Optimizing the power of human performed audio surveys for monitoring the endangered Houston toad using automated recording devices

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Knowledge regarding the locations of populations of endangered species is a critical part of recovery and facilitates land use planning that avoids unnecessary impacts. Regulatory agencies often support the development of survey guidelines designed to standardize the methods and maximize the probability of detection, thereby avoiding incorrectly concluding a species is absent from a site. Here, using simulations with data collected using automated recording devices (ARDs) we evaluated the efficacy of the existing U.S. Fish and Wildlife Service's survey requirements for the endangered Houston Toad (*Bufo* [=*Anaxyrus*] *houstonensis*). We explored the effect of 1) increasing survey duration, 2) increasing the number of surveys, and 3) combinations of environmental conditions (e.g., temperature, humidity, rainfall) on the detection probability and the number of surveys needed to be 95% confident of absence. We found that increases in both the duration of the survey and the number of surveys conducted decreased the likelihood of incorrectly concluding the species was absent from the site, and that the number of surveys required to be 95% confident greatly exceeded the existing survey requirements. Targeting specific environmental conditions was also an effective way to decrease the number of surveys required but the infrequency in which these conditions occurred might make application difficult in some years. Overall, we suggest that the survey effort necessary to achieve confidence in the absence of Houston Toads at a site is more practically achievable with the use of ARDs, but this may not be suitable in all monitoring scenarios.

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Abstract

Knowledge regarding the locations of populations of endangered species is a critical part of recovery and facilitates land use planning that avoids unnecessary impacts. Regulatory agencies often support the development of survey guidelines designed to standardize the methods and maximize the probability of detection, thereby avoiding incorrectly concluding a species is absent from a site. Here, using simulations with data collected using automated recording devices (ARDs) we evaluated the efficacy of the existing U.S. Fish and Wildlife Service's survey requirements for the endangered Houston Toad (*Bufo* [=*Anaxyrus*] *houstonensis*). We explored the effect of 1) increasing survey duration, 2) increasing the number of surveys, and 3) combinations of environmental conditions (e.g., temperature, humidity, rainfall) on the detection probability and the number of surveys needed to be 95% confident of absence. We found that increases in both the duration of the survey and the number of surveys conducted decreased the likelihood of incorrectly concluding the species was absent from the site, and that the number of surveys required to be 95% confident greatly exceeded the existing survey requirements. Targeting specific environmental conditions was also an effective way to decrease the number of surveys required but the infrequency in which these conditions occurred might make application difficult in some years. Overall, we suggest that the survey effort necessary to achieve confidence in the absence of Houston Toads at a site is more practically achievable with the use of ARDs, but this may not be suitable in all monitoring scenarios.

