

# Counting chirps: acoustic monitoring of cryptic frogs

G. John Measey<sup>1\*</sup>, Ben C. Stevenson<sup>2</sup>, Tanya Scott<sup>3</sup>, Res Altwegg<sup>3,4</sup> and David L. Borchers<sup>2</sup>

<sup>1</sup>Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Natural Sciences Building, Private Bag X1, Matieland, Stellenbosch 7602, South Africa; <sup>2</sup>Centre for Research into Ecological and Environmental Modelling, University of St Andrews, The Observatory, Buchanan Gardens, St Andrews, Fife KY16 9LZ, UK; <sup>3</sup>Statistics in Ecology, Environment and Conservation, Department of Statistical Sciences, University of Cape Town, Rondebosch 7701, South Africa; and <sup>4</sup>African Climate and Development Initiative, University of Cape Town, Rondebosch, South Africa

## Summary

1. Global amphibian declines have resulted in a vital need for monitoring programmes that follow population trends. Monitoring using advertisement calls is ideal as choruses are undisturbed during data collection. However, methods currently employed by managers frequently rely on trained observers and/or do not provide density data on which to base trends.

2. This study explores the utility of monitoring using acoustic spatially explicit capture–recapture (aSCR) with time of arrival (ToA) and signal strength (SS) as a quantitative monitoring technique to measure call density of a threatened but visually cryptic anuran, the Cape peninsula moss frog *Arthroleptella lightfooti*.

3. The relationships between temporal and climatic variables (date, rainfall, temperature) and *A. lightfooti* call density at three study sites on the Cape peninsula, South Africa, were examined. Acoustic data, collected from an array of six microphones over 4 months during the winter breeding season, provided a time series of call density estimates.

4. Model selection indicated that call density was primarily associated with seasonality fitted as a quadratic function. Call density peaked mid-breeding season. At the main study site, the lowest recorded mean call density (0.160 calls m<sup>-2</sup> min<sup>-1</sup>) occurred in May and reached its peak mid-July (1.259 calls m<sup>-2</sup> min<sup>-1</sup>). The sites differed in call density, but also the effective sampling area.

5. *Synthesis and applications.* The monitoring technique, acoustic spatially explicit capture–recapture (aSCR), quantitatively estimates call density of calling animals without disturbing them or their environment. In addition, time of arrival (ToA) and signal strength (SS) data significantly add to the accuracy of call localization, which in turn increases precision of call density estimates without the need for specialist field staff. This technique appears ideally suited to aid the monitoring of visually cryptic, acoustically active species.

**Key-words:** acoustic array, acoustic spatially explicit capture–recapture, anurans, call density, non-invasive sampling, population monitoring, sensor networks, signal strength, time of arrival, triangulation

## Introduction

Monitoring the sizes and trends of wild populations is important for understanding a species' ecology and to guide conservation actions (Hellawell 1991; Fasham & Mustoe 2005; Tucker *et al.* 2005). Effective monitoring techniques should provide reliable and quantitative

estimates of abundance so that trends can be quantified (Legg & Nagy 2006). Assessment of population size can be used to detect species' responses to established or incipient environmental change (Gibbons *et al.* 2000); determine the conservation status of a particular species; identify conservation needs of species, communities or habitats; assess the ecological state of ecosystems; and evaluate the effectiveness of existing conservation measures (Hellawell 1991; Downes *et al.* 2002). Lack of monitoring success has been attributed to (amongst others)

\*Correspondence author. E-mail: [john@measey.com](mailto:john@measey.com)

insufficient statistical power, loss of key personnel and loss of integrity of the long-term data record (Legg & Nagy 2006; Lindenmayer & Likens 2010). Rectification of these failures has already been substantially aided by the digitization of traditional data collection, including images and sound recordings, but new analytical tools are required to realize the full potential of this digital data revolution.

Amphibian populations have been declining world-wide since the 1970s with increased documentation in the 1980s and 1990s (Stuart *et al.* 2004). The Global Amphibian Assessment revealed that amphibians are more threatened and are declining more rapidly than any other vertebrate class. Of amphibian species, 32.5% are threatened globally and at least 48.2% have populations that are in decline (Stuart *et al.* 2004). In the decade following these findings, research efforts have focussed on determining the proximate causes of declines, as well as initiating monitoring schemes as early warning systems. Traditional methods of monitoring amphibian populations by estimating population size can be laborious and usually involve capturing, marking and recapturing individual animals. Although there are different methods for targeting different species and different life-history strategies (e.g. Fasham & Mustoe 2005), most of these methods are labour intensive and many involve direct handling, which is stressful to the animals.

