Searching for an Elusive Anuran: A Detection Model Based on Weather Forecasting for the Tandilean Red-belly Toad

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Abstract.—The study of elusive anurans requires efficient approaches to monitoring. We describe climatic variables associated with the probability of detection of the Tandilean Red-belly Toad (*Melanophryniscus* aff. *montevidensis*), an elusive, threatened, and endemic species of highland grasslands of Argentine Pampas. As expected for an explosive breeder, the probability of detection was strongly associated with rainfall during the previous day. Soil water storage and daily temperatures influenced detection probability. We did not find a link between the season in which surveys were conducted and detection probability. The low number of days during the year when conditions are suitable for breeding by Tandilean Red-belly Toad requires the development of an efficient field survey. The tool we have developed using weather forecast to predict detection probability provides a practical approach to effectively sampling this elusive species of conservation concern.

Key Words.-grassland; modeling; monitoring; occupancy

INTRODUCTION

Because anurans rely on vocalization for most communication, detection of calls provides a relatively efficient mechanism for studying and evaluating the status of anuran populations (Dorcas et al. 2009). However, some anuran species only vocalize during explosive breeding events, which occur in a short period of time, typically when rainfall of snowmelt create suitable breeding conditions (Gottsberger and Gruber 2004). Monitoring is particularly challenging for those explosive breeders that are also rare in the landscape and poorly documented (Thompson 2013).

Red-belly toads (*Melanophryniscus* spp.) are highly endemic to small areas, in some cases limited to only one or two known locations (Zank et al. 2014). In addition, these toads are typically difficult to find during most of the year and are usually detected only during explosive breeding events (Goldberg et al. 2006; Santos and Grant 2010; Pereyra et al. 2011). Precipitation and temperature appear to strongly influence the activity patterns of the species in this genus (Vaira 2005; Cairo et al. 2008).

The Tandilean Red-belly Toad (part of the Melanophryniscus stelzneri group) exemplifies these characteristics, occurring in small and fragmented remnants of highland grasslands of Argentinean Pampa (Fig.1; Vega and Bellagamba 1990; Soler et al. 2014). The remnants of highland grasslands of Tandilia Mountains belong to the Pampa, one of the highest priority conservation eco-region due to habitat loss, biological uniqueness, and absence of protected areas (Bilenca and Miñarro 2004). The fragmentation and modification of highland grasslands has been demonstrated to reduce dispersal of toads and the connectivity of breeding sites (Cairo and Zalba 2007). In consequence, the populations of Tandilean Red-belly Toad are threatened and have high conservation priority listed as Vulnerable by the International Union for Conservation of Nature (Vaira et al. 2012; Zank et al. 2014). Moreover, projected changes in the climatic suitability for known populations suggest that the Tandilean Red-belly Toad will lose areas with suitable climatic conditions in the near future (Zank et al. 2014). The taxonomy of this toad is still debated and most specialists refer to this species as Melanophryniscus aff. montevidensis or Melanophryniscus sp.3 (Kwet et al. 2005; Vaira et al. 2012; Zank et al. 2014). Given predicted climate change and existing habitat fragmentation, a solid monitoring program for populations of Tandilean Red-belly Toad is imperative. Our objectives

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FIGURE 1. Tandilean Red-belly Toad, *Melanophryniscus* aff. *montevidensis* (2.5 cm snout-vent length) in highland grasslands of the Pampa ecoregion, Argentina. (Photographed by Gabriela Soler).

were to explore which weather variables maximize the probability of detection of Tandilean Red-belly Toads, and to develop a tool based on weather forecasting to select optimal survey dates.

MATERIALS AND METHODS

Study site.—Our study site was located in the La Poligonal, a recently created 141.6 km² protected area adjacent to Tandil City, in the province of Buenos Aires, Argentina. La Poligonal consists of a mosaic of highland grassland fragments that have experienced a variety of habitat disturbances including stone quarrying, cattle ranching, introduction of invasive species (e.g., pine, *Pinus* sp., and Spanish Broom, *Spartium junceum*), and development for tourism (Bilenca and Miñarro 2004).