Introduction

39   Monitoring of endangered anuran populations is required to gain an understanding of
40 population dynamics (Pechmann et al. 1991) and the effects of management actions (Walls et al.
41 2014). Researchers commonly conduct auditory surveys to determine presence or absence of
42 anuran species (Bridges & Dorcas 2000; Crouch & Paton 2002; Schmidt 2003; Pierce &
43 Gutzweiller 2004; Weir et al. 2005; Jackson et al. 2006). Data from these surveys can also be
44 used for estimates of the relative abundance of calling male anurans (Zimmerman 1994) or for
45 determining the cadence or phenology of chorusing behavior (Saenz et al. 2006). These surveys
46 are also used to inform land use and development decisions, in addition to ecological research
47 and endangered species management.
48   There are presently 14 native anurans, with the inclusion of Puerto Rico, classified by the
49 United States Fish and Wildlife Service (USFWS , hereafter) as threatened and endangered
50 (*Anaxyrus californicus*, *Anaxyrus canorus*, *Bufo hemiophrys baxterii*, *Bufo houstonensis*,
51 *Eleutherodactylus cooki*, *Eleutherodactylus jasper*, *Eleutherodactylus juanariveroi*, *Peltophryne
52 lemur*, *Rana chiricahua*, *Rana draytonii*, *Rana muscosa*, *Rana pretiosa*, *Rana sierrae,* and *Rana
53 sevosa*). The USFWS has published guidelines for conducting detection/non-detection surveys
54 for only four of these 14 (*B. houstonensis, A. californicus, R. chiricahua,* and *R. draytonii*;
55 USFWS 1999; USFWS 2005; USFWS 2006; USFWS 2007). Two species (*R. pretiosa* and *R.
56 sierrae*) have general overviews of currently applied survey methods, authored by the United
57 States Forest Service, but these have not been established as approved policy guidelines. Federal
58 guidelines for conducting surveys of an additional two species (*A. canorus* and *R. muscosa*) are
59 reported to be pending approval. The remaining six species lack formal protocols to confirm
60 species presence at a site. These guidelines are intended to ensure that independent researchers
61 are performing standardized surveys designed to maximize the likelihood of detecting the species
62 when present. This lack of survey guidelines, or the existence of poorly designed guidelines, can
63 have serious negative consequences for populations of endangered species. Incorrectly
64 concluding a population of an endangered species is absent from a site can lead to "take" by
65 development of the site through the loss of breeding wetlands and/or upland habitat, as well as
66 potential mortality caused by having individuals present and active in the development site,
67 resulting in preventable losses to the species and expensive fines and protracted delays for the
68 development projects.
69   Among the existing published guidelines, recommendations for surveying Houston Toads
70 (*B. houstonensis*) are some of the most specific (USFWS 2007). These guidelines dictate that at
71 minimum, six 5-minute audio surveys, per year, are required at each listening post (i.e. potential
72 breeding location); surveys must be conducted for three consecutive years; surveys should be
73 spread out between February through April; temperatures must be at or above 57°F; surveys do
74 not begin until about 30 minutes after sundown and cease if a drop in temperature occurs
75 (presumably below 57°F, however, this is unclear); and wind speeds must not exceed 15 miles
76 per hour (USFWS 2007). These guidelines also include less quantitative recommendations
77 intended to increase the likelihood that Houston Toads are chorusing on nights that are chosen to
78 survey. These include nights in which humidity is greater than 70%; cloud cover is present or the

79   moon is not full; and rainfall occurring or recent rainfall has occurred. The efficacy of these
80   guidelines has been studied previously; as a result the updated policy now recommends
81   increasing the number of surveys necessary within each season from six to twelve (Jackson et al.
82   2006; USFWS 2007).
83   Many studies have revealed the advantages of automated audio recording systems in
84   determining the influential exogenous environmental factors associated with vocalizing behavior
85   when compared to manual call surveys (Bridges & Dorcas 2000; Oseen & Wassersug 2002; Hsu
86   et al. 2005; Acevado & Villanueva-Rivera 2006; Dorcas et al. 2009; Willacy et al. 2015;
87   MacLaren et al. 2018B). In general, automated approaches have the capacity to easily collect
88   significantly more survey data over a wider range of environmental conditions and accordingly
89   provide greater statistical power in examining these relationships over manual surveys. Recently,
90   the use of automated monitoring methods has been more formally recommended for Houston
91   Toad detection/non-detection surveys (USFWS 2020). Thus, the data provided by automated
92   methods provides an excellent opportunity to investigate the true efficacy of manual survey as
93   recommended by the USFWS (2007) guidelines. We are unaware of other studies that model the
94   potential outcomes of a manual survey protocol using data acquired from an automated recording
95   system, but this information is critical to better understand how well the recommended survey
96   guidelines are detecting Houston Toads in particular, and other endangered anurans in general.
97   The use of automated recording methods allows us to investigate the consequences of various
98   important choices in monitoring program design in a systematic and unbiased manner. Thus, the
99   purpose of this study is to 1) evaluate the efficacy of the existing Houston Toad survey
100   guidelines, 2) to investigate any opportunity to improve manual surveys by increasing the
101   duration of each survey and the total number of surveys conducted each year, and 3) to provide
102   updated recommendations for Houston Toad surveys.
103