Auditory monitoring techniques, such as estimating the number of calling males, are non-invasive and can be used to estimate population size. These techniques include manual calling surveys (MCS) involving human observers and automated recording systems (ARS). MCS are subject to imperfect detection, misidentifications and substantial observer bias. ARS allow collection of call data without the observer present, allow data to be collected by staff unskilled in identifying species-specific calls and provide a permanent record of the sampling occasion that can be reinterpreted later. Managers are increasingly instigating MCS and ARS methods to yield site occupancy data, inventories of anuran species and qualitative count data indexing abundance. Sampling area however is not clearly defined in these call survey methods, and so, it is not known what area the estimation of the target population covers (Stevens, Diamond & Gabor 2002; De Solla *et al.* 2006; Dorcas *et al.* 2009).

Spatially explicit capture–recapture (SCR; Efford 2004; Borchers & Efford 2008) combines capture–recapture and distance sampling methods (Buckland *et al.* 2001) and was originally developed for studies in which the target animals are physically captured. However, SCR can be used when the same individual is perceived by more than one detector on a single occasion, thus avoiding the need for them to be physically captured (Borchers 2012). This is why SCR can also be used with arrays of fixed microphones resembling trapping grids to estimate population density of vocalizing individuals, if individuals are identifiable from calls, and to estimate density of calls per unit

time if individual animals cannot be identified from their calls, but individual calls can be distinguished from one another (Dawson & Efford 2009; Efford, Dawson & Borchers 2009; Stevenson *et al.* 2015; Kidney *et al.* 2016). Each microphone represents a detector of known location, where detections of an individual call on one or more microphones constitute the ‘captures’; these records are used to estimate a distance-based detection probability surface. Acoustic data offer the advantage over physical capture in that they contain additional information about the detection process, namely signal strength (relative amplitude) and, in the case of calls that were recorded at more than one microphone, relative time of arrival. Novel statistical techniques allow all information to be combined to give greater accuracy on the location of the sound source and allow the parameters of the detection function, and therefore call density, to be estimated more precisely (Stevenson *et al.* 2015). Given the estimated detection function and the observed detections and non-detections of individual calls at the different microphones, one can estimate call density and the unobserved locations of the calling individuals (Borchers 2012).

Acoustic SCR (aSCR) is an appealing technique for monitoring vocalizing species because large volumes of acoustic data can be collected in a short amount of time over a known area, ensuring both the integrity of the data record and statistical power without the need for key personnel. It should be noted that no marking or recognition of individuals is required for aSCR; instead, each microphone acts as a proximity detector (Efford, Dawson & Borchers 2009; Borchers 2012; Stevenson *et al.* 2015). Moreover, at present, it is the only method that is capable of generating both point and interval estimates of either call density or calling male density in a statistically rigorous manner. The aim of this study was twofold, first to assess the practical use of aSCR with time of arrival (ToA) and signal strength (SS or call amplitude) data collected by the sequential deployment of a microphone array in the field. Secondly, we use these data to assess changes in calling density across a complete calling season in the Cape peninsula moss frog *Athroleptella lightfooti*, obtaining quantitative estimates of call density using non-invasive audio recordings. Our goal was to determine the conditions under which this monitoring technique should be carried out to capture the majority of a population of calling males. We demonstrate both the practicality of this approach and the period of peak calling density for *A. lightfooti* males.

## Materials and methods

### STUDY SPECIES

*Athroleptella* is a genus of frogs from the family Pyxicephalidae endemic to south-western South Africa and currently comprises seven described species. They are found in populations associated with mossy seepages in mountainous fynbos areas (Channing

2004). The different species are morphologically similar, but can be reliably distinguished by their advertisement calls (Turner *et al.* 2004; Turner & Channing 2008). Their limited distributions in montane fynbos habitat make them susceptible to invasions from alien plants (mostly pines and Australian acacias) that are prominent in this area (Wilson *et al.* 2014) and have resulted in high threat levels for many of the species (Measey 2011). Fynbos is a fire-dependent ecosystem, and woody invasive species increase fire temperatures and shorten fire return intervals (Kraaij & van Wilgen 2014) threatening moss frogs and other endemic species. The region in which these species occur is expected to undergo climatic change, especially linked to rainfall patterns and temperature (Altwegg *et al.* 2014).

The *Arthroleptella lightfooti* adults are cryptically coloured and small; females can attain a snout-vent length of up to 22 mm, while males are smaller (Channing 2004). The advertisement call of *A. lightfooti* is a short chirp consisting of three short pulses. The call has an emphasized frequency of 3 754 Hz (Turner & Channing 2008). This species is not sympatric with any other *Arthroleptella* species (Channing 2004).