Anuran surveys.—Because the small ephemeral wetlands used by the focal species are not currently mapped, our approach to sampling was to systematically survey the sample area. We established a regular grid (358 cells of 12.8 ha each) over the study area and randomly surveyed 38 cells (Fig. 2). We conducted daily surveys (between 1000 and 1700) during the

breeding season of Tandilean Red-belly Toads between August 2012 and March 2014. During each survey, a team of four surveyors hiked the whole survey cell looking for the presence of toads by performing a combination of male vocalization and visual encounter surveys (Crump and Scott 1994). In 25 of the 38 survey cells, we conducted four visits on different days throughout the season; the challenges of accessing remote sites prevented us from completing four visits at each one of the remaining 13 cells. Missing observations are acceptable in single-season models and equal sampling effort is not required across all sites (MacKenzie and Bailey 2004). For a missing observation, we did not collect information regarding detection (or non-detection) of the species, and the observation was not considered in the analysis.

Covariates.—We quantified six potential covariates in the field that seemed likely to influence the timing of breeding of the Tandilean Red-belly Toad, including a combination of weather variables and soil water storage (Table 1). We obtained maximum and minimum daily temperatures and cumulative precipitation from the closest weather station reporting to WindGuru web-



FIGURE 2. Highland grassland fragments in the La Poligonal protected area, province of Buenos Aires, Argentina. The regular grid includes 358 cells (12.8 ha each) with highland grassland. Inset map shows location of Pampa eco-region.

site (http://www.windguru.com); we obtained soil water storage from an agricultural governmental site (http:// www.ora.gov.ar). For details about how soil water storage is calculated, see Lay et al. (2008). To avoid including correlated covariates, we conducted pairwise comparisons using Pearson's correlation coefficient; all comparisons showed low coefficient values (i.e., $r \le$ 0.7). We hypothesized that (1) breeding activity would decrease as temperature increased; (2) breeding is more likely to occur when minimum daily temperatures are higher; (3) heavy rainfall and high levels of soil water storage create the temporary wetlands needed for reproduction and therefore encourage breeding; and (4) the breeding activity of toads decreases as the season progresses (i.e., time of season).

Modeling.—We employed occupancy modelling to estimate the probability of detecting the focal species during surveys (MacKenzie et al. 2006). Occupancy (ψ) is defined as the proportion of sites occupied, and detection probability (p) is the probability that a species will be detected within a sample area, given that it is present within that sample area (MacKenzie and Bailey 2004). The probability of detecting a toad at a survey cell in a given survey is defined as the product of the probability that the toad uses the surveyd cell during

the season and the probability of detecting the toad during the survey, given that it was physically present in the surveyed cell. Our estimates of detection probability are conditional on both the presence of a species and the availability of that species during the survey period (Bailey et al. 2014).

We developed a model set based on *a priori* hypotheses that the probability of detection may be affected by weather conditions, soil condition, and time of the season. We used the package Unmarked in R (Fiske and Chandler 2011) to perform single-season occupancy models. We considered models with up to four parameters (including the intercept and probability of detection) to avoid the occurrence of spurious results (Burnham and Anderson 2002). We fitted the data to a baseline model in which occupancy was constant across all survey cells [denoted as ψ (.)].

We used Akaike's Information Criterion (AIC) to compare among models (Burnham and Anderson 2002). To identify which covariates in our models were good predictors of detection, we assessed the strength of evidence from our model-selection results and from model estimates of effects of the covariates. First, we examined whether the best models in the set were better than the constant-detection model [i.e., $\psi(.) p(.)$]. Next, we identified which covariates were consistently included in the set of best models (i.e., models that are within two AIC units $[\Delta AIC < 2]$ of the top-supported model). For covariates we calculated the estimates of parameters (β) and their standard errors. For determining the overall level of support for each covariate (given the model set), we added the model parameter estimates for each of the respective models (Burnham and Anderson 2004). We used the best model to estimate the daily probability of detection for the 2012-2013 and 2013-2014 breeding seasons (i.e., 244 d each), and to predict the probability of detection from weather forecast by building an algorithm in R software (Appendix). This approach used the weather forecasted from the WindGuru website (http:// www.windguru.cz/) to predict the daily probability of detection, allowing us to select the best survey dates. Because the breeding activity of Tandilean Red-belly Toads usually begins when maximum daily temperature exceeds 20° C (Cairo et al. 2008), we excluded days when maximum daily temperature was below 18° C from our estimations.

RESULTS

Our top-ranked models included daily temperature, amount of precipitation, and soil water storage as covariates for detection (Table 2). Model parameter estimates indicated that the probability of detecting toads decreased as daily maximum temperature increased (Table 3). Conversely, detection probability increased at higher minimum daily temperature, and was positively correlated with rainfall on the day(s) preceding the survey and higher soil water storage. Time of season was not included in the top-ranked models. Our best model predicted 13 d during the 2012-2013 season and 9 d during the 2013-2014 season with an estimated detection probability > 0.9 (Fig. 3).

