Acoustic Spatial Capture-Recapture (aSCR) and the Cryptic Cape Peninsula Moss Frog *Arthroleptella lightfooti*

Marike Louw

Thesis presented in partial fulfilment of the requirements for the degree

Master of Science in Zoology

at Stellenbosch University

Supervisor: Dr. John Measey

Faculty of Science

Department of Botany and Zoology

University of Stellenbosch

December 2018

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2018

Marike Louw

ABSTRACT

Quantitative measurements of wildlife populations, such as population density, are quintessential for management and conservation. Acoustic Spatial Capture-recapture (aSCR) is a technique that is used to estimate the densities of acoustically active animals. It is advantageous to use when animals defy the use of traditional methods of population estimation by being very visually cryptic, but remaining acoustically active. *Arthroleptella lightfooti* is a visually cryptic moss frog with an average snout to vent length of 14.5 mm. The males call during the austral winter from seepages within a restricted range across the Cape Peninsula. The species has an IUCN status of Near Threatened.

Here, the population densities of the endemic *A. lightfooti* are estimated across their range on the Cape Peninsula for the first time using aSCR. Multiple microphones, termed "acoustic arrays", are deployed in the field to record the calls of the frogs. I assess the use of aSCR in terms of reliability of the density estimates by examining the standard errors as coefficients of variation (CVs) of the density estimates. A density estimate with a CV above 30% was considered unreliable. Recording calls for the aSCR analyses involved visiting more than 200 sites during 2016 and 2017, and deploying acoustic arrays at a total of 149 sites, of which a subset of 85 sampling sites was used. I examined the influence of different variables on the size of the CV, namely: the average number of calls received by the acoustic array per minute, the array formation, and the detector frequencies (the combination of different numbers of microphones across which calls were heard). In addition, I made use of an output from aSCR that is an aerial view of the estimated calling locations of frogs relative to the acoustic arrays. I overlaid this output with aerial images taken at three different sites using a drone, and I examined the microhabitat features that relate most significantly to the presence of calling *A. lightfooti.*

When there were less than 111call.min^{-1} received by the array, density estimates had CVs that exceeded 30% and were therefore considered unreliable. Above this threshold, 91% of density estimates were acceptable. When calls were heard on mostly one microphone, and decreasingly heard across two, three, four, five and six microphones, the density estimates were more reliable. However, when calls were mostly picked up across a combination of one and six microphones, density estimates became less reliable. This suggests that array formations should have the microphones spaced in such a way that not all calls are detected across all the microphones or only one microphone. The presence of calling frogs was significantly related to the presence of wet, seepy patches in the microhabitat and to the absence of standing water. This is consistent with observations in the field and reflects the biological needs of the species: it has no life stages in water but needs moist areas for eggs and tadpoles to develop. The successful application of aSCR to *A. lightfooti* is promising in the field of population studies on cryptic species, as it can be used to evaluate the populations of other calling taxa, which holds important implications for conservation and management.

OPSOMMING

Kwalitatiewe metings van diere-bevolkings soos byvoorbeeld bevolkingsdigtheid, is belangrik vir die bestuur en bewaring van natuurlike fauna. Akoestiese Ruimtelike Opname-Heropname (ARO) is 'n tegniek wat gebruik word om die digtheid van dié bevolkings wat akoesties aktief is, en dus maklik hoorbaar is, te skat. Dit is veral voordelig om te gebruik wanneer die diere moeilik is om op te spoor en raak te sien en tradisionele metodes van bevolkingsberaming nie gebruik kan word nie. *Arthroleptella lightfooti* is n paddatjie met 'n gemiddelde lengte van nét 14,5mm. Die mannetjies roep tydens die wintermaande vanuit vogtige gebied binne beperkte areas van die Kaapse Skiereiland. Die spesie is dus endemies aan die Kaapse Skiereiland en het 'n IUCN status van 'n Amper-Bedreigde-Spesie.