These moss frogs aestivate during the dry season (austral summer) and become active, breed and develop choruses from April to December during the rainy season (Channing 2004). The adult males call during the day to attract females to egg deposition sites. The females lay clutches of 5–12 eggs in mossy areas, under thick vegetation or at the bases of grass tufts (Channing 2004). Even though little is known about the calling ecology of these species, we expected that the number of calling males may vary with changes in temperature (Navas 1996; Oseen & Wassersug 2002; Murphy 2003; Hauselberger & Alford 2005; Weir *et al.* 2005; Kirlin *et al.* 2006; Saenz *et al.* 2006), and/or rainfall (Oseen & Wassersug 2002; Murphy 2003; Hauselberger & Alford 2005; Weir *et al.* 2005; Kirlin *et al.* 2006; Saenz *et al.* 2006). Moss frogs stop calling if disturbed, but generally start calling again after about five minutes once the disturbance has ceased. This species is a Cape peninsula endemic within Table Mountain National Park and has a IUCN Near Threatened status as it has a restricted distribution, but it is not known whether populations are in decline. It has been identified for monitoring as it occurs throughout the Cape peninsula indicating the presence of vulnerable seepage habitats, which also host a variety of threatened plant species (Measey 2011). In addition, other species in the genus are more highly threatened with alien invasive trees as well as in increased fire intensity and return rate (Measey 2011).

#### SITE DESCRIPTION

Three sites (referred to as 'Site 1', 'Site 2' and 'Site 3' below) situated on Steenberg Plateau in Silvermine Nature Reserve, Table Mountain National Park on the Cape peninsula were sampled in 2012 from May to September to coincide with the breeding season of *A. lightfooti*. The sites were approximately 300 m from each other and chosen based on known presence of *A. lightfooti* and reasonable access. Due to time constraints, at most two of the sites could be sampled on any given day. Site 1 (34°06'03.5" S; 18°26'55.2" E) was sampled on fortnightly visits, and Site 2 (34° 05'51.0" S; 18°26'56.8" E) and Site 3 (34°05'57.7" S; 18°27'03.8" E) were sampled on alternate visits. The 17 visits were made between 10.00 and 14.00 h (the frogs call steadily between sunrise and sunset), avoiding rain and high winds due to use of unprotected electronic equipment. The vegetation type growing on Steenberg Plateau is Cape peninsula

sandstone fynbos. The vegetation consists of tall (1–2 m) proteoid shrubland over dense, shorter (<0.5 m) ericoid shrubland (Rebello *et al.* 2006).

#### ENVIRONMENTAL DATA COLLECTION

Factors that potentially influence anuran calling explored in this study were time of the season (date), precipitation during the days prior and the day of the recording, and ground and air temperature at the time of the recording. These small ectotherms are more likely to be affected immediately by temperature, whereas prolonged rain is important for reproduction. One temperature logger (iButton in silicon holder), measuring ground (2 cm below-ground) and air temperature (10 cm above-ground) every hour, was placed at Site 1 throughout the sampling period. It was considered that the temperature the frogs experience was most likely to be closest to this position as the frogs occupy concealed moist locations at the base of and under vegetation. Rainfall (millimetres of precipitation per day) was measured using a rain gauge (situated approximately 3 km west-north-west of the study sites) at 08.00 h each day and recorded by park rangers.

#### MICROPHONE DEPLOYMENT AND SOUND RECORDINGS

We used a DR-680 6-Track Portable Field Audio Recorder (Tascam; TEAC, Wiesbaden, Germany) with six Audio-Technica AT8004 Handheld Omni-directional Dynamic Microphones (Audio-Technica, Leeds, UK). At each site, six labelled microphones on 1-m wooden dowels with a microphone holder attached to one end with duct tape were placed in an array approximately 4 m from the recorder and 2 to 5 m from each other in a rough circle, but without regular spacing: close enough so that some calls are heard on more than one microphone, but not so close that all calls are heard on all microphones. The positions of the dowels were kept constant by inserting them into plastic tubes that were left in the ground between visits. The straight-line distance from each microphone to every other microphone in the array was measured to the nearest centimetre using a measuring tape. Vocalizing moss frogs were recorded for 40 min on each visit. The area around the site (200 m) was vacated for the duration of the recording. The six microphones recorded on independent tracks with a resolution of 24-bit and a recording frequency of 48 kHz. Inclement weather was deliberately avoided for recordings as this equipment is not weather-proof.

#### SOUND PROCESSING INTO A NUMERICAL DATA BASE

The stereo recordings were pre-processed, before they were statistically analysed, to identify individual calls of *A. lightfooti*. Call recognition routines were constructed for *A. lightfooti*, and then, the recordings were processed using these call recognition routines. Call recognition and pre-processing was done using PAMGUARD (version 1.11.00 BETA; Miller *et al.* 2014; www.pamguard.org), which also allowed us to check recognitions. *Arthroleptella lightfooti* calls consist of three pulses that make up a single note and are identified as a single frog call using a click detector in PAMGUARD (see Stevenson *et al.* 2015 for more details). The data captured from PAMGUARD comprise the start time of each call (in seconds), the signal strength and the microphone on which the call was heard. The data were captured to an

accuracy of  $2.083 \times 10^{-5}$  s. The first 10 min of each recording was omitted from the pre-processed data, prior to statistical analysis, to remove any disturbance of the site while setting up the microphone array, leaving 30 min of acoustic data per visit for the analysis. Ten minutes appeared sufficient time to allow normal frog calling activity to resume. All files of call data were deposited with the South African Environmental Observatory Network ([www.saeon.ac.za](http://www.saeon.ac.za)) and are available on request.