104   **Materials & Methods**
105   _Study Site_
106   We carried out this study utilizing data gathered from the Griffith League Ranch (GLR),
107   located in Bastrop County, Texas, USA. The GLR is a private property owned and operated by
108   the Boy Scouts of America. This property is commonly represented as the primary recovery site
109   for the Houston Toad (Duarte et al. 2014), and received population supplementation through
110   captive propagation efforts both during and prior to when this study was conducted. Audio
111   collected from two Houston Toad breeding locations was used. These sites are separated by 2.37
112   kilometers and are acoustically independent (see MacLaren et al. 2018C). All work conducted to
113   complete this study was done under scientific permit TE-039544-1 issued by the USFWS.
114

115   _Audio Recording and Analysis_
116   We used Song Meter SM3 (Wildlife Acoustics, Maynard, MA) audio recording devices
117   to monitor for the call of male Houston Toads at the two breeding locations on the GLR. Song
118   Meters were programmed to record continuously, beginning in January and ending in July, for

119   four years (2015-2018). We powered the Song Meters using rechargeable sealed lead acid
120   batteries (Power Sonic PS-6360 NB, 6V, 36.0 AH). We stored the external batteries in plastic
121   cases, secured to a structure adjacent to each Song Meter. We equipped each Song Meter with
122   four 64GB SD cards for media storage. The additional costs and data storage requirements
123   associated with continuously monitoring limited us to only two locations. We selected
124   monitoring locations based on their history of maintaining a large number of chorusing male
125   Houston Toads relative to other documented Houston Toad chorusing ponds within the GLR.
126   To analyze the large quantity of audio files collected, we trained an audio classifier using
127   the software Kaleidoscope version 4.3.1 (Wildlife Acoustics). We followed the steps outlined by
128   the manufacturer for completing this process (Wildlife Acoustics 2017) and used the audio
129   training data provided by MacLaren et al. (2018A) for the call of the Houston Toad. We chose to
130   simply train towards two "clusters", Houston Toad vocalizations, and anything that is not a
131   Houston Toad vocalization. This was efficient, and we achieved 100% detection of training
132   vocalizations within a single round of training. However, as discussed by MacLaren et al.
133   (2018A) focusing on eliminating false negatives (i.e., a Houston Toad vocalization classified in
134   the "not Houston Toad" group) results in a classifier that is permissible to many false positives
135   that must be manually verified as such. This Kaleidoscope cluster was applied to filter all audio
136   recordings for Houston Toad vocalizations. All detections made by the software were manually
137   verified by ARM. We observed during training that detections below 3 seconds in duration were
138   overwhelmingly false positives, and extremely abundant throughout these data, so we excluded
139   these from Kaleidoscope output prior to manual review. Ultimately, false positive rate for
140   detections greater than 3 seconds in duration was 0.54, indicating just over half of all detections
141   classified as Houston Toad were not true positives, emphasizing the necessity of manually
142   reviewing all results. We binned detections into 5-minute time intervals, and summarized them
143   as binomial, where 1 and 0 indicate detection and non-detection of Houston Toads, respectively.
144
145   *Simulation*
146   We simulated Houston Toad audio surveys under three sampling scenarios, each
147   representing a unique approach to subsampling our complete dataset. First, by randomly
148   selecting survey data from the complete pool of recordings from both sites across all years.
149   Second, by restricting available survey data according to environmental conditions presented in
150   the USFWS protocol for conducting surveys for this species (USFWS 2007). Last, we sought to
151   identify the environmental conditions, if any, that maximize the probability of detecting Houston
152   Toads. For environmental variables we used the National Oceanic and Atmospheric
153   Administration's quality controlled local climatic dataset, measured at Giddings, Texas, USA,
154   ca. 25 km East of our sites (WBAN 53979). We utilized moon illumination measured by the U.S.
155   naval observatory (USNO) for Central Time Zones.
156   The decision to conduct Houston Toad audio surveys is often made in advance and
157   anticipation of appropriate environmental conditions occurring, based largely on weather
158   forecasts. To reflect the uncertainty implicit in this practice we assumed that if environmental

