Gerhardt and Huber, 2002). A parallel example from the visual modality comes from the bioluminescent flashes of fireflies; multiple males that produce their bioluminescent simultaneously may generate a “beacon effect” making the signal detectable at much greater distances (Buck and Buck, 1968). In the acoustic modality, this phenomenon has been called “signal enhancement” with respect to the behaviour of some insects (Otte, 1974). The potential for multiple concurrent calls to increase the active space of an acoustic signal has been alluded to in some reviews (Bradbury, 1981; Gerhardt and Huber, 2002; Gerhardt and Bee, 2007) and has been quantified in some invertebrates (e.g., Hartbauer, 2014), but has not been experimentally quantified in vertebrates. Nevertheless, behavioural evidence suggests that chorus sounds are important for both males and females: in three different species, females appear to be able to locate temporary aggregations based on conspecific choruses (Gerhardt and Klump, 1988; Swanson et al., 2007; Buxton et al., 2015); in a fourth species, males reliably migrate toward conspecific choruses (Bee, 2007b).

Another problem associated with explosive breeding is the short time window to find mates within the dense breeding aggregation. Explosive breeders often engage in scramble competition for mates; instead of producing an advertisement call from a fixed location and waiting for a female to choose (as in prolonged breeders), males actively
search to secure a breeding partner before rivals (Thornhill and Alcock, 1983; Wells, 2007). Scramble competition selects for attributes that allow animals to rapidly acquire mates (Thornhill and Alcock, 1983) such as high mobility and locomotion (Schwagmeyer, 1988; Dickinson, 1992), well-developed sensory organs (Andersson and Iwasa, 1996), and sexually dimorphic features (Able, 1999). Dichromatism is a common type of sexual dimorphism, where females and males exhibit differences in colouration (Endler, 1984; Badyaev and Hill, 2003). Some explosively breeding frogs have been documented to exhibit dynamic sexual dichromatism (Bell and Zamudio, 2012). For example, in the European moor frog (*Rana arvalis*), males shift from brown to blue during the brief breeding season (Ries et al., 2008). One study determined that the rapid colour change functioned in a sex recognition context, facilitating the differentiation of mates and rivals (Sztatecsny et al., 2012). As a consequence of their high density aggregations and brief breeding periods, it is likely that explosive breeders are under intense selection pressure to discriminate between mates and rivals through a rapid colour dimorphism (Bell and Zamudio, 2012).

**Study species**

My thesis focuses on communication in the little-studied Neotropical Yellow Toad, *Incilius luetkenii* (previously referred to as *Bufo luetkenii*, *Cranopsis luetkenii*, or *Ollotis luetkenii*). This species is distributed in the Central American Pacific lowlands from Chiapas, Mexico to central Costa Rica (Savage, 2002). Yellow Toads inhabit dry forests and are active for the first half of the rainy season (May to August) before they
retreat to underground hideaways during the dry season (Savage, 2002). After the first substantial rain of the wet season, individuals aggregate and vocalize around newly formed bodies of water for a brief period of 1-3 days (Savage, 2002; Doucet and Mennill, 2010). Yellow Toads breed explosively, with the timing of their breeding events being driven by unpredictable environmental conditions (Wells, 1977) like those observed in the lowland dry forests of Central America. Many explosively breeding species engage in scramble competition for mates whereby males actively search within the aggregation for females while engaging in many physical encounters with rivals (Wells, 1977). This tactic appears to be followed by Yellow Toads, although their breeding behaviour has not been studied previously.

In northwestern Costa Rica, I have found Yellow Toad aggregations to persist for the first 1-3 days following the first significant rain of the year. Annually, I witness hundreds of individuals aggregating in multiple seasonally dry creek beds throughout the forest that have been filled by the first rain. I have observed these aggregations form during the evening of the first rain, peak in number the following morning, then decline over the following two days. Amplexus with females is axillary and persists throughout the morning. I have observed female ovipositing only after most males have either entered amplexus or left the pond.

The advertisement call of male Yellow Toads has been described in basic detail as “a short trill lasting about 4 seconds repeated after intervals of 1 to 4 seconds [with a] dominant frequency is 1.6 to 1.95 kHz with a trill rate of 21 per second” (Savage, 2002, p. 208). This existing description is based on a very small sample of 4 toads (Porter,
1966); a rigorous study of their vocal behaviour has never been conducted. The high number of male *I. luetkenii* in breeding aggregations produces a chorus from the overlapping sounds of many males vocalizing concurrently. Whether this chorus of overlapping male vocalizations transmits far in the natural environment has yet to be tested.

One remarkable aspect of male Yellow Toads is their colour; both sexes are a brown colouration throughout the year, but males change to a bright lemon yellow colour during their brief mating events (Doucet and Mennill, 2010). This dramatic colour dimorphism observed in male Yellow Toads and other species was studied in a recent review of anuran sexual dichromatism by Bell and Zamudio (2012). Yellow Toads were described as being one of only 31 species known to exhibit dynamic sexual dichromatism (Bell and Zamudio, 2012). Bell and Zamudio (2012) outline the possible evolutionary mechanisms underlying dynamic sexual dichromatism in frogs, noting that this phenomenon is likely driven by sexual selection because colour change is only observed in males and coincides with the breeding season. The duration of colour change has been documented as lasting from only a few hours in some species to weeks in others (Bell and Zamudio, 2012). Male Yellow Toads are at the shortest duration of this continuum, having been documented to significantly change from yellow to brown over only 4 hours in amplexus (Doucet and Mennill, 2010). The function of this conspicuous, dynamic colour signal in Yellow Toads has yet to be studied.

**Thesis goals**
In this thesis, I explore the vocal and visual signals of Neotropical Yellow Toads. In my first data chapter (Chapter 2), my goal is to provide the first quantitative investigation of *I. luetkenii* vocalizations and vocal behaviour. I describe spectro-temporal features of male advertisement calls, I test the hypothesis that body size correlates with acoustic features, and I describe the context of male vocalizations. In my second data chapter (Chapter 3), I test the hypothesis that the vocal overlap observed in breeding aggregations enhances the signal amplitude of the overall chorus. My goal is to explore how well male *I. luetkenii* advertisement calls transmit through the natural environment as a function of the number of signallers, the distance away from the signal source, and the spectral frequency of the chorus. In my third data chapter (Chapter 4), I explore the signal function of the dynamic yellow colouration observed in male *I. luetkenii*. I test the hypothesis that males use their colouration to discriminate between prospective mates and rival males during their brief breeding season. I test this idea during both night and day to determine if male colouration is important in both high- and low-light conditions.
References


Fig. 1.1. Male Neotropical Yellow Toads, *Incilius luetkenii*, exhibit a bright lemon yellow colouration during the explosive breeding season (A), but rapidly change back to an olive or brown colour (top, B) after entering amplexus with a female (bottom, B). The breeding aggregation hosts many males (C) that engage in active mate searching while producing loud calls.
Chapter 2

Vocal behaviour of the explosively breeding Neotropical Yellow Toad, *Incilius luetkenii*
Chapter Summary

Frogs and toads exhibit complex vocal behaviour, often in the context of mating and territory defense. Although over 6500 species of frogs and toads have been described, detailed accounts of vocal behaviour exist for very few species. In this study, we provide the first detailed investigation of the vocal behaviour of Neotropical Yellow Toads, *Incilius luetkenii*, based on detailed recordings collected from animals in northwestern Costa Rica. We provide quantitative measurements of two types of calls, 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 behaviour in the framework of an aggressive scramble competition mating system. We found that male *I. luetkenii* produce two vocalizations: advertisement calls and release calls. Advertisement calls are loud, 2.5-second long vocalizations comprised of a series of pulses that rise and fall in amplitude and are produced by males as they inflate their vocal sac. Release calls are less common than advertisement calls, and are 0.6-second long vocalizations comprised of short pulses and produced by males when other males attempt amplexus on them. As predicted, spectral properties of advertisement calls are correlated with body size, with larger males producing lower frequency vocalizations. Video analysis of male calling behaviour revealed a call rate of 0.58 calls per minute and revealed that most advertisement calls occur within 2 seconds following a contact with another male. In light of the scramble competition mating system, our findings suggest that male advertisement calls are associated with aggression between males and that males communicate acoustic information about their body size during male-male
encounters. By studying anuran vocal behaviour, we can better understand the
behavioural ecology of these animals as well as guide conservation efforts.
Introduction

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 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., 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 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 anurans.
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 behaviour, ecology, and conservation of anurans, relatively few species’ vocalizations have been described in detail. A recent resurgence of rigorous quantifications of anuran vocal behaviour 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, it is increasingly important to understand the behaviour and ecology of anurans in these environments to facilitate conservation action (Whitfield et al., 2007).

In this study, our goal was to provide the first quantitative investigation of the vocalizations and vocal behaviour of Neotropical Yellow Toads, *Incilius 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 behaviour. We predicted that, as in other anurans (e.g. Gingras et al., 2013) and non-anuran taxa (e.g., Fitch, 1997), *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 UNESCO World Heritage Site in northwestern Costa Rica that is dominated by Neotropical dry forest habitat. 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). Annually, we witness hundreds of individuals aggregating in seasonally dry creek beds that have been filled by the first significant rain, which occur at unpredictable times, usually in the month of May at our study site (range: 28 April to 6 June over the last 8 years). 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 one to two 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, producing at least two types of calls, only one of which – the advertisement call – has been described, and only in rudimentary detail (Porter, 1966; Savage, 2002). When a male successfully enters amplexus, the pair remains this way throughout the morning, or longer. We have observed egg laying only after most bachelors 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. Males do not hold resources, but actively compete with rival males by engaging in male-male displacement behaviour while searching for incoming females (Wells, 1977). Notably, males exhibit bright yellow colouration during their breeding event, and have been documented to rapidly shift to a cryptic female-like brown colouration following amplexus (Doucet and Mennill, 2010).

