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CALL LATENCY AS A MEASURE OF CALLING INTENSITY IN ANURAN AUDITORY SURVEYS

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***Abstract.*—Anuran auditory surveys are increasingly being used in studies of amphibian ecology, conservation, and management. Most call surveys record some measure of calling intensity, most commonly the amphibian calling index (ACI). Previous studies have shown that the ACI exhibits poor interobserver agreement, and ACI is an ordinal measure, limiting its statistical analysis. We examined call latency (defined as the length of time between the start of the survey and the detection of the first call of a species) as a potential measure of calling intensity in 530 frog call surveys conducted in central Texas from 2007 to 2010. Call latency significantly differed among species and negatively correlated with ACI in seven of nine species examined. Call latency also negatively correlated with estimated maximum number of frogs calling in eight of nine species. There was no significant correlation between air temperature and call latency in any of the species examined. We found that call latency was negatively correlated with road noise in only one species. We observed that call latency was longer in windy surveys for two of the species examined. We conclude that call latency is a potential measure of calling intensity that has certain methodological advantages over ACI.**

Key Words.—anurans; call index; call latency; call surveys; survey techniques; temperature

INTRODUCTION

Recognition that amphibians worldwide are experiencing significant declines (Stuart et al. 2004; Lannoo 2005) has stimulated interest in developing better sampling techniques for determining the occurrence and abundance of frogs and toads. Anuran auditory surveys (frog call surveys) are a sampling technique gaining wide application in amphibian ecology, conservation, and management. This technique is based on detection of species-specific calls produced by male frogs during the breeding season and can be applied to many different field situations, requires little equipment, is cost-effective, and can be carried out by trained volunteers (Weir and Mossman 2005; Dorcas et al. 2009). Frog call surveys have provided important information for many topics of amphibian study, including landscape ecology (Knutson et al. 1999; Carr and Fahrig 2001; Naugel et al. 2005), the distribution and status of amphibian populations (Mossman et al. 1998; Weir et al. 2009), effects of climate change and urbanization on amphibians (Gibbs and Breish 2001; Pillsbury and Miller 2008), and conservation management (Stevens et al. 2002; Jackson et al. 2006).

The use of frog call surveys for amphibian research and monitoring has been facilitated by the establishment of a standardized protocol for surveys (Weir and Mossman 2005). The North American Amphibian Monitoring Program protocol specifies 5-min listening surveys at 10 designated sites along randomly selected roadside transects. At each site, an observer records what species are calling and assigns to each species a call index based on the size and calling activity of the amphibian chorus. The amphibian calling index (ACI) ranges from 1 to 3: 1 is assigned when calls are individually distinct with space between calls; 2 is assigned when calls of individuals can be distinguished but there is some overlapping of calls; and 3 is assigned to a full chorus with constant, continuous, overlapping calls (Weir and Mossman 2005).

There are two problems with using the ACI to assess amphibian abundance. First, because ACI is an ordinal measure, it cannot be analyzed with statistical tests that require continuous data. Second, several studies have reported high interobserver variation in assignment of the ACI to amphibian choruses. Using recordings of frog calls, Genet and Sargent (2003) evaluated the accuracy of trained volunteers in determining the correct species and call index of amphibians in Michigan. They found that while most observers correctly identified the species, they varied dramatically in assignment of ACI. In two audio recordings that were exact replicas, only 43.8% of observers assigned the same ACI in both recordings. Most of this variability occurred because of difficulty in assigning ACIs of 2 and 3. Under field conditions, Pierce and Gutzwiller (2007) found that two observers standing 10 m apart listening simultaneously agreed on the assignment of ACI only 51.3% of the time in 5 min surveys and 48.1% of the time in 30 min surveys. Shirose et al. (1997) found that volunteers agreed on assignment of ACI 47% to 83% of the time and Bishop et al. (1997) observed 56% to 86%

agreement between ACIs assigned by different observers.