#### SPATIALLY EXPLICIT CAPTURE–RECAPTURE (SCR)

The methodology of Stevenson *et al.* (2015) was used to estimate call density (in frog calls per hectare per minute) and is briefly described below. These methods are an extension of those set out by Efford, Dawson & Borchers (2009), and allow for the incorporation of both ToA and signal strength information into SCR analyses.

#### ACOUSTIC SCR

Frog calls detected across the microphone array can be seen as capture–recapture data: calls can potentially be detected at each microphone, analogous to how individuals can potentially be detected on each occasion during a traditional live-trapping capture–recapture survey. The data required to estimate density using aSCR are the capture histories, a record of which microphones detected each identified frog call. The patterns of detections and non-detections at the different microphones allow the unobserved locations of the frog calls to be estimated with an associated measurement error.

A frog call's probability of detection at a particular microphone is a decreasing function of the horizontal distance between the locations of the source of the call and the microphone; that is, the further a microphone is located from the source of a call, the less likely it is to detect the call. SCR methods use the locations of the microphones relative to the estimated sources of the detected calls to estimate the parameters of a detection function, describing how detectability declines with increasing distance (see Borchers 2012 for further details).

Conditional on their locations, frog calls are assumed to be detected independently across the microphones, and there is uniformity in the sensitivity across the microphones. For any given point in the survey area, the estimated detection function allows calculation of the probability that a call emitted from this location is detected by the array (i.e. that it is heard by one or more microphones). The proportion of calls detected and the effective survey area (ESA),  $a$ , can then be calculated. The latter is the area in which it is estimated that  $n$  calls (detected or otherwise) were made over the course of the survey, where  $n$  is the total number of detected calls. For example, if the survey area is 1 ha, and it is estimated that a quarter of all calls are detected, then  $\hat{a} = 0.25$  ha. Dividing the total number of detected calls by both the estimated ESA and the survey length,  $t$ , gives rise to the call density estimate,  $D$ ; that is,  $\hat{D} = n/(\hat{a} * t)$ .

Frogs are assumed to be located uniformly throughout the survey area, and so, the density of frog calls is also uniform. The initial acoustic SCR methodology of Efford, Dawson & Borchers (2009) assumed that call source locations were independent of one another; however, this rarely holds in practice: individuals may emit many calls over the course of the survey, and the locations of calls made by the same animal are likely to have the

same (or similar) source locations. Stevenson *et al.* (2015) showed that bias in point density estimates is negligible despite the violation of this assumption, though variance is typically underestimated. Correcting variance estimates is possible via a simulation approach if call rate data are available (see Stevenson *et al.* 2015). Although the effect of violation of the assumption of spatial uniformity with SCR estimators has not been thoroughly investigated, there are a number of studies that suggest that while violation of assumptions can result in bias in some parameters of the SCR model, and to biased inferences about distribution in space, SCR estimates of density itself appear to be remarkably robust to violation of the assumption (Efford, Borchers & Byrom 2009; Distiller & Borchers 2015).

#### INCORPORATION OF SIGNAL STRENGTH AND TIME OF ARRIVAL DATA

Increased precision in location estimates results in a more precise detection function estimate and this in turn propagates through to an estimation of call density. Efford, Dawson & Borchers (2009) recognized that signal strength data (or the relative amplitude of the call) can be informative about the location of a call's source: a call closer to microphones is likely to have higher received signal strength than a call further away. Borchers *et al.* (2015) made a full generalization, providing a framework under which supplementary spatial data informative about animal locations can be incorporated into SCR approaches. One such example is the use of ToA in aSCR. A call recorded on multiple microphones reaches the closest microphone slightly earlier than the others. The difference in ToA between microphones gives additional information about the location of a call above and beyond what is provided by signal strengths and the locations of the microphones that detected it. Incorporation of both SS and ToA information into aSCR can substantially increase the precision of the call density estimate (Borchers *et al.* 2015; Stevenson *et al.* 2015).

#### MODEL FITTING

Call density and the detection function estimates were obtained from the PAMGUARD output data using the ascr package (Stevenson 2016) in R (version 3.1.3; R Core Team, 2015) using a maximum-likelihood approach. The likelihood function is a version of what is now a standard likelihood function in capture–recapture studies, first developed by Borchers & Efford (2008). The distinguishing feature of such likelihoods is that they accommodate capture histories that consist of the *locations* (microphones) at which detections occurred rather than *occasions* on which captures occurred. In this context, each vocalization made by a frog generates a capture history (some of which are unobserved) and there is only one capture occasion. Because the locations of the animals themselves are not observed, they are treated as latent variables in SCR analyses. Acoustic SCR methods are further distinguished by the fact that acoustic capture histories include additional information on locations in the form of ToA data and received signal strength. The aSCR likelihood therefore includes statistical models for received signal strength and for ToA as functions of animal location, and as a result, distance and angle to animals is implicitly estimated (together with associated uncertainty) simultaneously with density. See Stevenson *et al.* (2015) for further details about this likelihood, along with example code.