Hier word die bevolkingsdigtheid van die endemiese *A. lightfooti* vir die eerste keer oor hul volle Kaapse Skiereiland habitat bepaal. 'n Groep mikrofone, bekend as 'n "akoestiese ARO-stel", is in die veld opgestel om die roepgeluide van die paddas in die bepaalde area aan te teken. Om die betroubaarheid van die digtheidskattings van die ARO te evalueer, ondersoek ek die standaardfout as koëffisient van variasie (KV's) van die digtheidskatting. 'n Digtheidsberaming met 'n KV van bo 30% , word as onbetroubaar beskou. Daar is meer as 200 geskikte areas tydens 2016 en 2017 besoek, en hiervan is 149 areas met die Akoestiese ARO-stelsel gedoen. 85 van hierdie opnames is gebruik vir hierdie tesis. Ek het die aspekte wat moontlik die KV van die digtheidsberaming kan beivloed, naamlik die gemiddelde aantal roepe per minuut ontvang deur die ARO-stelsel, die formasie waarin die mikrofone opgestel is in die veld, en die detektorfrekwensie (verskillende aantal mikrofone waarop die paddaroepe gehoor is), ondersoek en evalueer. Ek maak ook gebruik van die ARO-stelsel se funksie, wat 'n uitsig vanuit die lug van die roepgebied is. Hieroor oorvleuel ek n 'n lugfoto van die area, geneem vanuit n hommeltuig. Drie areas is so ondersoek om die eienskappe van die mikro-omgewing wat die paddas se roepgebiede die meeste beindvloed, te bepaal.

Wanneer daar minder as 111 roepe.min^{-1} deur die akoestiese ARO stelsel ontvang was, het digtheidskattings KVs gehad wat groter as 30% was, en is dus as onbetroubaar beskou. 91% Data bo hierdie drempel van digtheidskatting was wel aanvaarbaar. Wanneer roepgeluide meestal op een mikrofoon gehoor is, en dit geleidelik minder op die twee, drie, vier, vyf en ses mikrofone opgetel was, is die digtheidskatting betroubaar. Die digtheidskatting het egter minder betroubaar geword wanneer die roepgeluide op een sowel as ses mikrofone gekombineerd opgetel is, en die skatting was dus minder betroubaar. Dit dui daarop dat die formasie waarop die die mikrofone opgestel word belangrik is, sodat al die roepgeluide nie slegs of één mikrofoon, of gelyktydig op al die mikrofone opgeneem word nie. Die teenwoordigheid van manlike paddas wat roep, korreleer aansienlik met mikro leefgebiede wat klam en baie vogtig is, maar nie waar staande water voorkom nie. Dit is in ooreenstemming met veldwaarnemings en weerspieël die spesie se behoeftes*: A lightfooti* het geen lewensfases in water nie. Hulle benodig net vogtige areas vir eiers en die paddavissies om te ontwikkel. Die gebruik van ARO met *A. lightfooti* is belowend in die veld van bevolkingsdigtheid navorsing en kan gebruik word om ander roepende diere te bestudeer, wat belangrik kan wees vir die bestuur en bewaring van wild.

ACKNOWLEDGEMENTS

It is a pleasure to thank my supervisor John Measey for his exceptional guidance during this project. In addition to his commitment to helping his students with their work (always returning corrected work within mere hours), he made sure to introduce us to the world of science beyond our theses through lab projects, collaborative research, enjoyable [working] lab retreats, and encouragement to attend conferences and participate in popular science writing.

It was an honour to be part of the DST-NRF Centre of Excellence for Invasion Biology (C.I.B), and I am grateful for the MSc funding and travel award. From the C.I.B, I am in awe of Christy Momberg who is incredibly organized and whose “can-do” attitude I admire immensely. I thank her not only for all the valuable help over the two years but also for her advice and gentle support with life in general. Also for funding, I appreciate the National Geographic Society’s Committee for Research and Exploration (grant 9913-16). For permits, I thank CapeNature (Permit 0056-AAA007-00092) and the South African National Parks (SANParks; Permit CRC/2016-2017/001—2009/V2). From SANParks, I am especially grateful to Carly Cowell, Debbie Winterton, Zishan Ebrahim, Mathabatha Matjila, Chamell Pluim, Jaclyn Smith, Marisa Langton, and Justin Buchmann for their help during the period of fieldwork.

