Automated Monitoring Techniques Reveal New Proximate Cues of Houston Toad Chorusing Behavior

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Long-term monitoring of anuran populations is becoming routinely carried out using automated recording devices (ARDs hereafter; Oseen and Wassersug 2002; Saenz et al. 2006; Aide et al. 2013). These devices remotely collect audio of male anurans vocalizing to attract females during breeding periods (Bridges and Dorcas 2000). ARDs may be preferred when a large survey effort is required, when environments are inhospitable and difficult to access, or when seeking to avoid bias from human disturbance of vocalizing animals (Alldredge et al. 2007; Hutto and Stutzman 2009). ARDs range in cost and performance capabilities, and may be more affordable than manual call surveys (Charif and Pitzrick 2008; Rempel et al. 2013; Yip et al. 2017). Anuran breeding activity, and thereby male anuran chorusing, is strongly influenced by weather (Blair 1960, 1961; Blankenhorn 1972; Obert 1975; Saenz et al. 2006). Studies of the relationship between rainfall, temperature, and anuran breeding indicate that these correlates are unique for each species (Salvador and Carrascal 1990; Moriera and Lima 1991; Bertoluci 1998). With the ability to collect more complete data through the implementation of ARDs (Dorcas et al. 2010), studies of anuran breeding phenology and proximate cues for male anuran chorusing have increased in detail. In other words, ARDs allow us to monitor for longer periods of time, more frequently than manually performed surveys are commonly carried out. This refines our understanding of anuran natural history (Saenz et al. 2006; Williams et al. 2013; Willacy et al. 2015).

The Houston Toad, *Bufo houstonensis* (= *Anaxyrus houstonensis*) (Sanders 1953; Frost et al. 2006), is a rare anuran native to east central Texas, USA, that is listed as endangered at state, federal, and international levels (Gottschalk 1970; Honegger 1970; U.S. Endangered Species Act [ESA 1973, as amended]; Hammerson and Canseco-Márquez 2004). Drivers of population declines include habitat loss and fragmentation throughout its range, among other stressors (e.g., drought, wildfires, anthropogenic disturbance; Brown 1971; Potter et al. 1984; Duarte et al. 2014). Robust populations presently occur only in Bastrop and Robertson counties, Texas, USA.

Here we re-examine the meteorological variables associated with Houston Toad chorusing more completely and at a finer temporal resolution than is achievable through traditional manual call survey methods. To explore differences between conclusions drawn from traditional manual call surveys and our automated approach to monitoring Houston Toads we compared our findings to the United States Fish and Wildlife Service guidelines for conducting manual call surveys, which describe the putative ideal chorusing conditions for this species (USFWS, hereafter; USFWS 2007).

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Methods

Deployment of ARDs for this study occurred concurrent to those utilized in MacLaren et al. (2018), and thus what follows is a paraphrasing of those accounts. Data analyzed here were also utilized in the completion of the study by MacLaren et al. (2018). We deployed 20 ARDs (SM2, SM2+, and SM3; Wildlife Acoustics, Maynard, Massachusetts) at potential breeding locations in two counties of east central Texas, USA known to be occupied by the Houston Toad (N = 11 in Bastrop County and N = 9 in Robertson County). ARDs were monitored for chorusing events from 3 January to 12 July 2014. We mounted ARDs to structural objects within 10 m of the pond, drainage, or water body edge. We scheduled ARDs to record the first 10 min of every hour from 1800 to 0500 H. This resulted in 12, 10-min segments (120 min) of audio per ARD, per night. We collected audio in the WAC format, and reduced sample rate to 16 kHz, reducing the maximum frequency recorded to 8 kHz which is appropriate for most North American anuran vocalizations (Narins et al. 2004). ARDs required battery changes and SD card changes approximately every 40 days.