*Data Collection*

We recorded vocalizations 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 shotgun microphones (Audio-Technica 815b and Sennheiser ME66/K6) and solid-state digital recorders (Marantz PMD-660; 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 colour (Doucet and Mennill, 2010) and behaviour.

*Acoustic Quantification*

We measured nine spectro-temporal features of male advertisement calls: (1) call duration, (2) call rise time, (3) call fall time, (4) pulses per call, (5) pulse rate, (6)
pulse duration, (7) pulse period, (8) frequency of the first spectral peak, and (9) frequency of the second spectral peak (Table 2.1, Fig. 2.1). Each call was isolated and measured using Avisoft SasLab Pro (V5.2, R. Sprecht, Berlin, Germany), minimizing human subjectivity in call measurement. For temporal properties, we used the pulse-train analysis feature of AviSoft (settings: rectification + exponential decay envelope; peak search 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 6000Hz prior to measurement). We found that advertisement calls have two dominant spectral peaks (see Results), and substantial variation in regards 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. 2.1 f, 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 behaviour, we collected 10-minute video recordings of 15 males during the 2015 breeding event that took place June 7, 2015. We
followed each focal male from a distance of approximately 1 m, recording his behaviour with a video camera (Bell & Howell DNV16HDZ), 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 behaviours: (1) focal male produced an advertisement call (n = 76; numbers represent total number of instances of the behaviour across all of our video recordings), (2) non-focal male produced an advertisement call (n = 81), (3) focal male initiated contact with another male (n = 171), (4) non-focal male initiated contact with focal male (n = 116), (5) focal male approached within one body length of non-focal male without contacting him (n = 62), and (6) non-focal 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. In two of the 15 videos, the focal male did not call during the 10-minute trial and these trials were excluded from analyses.

Statistical Analysis

Our two body size measurements (snout-vent length and body size) were highly correlated (r = 0.82, N = 74) and therefore we conducted a principal component analysis with Varimax rotation on these two measurements of body size. This analysis yielded a single principal component that explained 91% of the variation in the two measurements; we named this principal component “body size”. We ran Pearson correlations to describe the association between our body size principal component
score and spectro-temporal properties of male calls. To maintain an overall significance threshold of 0.05 across these nine correlations, 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 behavioural video data by quantifying how many times each of the above six behaviours occurred, how many times each behaviour directly preceded a male call, and the average length of time between the focal male call and the behaviour that directly preceded it.

We calculated coefficients of variation (CV = 100% x standard deviation / mean) to locate the sources of call property variation (Gerhardt, 1991; Gerhardt and Huber, 2002). We were interested in sources of within-individual variation and between-individual variation (Bee et al., 2013a,b). We computed within-individual variation (CVw) from all males that produced more than one call (N = 59, range: 2-11 calls). CVw was calculated using the average and standard deviation for each male, and then averaged to get a single mean CVw for each call property. We calculated between-individual CV (CVb) by using the overall average and standard deviation from all males to create a single CVb value for each call property.

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

Call Properties

Male *I. luetkenii* produce advertisement calls (Fig. 2.1) that last for 2.5 ± 0.7 s and consist of a series of rapid pulses (pulse duration: 22.5 ± 0.3 ms) with a pulse rate of 22.1 ± 0.3 pulses per second. The call generally reaches its peak amplitude after 1.75 ± 0.07 s and persists for 0.75 ± 0.06 s 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 2.2).

Male advertisement calls contained some properties that showed high coefficients of variation and some with low coefficients of variation. Call duration, call rise time, fall time, and number of pulses per call were all highly variable both between and within individuals (i.e. CVs > 24%; Table 2.2). 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. CVw < 6%; Table 2.2).

Males also produce release calls (Fig. 2.2). These calls were produced much less frequently; we recorded this call in 8 instances from 5 different individuals. From this small sample, we found an average call duration of 0.58 ± 0.05 s, 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 to 93.5 mm) and weighed 47.5 ± 1.0 g (range: 31 to 72 g; *N* = 74). The spectral features of male *I. luetkenii* advertisement calls showed a significant inverse relationship with body size (Table 2.3, Fig. 2.3), with larger males producing lower-frequency calls. We found no significant correlations between temporal measurements and body size (all *r* < 0.25, *p* > 0.11; *N* = 74).

**Video Analysis of Calling Behaviour**

Video analysis revealed that male advertisement calls were always associated with inflation of the male vocal sac (Fig. 2.4). Males produce advertisement calls at a rate of 0.58 ± 0.16 calls per minute (*n* = 76 calls from 13 males). In 50 of 76 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 s between contact and vocalization); in 9 of 76 cases, the focal male called immediately after making contact with another male (average time interval: 1.67 ± 0.26 s between contact and vocalization); in 9 of 76 cases, the focal male called immediately after another male within frame produced an advertisement call (average time interval: 1.79 ± 0.11 s between call 1 and call 2); in 7 of 76 cases, the focal male’s call was preceded only by his previous call (average time interval: 3.28 ± 0.44 s between calls); in the final case, the focal male called after approaching a non-focal male within one body length (time interval: 3.42 s).
Discussion

In this study, we described the vocalizations and the vocal behaviour of male Neotropical Yellow Toads, *I. luetkenii*. We provided quantitative measurements of the spectro-temporal properties of *I. luetkenii* advertisement calls and release calls. We also reported 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 quantified the context of male advertisement calls using video analysis of vocal behaviour, and found an association between vocalizations and male-male physical interactions. Together, this work provides the first comprehensive account of the vocal behaviour of this little-studied Neotropical toad species.

Our rigorous quantification of the advertisement calls of *I. luetkenii* expands our understanding of this species’ calls beyond previous anecdotal descriptions. To date, the advertisement calls of *I. luetkenii* have been described as being “about 4 seconds” in duration (Savage, 2002, p. 208), with a dominant frequency of 1.6 to 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-seconds long with two spectral peaks at 1.4kHz and 1.6kHz 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). 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<sub>w</sub> < 5%) and are generally constrained by vocal morphology and used in species recognition, whereas dynamic properties exhibit high coefficients of variation (i.e. CV<sub>w</sub> > 12%) and are often 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 influenced 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 may therefore 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 may also use call dominant frequency as a signal to assess the fighting ability of rival males. Male *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 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 based on other factors such as delay in 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 exhibit scramble competition for mates, breeding aggregations exhibit significant male-male aggression and some lack acoustic chorusing behaviour (Höglund and Robertson, 1988). In contrast, male *I. luetkenii* seem to chorus loudly and often at high densities. Our findings suggest that chorusing behaviour may be strongly influenced by 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 behaviour. Indeed, 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 only be accurately identified by their vocalizations. Savage (2002) reports three congeners that are visually similar to *I. luetkenii* and have overlapping distributions: *Incilius melanochlorus*, *I. valliceps*, and *I. 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 provided the first rigorous quantitative description of male *I. luetkenii* vocalizations. We presented detailed information on the acoustic properties of male advertisement calls, and confirmed 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 behaviour due to the high rate of aggressive interactions. It is important to understand the ecology and behaviour of anurans 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 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.

Acknowledgements

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References


# Tables and Figures

**Table 2.1.** Description of nine spectro-temporal properties of male *Incilius luetkenii* advertisement calls that were measured in this study.

<table>
<thead>
<tr>
<th>Type of acoustic property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temporal properties</strong></td>
<td></td>
</tr>
<tr>
<td>Call duration (s)</td>
<td>Time between onset of first pulse(^1) and offset of the last pulse in a call</td>
</tr>
<tr>
<td>Call rise time (s)</td>
<td>Time between onset of first pulse and the onset of the pulse with maximum amplitude</td>
</tr>
<tr>
<td>Call fall time (s)</td>
<td>Time between onset of the pulse with maximum amplitude and offset of the last pulse</td>
</tr>
<tr>
<td>Pulses per call</td>
<td>Number of pulses in a call ((k))</td>
</tr>
<tr>
<td>Pulse rate (pulses/s)</td>
<td>Number of pulses minus 1 ((k – 1)) divided by the time between call onset and onset of last pulse</td>
</tr>
<tr>
<td>Pulse duration (s)</td>
<td>Time between pulse onset and pulse offset</td>
</tr>
<tr>
<td>Pulse period (s)</td>
<td>Time between pulse onset and onset of the next pulse in a call</td>
</tr>
<tr>
<td><strong>Spectral properties</strong></td>
<td></td>
</tr>
<tr>
<td>Dominant frequency of first spectral peak (Hz)</td>
<td>Frequency at each pulse's first spectral peak, averaged across all pulses in a call</td>
</tr>
<tr>
<td>Dominant frequency of second spectral peak (Hz)</td>
<td>Frequency at each pulse's second spectral peak, averaged across all pulses in a call</td>
</tr>
</tbody>
</table>

\(^1\) First pulse was determined as the first pulse that rose above background noise in a continuous bout of pulses within a call.
Table 2.2. Mean and standard errors of nine spectro-temporal properties of the advertisement calls of male *Incilius luetkenii* \((N = 74)\). Coefficients of variation (CV) are presented for nine spectro-temporal properties for between individuals (CVb) and within individuals (CVw) \((N = 59)\).