Model fitting was computationally intensive, so to obtain a good representation of the call density during the 30min sampling period, 10 subsamples of 1 min were taken at three-minute intervals. A single estimate of call density from each recording was then obtained by averaging over those obtained from the subsamples. The unit of replication in the following analysis is therefore the recording, that is a single visit to a particular site.

#### CORRELATES OF CALLING DENSITY

We then examined variation in call density estimates using linear models. Model diagnostics indicated that the most parsimonious models involved a log transformation of the estimated call densities. Although this did help in stabilizing error variance, residuals nevertheless showed heteroscedasticity. These linear models were therefore fitted using generalized least squares, implemented in the R function `gls` from the `nlme` package. The response variable was estimated frog call density, and the covariates were site, date, rainfall and temperature (see Environmental Data Collection section, above). 'Site' entered as a factor in each model because frog call density was likely to vary between the three sites. In addition, we investigated models that included a site/time interaction effect, but these were found to increase the AIC score indicating more support for models with the same quadratic effect across sites. We therefore did not include interaction models in the following model-selection process.

Models were fitted that encompassed all possible subsets of the covariates. For each model, the value of the maximized log-likelihood, the number of parameters, Akaike second-order Information Criterion (AIC<sub>c</sub>) and their differences, and Akaike weights were calculated (Table 1). Akaike weights are a measure of the weight of the evidence that the particular model is the best model in the set (Anderson, Burnham & Thompson 2000). The aSCR models, fitted to each one-minute subsample, estimate a parameter that measures the range of detectability of frog calls. It was of

**Table 1.** Model-selection Table: The 10 most preferred models (by AIC<sub>c</sub>). All include effects due to the site, and both linear and quadratic time effects. The model with the most support does not include any other variables. The 'delta' column provides the AIC<sub>c</sub> difference from this model

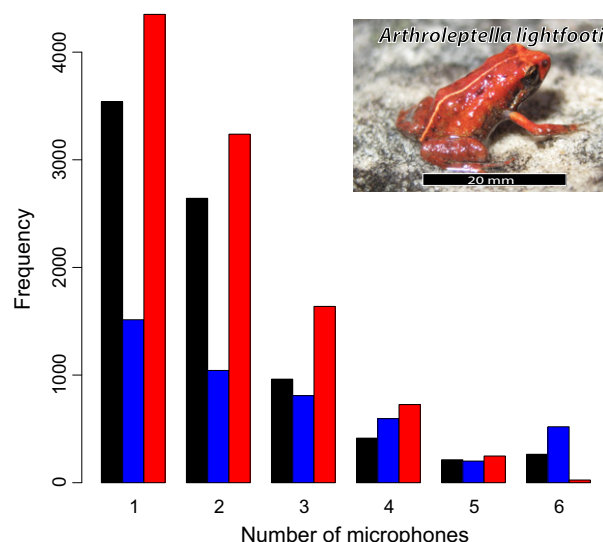
Additional variables	Model degrees of freedom	logLik	AIC <sub>c</sub>	delta AIC <sub>c</sub>	Weight
None	7	-6.721	32.3	0.00	0.295
Air temp	8	-5.451	33.4	1.14	0.167
Ground temp	8	-6.117	34.8	2.47	0.086
Total rain	8	-6.428	35.4	3.09	0.063
Rain 0 days prior	8	-6.603	35.8	3.44	0.053
Rain 1 day prior	8	-6.662	35.9	3.56	0.050
Air temp, rain 0 days prior	9	-5.023	36.6	4.31	0.034
Air temp, rain 1 day prior	9	-5.177	36.9	4.61	0.029
Air temp, ground temp	9	-5.262	37.1	4.78	0.027
Air temp, total rain	9	-5.270	37.1	4.80	0.027

interest to determine whether frog call detectability varied across surveys and/or sites. A mixed-effects model was fitted with these parameter estimates as the response variable, survey as a random effect and site as a fixed effect. Restricted maximum-likelihood and Wald chi-square tests, respectively, were used to assess significance of these effects.