We used a previously designed and tested Houston Toad call recognizer (MacLaren et al. 2018) within the program Song-Scope (Version 4.1.3A), a bioacoustics software (Wildlife Acoustics), to analyze data from 1 March to 31 March 2014. We limited our data to one month to be both analytically manageable, and represent the annual peak of chorusing events (Brown et al. 2013). We used iButton Hygrochrons (Maxim Integrated, San Jose, California) to collect temperature and relative humidity in one-hour increments at each site. We obtained hourly measurements of wind speed, barometric pressure at sea level, precipitation, and moon illumination from the nearest weather station (~10 km away; Weather Underground, stations KGYB Bastrop, and KLHB Hearne, Texas, USA). To explore relationships among Houston Toad chorusing and the above environmental variables we conducted a principal components analysis using Program R v3.1.2 (Gottelli and Ellison 2004; R Core Team 2018). Predictors estimated included date (1 to 31, as categories), hour of night (1800 to 0500 H overnight [coded sequentially]), temperature (°C), percent relative humidity, percent moon illumination, 24-h cumulative precipitation (mm), barometric pressure at sea level (mmHg), and wind speed (kmph), with the occurrence of Houston Toad vocalizations used categorically as the response variable (1 = detected, 0 = not detected). Our principal components analysis includes environmental data coincident to the 12 h ARDs were in operation only. We normalized all variables by centering, such that their mean = 0, and scaling by dividing each category by its standard deviation within the R function "prcomp" (R Core Team 2018). We also calculated the mean, median, and range for each environmental variable observed during choruses detected via automated techniques and compared these values to the suggested conditions for manual call surveys (USFWS 2007).

Table 1. Variable loadings and variance explained by each principal component for environmental conditions observed during Houston Toad (*Bufo houstonensis*) chorusing activity detected by audio recording devices and automated recognition. Houston Toad vocalizations used in this analysis were collected in March 2014 from Bastrop and Robertson counties, Texas.

	Principal Component						
Parameter	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Hour	0.29	-0.226	0.435	0.368	0.713	-0.179	-0.018
Temperature	-0.595	-0.118	0.078	0.234	-0.032	-0.336	0.677
Humidity	0.447	-0.41	-0.025	0.349	-0.351	0.462	0.417
Wind speed	-0.078	-0.101	-0.824	0.083	0.507	0.18	0.094
Moon illumination	-0.182	-0.417	0.254	-0.702	0.249	0.377	0.179
Date	-0.403	0.408	0.246	0.344	0.135	0.683	-0.085
Barometric pressure	0.401	0.646	0.031	-0.264	0.176	0.009	0.566
Standard deviation	1.463	1.079	1.052	1.033	0.876	0.731	0.468
Proportion of variance	0.306	0.166	0.158	0.152	0.11	0.076	0.031
Cumulative proportion	0.306	0.472	0.631	0.783	0.892	0.969	1

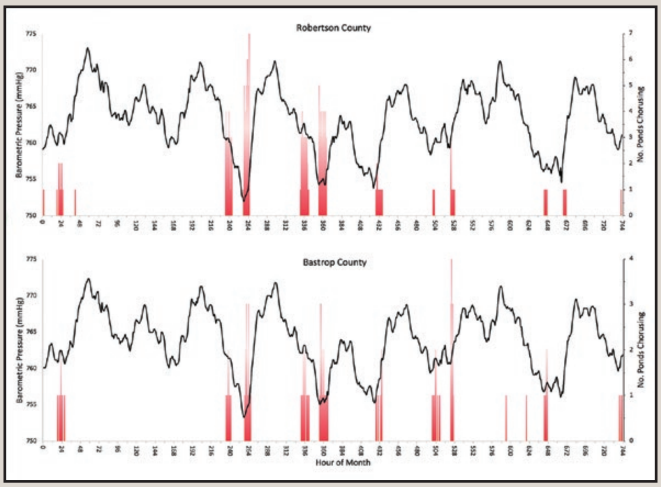

Fig. 1. Houston Toad (*Bufo houstonensis*) detections (left axis) coincident to troughs in barometric pressure (mmHg; right axis) in Robertson (top panel) and Bastrop (bottom) counties, Texas. Pink bars indicate the number of ponds with toads chorusing, and the black line depicts barometric pressure, for each hour of the month of March 2014 (N = 744). Detections of toads were made using SongScope bioacoustics software (Wildlife Acoustics). Recording devices monitored for 10 minutes at the beginning of every hour from 1800 to 0500 H each day. Barometric pressure was gathered from one weather station within ~10 km of each group of ponds monitored remotely in Bastrop and Robertson counties, Texas (Weather Underground, stations KGYB Bastrop, and KLHB Hearne, Texas).