<table>
<thead>
<tr>
<th>Type of acoustic property</th>
<th>Property</th>
<th>Mean</th>
<th>SE</th>
<th>CVb</th>
<th>CVw</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temporal properties</strong></td>
<td>Call duration (s)</td>
<td>2.52</td>
<td>0.1</td>
<td>26.3</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>Call rise time (s)</td>
<td>1.76</td>
<td>0.1</td>
<td>33.8</td>
<td>41.0</td>
</tr>
<tr>
<td></td>
<td>Call fall time (s)</td>
<td>0.76</td>
<td>0.1</td>
<td>62.5</td>
<td>69.9</td>
</tr>
<tr>
<td></td>
<td>Pulses per call</td>
<td>55.7</td>
<td>1.5</td>
<td>24.5</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td>Pulse rate (pulses/s)</td>
<td>22.1</td>
<td>0.3</td>
<td>11.3</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Pulse duration (s)</td>
<td>0.023</td>
<td>0.003</td>
<td>12.9</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Pulse period (s)</td>
<td>0.046</td>
<td>0.005</td>
<td>11.2</td>
<td>2.3</td>
</tr>
<tr>
<td><strong>Spectral properties</strong></td>
<td>Frequency of first spectral peak (kHz)</td>
<td>1.42</td>
<td>0.01</td>
<td>6.1</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Frequency of second spectral peak (kHz)</td>
<td>1.56</td>
<td>0.01</td>
<td>5.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Table 2.3. Pearson correlations between body size (a principal component score summarizing variation in snout-vent length and body mass) and nine spectro-temporal properties of advertisement calls of male *Incilius luetkenii* (*N* = 74).

<table>
<thead>
<tr>
<th>Type of acoustic property</th>
<th>Property</th>
<th>PC (Body Size)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>r</em></td>
</tr>
<tr>
<td><strong>Temporal Properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Call duration (s)</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Call rise time (s)</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Call fall time (s)</td>
<td>-0.10</td>
</tr>
<tr>
<td></td>
<td>Pulses per call</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Pulse rate (pulses/s)</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>Pulse duration (s)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Pulse period (s)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Spectral Properties</strong></td>
<td>Frequency of first spectral peak (kHz)</td>
<td>-0.51</td>
</tr>
<tr>
<td></td>
<td>Frequency of second spectral peak (kHz)</td>
<td>-0.48</td>
</tr>
</tbody>
</table>
Fig. 2.1. Waveform (top) and spectrogram (bottom) of a typical male *Incilius luetkenii* advertisement call depicting properties that were measured: (a) pulse duration, (b) pulse period, (c) pulse rise time, (e) pulse fall time, (f) frequency of first spectral peak, and (g) frequency of second spectral peak. The location of pulse of maximum amplitude (d) determined call rise and fall time, whereas call duration was the length of the entire call.
**Fig. 2.2.** Waveform (top) and spectrogram (bottom) of a male *Incilius luetkenii* release call.
Fig. 2.3. Male *Incilius luetkenii* body size is inversely related to call frequency. Here we show this relationship between body size (a principal component score summarizing variation in snout-vent length and mass) and the dominant frequency of the first spectral peak.
Fig. 2.4. 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.
Chapter 3

Overlapping male toad calls produce a far-reaching chorus
Chapter Summary

Animals that breed in aggregations often produce sexual signals concurrently, called a “chorus”. One potential benefit of concurrent signalling is the enhancement of overall signal amplitude. The transmission of acoustic choruses has been quantified in some invertebrates, but has never been experimentally quantified in relation to the number of individuals participating in the chorus in vertebrates. In this study, we test the hypothesis that the overlapping sounds produced by a breeding aggregation of toads enhances the transmission properties of the chorus. We test this hypothesis using multi-speaker playback of the calls of male Neotropical Yellow Toads (*Incilius luetkenii*) re-recorded with different numbers of signallers, at different distances, and at different spectral frequencies. We found that as the number of calling males increased, the propagation distance of their chorus signal also increased. This toad species exhibits variation in call frequency that corresponds with body size; we found that the signal enhancement effect is further pronounced when overlapping calls have low frequency compared to medium- or high-frequency. Our findings represent the first experimental demonstration of chorus-level vocal signal enhancement in vertebrates. By contributing advertisement calls to a chorus, a male enhances the signal strength of the overall chorus and thereby increases the chance that conspecifics will perceive the chorus signal, which may attract both additional male signallers as well as potential mates.
Introduction

Many animals breed in large aggregations, which makes them subject to intense sexual selection through female mate choice and male-male competition (Emlen and Owring, 1977). Animals in these aggregations often produce visual and acoustic displays to attract mates or defend display territories (Gibson and Bradbury, 1985). Signal timing in these aggregations can vary anywhere within the spectrum of “perfect synchrony” wherein individuals signal at the exact same time, to “perfect alternation” wherein individuals do not overlap their calls at all (reviewed in Greenfield, 1994). Many animals attempt to avoid signal overlap with neighbors in order to avoid signal masking (reviewed in Gerhardt and Huber, 2002), whereas other animals compete to produce signals simultaneously with neighbors and consequently “jam” their neighbour’s signal, resulting in synchrony (Greenfield and Roizen, 1993). High rates of signal overlap are observed in many aggregating species (e.g., Lampyrid beetles, katydids, and anurans; reviewed in Greenfield, 1994).

One major benefit of signal overlap is the potential to increase overall signal amplitude (Hartbauer et al., 2014), a phenomenon called “signal enhancement” (Otte, 1974). In an analogous phenomenon from the field of visual signals, concurrently signalling bioluminescent fireflies exhibit a “beacon effect” where the simultaneous flashes enhance the transmission properties of a group of males beyond what is possible for one male alone (Buck and Buck, 1968). Therefore, concurrent signalling may increase the active space of signals, allowing other animals to perceive the group signallers from greater distances (Hartbauer et al., 2014). Many reviews of communication in
aggregating vertebrates allude to this phenomenon, but as far as we are aware, it has never been quantified in vertebrates. Bradbury (1981, p. 140) states that “each male added [to the chorus] would increase the maximum range that the signals would be detectable.” Similarly, Gerhardt and Huber (2002, p. 253) state that “the range of attraction of grouped signalers is also likely to be somewhat greater than that of single individuals.” Furthermore, Gerhardt and Bee (2007, p. 115) imply that sound intensity increases with group size, and suggest that “sound intensity does not increase linearly as more males call.” A study that experimentally demonstrates this effect is clearly needed in vertebrates.

Concurrent acoustic signalling by more than two individuals is called a chorus. Choruses are observed across diverse taxa, including birds (Todt and Naguib, 2000), insects (Walker, 1969), fish (Amorim and Vasconcelos, 2008), mammals (Lammers et al., 2003), and anurans (Gerhardt and Huber, 2002). Considerable research has been devoted to exploring how animals resolve acoustic problems associated with choruses (e.g., how to discriminate individuals in a cacophony of chorus noise; Vélez et al., 2013). Most research on chorus function come from studies of prolonged breeders (i.e., animals that breed for a long duration), where choruses are understood to be important in group-level advertisement to rivals (e.g., Black-breasted wood quail; Hale, 2006), territory defense (e.g., Rufous-naped wrens; Bradley and Mennill, 2009), and mate attraction (e.g., katydids; Hartbauer et al., 2014). However, less is known about the chorus of explosive breeders (i.e., animals that breed for a very short duration; Wells, 1977).
Animals that breed in aggregations or breed explosively often produce choruses. Explosive breeding is associated with the challenge of locating conspecific mating aggregations in a short time window (Wells, 1977) – sometimes only a few hours – and therefore animals using this system may exploit chorus signals to locate these aggregations (Gerhardt and Klump, 1988). Females may locate temporary aggregations of males based on conspecific chorus noise (Swanson et al., 2007; Buxton et al., 2015). Under these conditions, males may gain a fitness advantage by joining a temporary aggregation and vocalizing. Males of some explosively breeding species do reliably migrate toward the sound of conspecific choruses, suggesting that a benefit arises from joining a chorus (e.g., Bee, 2007).

Spectral frequency plays an important role in acoustic communication. Low-frequency sounds travel greater distances because they are less vulnerable to distortion by atmospheric absorption, ground effects, and scattering compared to higher-frequency signals (Bradbury and Vehrencamp, 2011; Forrest, 1994). In vocal communication, the frequency of the signal is also closely tied to the body size of the animal producing the sound (e.g., Mammals: Fitch, 1997; Anurans: Gingras et al., 2013; Birds: Martin et al., 2011). In anurans, this frequency relationship with body size can largely be explained by vocal fold dimensions and laryngeal allometry (Gingras et al., 2013). Rival males and choosy females may be able to assess the quality of an individual based on vocal frequency. Male common toads (Bufo bufo) can assess the fighting ability of other male toads based solely on vocalizations (Davies and Halliday, 1978). Likewise, in red deer, stags are able to use formant frequency as assessment cues during
aggressive encounters, and females prefer the roars of larger males (Charlton et al., 2007; Reby et al., 2006). It may therefore stand to reason that larger-bodied animals may have a disproportionate effect in the transmission of a chorus signal.

Anurans provide an ideal system for studying chorusing behaviour in large aggregations. Male vocalizations are driven by sexual selection through female choice and male-male competition (reviewed in Gerhardt, 1994). Our study species, the Neotropical Yellow Toad (*Incilius luetkenii*), breeds explosively after the first substantial rain of the wet season. Males produce loud and complex advertisement calls in their aggregation (Chapter 2), where hundreds of males can be found in small ponds and can be heard from far away (Doucet and Mennill, 2010). The dominant frequency of male Yellow Toads advertisement varies between males and is highly correlated with body size (Chapter 2).