DISCUSSION

As expected for an explosive breeder, the probability of detection of the elusive Tandilean Red-belly Toad was largely associated with the amount of precipitation during the previous day. Highest detection probabilities occurred usually, but not always, after heavy rains. This finding is similar to that for other *Melanophryniscus* species where breeding activity has been shown to be concentrated during the first two or three days after a heavy rainfall event (Vaira 2005; Cairo and Zalba 2007).

The amount of precipitation, daily temperature, and soil water storage influence hydroperiod (i.e., breeding conditions). High summer temperatures increase evaporation, reducing soil moisture and persistence of ephemeral wetlands. High levels of soil moisture are necessary for the establishment and persistence of the temporary ponds in which this species breeds (Williams 2005).

Most Melanophryniscus species are diurnal, and as expected for most ectotherms, the daily temperature constrained the probability of detection of Tandilean Red-belly Toads (Rohr and Malone 2001). Increasing levels of minimum daily temperature (warm nights) had a positive effect on the probability of detecting toads. Conversely, increasing levels of maximum daily temperature (hot days) had a negative effect on detection. High temperatures may cause toads to retreat into the vegetation, and reduce calling activity (Ospina et al. 2013). Even though the reproductive activity of toads seems concentrated in spring and the end of the summer, we found no effect of time of the season in the probability of detection. In addition to precipitation and daily temperatures, soil water storage was also found to be positively associated with detection probability.

TABLE 1. Candidate covariates included in models assessing drivers of detection probability of the Tandilean Red-belly Toad (*Melanophryniscus* aff. *montevidensis*) in highland grasslands of the Pampa ecoregion, Argentina. Values are mean \pm standard deviation (SD), minimum, and maximum values of covariates during survey dates, and probability of detection (*p*).

Variable	Mean ± SD (Min-Max)	Hypothesis
Max (Maximum daily temperature)	24.6 ± 3.7° C (19.0–33.0° C)	Breeding activity <i>p</i> (-)
Min (Minimum daily temperature)	9.6 ± 4.2° C (2.5–18° C)	Breeding activity $p(+)$
Pp-24 (Amount of precipitation during the last 24 h)	3.1 ± 11.0 mm (0–70 mm)	Breeding conditions $p(+)$
Pp-72 (Amount of precipitation during the last 72 h)	18.9 ± 21.7 mm (0–97.8 mm)	Breeding conditions $p(+)$
SWS (Soil water storage)	71.1 ± 10.9 mm (54.6–90.8 mm)	Breeding habitat availability $p(+)$
Time (Time of season)	From day 1 to day 244	Breeding activity p (-)



FIGURE 3. Daily predicted probabilities of detection of Tandilean Red-belly Toad (*Melanophryniscus* aff. *montevidensis*) in highland grasslands of the Pampa ecoregion, Argentina for (A) 2013–2014, and (B) 2014–2015 breeding seasons. We estimated probabilities considering the best detection model. The horizontal dotted line indicates a reference value for the probability of detection.

The strong relationship between weather and probability of detection allowed us to estimate how many breeding opportunities occurred in each of the two seasons we sampled. Even though the toads may breed during a prolonged season, there are actually very few days when conditions are optimal. We found only nine and 13 d per season when weather conditions were optimal for the detection of toads. A similar number of opportunities (four and six breeding opportunities per season) were reported in *Melanophryniscus rubiventris* (Vaira 2005). Not considering detection probability will likely lead to much wasted surveying effort. Moreover, potentially changes in the environment such as shifts in climate may mean that these few days are lost. By integrating our results with climate data, models could be used to evaluate whether the number of breeding opportunities of each season are stable, increasing, or decreasing. Climate change predictions in the Pampa

TABLE 2. Top performing site detection models ($\Delta AIC < 2$) of highland grasslands survey cells occupied [ψ (.)] by the Tandilean Red-belly Toad (*Melanophryniscus* aff. *montevidensis*) in the Pampa ecoregion, Argentina. Variables as defined in Table 1.

Model	df	ΔAIC	Weight	-2 LogLikelihood
ψ (.) <i>p</i> (Max + Pp-24 + Pp-72)	5	0.00	0.46	123.5
ψ (.) p (Max +Pp-24 + SWS)	5	1.35	0.24	124.9
ψ (.) p (Max + Min + Pp-24)	5	1.91	0.18	125.4
ψ(.) <i>p</i> (.)	2	38.64	0.00	168.2

region included an increase of temperature and length of the dry season, a decrease of soil moisture, and greater inter-annual variation in rainfall, which will result in a reduction of habitat availability for ephemeral breeding anurans (Corn 2005; Thomas et al. 2014). A recent estimation of climate change impact predicted a 60% decrease in the area of suitable habitat for the Tandilean Red-belly Toad by 2080 (Zank et al. 2014).