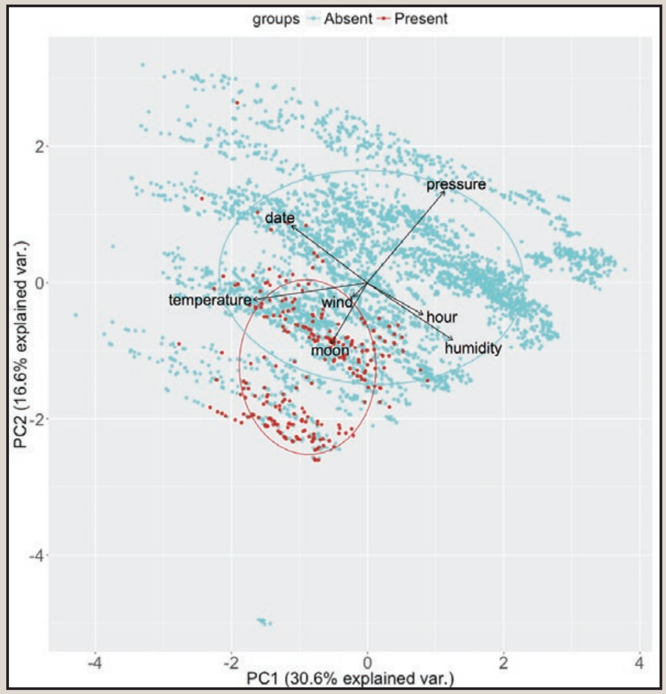

Fig. 2. Biplot of environmental variables observed in March 2014 in Bastrop and Robertson counties, Texas, in response to detection (red) or non-detection (blue) of Houston Toad (*Bufo houstonensis*) vocalizations. The first two principal components, each explaining 30.6% and 16.6% of the variation, respectively, illustrating the influence of date, hour of night, temperature, relative humidity, moon illumination, barometric pressure, and wind speed on the detectability of Houston Toads. Factors with the greatest positive and negative influence are temperature and barometric pressure, respectively. Ellipses represent the area of one standard deviation around the centroid of each categorical response variable (i.e., detection [red], non-detection [blue]).

Results

Each ARD (N = 20) carried out 372 surveys within March 2014. We detected Houston Toads chorusing at four of the 11 locations monitored in Bastrop County. We detected Houston Toad vocalizations from at least one site in Bastrop County on 16 of 31 dates in March 2014. The greatest number of surveys containing Houston Toad vocalizations from a single location within Bastrop County was 52. Figure 1 illustrates that in Bastrop County the Houston Toad breeding events clearly coincide with cyclical periods of low barometric pressure. We detected Houston Toads at seven of nine locations monitored in Robertson County. Again, we detected Houston Toad vocalizations from at least one site within Robertson County on 16 of 31 dates in March 2014. These dates differ from those in Bastrop County on only two occasions; detections occurred on 23 and 28 March within Robertson, but not Bastrop, whereas 25 and 26 March yielded detections in Bastrop County, but not Robertson. Nevertheless, these data show a general synchronicity across the two Texas counties. The greatest number of surveys containing Houston Toad vocalizations from a single site within Robertson County was 45. Figure 1 illustrates the

Table 2. Comparison of observed environmental conditions during Houston Toad (*Bufo houstonensis*) chorusing when detected by audio recording devices and automated detection versus the United States Fish and Wildlife Service (USFWS) manual call survey protocol. Houston Toad vocalizations used in this comparison were collected in March 2014 from Bastrop and Robertson counties, Texas.

Parameter	Observed conditions				USFWS
	Min.	Mean	Median	Max.	
Temperature (°C)	11.73	18.06	18.14	25.95	> 14.0
Relative humidity (%)	37.75	88.10	89.56	102.10	> 70.0
Wind speed (kmph)	0.00	10.47	11.10	24.14	< 24.14
Moon illumination (%)	0.00	29.61	78.00	100.00	"dark"
Barometric pressure (mmHg)	752.09	758.24	759.71	768.60	—
Precipitation (mm/day)	0.00	0.01	0.00	1.52	"recent"

same coincident relationship between low barometric pressure and calling activity in Robertson County as was found in Bastrop County.