159   thresholds were met at any point within a calendar date, then all data for this date may be
160   surveyed. This is reflected in the results as "dates surveyable" under each scenario. Each of our
161   three survey scenarios selected only for intervals occurring in the months February, March, and
162   April, before 0600 and after 1800 hours of each date. This not only reflects roughly what is
163   currently required (USFWS 2007), it also coincides with peak chorusing activity for the Houston
164   Toad. To implement a random survey selection scenario, all 5-minute intervals within this time
165   frame were considered. To replicate the restrictions within USFWS (2007) we removed dates
166   wherein environmental variables failed to meet the following thresholds: temperature > 14 °C,
167   relative humidity > 70%, wind speeds < 24 kmph, and percent moon illumination < 0.5. For our
168   final scenario we searched for alternative environmental thresholds by which researchers may
169   improve success when conducting a human-performed audio survey for the Houston Toad. We
170   calculated summary statistics for the following environmental variables, when Houston Toad
171   vocalizations were detected: temperature (°C), relative humidity (%), wind speed (kmph), moon
172   illumination (%), hourly precipitation (mm), cumulative precipitation over the previous 24 hours
173   (mm), barometric pressure (mmHg at sea level), difference in barometric pressure across 24
174   hours (mmHg at sea level). We then examined which, if any, of these variables offered
175   thresholds that resulted in eliminating the number of dates containing non-detections. We
176   calculated the ratio of detections/non-detections for all combinations of thresholds both above
177   and below all values of temperature, the change in barometric pressure over 24 hours, and
178   cumulative precipitation over the previous 24 hours. This allowed us to identify which thresholds
179   excluded large periods of inactivity within the breeding season. We then applied these thresholds
180   in the same way as described above and carried out the simulation under these new restrictions.
181   We removed all instances in which the Song Meters did not record, then pooled all
182   Houston Toad detection data (e.g., 5-minute intervals) across the four years and the two sites
183   (N=92,652). We randomly selected one 5-minute interval for every date (N=433) without
184   replacement within the pooled dataset and repeated this 1,000 times. This was done to eliminate
185   the possibility of randomly selecting multiple surveys within the same date, which also more
186   correctly reflects how manual surveys are conducted in practice. We calculated the detection
187   probability as the proportion of positive detections out of the total number of files sampled
188   within each permutation, resulting in 1,000 estimates for detection probability for each
189   simulation. From these 1,000 estimates of detection probability, we calculated the mean and 95%
190   confidence intervals (2.5th and 97.5th quantile) for each distribution of probabilities. We
191   conducted separate simulations for survey scenarios of length 5 minutes, through 60 minutes, by
192   increasing 5-minute intervals, resulting in 12 distributions per scenario. We used the formula
193   provided by Pellet and Schmidt (2005) for calculating the minimum number of surveys required
194   to be 95% confident in Houston Toad absence, $N_{min} = \frac{\log(0.05)}{\log(1-p)}$, were p = the simulated estimate
195   of the detection probability for the particular survey scenario.
196   The detection process consists of many components. First, each site must be occupied
197   during the survey window, then individuals must chorus during the survey window in order for
198   detections to take place. For this study, we assume that for human observers and ARDs this