Because of these limitations of ACI, we were motivated to search for other measures of call intensity that could be easily recorded during frog call surveys. One measure that has not been examined is call latency, defined here as the length of time in seconds between the start of the survey and the detection of the first call of a species. Like ACI, call latency is a measure of call intensity. Both parameters are affected by two aspects of calling behavior, which are often associated: the number of frogs calling and the calling frequency of individual frogs. As the number of frogs calling increases, call latency will decrease. Similarly, as the calling frequency of individual frogs increases, call latency will decrease. Thus, call latency, like ACI, provides information about chorus size and frog abundance at particular sites. A few studies have examined call latency in frogs in relation to the threat of predation (Tuttle et al. 1982; Lahanas 1995; Phelps et al. 2007), but to our knowledge this parameter has not been used to assess calling intensity in call surveys.

In this study, we recorded call latency of 10 species of frogs in 530 call surveys conducted in central Texas from 2007 to 2010. We examined how call latency varies among species and with air temperature. We also compared call latency with ACI and estimated numbers of frogs calling. Because some previous studies have asked whether wind and road noise affect detection in frog call surveys (Johnson and Batie 2001; Oseen and Wassersug 2002; Weir et al. 2005), we applied call latency to the question of whether these factors affect calling intensity in frog call surveys.

Materials and Methods

***Data collection*.**—We conducted frog call surveys weekly from late February to mid July from 2007 to 2010 using the North American Amphibian Monitoring Program (NAAMP) protocol (Weir and Mossman 2005), except that we conducted surveys under all wind conditions because we were interested in the effect of wind on calling behavior. Using the NAAMP procedure, we selected four routes along secondary roads within 100 km of Georgetown, Texas (30° 38'N, 97° 39'W). We surveyed each route approximately once per month during the estimated amphibian breeding season.

Each route consisted of 10 listening stations, each within 200 m of a potential amphibian habitat (pond, creek, lake, river, or wetland). All listening stations on a route were spaced a minimum of 0.81 km (0.5 miles) apart. Surveys were conducted between 1900 and 2400 by a team of two or three observers trained to recognize the calls of frog species that might occur in the survey area. Observers were field-trained in survey methods and tested weekly on call identification.

Upon arriving at each listening station, observers first recorded survey time and then measured air temperature (°C), wind speed (m/s) averaged over 1 min, relative humidity (percentage), and barometric pressure (hPa) with a Kestrel 4000 Pocket Weather Tracker (Nielsen-Kellerman Company, Boothwyn, Pennsylvania, USA). The observers visually estimated cloud cover (percentage), and noted the presence of any precipitation. As specified by the NAAMP procedure, the listening survey then began immediately, without any initial waiting period. At each site, one observer was designated the primary observer. The primary observer listened for 5 min and recorded the following variables for each species heard: NAAMP ACI (1, 2, or 3), estimated maximum number of individuals calling (1, 2, 3–5, 6–10, or > 10), call latency (length of time in seconds between the start of the survey and the detection of the first call of a species), and whether frogs called from the designated amphibian habitat. The observer also recorded the number of vehicles that passed during the 5-min survey. The amount of time that elapsed between arrival at the listening station and the initiation of the listening survey varied, but was typically 3–5 min.

***Data analyses*.**—We sought to uncover whether call latency varied between species and to describe how call latency was associated with NAAMP calling variables. Because call latency and call index were not normally distributed, we used nonparametric statistics for all analyses. We used Kruskal-Wallis tests to determine if call latency and ACI differed between species. We used two-tailed Spearman's rank correlations to identify associations between call latency and ACI, and between call latency and maximum number of individuals heard calling. To determine if call latency was associated with wind or road noise, we used two-tailed Spearman's rank correlations to uncover associations between call latency and average wind speed, and between call latency and the number of cars that passed during a survey (i.e., road noise). We performed all correlations on each species separately, with the exception of combining *Hyla chrysoscelis* and *H. versicolor*, as described below. Correlations with $P \leq 0.05$ were considered significant.