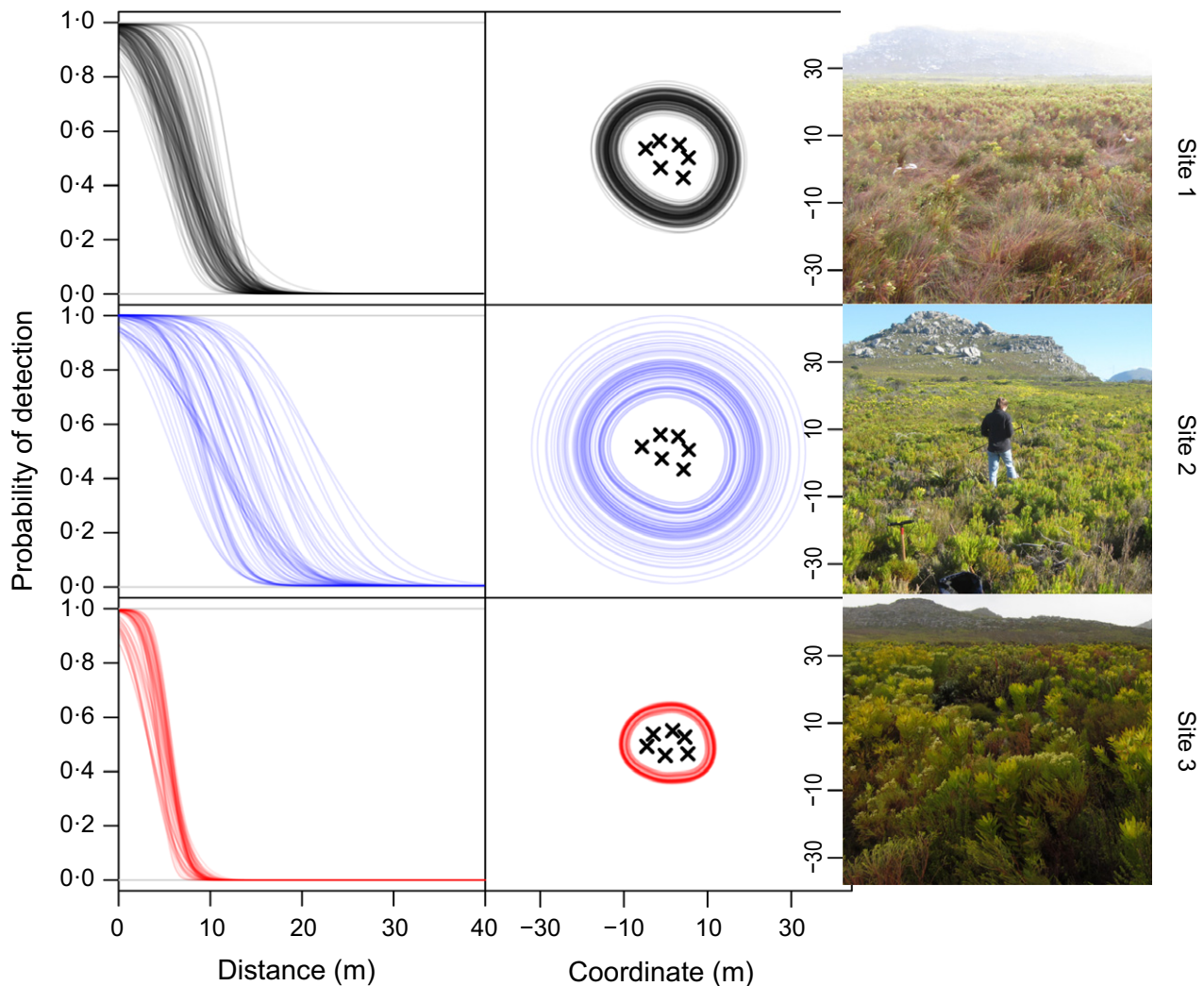
#### Results

The equipment was swift to deploy at the field site with a set-up time of approximately 10 min prior to recording at each site. Inserting dowels into tubes left at the site between recordings made the microphone locations constant and simple to replicate on each visit. The number of calls detected by PAMGUARD during the entire 30 min of recording varied between 5 842 and 30 036 (mean  $17\,854 \pm 1014.3$ ). Most calls were detected on a single microphone. Calls that were heard on more than a single microphone show a steep reduction in frequency with only a small proportion heard on all six microphones (Fig. 1).

There was strong evidence to suggest that detectability varied across both surveys (restricted maximum-likelihood test statistic = 227.93,  $P < 0.0001$ ) and sites (chi-square test statistic = 35.97,  $P < 0.0001$ ). Frog calls were most difficult to detect at Site 1, while those at Site 2, on average, were detectable over the greatest distances. Site 2 also had the greatest variation in the estimated detection function (Fig. 2). While the three sites did differ in vegetation (Site 3 had markedly higher vegetation), it was not predicted that this would affect the detection function to such an extent. Similarly, as windy and rainy days were



**Fig. 1.** The number of calls (frequency) detected on microphones (Silvermine on 11 July 2012; Site 1 black; Site 2 blue; and Site 3 red) impacts on the type of analysis that can be made. Two or more calls are required to get data from ToA and SS data types. Conversely, aSCR generates data from calls irrespective of how many microphones they are heard on. Inset, a male *Arthroleptella lightfooti* is (on average) 20 mm in snout-vent length.



**Fig. 2.** Call detection function (first column) and a measure of the effective sampling area (ESA: second column), constituting the range of detectability of frog calls of three sites (third column) recorded for calls of *Arthroleptella lightfooti* in Silvermine, Table Mountain National Park in 2012. Crosses (second column) represent the relative positions of the microphones at each site (with microphone 1 at 0,0), and lines indicate one-minute samples in the survey area at which the probability of detection by at least one microphone is estimated to be 0.05 (and so any calls emitted beyond this are unlikely to be detected). There was a large amount of variation in the ESA by the acoustic spatially explicit capture–recapture method at each site, which may correspond to the height of the vegetation.