The low detectability of the Tandilean Red-belly Toad requires an efficient field survey program. Our predictive tool, based on weather forecasting, is an effective approach to selecting the best survey dates. While precipitation is certainly a factor influencing the detection probability of many anuran species, we also found other important covariates. Anuran species that are only detected during infrequent explosive breeding events during a prolonged potential breeding season, require accurate estimation to determine when the temporary aquatic habitat will be created, and this does not always occur after intense rainfall. In the case of our focal species, the level of soil water storage and daily temperatures also play an important role in the probability of detection and need to be included in predictive models. By developing a predictive tool based on freely available weather data, coupled with open-source R code (Appendix), we have provided a practical solution to effective sampling of an elusive species of conservation concern that could be readily adapted for other similar species.

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	Parameter estimates (β)						
Model	Max	Min	Pp-24	Pp-72	SWS		
<i>p</i> (Max + Pp-24 + Pp-72)	$\begin{array}{c} -1.45 \pm 0.32 \\ (-1.98, -0.92) \end{array}$		$\begin{array}{c} 2.58 \pm 1.70 \\ (\text{-}0.22, 5.39) \end{array}$	$\begin{array}{c} 1.00 \pm 0.29 \\ (0.52, 1.48) \end{array}$			
p (Max +Pp-24 + SWS)	-1.41 ± 0.32 (-1.93, -0.88)		$\begin{array}{c} 1.38 \pm 1.20 \\ (-0.61, 3.37) \end{array}$		$\begin{array}{c} 0.91 \pm 0.27 \\ (0.47, 1.35) \end{array}$		
p (Max + Min + Pp-24)	-1.67 ± 0.36 (-2.25, -1.09)	$\begin{array}{c} 1.08 \pm 0.34 \\ (0.53, 1.64) \end{array}$	3.00 ± 1.80 (0.04, 5.95)				

TABLE 3. Model parameter estimates (β), standard error (SE) and 90% confidence intervals (low, high) of top-ranked models (Δ AIC < 2) for the Tandilean Red-belly Toad (*Melanophryniscus* aff. *montevidensis*) in highland grasslands of the Pampa ecoregion, Argentina. Estimates ± standard error are on the logit scale. Variables as defined in Table 1.

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Herpetological Conservation and Biology

APPENDIX.—R IMPLEMENTATION

Here we present the functions in the R statistical language used to estimate the probability of detection of the Tandilean Red Belly Toad in highland grasslands of the Pampa ecoregion, Argentina, based on weather forecast information. Throughout the code we include comments so the reader can understand every step and modify it for application to other data.

The code presented below requires the installation of the following R packages:

- *unmarked*: Is a complete environment for the statistical analysis of data from surveys of unmarked animals. It includes the fitting and prediction functions, and also the model selection framework for the occupation models and other hierarchical models of animal abundance and occurrence.
- *XML*: We use it for reading the HTML document from the weather service URL and for searching through its attributes to find the data we need.
- *xlsx*: Provides R functions to read/write/format Excel file formats. The user can choose between a wide range of other packages depending on the user's preferences and the format of the data.
- ggplot2: Is a plotting system for R, based on the grammar of graphics. It provides a powerful model of graphics that makes it easy to produce complex multi-layered graphics.

Function: estimDet

> estimDet=function(formula,SWSVal=c(NA)){
#1. IMPORT WEATHER DATA
library(XML)
#Import the URL where the weather data is allocated
doc = htmlTreeParse("http://www.windguru.cz/es/index.php?sc=53104", useInternalNodes = TRUE)

#Find the character string that includes the data tables<-xpathSApply(doc, "//*/script[@type='text/javascript']", xmlValue) wndgru<-grep('wg_fcst_tab_data_l',tables,value=T)

#Extract temperatures TMPw<-strsplit(wndgru, '\"TMP\":[',fixed=TRUE)[[1]][2] TMPw<-strsplit(TMPw, ']',fixed=TRUE)[[1]][1] TMP<-strsplit(TMPw, ', ',fixed=TRUE)[[1]] TMP<-as.numeric(TMP)

#Extract precipitation data PCPw<-strsplit(wndgru, '\"APCP\":['fixed=TRUE)[[1]][2] PCPw<-strsplit(PCPw, ']'fixed=TRUE)[[1]][1] PCP<-strsplit(PCPw, ', 'fixed=TRUE)[[1]] PCP<-suppressWarnings(as.numeric(PCP))