An important issue in amphibian call surveys (and in all wildlife monitoring methods) is the problem of imperfect detection (MacKenzie et al. 2006; Mazerolle et al. 2007). For example, the absence of a species call during a survey may result from the fact that the species is truly absent from the site or from the inability to detect it either because it is present but not calling or because the observer does not hear the call. To avoid problems with incomplete detection, we analyzed the effects of

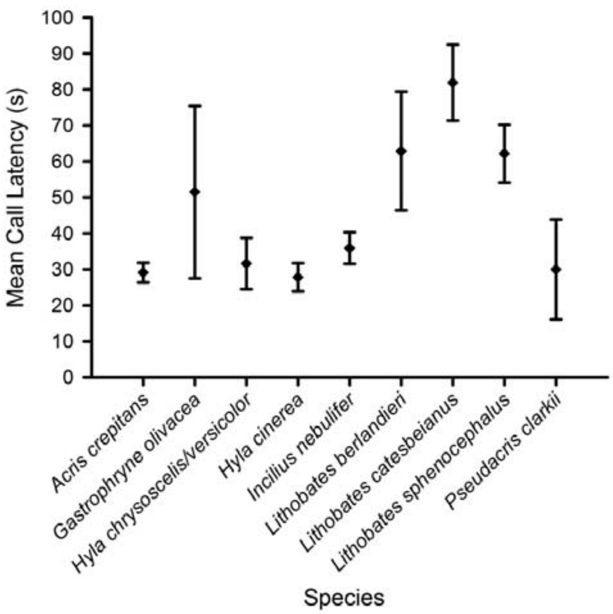

FIGURE 1. The average time in seconds (± 1 SE) between call survey start time and hearing the respective anuran species for the first time (i.e., call latency). Overall, call latency varied across these nine species ($H = 96.18$, df = 8, $P < 0.001$).

call latency on each species separately, and we only included surveys in which at least one frog of the analyzed species called. We combined data for *H. versicolor* and *H. chrysoscelis* in all analyses due to difficulty separating these calls in the field (Saenz et al. 2006; Pierce and Gutzwiller 2007). We used SPSS statistical software (SPSS 19 for Windows, Release 19.0.0, SPSS Inc., Chicago, Illinois, USA) to analyze data.

RESULTS

We conducted 689 call surveys between 2007 and 2010. We detected at least one species on 530 of these surveys; our analyses are limited to these surveys in which one or more frogs called. Overall, we heard 10 species calling: Blanchard's Cricket Frogs (*Acris crepitans*), Great Plains Narrowmouth Toads (*Gastrophryne olivacea*), Cope's Gray Treefrogs (*Hyla chrysoscelis*), Gray Treefrogs (*H. versicolor*), Green Tree Frogs (*H. cinerea*), Gulf Coast Toads (*Incilius nebulifer*), Rio Grande Leopard Frogs (*Lithobates berlandieri*), American Bullfrogs (*L. catesbeianus*), Southern Leopard Frogs (*L. sphenocephalus*), and Spotted Chorus Frogs (*Pseudacris clarkii*). Average call latency differed among species ($H = 96.18$, df = 8, $P < 0.001$; Fig. 1). Average ACI also differed among species ($H = 493.214$, df = 8, $P < 0.001$). Average call latency was longest in the three species of *Lithobates* (see Fig. 1).

Call latency negatively correlated with ACI in all species and this association was significant in seven of the nine species tested (Table 1). Call latency also negatively correlated with estimated maximum number of individuals calling in all species; this correlation was significant in eight of the nine species tested (Table 1). Call latency was not significantly correlated with air temperature in any of the species studied. Call latency positively correlated with road noise in only one species, *H. cinerea* ($\rho = 0.163$, df = 175, $P = 0.029$). Call latency also positively correlated with average wind speed in two species: *H. cinerea* ($\rho = 0.198$, df = 175, $P = 0.008$) and *L. sphenocephalus* ($\rho = 0.337$, df = 76, $P = 0.003$). Summary statistics for calling and environmental measurements by frog species is presented in the Appendix.

DISCUSSION

In this study, we examined call latency as a possible alternative measure of calling intensity in amphibian call surveys. The North American Amphibian Monitoring Program (Weir and Mossman 2005) currently uses ACI as a measure of calling intensity. The function of the ACI is to provide an estimate of the relative abundance of individual species at particular sites. Indeed, Nelson and Graves (2004) found a positive correlation between ACI and abundance as measured by mark recapture studies for *Rana clamitans*, but other studies (Goldberg and Schwalbe 2004; Corn et al. 2011) found that ACI was a poor predictor of population size.