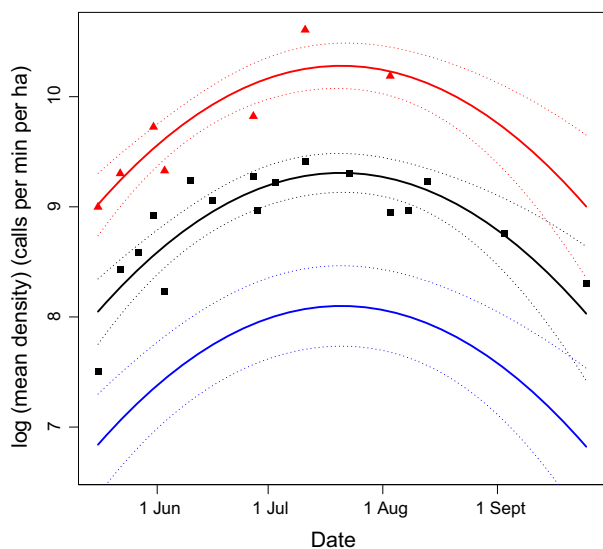
not suitable for recording using our electronic equipment, we had assumed that call detection would be very similar over time. However, our finding that both these assumptions were incorrect did not affect our ability to estimate calling density, unlike previous methods.

Call density was significantly different at each of the sites and was found to change significantly throughout the season (Fig. 3; Table 1). Site 3 consistently had the least calls per minute per hectare, while Site 2 had the highest densities. A quadratic model was the best fit for call density at all three sites (Fig. 3) resulting in a clear peak in calling in mid-July. The model with date and site fitted our data better than those that included any other covariates (temperature, rainfall). Our data do show clear seasonal variation in the call density of *A. lightfooti* with the same peak in activity at all three sites. This indicates

that for monitoring purposes, recordings made during July should be indicative of maximum call densities.

## Discussion

In this study, we show a practical application of aSCR to determine seasonality in the calling ecology of the Cape peninsula moss frog, *A. lightfooti*. Moreover, we demonstrate the importance of including a call detection function when monitoring frogs. Our acoustic data from (for example) Site 1 monitored an effective sampling area (ESA) estimated by aSCR of between 431.46 and 783.51 m<sup>2</sup>. Without using aSCR to generate this estimate, conventional ARS would likely have resulted in a dramatic change in calls. We do not know why our call detection functions varied so much, although the



**Fig. 3.** Density of *Arthroleptella lightfooti* calls recorded at Silvermine, Table Mountain National Park in 2012. The symbols are means of 10 1-min recordings. The lines show the best linear model (see Table 1) for each site (Site 1: black squares; Site 2: blue crosses; and Site 3 red triangles), explaining log (call density) as a quadratic function of date with site as a factor variable.

vegetation height appeared to make a consistent contribution and further variance may have been due to subtle differences in ambient conditions during recording. While the application of acoustic arrays to monitor acoustically active animals is not new (e.g. Blumstein *et al.* 2011; Mennill *et al.* 2012), we demonstrate here that without the statistical application of aSCR, these methods cannot reasonably estimate their audible footprint, in effect rendering them (and single or dual microphone systems) unrepeatable for monitoring purposes. Our methodology is particularly appealing for assessing call density of cryptic species, like the Cape peninsula moss frog, but it may be found that it is of use for monitoring a much greater range of vocalizing species.

The calling behaviour of male moss frogs (*A. lightfooti*) at the three Silvermine study sites showed strong seasonality in calling ecology. Calling increased early in the breeding season, peaked mid-season and then declined towards the end of the breeding season. This result is consistent with other studies on anuran calling ecology (e.g. Hauselberger & Alford 2005; Weir *et al.* 2005). In our study, the quadratic season effect was found to explain a substantial portion of the variation in call density in *A. lightfooti*. Qualitative estimates of call density for frog populations have been found to correlate well with capture–recapture estimates (Grafe & Meuche 2005), and as a result, call density is often used as a proxy for frog density (e.g. Corn, Muths & Iko 2000). A monitoring study should attempt to capture peak activity of the vocalizing species, to ensure that the maximum number of calling males in the population is enumerated. This may require a prior assessment of the entire breeding season to determine the

most appropriate monitoring period (as with our example), or this could be negated if the monitoring period is constrained by brief calling activity. Once the peak in call density is known, call rates during this period can be used to determine the population size of calling males. For our example, we used a sample of call rates from eight frogs (mean of 16.25 calls per individual per minute, standard deviation of 0.886) to estimate the density of calling males (see Stevenson *et al.* 2015) to be 712.32 per hectare at Site 1 on 11 July 2012 (95% CI: (487.26, 937.38), corresponding to one frog every 14.04 m<sup>2</sup>), 348.44 per hectare at Site 2 on 23 July 2012 (95% CI: (220.67, 476.20), corresponding to one frog every 28.70 m<sup>2</sup>) and 2 485.22 per hectare at Site 3 on 11 July 2012 (95% CI: (1 890.17, 3 080.27), corresponding to one frog every 4.02 m<sup>2</sup>).