Ben Stevenson has been remarkable in his help and patience with this project, explaining difficult concepts more than once with thorough and clear emails. He has always been available to help, despite the outrageous time differences, taking up a lecturing position, and various other academic demands that accompany being brilliant.

I thank David Borchers for his help during my visit to St. Andrews, and I would like to thank everybody at the Centre for Research into Ecological and Environmental Modelling (CREEM) for welcoming me so warmly and for introducing me into their cheery cake-consuming culture. Jasper Slingsby provided important information and help at the start of this project, and Res Altwegg has helped throughout the MSc. Francois Becker helped to ease my first field outings into manageable experiences with his expertise in the field and has continued to be a valuable source of information and friendship. I would also like to thank Andrew Turner for his ready help.

My gratitude goes out to all the field assistants who have ventured into the field armed with microphones and recorders: Steve Avidon, Michael Brits, Brent Eachells, Arjan Engelen, Oliver Freyer, Jonathan Herring, Ashley Koopman, Leila Mitrani, Gemma Rashley, Tsilavo Razafimanantsoa, Brittany Schultz, Ross Soller, Philip Uken, David Wright, Lily Bovim and Mujaahid Philander. It was not easy work, and I commend everyone who stuck through it. The stoicism in the field was also accompanied with silliness, and even the most challenging days make for hilarious stories now. A special mention is required for Mujaahid Philander, Lily Bovim, Michael Brits and Ashley Koopman for their leadership in the field and commitment to fieldwork.

Life would have been dreary in the office without the succession of officemates during my time at Stellenbosch: Giovanni for our discussions and debates that broke the monotony of work (often for hours, woops), Nitya for his invaluable advice, emotional support (stocking up the office tissues regularly!), wit and sporadic dance sessions, and Natasha for her ever-ready kindness and provision of comic relief. I am indebted to Alex Rebelo for helping me with aspects of my project both in the office and in the field, and for his friendship.

I am eternally grateful to my best friend, Laura Cussen, for being a spectacular human being. Emma Snyman and Johan van Wyk also deserve special mention for their love, support and patience.

The most special thanks goes out to my parents, Issabel and Pieter, who have always been a source of infinite love and support. While my dad could not see the termination of this thesis or any challenges that lay in my future, his endless encouragement during his days on Earth is the fuel that will drive me to follow through with any dream.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1

General Introduction

Quantitatively estimating the abundance of animals in a given area is central to wildlife management and conservation (Skalski and Robson 1992) and is imperative as the increase in human population and activities put natural areas at risk (Cincotta *et al.* 2000; Holdren and Ehrlich 1974). Population density studies normally make use of distance sampling or capture-recapture techniques (Seber 1986; Borchers *et al.* 2015). Distance sampling typically involves line-transects along which an observer walks, and the distances from the line transect to target animals are recorded (Buckland *et al.* 1993). Detection functions are determined from the distances at which target species are spotted along the transect line, which model the probability of detecting an animal depending on its perpendicular distance from the transect (Buckland *et al.* 1993). This is used to account for the animals missed during the sampling occasion and allows population density to be estimated (Buckland *et al.* 1993; Burnham *et al.* 1980). The basic capture-recapture procedure involves the deployment of traps in which individuals are caught (Skalski and Robons 1992). The individuals are marked and released, and the traps are left open to catch animals on more occasions (Pollock *et al.* 1990). The proportion of recaptures (marked animals caught across multiple sampling occasions) and the proportions of newly captured animals across sampling occasions are used to estimate animal abundance (Pollock *et al.* 1990). To convert this to a population density value, the area that the traps cover needs to be determined. This area is often arbitrarily selected, resulting in a lack of statistically rigorous means to estimate density in classical capture-recapture studies (Efford *et al.* 2009a). In addition, animals that are closer to traps will have a higher probability of being detected, which is not addressed in classic capture-recapture studies (Efford *et al.* 2009a; Efford *et al.* 2004). However, by combining capture-recapture techniques with distance sampling techniques, the issues of an arbitrarily selected sampling area can be addressed and modelling the detectability of target animals based on distances from the traps can be incorporated. These models are known as spatial capture-recapture (SCR) models (Borchers *et al.* 2015; Borchers and Marques 2017).