In this study, we tested the hypothesis that vocal overlap of animal signals within aggregations enhances signal amplitude. We used a multi-speaker playback experiment to broadcast male Yellow Toad advertisement calls and rerecord the chorus signal from multiple fixed distances. Our goal was to explore how well these vocalizations transmit through the environment based on the number of signallers, the transmission distance, and the spectral frequency of the calls. We predicted that we would observe the highest signal-to-noise ratios (1) at the closest distance from the speakers, (2) when more vocalizations were being produced concurrently, and (3) when speakers broadcast the lowest frequency vocalizations.
Methods

Study site and species

We conducted this experiment in Sector Santa Rosa of the Guanacaste Conservation Area (10°40′ N, 85°30′ W), a seasonal dry Neotropical forest in northwestern Costa Rica. Yellow Toads are abundant at this field site, most commonly found during their explosive breeding events (Doucet and Mennill, 2010). Breeding coincides with the first major rainfall of the year, typically in the month of May, when males gather in newly-formed ponds. Within their breeding aggregations, males temporarily change from a female-like dull brown colour to an intense lemon yellow colour (Doucet and Mennill, 2010). The yellow males gather in breeding ponds and attempt to breed for 1-3 days following the first substantial rain of the year. Males gather in groups of several hundred individuals, where they call and interact aggressively with one other. The behaviour of these toads is understood as a scramble competition mating system (similar to some other bufonids, e.g., *Bufo bufo*; Höglund and Robertson, 1987), whereby males actively search to enter amplexus with incoming females and remain in amplexus until most bachelor males have left and females have spawned. After mating, males rapidly shift back to their cryptic brown colour and disperse from the breeding site (Doucet and Mennill, 2010; Savage, 2002).

Playback stimuli

To create playback stimuli, we isolated calls from ten individual toads that we recorded in 2013. Recordings were collected using Audio-Technica 815b and Sennheiser
ME66/K6 microphones and Marantz PMD660 solid-state digital recorders. We selected recordings with a high signal-to-noise ratio that were collected at similar distances from the recorded toad (approximately 1.0 m), and without interference from other toad calls or other overlapping sounds. Using the “normalize” feature of Audition software (V3.0, Adobe Systems, Mountain View, CA), we standardized these 10 calls to the same amplitude (-3 dB). These calls had an average length of 2.22 ± 0.24 s and an average dominant frequency of (mean ± SE = 1.54 ± 0.03 kHz). We also added a buffer of silence of at least 2 s at the end of each stimulus to create stimuli of equal duration.

In addition to the stimuli we broadcast at a natural frequency, we transposed each of the 10 calls to three different frequencies: high (1.57 kHz), medium (1.43 kHz), and low (1.25 kHz). We chose the three frequencies to correspond with the 90th, 50th, and 10th percentiles of male call frequency range, based on 279 advertisement calls from 74 toads recorded in 2013 (Chapter 2). We used the “transpose” function of Audition to raise or lower the frequency of each of the 10 calls and achieve the same dominant frequency as the high, medium, and low-frequency targets listed above.

*Playback procedure*

We broadcast and re-recorded the stimuli during five rain-free mornings in late April 2013 (specifically: April 20, 26, 27, 28, and 29). We used ten identical loudspeakers (Scorpion TX200, FOXPRO Inc.) each broadcasting the calls from one of the ten stimulus toads. We arranged the ten speakers in a linear array as close to each other as possible without touching. Speakers were placed on the ground, with the cone of the speaker facing outwards parallel to the ground at a height of 5cm (the height of a toad’s head).
We set the volume of each loudspeaker to the same level, which we measured at 92.7 dB (Sound Pressure Level at 100 cm; measured using a Casella sound level meter, Cel-24x, Bedford, UK; fast setting with C-weighting). This amplitude matched what we consider to be the typical amplitude of a calling male toad in the field.

Playback began with one loudspeaker broadcasting one call, then five loudspeakers broadcasting five different calls simultaneously, then ten loudspeakers broadcasting ten different calls simultaneously. We used a remote control to ensure a simultaneous start of all of the tracks from each of the speakers. Each sound was broadcast at least five times, to maximize the chance that we would collect a clear recording that was not overlapped by background noise. Playback of the natural-frequency sounds was followed by playback of the three manipulated chorus frequencies (low, medium, and high).

We recorded the broadcast sounds with microphones that we arranged in a transect stretching away from the loudspeakers at distances of 5, 10, 20, 40, 60, 80, 100, and 120 m. As with the loudspeakers, the microphones were positioned at a height of 5 cm from the ground (the height of a toad’s head). We used four identical omni-directional microphones (Sennhesier ME-62/K6) connected to the left- and right-channels of two identical digital recorders (Marantz PMD660 digital recorders; recording format: WAVE, 44.1 kHz sampling rate, 16-bit accuracy). Four distances were recorded at a time, with the four close distances and four far distances recorded first on alternating days.
We repeated this procedure across five different transects. We chose the five transects based on having observed toads at these locations in previous years. We situated the speakers in five different locations in the dry streambed and oriented the transect perpendicular to the streambed in the direction of the forested areas where we have seen migrating toads in previous years.

Acoustic analysis

We used Avisoft SasLab Pro (V5.2, R. Sprecht, Berlin, Germany) to measure the re-recorded sounds, focusing only on sounds that were not overlapped by background noise. We resampled sounds to 8000 Hz so that the spectrograms would fill most of the frequency domain in AviSoft. We used AviSoft’s automatic parameter measurement tool (spectrogram parameters: 512 Hz FFT length, 500-3600 Hz bandpass cutoffs, Hamming window, 25% frame size, 93.75% temporal overlap), thereby minimizing human subjectivity in sound measurement. After applying a 500-3600 Hz bandpass cutoff, we measured the peak amplitude in regular intervals of 100ms, starting 500 ms after the onset of the call and continuing for 1000 ms, yielding a total of 11 amplitude measurements. We removed the highest and lowest measurement values and calculated the signal amplitude as an average of the remaining 9 measurements. We followed a similar procedure to measure background noise, collecting 11 noise measurements during the silent period immediately preceding each signal. We subtracted the “noise” measurement from the “signal” measurement to determine the signal-to-noise ratio (in dB) for each sound.
Sample size and data analysis

In total, we collected 480 measurements: 8 distances between the speakers and the microphone x 4 frequency types (natural, low, medium, or high) x 3 speaker conditions (one-speaker, five-speaker, or ten-speaker playback) x 5 transects. Many of these measurements were based on an average signal-to-noise ratio from broadcasting each type of sound 5-22 times. For 7 of the 480 measurements, we failed to record a non-overlapped example, resulting in a final sample size of 473 signal-to-noise ratio measurements. We applied a square-root transformation to normalize the signal-to-noise ratio data, although we depict non-transformed values in figures.

We conducted two analyses of variance (ANOVA). First, to analyze signal-to-noise ratio of the calls broadcast at a natural frequency, we conducted an ANOVA with two fixed factors: distance from the speakers (eight levels: 5, 10, 20, 40, 60, 80, 100, and 120 m) and number of speakers broadcasting simultaneously (three levels: 1, 5, and 10 speakers). We included “transect” as a random effect not because we were interested in differences between the five sites, but to account for the repeated sampling that was conducted at different distances along each transect. Second, to analyze signal-to-noise ratio of the calls broadcast at the three transposed frequencies, we conducted an ANOVA with three fixed factors: distance from the speakers (eight levels: 5, 10, 20, 40, 60, 80, 100, and 120 m), number of speakers broadcasting simultaneously (three levels: 1, 5, and 10), and the type of transposition (three levels: high, medium, and low frequencies). In both analyses, we included all main effects and first-order interaction
terms. For all significant effects, we conducted pair-wise post-hoc Tukey tests of honestly significant differences.

All analyses were conducted in JMP 12 (SAS Institute, Cary, NC). All analyses are two-tailed. All values are presented as mean ± SE.

Results

Transmission of natural calls

The advertisement calls of Yellow Toads transmitted over long distances, and the integrity of the calls varied with broadcast distance and with the number of speakers broadcasting simultaneous sounds (Fig 3.1). Signal-to-noise ratio declined significantly with distance (Fig. 3.2; \( F_{7,89}=169.0, P < 0.0001 \)); post-hoc analyses revealed that most distances showed significantly different signal-to-noise ratios (each distance yielded a significantly different signal-to-noise ratio except for 60 vs 80m, 80 vs 100m, and 100 vs 120m). Signal-to-noise ratio increased significantly with the number of simulated toads (Fig. 3.2; \( F_{2,89} = 74.5, P < 0.0001 \)); post-hoc analyses revealed significant differences in pair-wise comparisons between ten, five, and one loudspeakers. There was no significant interaction effect between distance and number of speakers \((F_{14,89}=1.6, P = 0.08)\).

Transmission of frequency-transposed calls

We broadcast Yellow Toad advertisement calls that were frequency-transposed within the natural range of variation to explore whether high-, medium-, and low-frequency calls varied in transmission properties. As in our analysis of natural-frequency
calls, we found a significant effect of distance (Fig. 3.3; \( F_{7,308} = 377.2, P < 0.0001 \); post-hoc tests showed significant differences between all eight distances except 40 vs 60m and 80 vs 100m) and number of speakers (\( F_{7,308} = 252.0, P < 0.0001 \); post-hoc tests showed significant difference between all three levels). We also found a significant effect of frequency (\( F_{7,308} = 13.0, P < 0.0001 \); post-hoc analyses revealed that calls transposed to a low frequency had significantly higher signal-to-noise ratios than calls transposed to a medium or high frequency. There were no significant interaction effects between distance, number of speakers, and frequency (\( F < 2.3, P > 0.06 \)).