A large amount of variability was found in the estimated ESA between sites and between recording occasions. The fact that our technique allows calculation of the ESA enables us to continue to estimate call density, even when conditions are not constant. Because SCR accounts for variation in the detection function and thus the ESA (Borchers 2012), the call density estimates are not affected by the variation in the detection process. The variation in ESA however does affect the number of calls or individuals actually recorded and therefore would pose a problem for methods that do not account for the detection process (conventional ARS). Most conventional monitoring methods that rely on calling depend to some extent on the number of individuals recorded but do not control for variation in the detection range. Due to the large variability in ESA recorded in this study, it is evident that methods that do not account for this variation may lead to biased estimates of trend. The area sampled could possibly have been affected by wind and/or vegetation structure. Wind reduces the detection probability of frog calls (Weir *et al.* 2005; De Solla *et al.* 2006), and subsequently, fewer frog calls, probably covering a smaller area, are detected. The implication being that conventional ARS methods do not produce robust indices of abundance, and should therefore be avoided (see Hayward *et al.* 2015) when ecologists need to compare estimates of call abundance from one recording session to the next. In contrast, aSCR explicitly accounts for detection probability and shows that it is important to do so to produce a robust estimate of call density. More work is needed to investigate the variability in ESA reported and the factors that contribute to its variability.

The call densities obtained of *A. lightfooti* using aSCR were quantitative and free from observer bias. Subsequently, the results should be robust, and application of the method by different investigators should yield the same results. Moreover, our method could reliably estimate call densities up to 1.259 calls m<sup>-2</sup> min<sup>-1</sup>. Conventional MCS cannot cope with such high call densities, and it is unlikely that ARS can accurately interpret such high call densities. This suggests that our methodology holds potential to be used in intense chorus situations, although



this has yet to be tested. Several issues remain that may limit the use of our method. First, monitoring the number of individuals relies on having a set call rate that can be reasonably used for the population. The method is applicable to anurans that practise call alternation (over call masking), and avoidance of calling in close proximity (Schwartz & Gerhardt 1989; Grafe 1996). Lastly, the method would be compromised by males that call from different localities throughout the survey period (but see Stevenson *et al.* 2015). Despite these and other caveats (see Stevenson *et al.* 2015), we feel that our method holds great potential for monitoring of many calling taxa and that some of the caveats could be overcome through additional research on the species to be monitored and the development of further statistical methodology.

It seems reasonable to assume that our method can be transferred to other visually cryptic, vocalizing species to monitor their populations and investigate their calling ecology. Acoustic SCR is a reasonably easy monitoring technique for conservation authorities to implement as personnel with relatively little training in the method are able to go out into the field and record the sound data needed for later quantitative analyses by researchers. The method is non-invasive and is therefore well suited to monitoring threatened species, or species in sensitive habitats. It can also be implemented in a range of habitat types that broadens its usage in terms of species numbers monitored using the technique. Despite the additional cost associated with microphones and recording equipment, the technique requires no additional travel or field access (the most significant cost in most monitoring protocols) and provides substantial benefits in terms of repeatability. However, our method also poses greater problems in terms of data storage, which will need to be addressed prior to starting to use the technique, preferably archived with an institutional repository (as here). For example, the acoustic data generated in this study amounted to 0.5 Tb.

## CONCLUSION

Many managers are now required to monitor species of special concern, but choosing monitoring methods is particularly problematic as many provide qualitative estimates that rely on trained staff. Our repeated acoustic surveys within a calling season demonstrate the practical application of aSCR for monitoring purposes. Each call density estimate can be meaningfully compared to prior and subsequent recordings, without the need for specialist field staff. Our method therefore meets several demands that are required of good monitoring: minimal physical impact to the site, adequate field markings, adequate spatial replication and the potential to integrate with other monitoring programmes (Legg & Nagy 2006). Our recording apparatus and subsequent processing treatment is capable of generating estimates of call density and can be transferred to other visually cryptic, vocalizing species, providing that species can be identified from their calls.

Realizing the full potential of aSCR methods requires further work on automated species identification, and we anticipate that this will be an area of substantial research activity in future. Indeed, with adequate archiving of sufficient recordings, our existing data could be re-analysed for any taxonomic group captured and a density estimate made. In addition, our approach lends itself particularly to automated recording projects in remote areas as no more is required in the field than existing ARS protocols.

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## Data accessibility

Original acoustic audio files: South African Environmental Observation Network (SAEON) Data Repository <http://dx.doi.org/10.15493/SAEON.METACAT.10000005> (Measey *et al.* 2016).

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