199 detection process is the same. In practice, human observers may disturb chorusing animals
200 leading to higher rates of false-negative errors, but we did not collect comparable data from
201 human observers in order to test for this potential difference in detection.
202
203 **Results**
204   We collected detection/non-detection data on 433 dates across all four years (86, 123,
205 101, and 123 dates for years 2015-2018, respectively). Out of 92,652 5-minute intervals
206 (intervals, hereafter) we detected Houston Toad vocalizations in 3,975 intervals (approximately
207 4%), occurring among 123 dates (18, 35, 37, and 33 dates for years 2015-2018, respectively).
208 Environmental variables measured for the dates which include these intervals are given in Table
209 1. Only 122 (~3%) of intervals containing Houston Toad vocalizations occurred outside of Feb-
210 April. All results henceforth refer to sampling within this peak chorusing period only (72,359
211 total intervals, 328 total dates, 3,853 intervals with detections).
212   In the first survey scenario, when we selected surveys randomly, the mean detection
213 probability was 0.063 (95% CIs 0.038 – 0.100) for surveys 5 minutes in duration, and 0.121
214 (95% CIs 0.088 – 0.171) for surveys 60 minutes in duration (Figure 1, Table 2). These
215 probabilities result in requiring a mean of 47 (range = 29-79) surveys to be 95% confident in
216 determining absence of the species when conducting 5-minute-long surveys, and on average 24
217 (range = 16-33) when conducting 60-minute-long surveys (Figure 1, Table 2).
218   Our second survey scenario, in which we removed all dates that did not reflect the
219 environmental thresholds given by USFWS (2007), resulted in reducing the number of available
220 survey dates to 118 out of 328. Under this scenario only 1,737 intervals containing detections are
221 available to be sampled, leaving 1,895 intervals (49%) known to possess Houston Toad
222 vocalizations unobservable to surveyors (3,853 intervals total). The mean detection probability
223 was 0.08 (95% CIs 0.042 – 0.119) for surveys 5 minutes in duration, and 0.142 (95% CIs 0.093 –
224 0.186) for surveys 60 minutes in duration (Figure 1, Table 2). These probabilities result in
225 requiring a mean of 36 (range = 24-70) surveys to be 95% confident in determining absence of
226 the species when conducting 5-minute-long surveys, and on average 20 (range = 15-31) when
227 conducting 60-minute-long surveys (Figure 1, Table 2).
228   In our final scenario, calculating the proportion of detections to non-detections over a
229 range of environmental thresholds revealed that a unique combination of temperature (> 16 °C),
230 precipitation (> 0 mm/day), and change in barometric pressure (< -0.07 mmHg) provided the
231 greatest advantage to surveyors. These thresholds allow 133 dates to be surveyable, comparable
232 to USFWS (2007), yet provide 2,569 intervals containing detections. Under this scenario, the
233 mean detection probability was 0.105 (95% CIs 0.066 – 0.146) for surveys 5 minutes in duration,
234 and 0.179 (95% CIs 0.133 – 0.229) for surveys 60 minutes in duration (Figure 1, Table 2). These
235 probabilities result in requiring a mean of 28 (range = 20-49) surveys to be 95% confident in
236 determining absence of the species when conducting 5-minute-long surveys, and a mean of 16
237 (range = 13-22) 60-minute-long surveys to be 95% confident in determining absence of the
238 species (Figure 1, Table 2).

239

Discussion

240

241   This study demonstrates that the existing USFWS (2007) guidelines for conducting
242 human performed surveys result in a likelihood of concluding absence of Houston Toads from a
243 site when they may be truly present. Given the endangered status of the species, and the
244 precedent set by Jackson et al. (2006), researchers should attempt to achieve 95% confidence in
245 conclusions of absence. Currently, 36 (12 per year, for 3 years) 5-minute-long surveys (180
246 minutes total) are required to determine absence (USFWS 2007). However, our simulation
247 reveals that up to 79 surveys (395 total minutes) of this duration may be required to adequately
248 determine Houston Toad occurrence at a single site. Failing to detect Houston Toads when they
249 are truly present ultimately results in undetected populations, which in the event that the
250 monitoring is being performed as part of a development project, may contribute to local
251 extirpation events. This is especially true for populations outside of our study site, which do not
252 receive any form of population supplementation, and may only support a few individuals. It is
253 also important to add that our study, and the study conducted by Jackson et al. (2006), were
254 carried out using data collected from locations where Houston Toads are likely at, or near, their
255 highest abundance, and as such the data presented here could underestimate of the survey
256 requirements necessary to detect other, smaller populations (Tanadini and Schmidt 2011).
257   Our approach demonstrates that the false negative errors associated with manual surveys
258 can be reduced in three main ways. First, manual surveys of longer duration can be performed. In
259 each of our scenarios, the detection probability increases as survey's duration lengthens. Second,
260 the number of surveys performed can be increased. Third, surveyors can target occasions in
261 which environmental conditions are most closely associated with chorusing behavior among
262 Houston Toads. The trade-offs associated with each of these methods of decreasing false
263 negative errors will depend on the timeframe and scope of the specific Houston Toad monitoring
264 project. Our results indicate human observers must, at minimum, more than double the amount
265 of time spent surveying in order to determine absence with 95% confidence. However, for
266 human observers these changes may be too onerous to allow manual methods to be feasible for
267 determining detection/non-detection from a site. An alternative approach would be the use of
268 automated recording devices (ARDs), as they are demonstrably effective at the task of detecting
269 anurans, especially when rare, due to the efficiency with which high cumulative detection
270 probabilities are achieved (Hsu et al. 2005; Acevado & Villanueva-Rivera 2006). Automated
271 audio recording devices designed for monitoring wildlife are becoming smaller, more affordable,
272 and are able to collect audio much more frequently than human observers (Saenz et al. 2006;
273 Aide et al. 2013; Willacy et al. 2015).
274   The goals of this study, and that of the federal protocol we evaluate (USFWS 2007;
275 USFWS 2020) are limited to determining occurrence of the Houston Toad at a single site (i.e.,
276 one human listening post or ARD location). It is our view that human observers should only be
277 employed towards site specific detection of Houston Toads when automated methods are not
278 suitable or potential recorder placement is not permitted. This is particularly relevant for the