**Discussion**

Using a multi-speaker playback experiment to simulate an aggregation of breeding toads, we demonstrated that the transmission properties of choruses improve with the number of animals contributing overlapping calls. For example, we observed similar signal-to-noise ratios at 80 m from a 10-toad chorus compared to a single toad at 20 m, suggesting that a chorus could travel at least four times as far as a single call. We showed that this effect is enhanced with lower-frequency signals, compared to medium- or high-frequency calls. The chorus signal that arises from vocal overlap in male Yellow Toads may be an important indicator of location to far away animals, such as females prospecting for males or males prospecting for other chorusing animals.

Our data suggest that male Yellow Toad advertisement calls interfere constructively to create chorus-level “signal enhancement”, an idea that has not been experimentally studied in vertebrates. The signal amplitude during breeding choruses
has been documented in some fish (e.g., an amplitude increase in breeding versus non-
breeding reef fish; Fish and Cummings, 1972; McCauley and Cato, 2000) and anurans
(e.g., sound amplitude of a natural chorus of barking treefrogs decreases at greater
distances away from the chorus; Gerhardt and Klump, 1988), but the relationship
between number of individuals concurrently signaling and chorus amplitude has not
been quantified in vertebrates. It has been hypothesized that the active space of a signal
increases with the number of individuals vocalizing concurrently (e.g., Bradbury, 1981),
but until recently, this has not been experimentally tested. Hartbauer et al. (2014)
simulated the active space of concurrent acoustic signalling in the katydid *Mecopoda
elongata*. They found that the broadcast area of four combined signals strongly
increased when compared to lone signallers, but as predicted by Bradbury (1981) the
per capita mating advantage in the simulation decreased (Hartbauer et al., 2014). The
current study provides the first experimental evidence of acoustic signal overlap
resulting in chorus-level signal enhancement in an explosively breeding animal, and the
first experimental evidence of this phenomenon in vertebrates.

Animals can use the far-reaching chorus signal of male Yellow Toads to locate
breeding aggregations. Given the extremely short duration of the breeding season (1-3
days) that occurs on an unpredictable date (range: 28 April to 6 June in the last 8 years
years at our study site), male and female Yellow Toads may use this chorus-level
information to quickly find viable mating locations and gain access to potential mates
(Bee, 2007; Swanson et al., 2007). Such a phenomenon has been observed in explosively
breeding wood frogs (*Lithobates sylvaticus*), where males reliably move toward
conspecific chorus noise (Bee, 2007). But there are also potential costs to signalling in a chorus. The opportunities to mate with females decrease with the number of rival males, as predicted by Bradbury (1981). Further, loud choruses can potentially be exploited by predators or parasites to locate an abundant and temporary herpetofaunal food source that is normally hidden for the rest of the year in the Neotropical dry forest (Savage, 2002). Many parasites and predators have been documented to locate prey animals by eavesdropping on chorus signals. For example, male field crickets (Teleogryllus oceanicus) in Hawaii have been heavily parasitized by an acoustically orienting parasitoid fly (Zuk et al., 1993). Likewise, eavesdropping frog-eating bats and blood-sucking flies can localize and judge the densities of male túngara frogs (Physalaemus pustulosus) based on the complexity of their advertisement calls (Bernal et al., 2007). More work is needed using phonotaxis experiments to understand whether conspecifics and predators use Yellow Toad chorus signals to locate breeding aggregations.

Frequency effects on acoustic transmission have been well documented in diverse types of animal sounds (Bradbury and Vehrencamp, 2011; Forrest, 1994; Wiley and Richards, 1978). Low frequency signals are less subject to excess attenuation, scattering due to obstacles, atmospheric absorption, and thus travel farther than high-frequency signals, which are more vulnerable to these effects (Forrest, 1994). Indeed, we found a significantly higher signal-to-noise ratio in the low-frequency chorus signal than the high- and medium- frequency chorus signals. This is particularly relevant in a taxon that has a known relationship between body size and peak frequency, such as
Yellow Toads (Chapter 2). More work is needed to determine the exact function of male
Yellow Toad vocalizations, but because they contain body size information (Chapter 2),
there is potential for animals to be attracted to a breeding aggregation with larger-
bonied animals.

In this study, we provided the first demonstration of chorus-level signal
enhancement in vertebrates. Our findings suggest that for animals breeding and
vocalizing in dense aggregations, the additive effects of vocal overlapping have the
potential to transmit over greater broadcast distances. Male vocalizations enhance the
signal strength of the overall chorus and consequently increase the likelihood of
conspecifics perceiving the chorus signal. The resulting signal from advertisement call
overlap may attract additional male signallers, potential mates, and predators to the
explosive breeding aggregation from far away. We suggest that any animals that breed
in large aggregations and signal acoustically will experience this effect. Further, the
chorus-level signal enhancement found in this study has implications for the evolution
of vocal sound production and chorus-signal perception in diverse animal taxa.

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References


Figures

Fig. 3.1. Waveforms (top) and sound spectrograms (bottom) of the natural-frequency advertisement calls produced by one, five, or ten male Yellow Toads at four different distances between the broadcasting loudspeaker(s) and the microphone. Waveforms and spectrograms reveal more sound energy at closer distances and as the number of toads calling concurrently increases.
**Fig. 3.2.** The signal-to-noise ratio of the advertisement calls of Yellow Toads decreases with recording distance and increases with the number of loudspeakers producing simultaneous calls. For each distance we show the mean and SE for playback of one call from one loudspeaker (dotted line), five calls from five loudspeakers (dashed line) and ten calls from ten loudspeakers (solid line).
Fig. 3.3. When the pitch of the advertisement calls of Yellow Toads was adjusted to low, medium, and high frequencies – corresponding to the 10th, 50th, and 90th percentiles of natural values for this species – they showed significantly higher signal-to-noise ratios at low frequencies (solid lines) than at medium (dashed lines) and high frequencies (dotted lines). Each line shows the mean and SE.
Chapter 4

Dude looks like a lady: Experimental evidence that dynamic colour dimorphism

produces a sex signal in an explosively breeding Neotropical toad
Chapter Summary

Many animals breed in large mating aggregations, where males must rapidly discriminate between prospective mates and rivals. Selection may favour features that facilitate rapid discrimination in these aggregations. The explosively breeding Neotropical Yellow Toad, *Incilius luetkenii*, exhibits a rapid and dramatic colour change; males change from a cryptic brown to a conspicuous lemon yellow for their brief breeding period. Females, in contrast, remain cryptic brown throughout the year. The function of this temporary, sex-specific colour change is unknown. We tested the hypothesis that yellow colouration displayed by male *I. luetkenii* facilitates sex recognition during both daytime and nighttime mating aggregations. We created yellow and brown model toads and presented them to males during a breeding event. Male *I. luetkenii* responded significantly more intensely to brown models compared to yellow models, approaching them and making more amplexus attempts on the brown versus yellow models. This strong pattern held true regardless of ambient light intensity, making this the first study to expose a dynamic colour signal that operates at both day and night. Our results indicate that male *I. luetkenii* use colouration to quickly discriminate between males and females during their brief, explosive mating aggregations. Our findings suggest that the rapid, dramatic colour change of male *I. luetkenii* facilitates sex recognition, which could provide a significant fitness advantage to males in the form of reduced energy expenditure and reduced risk of injury by other males. Dynamic dichromatism may provide similar fitness benefits in any organisms that mate in large, competitive aggregations.
Introduction

Many animals breed in dense mating aggregations comprising tens to thousands of individuals, including diverse insects (Antolin and Strand, 1992; Beani and Turillazzi, 1990), fish (Foote et al., 1997), anurans (Wells, 1977), mammals (Orbach et al., 2014), and birds (Post and Greenlaw, 1982). Within these aggregations, males often engage in scramble competition mating, roaming to find and secure a breeding partner before their rivals do (Thornhill and Alcock, 1983). Scramble competition should select for attributes that allow animals to rapidly acquire mates (Thornhill and Alcock, 1983), such as high mobility and locomotion (Dickinson, 1992; Schwagmeyer, 1988), well-developed sensory organs (Andersson and Iwasa, 1996), and sexually dimorphic features (Able, 1999). Dichromatism is a common type of sexual dimorphism, wherein females and males exhibit differences in colouration (Badyaev and Hill, 2003; Endler, 1984).

Sexual dichromatism may arise from a combination of sexual selection for bright colouration in males and natural selection for cryptic colouration in females (Andersson, 1994). Bright male colouration should be favoured if it enhances a male’s ability to repel rivals or attract mates (Andersson, 1994). Sexual dichromatism has most commonly been studied in birds (e.g., Badyaev and Hill, 2003), fishes (e.g., Kodric-Brown, 1998), lizards (e.g., Macedonia et al., 2003), and butterflies (e.g., Allen et al., 2011). In these groups, bright male colouration has been shown to signal important information about males, including physiological condition (e.g., Thompson et al., 1997), dominance (e.g., Kodric-Brown, 1998), and fighting ability (e.g., Olsson, 1994). Sexual dichromatism has recently garnered attention in anurans (e.g., Bell and Zamudio, 2012; Doucet and
Mennill, 2010; Maan and Cummings, 2009; Ries et al., 2008). Bell and Zamudio (2012) distinguish two classes of sexual dichromatism in anurans: ontogenetic dichromatism and dynamic dichromatism. Although sexual dichromatism is relatively rare in anurans, having been documented in only 2% of species (Bell and Zamudio, 2012). Of the dichromatic species, ontogenetic dichromatism is more common than dynamic dichromatism (at least 92 species documented from 18 families and subfamilies exhibit ontogenetic dichromatism; Bell and Zamudio, 2012) and it occurs when females and males are permanently different in colour. Dynamic dichromatism is uncommon (31 species documented from 9 families and subfamilies; Bell and Zamudio, 2012), occurring when males experience a temporary change in colour, typically during the breeding season (e.g. the European moor frog, *Rana arvalis*; Ries et al., 2008).