Call latency, like ACI, is a measure of calling intensity, but call latency has several methodological advantages over ACI. Call latency is easy to measure objectively with a stop watch, requiring only that one

TABLE 1. Spearman's rho correlations between 1) call latency and amphibian call index (ACI) and between 2) call latency and maximum number of individuals heard calling.

	Call Index			Number Calling		
Species	*n*	Rho	*P*	*n*	Rho	*P*
Acris crepitans	415	-0.398	< 0.001	414	-0.490	< 0.001
Gastrophryne olivacea	12	-0.697	0.012	12	-0.833	< 0.001
Hyla chrysoscelis/ versicolor	87	-0.512	< 0.001	87	-0.492	< 0.001
H. cinerea	179	-0.102	0.173	179	-0.382	< 0.001
Incilius nebulifer	172	-0.547	< 0.001	172	-0.567	< 0.001
Lithobates berlandieri	20	-0.585	0.007	20	-0.480	0.032
L. catesbeianus	56	-0.286	0.033	56	-0.462	< 0.001
L. sphenocephalus	79	-0.531	< 0.001	79	-0.590	< 0.001
Pseudacris clarkii	19	-0.225	0.355	19	-0.302	0.209

detect the presence of a frog call. ACI, on the other hand, requires both the detection of a call and a subjective assignment of a value of the intensity of calling. Our measures of ACI were potentially subject to the same variation as seen in other studies (Bishop et al. 1997; Shirose et al. 1997; Genet and Sargent 2003; Pierce and Gutzwiller 2007). Although we did not measure interobserver variation of call latency or ACI, we assume that interobserver variation in call latency is much less than interobserver variation in ACI because no subjective evaluation of calling intensity is required with call latency. Future studies that compare interobserver variation in call latency and ACI would be beneficial. Additionally, call latency is a continuous variable, whereas call index is an ordinal variable with only three levels. The use of continuous variables allows for a wider range of statistical analyses.

We found that call latency significantly varied among the 10 species from central Texas that we detected in our surveys. In our study, the three species of *Lithobates* (formerly *Rana*) had longer call latencies compared with the other species examined. The average call latencies generally matched with the different calling characteristics of the species. For example, *L. catesbeianus*, which had the longest average calling latency in our study, typically gives loud and interspersed calls, whereas *Acris crepitans*, which had a much shorter call latency, is characterized by short, rapidly-attenuating calls that are more closely spaced. The different average call latencies also reflect differences in average chorus sizes. In our area, the three species of *Lithobates*, which have long call latencies, often call in small groups; several of the species with short call latencies (e.g., *A. crepitans* and *H. cinerea*) frequently form large choruses in our survey areas. The large standard error for the mean call latency of *G. olivacea* and *P. clarkii* reflects the relatively small number of observations we have for these species.

We found that call latency was correlated with ACI; the correlation was negative for all species examined and significant in seven of nine species examined. The negative correlation suggests that the time to detection of the first call was shorter when the chorus intensity (as measured by ACI) was greater. Call latency and ACI are probably correlated because both measures are related to the number and calling intensity of the frogs present in a chorus. Because ACI and call latency were both measured by the same observer, we cannot eliminate the possibility that a longer call latency biases the observer into estimating a smaller ACI, but we think this is unlikely, given that these two measures are based on different aspects of calling behavior (seconds to first call for call latency and overlap in calls for ACI).

Call latency is expected to be inversely related to the number of calling frogs in a chorus. As the number of frogs calling increases, the time between individual calls will shorten and call latency is expected to decrease. We found significant negative correlations between call latency and estimated numbers of frogs calling in eight out of nine species, and the correlation was negative, though not significant, in the other species. These correlations suggest that call latency provides an estimate of frog abundance. However, estimating the number of frogs calling in a call survey is often difficult, especially when there are large numbers of frogs in a chorus. The association between frog abundance and call latency could be better assessed by measuring call latency at a series of sites and then independently estimating the number of frogs present at each site with mark-recapture studies. Call latency (as well as ACI) is also potentially affected by variables that influence the frequency of calling by individual frogs, such as threat of predation, disturbance, recent rainfall, and season.

Because amphibians are ectotherms, calling behavior is often associated with temperature. Temperature affects both the timing of reproduction and calling frequency, and several studies have demonstrated an association between air temperature and species detection in call surveys (Todd et al. 2003; Weir et al. 2005; Kirlin et al. 2006). However, we did not find a correlation between call latency and air temperature for any of the examined species.