Surveys considered for this study totaled 4464 presence/absence points. Houston Toads were detected in 264 surveys. Our principal components analysis illustrates relationships between Houston Toad detectability (i.e., vocalizations) and environmental conditions (Figs. 1 and 2; Table 1). The variation within the data accounted for by the first two principal components was 30.6% and 16.6%, respectively (Table 1, Fig. 2). Temperature and barometric pressure are oriented linearly relative to the detection or non-detection of Houston Toads, indicating a positive relationship between temperature and chorusing, and a negative relationship between barometric pressure and chorusing (Fig. 2). Temperature contributed the greatest to the first principal component with a loading of -0.595, whereas barometric pressure contributed the greatest to the second principal component with a loading of 0.646 (Table 1, Fig. 2). Fig. 1 illustrates bouts of chorusing occurred within troughs of barometric pressure repeatedly within March 2014 across both Bastrop and Robertson counties. These two groups of ponds are separated by ~100 km, but experience very similar patterns of change in barometric pressure (Fig. 1). Consequent to this phenomenon, periods of toad chorusing are coincident between these two distant sites (Fig. 1). Precipitation averaged 0.01 mm per day across both counties during our study, and was excluded as a predictor within our principal components analysis for this reason (Fig. 2). Our comparison between the suggested conditions appropriate for manual call surveys within the federal guidelines and the conditions in which ARDs detected chorusing reveal inconsistencies for temperature, humidity, moon illumination, and precipitation (Table 2). The USFWS does not include barometric pressure within their guidelines (USFWS 2007).

Discussion

Manual call surveys for Houston Toads are generally restricted to the environmental conditions that putatively influence chorusing among Houston Toads (USFWS 2007). What follows, we believe, are the apparent sources of that information, given that USFWS (2007) lacks a conventional works cited; a similar summary appears in Potter et al. (1984). Hillis et al. (1984) and Jacobson (1989) note temperatures above 14°C influence chorusing; however, subsequent studies have reported chorusing at lower thresholds (minimum of 7°C; Dixon et al. 1990; Price 2003; Brown et al. 2013). Price (2003) reports ≥ 70% relative humidity attributed to chorusing. The USFWS (2007) statements regarding moon illumination most closely resemble those of Price (2003; i.e., cloud cover present as moon approaches full), however the influence of this variable is inconsistent among comparable studies (Dixon et al. 1990; Swannack et al. 2009). Our findings with respect to moon illumination may be biased relative to previous findings because we did not account for cloud cover or other factors that might obscure the moon's perceived effect at each pond. Previous studies that rely solely on manual call surveys, of varying effort, document Houston Toad chorus detectability rather than true call occurrence. In other words, Houston Toads may chorus under conditions in which human observers would not detect them. Thresholds reported for wind speed exemplify this and could likely represent the maximum threshold at which human observers are able to detect chorusing. This is particularly relevant given that the majority of Houston Toad audio surveys conducted for regulatory monitoring are performed by human observers without specifically visiting each of the potential chorusing sites, but rather from public roadways that are nearest to the sites of interest. Our findings indicate that ARDs are useful in overcoming some, but not all, of these obstacles, due to the fact that they are able to monitor more frequently and in more extreme weather. For instance, we detected toads chorusing during periods of increased wind speed because ARDs are not as sensitive to this disruptive confound as human observers appear to be. That is not to say that ARDs are infallible, as this advantage is not consistent across all studies utilizing ARDs (Shearin et al. 2012). Additionally, our automated detection software protocol ensures that if a toad vocalized there is a near perfect probability of that call being detected (MacLaren et al. 2018).