Applying our knowledge of the mating tactics of species exhibiting sexual dichromatism may advance our understanding of the selective pressures favouring these colour differences. In animals that breed explosively (i.e., for a short duration when environmental conditions are favourable), differences in colouration between viable, opposite-sex mates and non-viable, same-sex mates should facilitate reproduction (Sztatecsny et al., 2012). Dynamic dichromatism, rather than ontogenetic dichromatism, may evolve when signals of sex are important for only an extremely brief period of an animal’s life cycle (i.e., a brief breeding season), especially if the conspicuous colouration is costly to produce or maintain.

The Neotropical Yellow Toad, *Incilius (Bufo) luetkenii*, is an explosively breeding species that exhibits dramatic dynamic sexual dichromatism in a scramble competition
mating system (Doucet and Mennill, 2010). Males rapidly shift from a cryptic brown to conspicuous lemon yellow at the onset of their very brief mating event, coincident with the first rain of the year in their dry forest habitat (Doucet and Mennill, 2010). After males enter amplexus, they quickly return to their cryptic colouration until females complete egg laying, and then disperse (Doucet and Mennill, 2010). With a short breeding season that lasts just one to three days, a rapid shift toward conspicuous colouration in males may facilitate quick discrimination between sexes while reducing risk of injury within these animals’ large mating aggregations (Sztatecsny et al., 2012).

In this study, we tested the hypothesis that male *I. luetkenii* use conspicuous yellow colouration to discriminate between prospective female breeding partners and rival males. To test this hypothesis, we presented males with models of yellow toads – simulating the colour of breeding males – and models of brown toads – simulating the colour of non-breeding males and breeding females. If this short-lived colour dimorphism is important in sex discrimination, we predicted that males would approach the brown models more frequently than yellow models, and that males would attempt to mate with the brown models more frequently than the yellow models. Furthermore, *I. luetkenii* aggregate during both day and night at the onset of the rainy season, which may have selected for colour signals that are effective in both high- and low-light conditions. We therefore predicted that discrimination between yellow and brown models would be highest during the day, but would still be present, albeit at lower levels, at night.
Methods

Study site and species

We conducted this experiment in Sector Santa Rosa of the Guanacaste Conservation Area, a UNESCO World Heritage Site in northwestern Costa Rica (10°40’ N, 85°30’ W). This site is dominated by Neotropical dry forest, where the climate is defined by little-to-no rain during the dry season from mid-November to mid-May, and abundant rain for the rest of the year (Campos and Fedigan, 2013). *Incilius luetkenii* are distributed across the Pacific lowlands from southern Mexico to central Costa Rica (Savage, 2002) and are common in Sector Santa Rosa (Doucet and Mennill, 2010). *Incilius luetkenii* are understood to emerge from underground hideaways and form breeding aggregations at the onset of the rainy season (Savage, 2002). We have found these aggregations to persist for the first 1-3 days following the first significant rain of the year at our study site. Annually, we witness hundreds of individuals aggregating in seasonally dry creek beds that have been filled by the first rain. The aggregations form during the evening of the first rain, peak in number the following morning, then decline but remain for up to two more days. Males scramble to enter amplexus with females when they arrive at the mating aggregation, and remain in amplexus throughout the morning. Egg laying occurs after most males have either entered amplexus or left the pond. Our observations suggest that the mating aggregations of *I. luetkenii* can be best understood as scramble competition mating, given that males do not hold resources and actively compete with rival males by engaging in searching and male-male displacement behaviour for incoming females at these sites (Wells, 1977).
Male *I. luetkenii* exhibit conspicuous yellow colouration during their short breeding events, whereas females are generally cryptic and dull in colour (Fig. 4.1 a, e). Previous work has shown that males rapidly change back from yellow to brown following amplexus (Doucet and Mennill, 2010). In designing this experiment, we chose to present models to males rather than females, because some authors argue that females exhibit little choice in scramble competition mating systems (Rausch et al., 2014), a notion that matches our observations that females undergo intense harassment by many males as they approach the mating aggregation.

All work was approved by our university’s animal care committee (Animal Care Permit #AUPP-13-16) and the government of Costa Rica (MINAE).

**Model design**

We created six yellow and six brown model toads from non-toxic modeling clay (Craftsmart Polymer Clay). We based the shape of the models on photographs taken at our study site in previous years. We used the 82 mm average snout-to-vent length of 81 males that we measured in 2013; this falls within the published size range of both males and females, though females are generally larger than males (77 to 99 mm for males, 73 to 107 mm for females; Savage, 2002). We baked the clay to harden it and used nontoxic acrylic paint to colour the models (Craftsmart Acrylic Paint). We painted brown models from white clay (7:2:1 Golden Brown:Olive:Black; numbers refer to colour ratios, and colours refer to the respective Craftsmart Acrylic Paint name). We produced yellow models from yellow clay that did not require base paint. We used paint to highlight prominent features on both the brown and yellow models, including tympana (9:1...
Olive:Black), parotid gland outlines (Black), eye outlines (Black), and throat (9:1 White:Beige). We glued plastic eyes into eye cavities of the models, using identical eyes for the yellow and brown models (model: ISPE-1, 10 mm, brown; glasseyesonline.com). We made all models resistant to water damage by coating the models with nontoxic sealer (Outdoor Mod Podge Waterproof Sealer). Yellow models were designed to simulate males in the breeding season whereas brown models were designed to simulate the colour of males outside of the breeding season, or females throughout the year. The only major difference between the yellow and brown models was the base colour (Fig. 4.1 b, c).

Model presentation

We conducted a total of 114 trials by presenting pairs of yellow and brown models to live toads following the first significant rain of the year on 10 May 2014. The breeding event started in the evening of 10 May ($N$ trials = 9) and persisted across 11 May ($N$ = 64), and 12 May ($N$ = 20), concluding in the afternoon of 13 May 2014 ($N$ = 10). A small number of trials were conducted during 31 May 2014 ($N$ = 11), when a substantial rain that followed a 20-day dry period elicited an additional $I. luetkenii$ mating event. Each trial consisted of the presentation of one brown and one yellow model for 5 minutes. Models were separated by 0.5 m and situated near the edge of three different ponds. We affixed fishing line to the bodies of the models and moved them in a lifelike manner, with short simulated hops up and down (as in Sztatecsny et al., 2012). If a model fell on its side or back and could not be moved upright using the fishing line, we paused the trial, placed the model upright, then resumed the trial. Upon
trial completion, we moved the pair of models to a new location, at least 3 m from the preceding location, moving clockwise around the pond to avoid sampling the same toads repeatedly. Given that our subjects were unmarked, we cannot rule out that some individuals were sampled repeatedly. There were, however, hundreds of individuals at each of the three ponds and we moved systematically away from the subjects around the perimeter of the breeding aggregation. Because of this, we are confident that repeated sampling was very minimal. We used video cameras with infrared features to record each trial, allowing us to record across light and dark conditions (Bell & Howell DNV16HDZ digital video recorder).

*Day versus night*

We conducted this experiment during both daytime and nighttime hours, because *I. luetkenii* mate during both day and night, and because we were interested in documenting any changes in model preference as a function of ambient light intensity. We measured the intensity of light every 15 minutes throughout the breeding event to document changes in ambient light (EXTECH EasyView Digital Light Meter). Daytime trials (*N* = 61) were conducted between 05:25 h and 11:00 h and were defined by greater than 1.0 lux of ambient light intensity (Fig. 4.2). Nighttime trials (*N* = 39) were conducted between 23:30 h and 05:00 h and were defined by 1.0 lux or less of ambient light intensity (Fig. 4.2). During nighttime data collection, there was very little ambient light, with light from the moon and stars making it barely possible for us to make out the shapes of animals on the ground. We minimized the use of artificial lights to conduct the
experiment by avoiding the use of flashlights and relying on the infrared feature on our video cameras.

Evaluation of trials

We quantified the toads’ responses to the model presentations based on video recordings using JWatcher software (v.1.0; Blumstein and Daniel, 2007). We evaluated four behaviours exhibited by male toads towards the models: number of physical contacts with each model, time in contact with each model, number of amplexus attempts with each model, and time in amplexus with each model. Amplexus attempts were scored when a male toad was on the model’s back and had both arms around the sides of the model. Temporal measurements were calculated as the difference between the time of contact termination and the time of contact initiation.

Statistical analysis

Our data for all four behavioural responses were non-normal owing to a preponderance of values concentrated around zero. We used Wilcoxon signed-rank tests to compare the number of contacts, time in contact, number of amplexus attempts, and time in amplexus between yellow and brown models.

We were interested in determining whether there were differences in male colour preference for each behavioural measure in daytime versus nighttime trials. To analyze this, we split each response variable into responses to yellow models and responses to brown models (i.e., number of contacts with brown model, time in contact with brown model, number of amplexus attempts with brown model, time in amplexus with brown model, number of contacts with yellow model, time in contact with yellow
model, number of amplexus attempts with yellow model, time in amplexus with yellow model). We then compared responses in the daytime versus the nighttime for each of those eight variables. We used Mann-Whitney U tests to compare the response measures between night and day.

All statistical tests were conducted using SPSS (version 20; IBM, Chicago, IL, U.S.A.). All tests of significance were two-tailed, with a threshold of 0.05.

Results

Toads responded strongly to the presentation of the clay models, hopping towards at least one of the models in the majority of trials (\( N = 100 \) of 114 trials). We excluded fourteen trials from subsequent analysis because no toads responded to either the brown or yellow model.

Daytime trials

Male *I. luetkenii* responded significantly more intensely to brown models compared to yellow models across all behavioural measures during daytime trials (Fig. 4.3; Wilcoxon signed-ranks tests for number of contacts, time in contact, number of amplexus attempts, time in amplexus: all \( Z > 5.2, N = 61, P < 0.001 \)).