SCR models make use of distance-based detection functions from distance sampling, and the multiple detector layout from capture-recapture studies (Borchers *et al.* 2015; Borchers and Marques 2017). In SCR studies, the detectors have known locations and the distances between the traps which did and did not detect an animal are used to estimate detection probabilities of target species (Borchers *et al.* 2015). The SCR models therefore use spatial information to take into account that animals closer to traps are more likely to be detected than those further away (Borchers 2012; Borchers and Marques 2017). The detection probabilities are used to create a detection probability surface associated with the traps, and from this, an effective sampling area is automatically determined (Borchers 2012; Borchers and Marques 2017). A variety of taxa have been studied using SCR with a couple of early studies being on possums using cage traps and birds using mist nets (Borchers 2012; Efford *et al* 2005; Efford *et al.* 2004). However, traps do not necessarily have to physically capture and contain an animal, because SCR can make use of traps that detect animals via camera traps, hair snares or microphones (Royle *et al.* 2009; Kery *et al.* 2011; Marques *et al.* 2013). This class of traps are known as “proximity” detectors, and they note an animal’s presence without holding the animal, allowing individuals to be detected on more than one detector during a sampling occasion (Efford *et al.* 2009b). Proximity detectors are advantageous because they are a non-invasive means of collecting data and the behaviour of the study animal is not influenced by their presence (Efford *et al.* 2009b; Borchers 2012; Measey *et al.* 2017).

Acoustic Spatial Capture-recapture (aSCR) makes use of proximity detectors in the form of multiple microphones that detect the presence of acoustically active animals (Efford *et al.* 2004; Marques *et al.* 2013). These recorded calls are run through an acoustic SCR model in order to obtain density estimates of the calling animals. In the field, acoustic data is gathered during a sampling occasion by arranging microphones into an array and recording the calls of a target species (Marques *et al.* 2013). For every call detected across the microphones, the following information is gathered into a "capture history" for that call: the microphones which did and did not detect the call, the exact times at which the call was detected across different microphones, and the signal strength of the call at the different microphones (Stevenson *et al.* 2015). Knowing which microphones did and did not detect a call, and the information associated with the detections of the call, is informative about the probable location of the emitted call (Efford *et al.* 2009b; Stevenson *et al.* 2015). The estimated calling locations are used in the aSCR model to create a detection function associated with the sampling occasion, which in turn can provide the estimated sampling area associated with that sampling occasion (Efford *et al.* 2004; Stevenson *et al.* 2015). Using passive acoustics to study animals is ideal when the target species is difficult to visually detect, but easy to hear.

The earliest published aSCR study was on a bird species, but since then, aSCR has been applied to cetaceans, a mammal species, and an amphibian species. Dawson and Efford (2009) used a set of four microphones and aSCR models to estimate the density of ovenbirds, *Seiurus aurocapilla,* in Maryland, USA. Marques *et al.* (2012) applied aSCR methods to data gathered from a set of 16 bottom-mounted hydrophones to study the analytical approaches in SCR (comparing Bayesian analyses to likelihood approaches) using Minke whales, *Balaenoptera acutorostrata*, as a focal study species. The aSCR model has also been applied to estimate the density of groups of gibbons in Cambodia where humans acted as detectors, and this study resulted in a new form of the aSCR likelihood that accounts for the random availability of animals (Kidney *et al.* 2016). The first application of aSCR to an amphibian appears in Borchers *et al.* (2015) and Stevenson *et al.* (2015) where 25 s of call data from *A. lightfooti* was used to test the application of aSCR. This was important in the development of the R package "ascr" (Stevenson and Borchers 2017) used in the statistical program R (v3.3.1, R Core Team, 2016). Measey *et al.* (2017) then applied aSCR to a study on the call densities of *A. lightfooti* at three calling sites to study change in call densities over a single season. They found that peak calling activity was reached during mid-breeding season.