Technology now enables advanced methods of characterizing chorusing events with increased temporal resolution. With the aid of ARDs we produced a detailed graphical representation of the conditions in which male Houston Toads were observed chorusing in March 2014 (Fig. 1). By viewing the subtle shifts in environmental conditions in sync with the chorusing activity of Houston Toads we observed relationships that have been overlooked or undiscovered in the past, such as the synchronicity of chorusing among two distantly located populations. Additionally, we discovered the impact barometric pressure may have on Houston Toad chorusing activity. This relationship was supported by the results of our principal components analysis (Fig. 2). The data here are extensive but still preliminary. This study represents the most extensive and intense monitoring effort of the species during a chorusing season to date. It is recommended that human observers perform 12 or more 5-min surveys per season, making the total effort required per site 60 min each season (USFWS 2007). For comparison, our study, that covered only one month rather than an entire breeding season, produced 120 min per night for 31 nights (i.e., 3720 min), a 62-fold increase in survey effort. Because these data do not explore broader interannual variation, particularly at small geographic scales often applied to regulatory decisions (i.e., within site variation), we do not offer survey revisions until further research across multiple years and geographic locations can be completed. We conclude that previous examinations of the environmental correlates to Houston Toad chorusing have been biased in two ways. First, by the limited number of surveys humans can conduct seasonally. Second, by the restriction of all previous call surveys to environmental conditions that were thought to be most influential at the time, which differ from the environmental cues we have described herein.

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Literature Cited

Aide, T. M., C. Corrada-Bravo, M. Campos-Cerqueira, C. Milan , G. Vega , and R. Alvarez. 2013. Real-time bioacoustics monitoring and automated species identification. PeerJ 1:e103.

Alldredge, M., K. Pollock, T. Simons, J. Collazo, S. Shriner, and D. Johnson. 2007. Time-of-detection method for estimating abundance from point-count surveys. Auk 124:653–664.

Bertoluci, J. 1998. Annual patterns of breeding activity in Atlantic rainforest anurans. J. Herpetol. 32:607–611.

Blair, W. F. 1960. A breeding population of the Mexican toad (*Bufo valliceps*) in relation to its environment. Ecology 41:165–174.

———. 1961. Calling and spawning seasons in a mixed population of anurans. Ecology 42:99–110.

Blankenhorn, H. J. 1972. Meteorological variables affecting onset and duration of calling in *Hyla arborea* and *Bufo calamita calamita*. Oecologia 9:223–234.

Bridges, A. S., and M. E. Dorcas. 2000. Temporal variation in anuran calling behavior: implications for surveys and monitoring programs. Copeia 2000:587–592.

Brown, D. J., T. M. Swannack, and M. R. J. Forstner. 2013. Predictive models for calling and movement activity of the endangered Houston toad. Am. Midl. Nat. 169:303–321.

Brown, L. E. 1971. Natural hybridization and trend toward extinction in some relict Texas toad populations. Southwest. Nat. 16:185–199.

Charif, R., and M. Pitzrick. 2008. Automated detection of Cerulean warbler songs using XBAT data template detector software. Preliminary Report. Bioacoustics Research Program Cornell Laboratory of Ornithology Technical Report 08-2.

Dixon, J. R., N. O. Dronen, J. C. Godwin, and M. A. Simmons. 1990. The amphibians, reptiles, and mammals of Bastrop and Buescher State Parks: with emphasis on the Houston toad (*Bufo houstonensis*) and the short-tailed shrew (*Blarina* sp.). Report of the Department of Wildlife and Fisheries Sciences at Texas A&M University to Texas Parks and Wildlife Department, Austin, Texas.

Dorcas, M. E., S. J. Price, S. C. Walls, and W. J. Barichivich. 2010. Auditory monitoring of anuran populations. *In* C. K. Dodd, Jr. (ed.), Amphibian Ecology and Conservation: a Handbook of Techniques, pp. 281–298. Oxford University Press, Oxford, U.K.

Duarte, A., D. J. Brown, and M. R. J. Forstner. 2014. Documenting extinction in real time: decline of the Houston toad on a primary recovery site. J. Fish Wildl. Manag. 5:363–371.

Frost, D. R., T. Grant, J. Faivovich, R. H. Bain, A. Haas, C. F. B. Haddad, R. O. de Sá , A. Channing, M. Wilkinson, S. C. Donnellan, C. J. Raxworthy, J. A. Campbell, B. L. Blotto, P. Moler, R. C. Drewes, R. A. Nussbaum, J. D. Lynch, D. M. Green, and W. C. Wheeler . 2006. The amphibian tree of life. Bull. Am. Mus. Nat. Hist. 297:1–370.