Nighttime trials

As in the daytime trials, male *I. luetkenii* responded significantly more intensely to brown models across all behavioural measures (Fig. 4.3; Wilcoxon signed-ranks tests for number of contacts, time in contact, number of amplexus attempts, time in amplexus: \( Z > 4.7, N = 39, P < 0.001 \)).
**Day versus night**

Male toads responded significantly more intensely to the models during the day versus night for two of eight trial response variables: number of contacts with the yellow model and time in contact with the yellow model (Mann-Whitney $U$ tests: number of contacts with yellow model: $U = 851, N1 = N2 = 100, P = 0.02$; time in contact with yellow model: $U = 830, N1 = N2 = 100, P = 0.01$). The six other measures were not significantly different (Mann-Whitney tests: $U > 930, N1 = N2 = 100, P > 0.05$).

**Discussion**

When presented with two model toads that differed only in base colouration, male *Incilius luetkenii* responded significantly more intensely to the brown female-like model compared to the yellow male-like model. Our results suggest that males use the conspicuous yellow colouration of other males as a signal of sex during the breeding period. Explosively breeding anurans that engage in scramble competition are expected to benefit from features that aid in rapid differentiation of mates versus rivals (Sztatecsny et al., 2012; Wells, 1977). Indeed, this phenomenon has been observed in a phylogenetically and geographically distant relative of *I. luetkenii*; the European moor frog (*Rana arvalis*) also exhibits dynamic sexual dichromatism, with males shifting to a conspicuous blue colouration during the breeding season (Ries et al., 2008). When male *R. arvalis* were presented with differently coloured models, they also preferred to clasp brown models compared to blue models (Sztatecsny et al., 2012). In contrast with *R. arvalis*, however, *I. luetkenii* rapidly return to pre-nuptial colouration after mating.
(Doucet and Mennill, 2010). This difference opens potential for future work to explore further the function of these differences across dynamically dichromatic species. Our findings represent the second demonstration of the use of dynamic sexual dichromatism as a visual signal used by male anurans to quickly discriminate between mates and rivals, and the first demonstration that this pattern holds true both in daylight and during the night.

Our data suggest that the rapid, dramatic colour change of male *I. luetkenii* facilitates sex recognition, which could provide a significant fitness advantage to males. Turning a conspicuous yellow colour is likely costly for males, perhaps due to the increased predation risk of being yellow, or due to the increased energy expenditure of turning from brown to yellow. The net benefit of turning yellow must therefore outweigh the cost of remaining brown. Indeed, brown female *I. luetkenii* undergo intense aggressive harassment during the breeding season, with many males attempting amplexus on a single female (unlike in *Bufo pardalis*, Cherry, 1992). This intense aggression routinely results in female death; in all of the large aggregations we have observed in *I. luetkenii*, there has been at least one female floating dead in the middle of the mating aggregation with many males continuing to attempt amplexus. Given the high similarity in male and female colouration outside the breeding season (Doucet and Mennill, 2010; Savage, 2002), males might face significant risk of injury or death by other males if their sex was misidentified in the context of the breeding aggregation. Thus the yellow colouration of males might both facilitate the rapid identification of females and while also preventing the risk of injury by other males.
The conspicuous yellow colouration of male *I. luetkenii* during the breeding event may also be important to females. For example, females might prefer “yellower” males, just as females prefer colourful males in other taxa (e.g. birds: Sætre et al., 1994; fish: Milinski and Bakker, 1990; insects: Kemp, 2007). Although we did not test this, female choice is likely constrained in *I. luetkenii* given their scramble competition mating system (Sullivan et al., 1995). In animals that engage in scramble competition mating, male mating success is closely tied to successful mate searching and winning overt male-male conflicts (e.g., thirteen-lined ground squirrels; Schwagmeyer and Woontner, 1986). Although males of some animal species do not engage in intrasexual struggles (Cherry, 1992), *I. luetkenii* males are intensely aggressive, which may limit active female mate choice. Indeed, Rausch et al. (2014) found that male *R. arvalis* nuptial colouration did not influence paternity success during scramble competition, and therefore was not a trait selected for by females. Females may still shape the outcome of successful amplexus based on other factors (e.g., female delay in oviposition; Eberhard, 1996), but females may have limited control over which males make contact with them and attempt amplexus with them.

Male toads could also use yellow colouration as a symbol of status; differences in yellow colour could signal fighting ability or condition. The intensity of the yellow colouration may be influenced by agonistic male-male interactions and may be similar to “badges” as signals of dominance in birds (Rohwer, 1982). Temporary colour change as a dominance signal has been observed in certain fishes (Kodric-Brown, 1998). For example, the intensity of temporary blue male nuptial colouration in pupfish
(Cyprinodon pecosensis) is influenced by both intersexual and intrasexual selection. Male pupfish are most intensely blue when they possess a territory, engage in agonistic male-male interactions, and when there are gravid females present (Kodric-Brown, 1996). Future work with male I. luetkenii should evaluate whether variation in male yellow colouration reflects male fighting ability, dominance status, or some other aspect of male quality.

We compared male I. luetkenii responses to male and female models during the day and night, making this the first study of the function of dynamic colour signals across extremes in lighting environments. Males clearly preferred the brown models during both the bright light of day and the very dim light of night, although the toads seemed to be more responsive overall during the day for two response measures: number of contacts with the yellow model, and time in contact with the yellow model. Interestingly, the magnitude of the response to the yellow and brown models appears similar across light conditions, suggesting that male I. luetkenii were able to discriminate between the models equally well during both the day and the night. Our findings show that male I. luetkenii perceive and use this visual signal of sex during both day and night.

Our findings are consistent with the assumption that scramble competition polygyny selects for high-functioning sensory systems (Andersson and Iwasa, 1996) and that toads (Bufo bufo) possess good vision under low-light conditions (Aho et al., 1993). It is unclear whether male I. luetkenii are attending to the colour (chromatic signal) or brightness (achromatic signal) of male skin during the night. Nocturnal anurans have rod classes (Liebman and Entine, 1968), and attending to chromatic versus achromatic
signals depends on whether the rod responses are summed or compared by the neural system (Gomez et al., 2010). Little is known about nocturnal colour vision in anurans; however, recent discoveries suggest colour vision in low-light conditions is not as rare as initially expected (Kelber and Roth, 2006). At least one anuran (Hyla arborea) is able to use colour signals during nighttime mating behaviour, with females preferring conspicuously coloured males (Gomez et al., 2009; Gomez et al., 2010). Males of 75% of the documented anuran species that exhibit dynamic colour changes during the breeding season tend to shift to become yellower, than females (Bell and Zamudio, 2012). This disproportionate amount of yellow colouration in frog dynamic dichromatism may be selected for by the environment; shifts toward yellow skin colour may reflect the nocturnal forest visual light environment, which has been documented as being yellow-green dominated with a peak flux of 560 nm (Veilleux and Cummings, 2012). Yellow colouration in animals is also relatively bright, and as such could effectively reflect the small amount of light available at night, even if the animals rely on the achromatic versus the chromatic part of the signal. More work is needed to understand the roles of scotopic and photopic vision in low-light situations, and the particular importance of the yellow colouration in I. luetkenii and similar anuran species.

Few anuran species have been shown to exhibit dynamic sexual dichromatism, and fewer still in such a dramatic fashion as I. luetkenii. The short, explosive breeding season of I. luetkenii results in costly mate-recognition challenges. Our findings suggest that the dynamic shift toward conspicuous yellow colouration by males serves to overcome these challenges by facilitating rapid discrimination between potential mates.
and rivals. This is the first documentation of this phenomenon in toads, and the first
documented tropical anuran to exhibit a dynamic and conspicuous visual signal of sex
recognition. Our data also reveal that males successfully use the conspicuous yellow
colouration during both daytime and nighttime breeding, suggesting that males may
have effective low-light vision. Sex-specific signals are common in nature, and often
appear to be a byproduct of stronger sexual selection on one sex (Andersson, 1994). Our
findings suggest that in species with competitive mating aggregations, divergence in
colouration between the sexes may be under direct selection to facilitate sex
identification and prevent costly mistakes in sex recognition. Dynamic dichromatism
should be favoured when the cost of remaining conspicuously coloured is high.

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References


Fig 4.1. Male Neotropical Yellow Toads, *Incilius luetkenii*, exhibit a conspicuous yellow colouration solely during their brief breeding event (a). We presented clay models of yellow toads (b) and brown toads (c) to males during their explosive mating aggregations. Males often responded by approaching and attempting amplexus with brown models (d), just as they do with live, brown females (e).
Fig. 4.2. Light intensity during nighttime trials and daytime trials of the model presentation experiment to Neotropical Yellow Toads, *Incilius luetkenii*. 
Fig. 4.3. Breeding male Neotropical Yellow Toads, *Incilius luetkenii*, respond significantly more intensely to brown models compared to yellow models for four behavioural measures: (a) number of contacts per trial, (b) time in contact per trial, (c) amplexus attempts per trial, and (d) time in amplexus per trial. We observed significantly higher response intensity during daytime trials compared to nighttime trials for 2 of 8 measures: number of contacts with the yellow model, and time in contact with the yellow model. Error bars represent standard error around the mean.
Chapter 5

General Discussion
Signals produced by males are often influenced by sexual selection through female choice and male-male competition (Emlen and Owring, 1977; Gerhardt, 1994). Anurans are a well-studied order of animals wherein breeding takes place in large aggregations while males vocalize intensely to attract females (Gerhardt and Huber, 2002). There are many communication challenges for the animals in these loud, competitive systems, including difficulty with sound localization and mate recognition (Gerhardt and Huber, 2002). These problems seem to become exacerbated in species that breed in aggregations that last for a very short duration; in such situations, animals must quickly finding a breeding aggregation and then finding a mate during a brief time window (Wells, 1977). These aptly named “explosive breeders” often engage in scramble competition for mates, whereby males actively search for females while fighting rivals (Wells, 1977). Signals that facilitate sex recognition (e.g., Sztatecsny et al., 2012), rival assessment (e.g., Davies and Halliday, 1978), and breeding localization (e.g., Hartbauer et al., 2014) should be selected for in such mating systems. In my thesis, I studied such signals in an explosively breeding Neotropical toad, *Incilius luetkenii*.