We applied call latency to the analysis of two environmental variables that have the potential to affect detection in frog call surveys: road noise and wind. Background noise resulting from road traffic and wind potentially affect frog calling behavior. This noise may also affect the ability of the observer to hear frog calls. Either situation could lower the probability of detecting frogs when they are present. In examining the effect of road noise on call surveys, Weir et al. (2005) observed that number of passing vehicles during their call surveys was associated with decreased probability of detecting frog calls in *Bufo americanus*, *H. chrysoscelis*, and *P. feriarum* and increased probability of detection in *H. versicolor* and *L. palustris*. In our study, call latency was correlated with road noise in only one species, *H. cinerea*. For this species, there was a negative correlation between call latency and road noise, meaning that when more cars passed, call latency increased. The differences between our results and those of Weir et al. (2005) may reflect differences in the levels of road noise or differences in the communities of frogs studied (although *H. chrysoscelis* and *H. versicolor* were common to both studies). The difference in the two studies may also be due to the fact that we omitted from our analysis surveys in which no frogs called.

Fewer frogs may call during high wind to avoid desiccation (Oseen and Wassersug 2002) and calls may be harder to detect because of wind noise. Because of concerns about the ability to detect frog calls under windy conditions, the NAAMP protocol specifies that call surveys must not be conducted when wind is high (for most regions, wind speed > 6 m/s; for Great Plains, wind speed > 8 m/s). For two species, *H. cinerea* and *L. sphenocephalus*, we found that call latency was longer as wind speed increased. The ACI did not differ between windy and calm conditions for any species. Several other studies found effects of wind on calling behavior and call detection (Johnson and Batie 2001; Oseen and Wassersug 2002; Weir et al. 2005). For example, Weir et al. (2005) found that wind negatively affected call detection in four of 10 species. It is unknown why, in our study, only *H. cinerea* and *L. sphenocephalus* exhibited a relationship between wind and call latency. How calling behavior and call detection relate during windy conditions warrants further study, especially as the NAAMP protocol does not currently allow surveys during periods of high wind.

Call surveys have become an increasingly important tool for understanding the distribution and status of amphibians, amphibian ecology, and management and conservation of amphibians (i.e., Naugel et al. 2005; Jackson et al. 2006; Weir et al. 2009). The use of call surveys has been facilitated by the development of a standardized protocol by the North American Amphibian Monitoring Program (Weir and Mossman 2005), which includes ACI as a measure of relative calling intensity. Our results suggest that call latency might be a useful measure of calling intensity that could easily be incorporated into frog call surveys. Call latency has some potential advantages over ACI, in that call latency is a continuous variable and a relatively objective measurement of call intensity. However, additional studies of call latency in anuran surveys are needed before more conclusive statements can be made about the value of call latency. We recommend that call latency be recorded during frog call surveys and that future studies examine the relationship between call latency and amphibian abundance.

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ALEXANDER S. HALL recently obtained his B.S. in Animal Behavior from Southwestern University where he worked on several amphibian ecology and behavior projects. Past projects include investigating how frogs might be affected by light pollution, refining aquatic salamander survey techniques, and determining how tadpoles carry learned associations through and after metamorphosis. Currently, he investigates squamate genome evolution at The University of Texas at Arlington (UTA). Most currently, he studies recombination within parthenogenetic whiptail lizards (*Aspidoscelis*) in pursuit of a Ph.D. in Quantitative Biology through UTA. (Photographed by Alex Hall)

APPENDIX 1. Summary statistics for calling and environmental measurements by frog species. IQ Range refers to interquartile range, i.e., 25th to 75th percentiles. Units for road noise are number of cars per survey.