Gotelli, N. J., and A. M. Ellison. 2004. A Primer of Ecological Statistics. Sinauer Associates, Inc., Sunderland, Massachusetts.

Gottschalk, J. S. 1970. United States list of endangered native fish and wildlife. Fed. Reg. 35:16047–16048.

Hammerson, G., and L. Canseco-Márquez. 2004. *Incilius nebulifer*. *In* IUCN 2009. IUCN Red List of Threatened Species. Version 2009.2. www.iucnredlist.org

Hillis, D. M., A. M. Hillis, and R. F. Martin. 1984. Reproductive ecology and hybridization of the endangered Houston toad (*Bufo houstonensis*). J. Herpetol. 18:56–72.

Honegger, R. E. 1970. Red Data Book. Vol. 3. Amphibia and Reptilia. Union for Conservation of Nature and Natural Resources. Survival Service Commission, Gland, Switzerland.

Hutto, R., and R. Stutzman. 2009. Humans versus autonomous recording units: a comparison of point-count results. J. Field Ornithol. 80:387–398.

Jacobson, N. L. 1989. Breeding dynamics of the Houston toad. Southwest. Nat. 34:374–380.

MacLaren, A. R., S. F. McCracken, and M. R. J. Forstner. 2018. Development and validation of automated detection tools for vocalizations of rare and endangered anurans. J. Fish. Wildl. Manag. 9:144–154.

Moriera, G., and A. P. Lima. 1991. Seasonal patterns of juvenile recruitment and reproduction in four species of leaf litter frogs in central Amazonia. Herpetologica 47:295–300.

Narins, P. M., A. S. Feng, W. Lin, H. U. Schnitzler, A. Denzinger, R. A. Suthers, and C. Xu. 2004. Old World frog and bird vocalizations contain prominent ultrasonic harmonics. J. Acoust. Soc. Am. 115:910– 913.

Obert, H. J. 1975. The dependence of calling activity in *Rana esculante* Linne 1758 and *Rana ridibunda* Pallas 1771 upon exogenous factors (Ranidae, Anura). Oecologia 18:317–328.

Oseen, K. L. , and R. J. Wassersug. 2002. Environmental factors influencing calling in sympatric anurans. Oecologia 133:616–625.

Potter, F. E. J., L. E. Brown, W. L. McClure, N. J. Scott, and R. A. Thomas. 1984. Recovery Plan for the Houston toad (*Bufo houstonensis*). U. S. Fish & Wildlife Service, Albuquerque, New Mexico. 73 pp.

Price, A. H. 2003. The Houston toad in Bastrop State Park 1990–2002: a narrative. Open-file report 03-0401, Texas Parks & Wildlife Department, Austin.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rempel, R., C. Francis, J. Robinson, and M. Campbell. 2013. Comparison of audio recording system performance for detecting and monitoring songbirds. J. Field Ornithol. 84:86–97.

Saenz, D., L. A. Fitzgerald, K. A. Baum, and R. N. Conner. 2006. Abiotic correlates of anuran calling phenology: the importance of rain, temperature, and season. Herpetol. Monogr. 20:64–82.

Salvador, A., and L. M. Carrascal. 1990. Reproductive phenology and temporal patterns of mate access in Mediterranean anurans. J. Herpetol. 24:438–441.

Sanders, O. 1953. A new species of toad, with a discussion of morphology of the bufonid skull. Herpetologica 9:25–47.

Shearin, A. F., A. J. K. Calhoun, and C. S. Loftin. 2012. Evaluation of listener-based anuran surveys with automated audio recording devices. Wetlands 32:737–751.

Swannack, T. M., W. E. Grant, and M. R. J. Forstner. 2009. Projecting population trends of endangered amphibian species in the face of uncertainty: a pattern-oriented approach. Ecol. Model. 220:148–159.

U.S. Endangered Species Act of 1973, as amended. 1973. Pub. L. No. 93-205, 87 Stat. 884 (Dec. 28, 1973).

U.S. Fish and Wildlife Service. 2007. Section 10(a)(1)(A) Scientific permit requirements for conducting Houston toad presence/absence surveys. U. S. Fish and Wildlife Service, Austin, Texas.