#Extract Days
Daysw<-strsplit(wndgru, '\"hr_weekday\":['fixed=TRUE)[[1]][2]
Daysw<-strsplit(Daysw,']'fixed=TRUE)[[1]][1]
Days<-strsplit(Daysw,','fixed=TRUE)[[1]]
Days<-as.numeric(Days)
IniDay<-strsplit(wndgru, 'initdate\":\"'fixed=TRUE)[[1]][2]
IniDay<-strsplit(IniDay, '12:00:00'fixed=TRUE)[[1]][1]
IniDay<-as.Date(IniDay)</pre>

#Split data by date TmpLst<-list(TMP[1]) PcpLst<-list(PCP[1]) day=1 for (i in 2:length(Days)) { if (Days[i]==Days[i-1]) { TmpLst[[day]]<-c(TmpLst[[day]],TMP[i]) PcpLst[[day]]<-c(PcpLst[[day]],PCP[i]) } else { day<-day+1 TmpLst<-c(TmpLst,TMP[i]) PcpLst<-c(PcpLst,PCP[i])}}</pre> Friedman et al.—A detection model for elusive anurans.

```
#Compute the maximum and minimum temperatures and the amount of precipitations

PcpLst[length(TmpLst)]<-NULL

TmpLst[1]<-NULL

MaxTmp<-unlist(lapply(TmpLst,max))

MinTmp<-unlist(lapply(TmpLst,min))

AcumPcp<-unlist(lapply(PcpLst,sum,na.rm=T))
```

#Create the dates vector Dates<-IniDay+1:length(TmpLst)

#2. RUN MODEL library(unmarked) library(xlsx)

#Import the data
toad<-read.xlsx("Toad.xls",1)</pre>

#Create the detection histories and covariate matrices and the occupation data frame y<-toad[,2:5] obsCovs<-list(Max=toad[,c(6:9)],Min=toad[,c(10:13)],Pp24=toad[,c(14:17)],SWS=toad[,c(18:21)]) toad2<-unmarkedFrameOccu(y=y,obsCovs=obsCovs)

#Create a matrix with the means and SDs of the covariates means&sds<-data.frame(data=matrix(nrow=2,ncol=4),row.names = c("Mean", "SD")) names(means&sds)<-names(obsCovs) with (toad2@obsCovs, { means&sds[1,]<<-c(mean(Max,na.rm=T),mean(Min,na.rm=T),mean(Pp24,na.rm=T),mean(SWS,na.rm=T)) means&sds[2,]<<-c(sd(Max,na.rm=T),sd(Min,na.rm=T),sd(Pp24,na.rm=T),sd(SWS,na.rm=T))})</pre>

#Standardize covariate and run the model obsCovs(toad2)<-scale(obsCovs(toad2)) fm1<-occu(formula(formula), toad2)

#3. ESTIMATE DETECTION FROM THE WEATHER DATA newdata = data,frame(Max=rep((MaxTmp -means&sds['Mean', 'Max'])/means&sds['SD', 'Max'],length(SWSVal)), Min=rep((MinTmp -means&sds['Mean', 'Min'])/means&sds['SD', 'Min'],length(SWSVal)), Pp24=rep((AcumPcp -means&sds['Mean', 'Pp24'])/means&sds['SD', 'Pp24'],length(SWSVal)), SWS=rep(SWSVal, each=length(MaxTmp))) pred<-predict(fm1, type = 'det', newdata = newdata, appendData=TRUE)

#4. GRAPH library("ggplot2") if (length(SWSVal)==1){ qplot(Dates, Predicted, data = pred,geom = "line")+ labs(x = "Days", y = "Estimated Detection") + geom_point() }else{ qplot(rep(Dates,length(SWSVal)), Predicted, data = pred, colour=as.character(SWS),geom = "line")+ labs(x = "Days", y = "Estimated Detection",colour='Soil Water Storage') + geom_point()}

Usage

The *estimDet* function requires the following arguments:

- *formula*: Is the model formula for the detection using the syntax: ~detection formula ~1.
- *SWSVal*: In case the model includes soil water storage (SWS) as a covariate, the function requires specific values of this variable in order to estimate the detection probability. SWSVal should be a vector of values in the standard scale.

Examples

```
With soil water storage as a covariate: > estimDet(~Max+SWS+Pp24~1, c(-1,0,1))
```

```
Without soil water storage as a covariate: > estimDet (~Max+Pp24~1)
```