Species	Variable	Mean	SE	Median	Min	IQ Range	Max	n
Acris crepitans	Call Latency (s)	29.12	2.693	0	0	0–30	300	417
	ACI	2.57	0.032	3	1	2–3	3	416
	Wind Speed (m/s)	1.296	0.063	1.00	0.00	0.100–2.20	6.60	415
	Air Temperature (°C)	23.05	0.200	23.65	8.60	20.90–26.20	32.10	418
	Road Noise	1.25	0.090	1	0	0–2	13	418
	Number Calling	11.880	0.470	8	1	4–20	50	410
Gastrophryne olivacea	Call Latency (s)	51.50	23.930	8.50	0	0–81.50	285	12
	ACI	1.83	0.207	2	1	1–2	3	12
	Wind Speed (m/s)	0.333	0.189	0.00	0.00	0.00–0.625	2.10	12
	Air Temperature (°C)	24.29	0.559	23.90	20.60	23.10–25.18	27.80	12
	Road Noise	0.58	0.193	0.50	0	0–1	2	12
	Number Calling	4.333	1.054	3	1	1–8	10	12
Hyla chrysoscelis/ versicolor	Call Latency (s)	31.64	7.105	0	0	0–30	280	87
	ACI	1.91	0.086	2	1	1–3	3	87
	Wind Speed (m/s)	1.02	0.113	0.700	0.00	0.05–1.90	4.00	85
	Air Temperature (°C)	22.26	0.379	22.00	14.50	19.70–24.80	29.00	87
	Road Noise	0.95	0.164	0	0	0–1	6	87
	Number Calling	8.02	0.878	5	1	4–8	30	87
Hyla cinerea	Call Latency (s)	27.85	3.891	0	0	0–34	240	179
	ACI	2.87	0.030	3	1	3–3	3	181
	Wind Speed (m/s)	0.926	0.068	0.80	0.00	0.00–1.50	3.40	179
	Air Temperature (°C)	26.00	0.168	26.20	18.50	24.50–27.70	30.60	181
	Road Noise	1.09	0.138	0	0	0–1	13	181
	Number Calling	22.04	0.893	20	1	10–30	50	181
Incilius nebulifer	Call Latency (s)	35.95	4.360	8	0	0–50	267	172
	ACI	1.69	0.057	2	0	1–2	3	173
	Wind Speed (m/s)	1.24	0.104	1.00	0.00	0.00–1.98	6.60	172
	Air Temperature (°C)	24.62	0.190	24.80	18.30	22.70–26.70	30.90	173
	Road Noise	1.09	0.100	1	0	0–2	6	173
	Number Calling	3.98	0.296	3	0	1–4	30	173
Lithobates berlandieri	Call Latency (s)	62.90	16.54	43	0	5.50–91.50	248	20
	ACI	1.14	0.078	1	1	1–1	2	21
	Wind Speed (m/s)	0.93	0.234	0.55	0.00	0.05–2.00	3.10	20
	Air Temperature (°C)	22.74	1.064	22.40	11.80	20.70–26.80	28.40	21
	Road Noise	1.10	0.300	1	0	0–2	4	21
	Number Calling	2.29	0.474	1	1	1–3	8	21
Lithobates catesbeianus	Call Latency	81.91	10.550	49	0	21.75–132.5	295	56
	ACI	1.11	0.042	1	1	1–1	2	56
	Wind Speed	0.77	0.142	0.50	0.00	0.00–1.20	5.80	55
	Air Temperature	23.83	0.505	24.25	13.60	21.80–26.48	30.50	56
	Road Noise	0.84	0.167	0	0	0–1	5	56
	Number Calling	1.48	0.117	1	1	1–2	4	56
Lithobates sphenocephalus	Call Latency	62.18	8.007	28.50	0	2.50–110.00	281	80
	ACI	1.22	0.054	1	1	1–1	3	83
	Wind Speed	0.976	0.103	0.80	0.00	0.10–1.40	4.30	82
	Air Temperature	19.11	0.449	18.15	0.70	16.50–21.98	27.10	84
	Road Noise	1.21	0.252	0	0	0–1.75	16	84
	Number Calling	3.08	0.357	2	1	1–4	25	83
Pseudacris clarkii	Call Latency	30.00	13.884	0.00	0	0.00–27.00	210	19
	ACI	2.26	0.168	2	1	2–3	3	19
	Wind Speed	1.268	0.253	1.20	0.00	0.00–2.30	2.80	19
	Air Temperature	18.847	0.757	19.20	12.40	16.00–20.60	24.20	19
	Road Noise	0.74	0.357	0	0	0–1	6	19
	Number Calling	8.21	1.70	4	1	3–20	20	19