279 Houston Toad as the majority of Texas is privately owned lands and public roadways enable
280 access across remaining habitat patches. Further, human observers might better be employed
281 conducting survey methods that cannot adequately be conducted using remote, passive, methods.
282 For example, in order to detect Houston Toads that do not chorus (i.e., subadults, females)
283 human observers may be required to employ drift fence arrays (Brown et al. 2013) or sample
284 aquatic habitats. Additionally, methods of determining anuran occurrence at larger scales (i.e.,
285 county or regional) often require visiting many sites in a single day, and have successfully been
286 implemented using human observers (Gorman 2009). Yantis and Price (1993) employed similar
287 methods to determine the distribution of the Houston Toad within Texas.
288   One critical aspect in the application of our findings is the frequency with which survey
289 dates containing suitable environmental conditions can be expected to occur. If we consider the
290 four years utilized in this study (2015-2018), we find that conditions permissible under the
291 currently accepted USFWS (2007) guidelines only occur on 27, 39, 39, and 31 dates within peak
292 chorusing period each year, respectively. Our simulation indicates that up to 79 5-minute-long
293 surveys should be conducted to minimize false negative errors, and accordingly surveyors would
294 be required to choose an alternative approach (i.e., surveys of longer duration) in order to
295 achieve confidence in their findings. If we consider the environmental conditions discussed in
296 our third scenario, we find that for years 2015-2018, these conditions only occur on 40, 53, 5,
297 and 50 dates within the peak chorusing period each year, respectively. While these results
298 illustrate that in certain years this increases the abundance of surveyable days, during 2017 this
299 method provides only five days of survey appropriate conditions, which reduces the practicality
300 of this approach considerably. Additionally, it would be unwise for researchers to restrict their
301 survey design to a limited set of environmental conditions when these events occur
302 stochastically, and are not guaranteed to occur the necessary number of times to have confidence
303 in conclusions of absence. A caveat within this scenario is that the range of conditions we
304 identified during are derived from our complete dataset, and have not been applied to an
305 independent group of data, and so our results from this scenario may be biased optimistically.
306   Given the environmental and temporal constraints we have identified, researchers have
307 much to consider when scheduling acoustic surveys for the Houston Toad. The cost to
308 researchers performing acoustic surveys using human observers is likely variable, and we will
309 not speculate on the individual cost of each survey. Although ARDs are, in the long term, less
310 expensive than human performed surveys, factors such as travel and wages can be minimized
311 (Williams et al. 2018). We believe our approach demonstrating detection probability as it
312 increases with survey duration provides researchers under variable circumstances options to
313 achieve confidence in absence determinations. For example, if travel costs are large (i.e.,
314 researchers must travel a great distance to reach their field sites) perhaps fewer surveys of greater
315 duration may be favored, whereas researchers with many sites to visit may prefer shorter
316 surveys, allowing more sites to be visited on each calendar date.
317
318 **Conclusions**