Willacy, R. J., M. Mahony, and D. A. Newell. 2015. If a frog calls in the forest: bioacoustic monitoring reveals the breeding phenology of the endangered Richmond Range mountain frog (*Philoria richmondensis*). Austral Ecol. 40:625–633.

Williams, P. J., N. J. Engbrecht, J. R. Robb, V. C. Terrell, and M. J. Lannoo. 2013. Surveying a threatened amphibian species through a narrow detection window. Copeia 2013:552–561.

Yip, D., L. Leston, E. Bayne, P. Sólymos, and A. Grover. 2017. Experimentally derived detection distances from audio recordings and human observers enable integrated analysis of point count data. Avian Conserv. Ecol. 12:11.

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The Function of Supplementary Notes in the Communication System of Johnstone's Whistling Frog, *Eleutherodactylus johnstonei*

Communication is a paramount component of animal behavior, since every interaction among individuals involves information exchange. In order for communication to successfully occur, the sender has to encode the message in a signal, which must be accurately transmitted to the receiver. This message can be sent using different mechanisms, including optical, chemical, electrical, and acoustic signals (Marler 1977). In anurans, the typical acoustic signal is the advertisement call, which has two important functions: (1) attracting potential mates and (2) mediating agonistic interactions (Wells 1977a; Duellman and Trueb 1986; Rand 1988). Advertisement calls can be classified as single or complex according to the number of components they have. Complex calls can be further divided into those that use a single sensory modality, referred to as "multi-component" signals (Hölldobler 1995), and those that use more than one sensory modality, referred to as "multimodal" signals (Guilford and Dawkins 1991; Rowe and Guilford 1999). Signals processed using more than one sensory modality may be more effective in producing a response from the receiver than those produced by a unimodal signal (de Luna et al. 2010; Higham and Hebets 2013; Preininger et al. 2013). While multimodal signals have been the subject of a large number of studies, the function of multi-component signals has received less attention (Elias et al. 2006). However, these kinds of signals may enhance communication in complex acoustic conditions (Richardson and Lengagne 2009).

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In several species, males may change the advertisement call by including additional components generally thought to be an aggressive response to potential competitors approaching (McDiarmid and Adler 1974). Other behavioral and evolutionary responses to acoustic competition among males include avoiding temporal overlap among calls (Rosen and Lemon 1974), alternating calls with competing males (Grafe 1995), increasing calling rates (Schwartz and Wells 1985; López and Narins 1991), modifying the peak frequency of the call (Given 1999), and increasing the number of aggressive calls (Schwartz and Wells 1984; Owen and Gordon 2005). Finally, males of some species may also modify their calls by adding notes that indicate greater aggressiveness (Arak 1983; Wells 1988; Wells and Schwartz 2007).

Advertisement calls in frogs may vary substantially within a species, among populations, and within a single individual depending on the social context (Ryan et al. 1996). Although the typical anuran advertisement call consists of a series of identical notes (Wells 1977b; Duellman and Trueb 1986), some species may emit a call composed of two notes that have differing acoustic properties (Duellman and Trueb 1986) that can be modified behaviorally. For example, the Túngara Frog (*Engystomops pustulosus*) has a complex advertisement call composed of a first component ('whine') that may be followed by shorter notes ('chucks') (Ryan 1985; Ryan and Rand 2003). Males of *E. pustulosus* add more chucks during interactions with other conspecific males than when calling alone and females have been found to prefer calls with more chucks (Gridi-Papp et al. 2006; Bernal et al. 2009; Akre and Ryan 2010). Another well-studied example is *Eleutherodactylus coqui*, which has a biphasic call where each component contains information addressed to receivers of a specific sex (Narins and Capranica 1978; Stewart and Rand 1991). The first component of the advertisement call is preferentially responded by males, while the second component seems to be used to attract mates (Narins and Capranica 1976; 1978; 1980). Although there are few well-studied systems, the analysis of the communicative function of additional notes in the genus *Eleutherodactylus* is still scarce. Moreover, given that calls bearing additional components are uttered occasionally, finding satisfactory answers has been a difficult task that requires extensive study.

The advertisement call of *Eleutherodactylus johnstonei* was initially described by Watkins et al. (1970) as a two-note call