The first data chapter of my thesis (Chapter 2) explored the vocal behaviour of male *I. luetkenii*. I recorded and measured 74 individuals from a population in northwestern Costa Rica and provided quantitative descriptions of the spectro-temporal properties of male advertisement calls and release calls. I also tested the hypothesis that spectral features of male advertisement calls are inversely related to male body size. Consistent with studies of animal vocalizations from diverse taxa (e.g., Fitch, 1997; Gingras et al., 2013), I found a strong negative correlation between spectral frequency
within male calls and their body size. Male and female receivers may use this size-related vocal signal to assess the attractiveness and fighting potential of the males (Davies and Halliday, 1978; Charlton et al., 2006). Additionally, I quantified the context of male advertisement calls. Based on video recordings of breeding males, I found that in most cases, males were in physical contact with other males directly before producing an advertisement call. This finding, along with our understanding the scramble competition mating system of *I. luetkenii*, suggests that male advertisement calls are associated with male-male aggression.

In my second data chapter (Chapter 3), I tested the hypothesis that overlapping advertisement calls produced by male *I. luetkenii* enhance the signal amplitude of the chorus. I broadcast natural and frequency-transposed toad calls from up to ten different speakers simultaneously and re-recorded the overall chorus signal at different distances. I found that the signal-to-noise ratio of the chorus was influenced by the distance from the chorus, the number of speakers broadcasting, and the frequency of toad calls the chorus comprised. This effect was further pronounced for low-frequency choruses compared to medium- and high-frequency choruses. By advertising in a breeding aggregation, male toad calls overlap to create a far-reaching chorus signal that has the potential to draw in females from greater distances than vocalizing as a lone male. The broadcast range of signal-enhanced chorusing has been demonstrated in some insects (e.g., Hartbaeur et al., 2014), but to my knowledge this is the first demonstration of chorus-level vocal signal enhancement in vertebrates.
In my third data chapter (chapter 4), I explored the function of the bright yellow colouration observed in male *I. luetkenii* during the breeding event. I tested the hypothesis that the yellow colouration facilitates sex recognition in males during both daytime and nighttime breeding aggregations. I conducted model presentation experiments wherein I presented male *I. luetkenii* with yellow and brown clay models that simulated males in and out of the breeding season, respectively. Males responded significantly more intensely to the brown models compared to yellow models, approaching and attempting to mate with the brown models significantly more often than the yellow models. This pattern held true during both daytime and nighttime trials. My findings suggest that males use this dynamic yellow colouration to discriminate between sexes, which likely provides a fitness advantage in the form of reduced energy expenditure and reduced risk of injury by other males. Dynamic dichromatism as a signal of sex has been studied once before in the European moor frog (Sztatecsny et al., 2012), but this is the first study to explore dynamic colour as a signal of sex in a Neotropical anuran, and the first to demonstrate this effect across light conditions.

When viewed together, the conclusions of my first two data chapters provide insight into the vocal behaviour of male Yellow Toads. My first data chapter revealed that individual male Yellow Toads produce advertisement calls primarily in aggressive contexts within their dense breeding aggregations. These signals advertise male body size and can potentially overlap to create a chorus signal that can presumably be detected by animals at far distances. I demonstrate in Chapter 2 that low-frequency advertisement calls travel farther than medium- and high-frequency signals. Therefore,
aggregations with larger calling males (and thus lower-frequency, farther-reaching calls) have the potential to attract females from greater distances than do aggregations with smaller males. Although this idea remains untested, females may be able to choose aggregations with the largest males based on low-frequency dominated chorus signals. Preference for large male size, as indicated through male vocalizations, is well established in other systems including red deer (Charlton et al., 2007). Furthermore, any male Yellow Toad may benefit from joining an aggregation with larger-bodied animals; in doing so, even a smaller toad may find itself in an aggregation that has the potential to attract more prospective breeding partners.

Taken together, the results of Chapter 2 and Chapter 4 point towards an important role of aggressive male-male interactions in the mating system of my study species. Male Yellow Toads engage in scramble competition for mates whereby they actively search for mates and interact aggressively with each other. Male colouration appears to help males discriminate between the sexes (Chapter 4). These animals routinely engage in male-male physical encounters (Chapter 2), and following these encounters, males routinely produce calls, which have the potential to signal fighting ability via body size (as in Davies and Halliday, 1978). By signalling body size after an aggressive interaction, males may deter future physical encounters with nearby males, thereby reducing energy expenditure and risk of injury. Future research should experimentally test the aggressiveness and fighting ability of males and relate this information to body size, spectral characteristics in their calls, variation in yellow colouration, and male physiology. This work can be further expanded by exploring the
function of male advertisement calls from the perspective of the receiver (males and females) through experimental playback.

Animal choruses may provide a signal to those animals attempting to locate breeding aggregations from some distance away. Females may migrate toward choruses for the potential to find high quality mates, whereas males may migrate toward choruses for a greater potential of finding a female (Ryan et al., 1981). Predators and parasites may also take advantage of this chorus signal as a means of locating prey that is normally cryptic and difficult to detect in the Neotropical dry forest (Bernal et al., 2007). Future studies should test the hypothesis that Yellow Toad choruses attract conspecific and heterospecific animals. This could be executed by broadcasting chorus signals from artificial ponds while toads are migrating from their hideaways to the breeding site (e.g., Buxton et al., 2015). Counting the number of males, females, and predators that arrive to these artificial ponds would provide evidence that animals are using these chorus signals.

The dynamic sexual dichromatism exhibited by male *I. luetteii* is a rare trait; among more than 6500 anurans (AmphibiaWeb, 2015) dynamic dichromatism has been described in only 31 species from 9 families and subfamilies (Bell and Zamudio, 2012). Yellow Toads stand out for the rapid speed of the transition between two male colour forms, which changes in a matter of minutes, whereas most others change over the course of days (Bell and Zamudio, 2012; Doucet and Mennill, 2010). Future research should study the mechanisms of rapid colour change in male Yellow Toads, including quantifying the rate of change from brown to yellow (yellow to brown documented in
Doucet and Mennill, 2010), determining what environmental factors are necessary to facilitate colour change, studying the specific mechanism that produces brown and yellow colouration, and quantifying neuro-hormonal regulation throughout the colour change process (as in Kindermann et al., 2013; Kindermann et al., 2014). My research adds to our growing understanding of this phenomenon by revealing a function that facilitates mate recognition in males.

In summary, my research provides insight into the vocal and visual signals produced in the explosive mating aggregations of Yellow Toads. My work in Chapter 2 provides a rigorous acoustic quantification of male advertisement calls that is becoming increasingly important in light of new acoustic monitoring techniques available (Blumstein et al., 2011) and a sharp decline in amphibian populations worldwide (Whitfield et al., 2007). The finding that vocal signal properties correlate to body size suggests a potential for males and females to assess rivals and mates based on vocalizations alone (Davies and Halliday, 1978). My work in Chapter 3 provides a test of chorus-level signal enhancement that had yet to be demonstrated in vertebrates. The far-reaching chorus signal that I have demonstrated here may be important for individuals attempting to locate the breeding aggregation, especially in Yellow Toads that have an brief, 1-3 day breeding season, and occurs with unpredictable timing in concert with the first rain of the year. My work in Chapter 4 elucidates the function of an uncommon male colour-changing phenomenon in anurans. The high density of males in the explosive breeding aggregation creates a sex-recognition problem that seems to be resolved through the dramatic sexual colour dimorphism of yellow males during the
breeding season. Taken together, my three data chapters have expanded our understanding of a mating system in which selection seems to have favoured signals that facilitate a rapid mating process.
References


### Vita Auctoris

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<thead>
<tr>
<th>Name</th>
<th>Nicolas Rehberg-Besler</th>
</tr>
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<tbody>
<tr>
<td><strong>Place of Birth</strong></td>
<td>Edmonton, Alberta</td>
</tr>
<tr>
<td><strong>Year of Birth</strong></td>
<td>1990</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
</tr>
<tr>
<td>Bev Facey Community High School, Sherwood Park, Alberta</td>
<td>2005 – 2008</td>
</tr>
<tr>
<td>MacEwan University</td>
<td>Edmonton, Alberta</td>
</tr>
<tr>
<td></td>
<td>2008 - 2013, B.A. [H], Psychology</td>
</tr>
<tr>
<td>University of Windsor</td>
<td>Windsor, Ontario</td>
</tr>
<tr>
<td></td>
<td>2013 - 2015, M.Sc., Biological Sciences</td>
</tr>
<tr>
<td></td>
<td>Yellow Toad Field Technician, Costa Rica, 2013-2015</td>
</tr>
<tr>
<td></td>
<td>Graduate Teaching Assistant, University of Windsor, 2014-2015</td>
</tr>
<tr>
<td></td>
<td>Ecology Lab Instructor, University of Windsor, 2013-2014</td>
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<tr>
<td></td>
<td>Bioacoustics Research Technician, Costa Rica, 2013</td>
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