319   Given the findings of our simulations, we strongly recommend that human observers
320   restrict their surveys to the peak of Houston toad activity that occurs during the 89 day period
321   between February 1 and April 30. While we believe it is wise to use *a priori* knowledge of the
322   environmental conditions in which chorusing generally takes place to improve the likelihood of
323   detecting Houston Toads (MacLaren et al. 2018B), our study reveals that these events are rare,
324   do not consistently elicit vocalization behavior, and may not allow for adequate effort to be put
325   forth by human observers in any given year. For these reasons we feel our first scenario is most
326   applicable, in which surveyors can choose to survey any date. Houston Toads are in decline
327   throughout their native range (Forstner and Dixon 2011) thus, we believe that, due to the serious
328   consequences of potential false negative errors, the upper 95% confidence interval for randomly
329   selected surveys be adopted as the minimum survey effort threshold (Table 2). Situations that
330   trigger the need to conduct surveys following USFWS (2007; 2020) are likely to occur in areas
331   where local occurrence is not known (e.g., marginal habitats). Marginal populations are in the
332   most need of stewardship, and maximum survey effort (i.e., the upper bounds of our confidence
333   intervals, or beyond) is likely necessary to detect these less abundant population remnants.
334   We found that previously suggested environmental correlates to chorusing among
335   Houston Toads offered improved detection probabilities over randomly selected surveys.
336   However, we found that not all suggested weather criteria within USFWS (2007) were useful,
337   specifically, moon illumination, humidity, and wind speed. This is either because these variables
338   share no true relationship with chorusing within Houston Toads, as is the case for moon
339   illumination, or because they do not serve as a hard boundary, as is the case for relative
340   humidity. For example, relative humidity may range between 10% and 90% within a given single
341   date in response to natural diel cycle. We identified definitive thresholds among temperature,
342   precipitation, and shifts in barometric pressure that improve the probability of detection for
343   Houston Toads beyond what USFWS (2007) currently suggests.
344   This study updates and expands upon the findings of Jackson et al. (2006). For
345   perspective this previous study (Jackson et al. 2006) utilized twenty 5-minute surveys (100
346   minutes) within a given year at a single site, whereas within a single year one ARD provided us
347   with approximately 60,000 minutes of audio from a single site. Using these vast and detailed
348   data we found that detection probabilities, for surveys of any length, and under any sampling
349   scenario, were lower than what has been previously estimated for this species (Jackson et al.
350   2006). By suggesting more accurate environmental thresholds under which surveys should be
351   conducted, and evaluating surveys of varying duration, we have provided researchers and
352   managers with an approach that should make the highest probability of detecting Houston Toads
353   possible. Our approach to simulating survey effort allows researchers to choose the combination
354   of survey duration and number of surveys they find most appropriate and maintain 95%
355   confidence in determinations of absence. Like Jackson et al. (2006) our results suggest that the
356   USFWS should modify the mandatory survey guidelines to require more surveys in each season
357   than is currently specified. Moreover, for surveys that are designed to determine presence or
358   absence towards regulatory decisions at a site, the conflict between the availability of suitable

359 environmental conditions and the importance of conducting sufficient surveys based on
360 environmental factors that increase the probability of survey success, ARDs should be strongly
361 considered where possible. Finally, it is critical to differentiate absence determinations made
362 from chorusing data from true absence of this endangered anuran from a potential disturbance
363 site given the underlying nature of juvenile amphibian dispersal and adult use of upland habitats.
364

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369

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Summary statistics of environmental variables during dates in which Houston Toads (*Bufo houstonensis*) were detected by automated audio recorders from 2015-2018 in Bastrop County, Texas.

1 Table 1. Summary statistics of environmental variables during dates in which Houston Toads
2 (*Bufo houstonensis*) were detected by automated audio recorders from 2015-2018 in Bastrop
3 County, Texas.

	MIN.	1ST QU.	MEDIAN	MEAN	3RD QU.	MAX.
TEMPERATURE (°C)	-1.15	16.63	19.20	19.24	22.00	31.40
RELATIVE HUMIDITY (%)	14.33	72.33	88.00	82.63	95.67	100.00
WIND SPEED (KMPH)	0.00	3.67	7.00	7.19	9.67	28.33
BAROMETRIC PRESSURE (MMHG)	29.06	29.38	29.47	29.47	29.57	29.99
PRESSURE CHANGE (MMHG)	-0.35	-0.11	-0.04	-0.03	0.04	0.38
PRECIPITATION (MM)	0.00	0.03	0.07	0.24	0.22	4.50
MOON ILLUMINATION (%)	0.00	11.00	40.00	48.76	91.00	100.00

Raw values of probability of detecting Houston Toads, and the number of surveys needed to infer absence, as duration of survey increases.

Mean and 95% confidence bounds for the probability of detection and number of surveys required to be 95% confident in absence of Houston Toads (*Bufo houstonensis*) during acoustic surveys, for three sampling paradigms, as duration of survey increases from 5 to 60 minutes.

1 Table 2. Mean and 95% confidence bounds for the probability of detection and number of
2 surveys required to be 95% confident in absence of Houston Toads (*Bufo houstonensis*) during
3 acoustic surveys, for three sampling paradigms, as duration of survey increases from 5 to 60
4 minutes.

Duration (mins)	Detection Probability			Number of Surveys		
	2.50%	Mean	97.50%	2.50%	Mean	97.50%
	Randomly Selected					
5	0.038	0.063	0.100	79	47	29
10	0.046	0.074	0.113	64	39	26
15	0.050	0.082	0.121	59	35	24
20	0.058	0.089	0.129	50	33	22
25	0.063	0.094	0.138	47	31	21
30	0.067	0.099	0.142	44	29	20
35	0.071	0.104	0.146	41	28	19
40	0.075	0.107	0.150	39	27	19
45	0.075	0.111	0.154	39	26	18
50	0.079	0.115	0.158	37	25	18
55	0.083	0.118	0.167	35	24	17
60	0.088	0.121	0.171	33	24	16
	USFWS Protocol					
5	0.042	0.080	0.119	70	37	24
10	0.051	0.094	0.136	58	31	21
15	0.059	0.103	0.153	49	28	19
20	0.068	0.110	0.153	43	26	19
25	0.076	0.116	0.161	38	25	18
30	0.076	0.121	0.169	38	24	17
35	0.085	0.126	0.169	34	23	17
40	0.085	0.129	0.178	34	22	16
45	0.085	0.133	0.178	34	21	16
50	0.093	0.136	0.178	31	21	16
55	0.093	0.140	0.186	31	20	15
60	0.093	0.143	0.186	31	20	15
	Optimized Protocol					
5	0.060	0.101	0.143	49	28	20
10	0.075	0.118	0.165	39	24	17
15	0.083	0.128	0.173	35	22	16
20	0.090	0.136	0.180	32	21	16
25	0.098	0.143	0.188	30	20	15
30	0.105	0.149	0.195	27	19	14
35	0.113	0.154	0.195	25	18	14
40	0.113	0.159	0.203	25	18	14
45	0.120	0.163	0.203	24	17	14
50	0.120	0.167	0.211	24	17	13
55	0.128	0.170	0.211	22	17	13
60	0.128	0.173	0.218	22	16	13

Figure 1

Probability of detecting Houston Toads, and the number of surveys needed to infer absence, as duration of survey increases.

Results of simulation to assess mean probability of detection of Houston Toads (*Bufo houstonensis*) (bottom panel), and the mean number of surveys necessary for a given probability of detection (top panel), and their 95% confidence bounds, as the length in minutes of each auditory survey increases along the x-axis, for three approaches to survey selection: Random selection (left), following USFWS 2007 (middle), and under our proposed optimization for survey selection (right).