<u>An application of aSCR in the conservation of a biodiversity hotspot</u>

The Greater Cape Floristic Region (GCFR) includes the Cape floristic, Hantam-Tangqua-Roggeveld and Namaqua regions and is a hotspot for species diversity (Born *et al.* 2007; Manning and Goldblatt 2012; Myers *et al.* 2000). The Core Cape Subregion (CCS) includes a region of the GCFR that is characterized by a schlerophyllous vegetation called fynbos and which has a unique degree of biodiversity compared to other ecoregions in Africa (Manning and Goldblatt 2012). Yet, the plants of the CCS constitute the highest proportion of threatened plants compared to other South African biomes (Rouget *et al.* 2014). It is also the most invaded terrestrial area in South Africa in terms of woody invasive plants (Wilson *et al.* 2014), and 30% of the CCS is currently transformed due to plantations, urbanization and self-sown invasive trees (Rouget *et al.* 2003). In addition, while fire is integral in the survival and persistence of fynbos vegetation (Kraaij and van Wilgen 2014), the deviation of natural fire regimes due to anthropogenic influence is also detrimental to fynbos biota (Kraaij and van Wilgen 2014; Keeley *et al.* 1999). The need to be informed about populations of biota that make up this exceptional and threatened region is evidently important for conservation efforts.

The diversity and endemicity of flora of the CCS is greater compared to the fauna (Manning and Goldblattt 2012), however, there are endemic fauna with very limited distributions that require conservation attention (Colville *et al.* 2014; Picker and Samways 1996). Much of the fauna is highly regionalized due to habitat suitability resulting in narrow ranges (Colville *et al.* 2014). Managing these ranges to avert the decline, or possible extinction, of these narrow-ranged species starts with the knowledge of the populations of these species within their ranges and establishing consistent records of population trends over time. There are more than 57 species of amphibians in the GCFR, of which 31 species are endemic (Colville *et al.* 2014). For the genus of moss frogs endemic to the GCFR, most research efforts have focussed on taxonomic work and apart from the seasonal density measurements by Measey *et al.* (2017), no efforts have been made to quantitatively estimate populations.

The anuran family Pyxicephalidae is highly diverse, with sizes of different species ranging from 15 mm to about 245 mm, and members occupying a wide variety of habitats (Van der Meijden *et al.* 2011). While pyxicephalids occur throughout Sub-Saharan Africa (Channing 2004), the highest diversity of species is in southern Africa (Van der Meijden *et al.* 2011). *Arthroleptella* is the genus of moss frogs and is endemic to the south western part of South Africa. *Arthroleptella* species are small, with mean snout to vent lengths under 20 mm (Turner and Channing 2017). In addition to their petite size, the moss frogs are visually cryptic, highly elusive and often inhabit dense vegetation associated with seepages (Turner 2010). Suitable habitat greatly defines the restricted range sizes of *Arthroleptella* (Turner and De Villiers 2007). For example, *Arthroleptella rugosa* only inhabits mountain seepages with dense vegetation on Klein Swartberg Mountain, occupying an area of 2.3 km^2 which is surrounded by unsuitable habitat (Turner and Channing 2008). Two species of the genus have IUCN statuses of Threatened (*A. villiersi* and *A. bicolor)*, three are Near Threatened (*A. landdrosia, A. lightfooti* and *A. drewesii),* and two are Critically Endangered (*A. rugosa* and *A. subvoce)* (IUCN 2017). While the IUCN statuses of three new species described, *A. draconella, A. atermina* and *A. kogelbergensis,* have not yet been assessed, the threat of invasive vegetation and frequent fires is present in their habitats (Turner and Channing 2017), and they are expected to receive a threatened status. Alien vegetation can change the microhabitats used by *Arthroleptella,* while too frequent fires can result in fluctuations in populations that may result in smaller populations failing to persist. However, to truly gauge the effects of these threats, accurate population estimates are necessary. *Arthroleptella lightfooti,* like the other members in the genus, is visually cryptic but easy to hear. I used aSCR to study populations of the species *A. lightfooti,* and the results are likely to be applicable to the other species of this genus, other frogs as well as many other calling taxa.

The use of *A. lighfooti* for aSCR studies is ideal for several reasons: the males emit an easily heard and simple chirp-like call that is distinguishable from any other calling species in its range. In addition, the frog species remain mostly stationary while calling, and these characteristics reduce complexities involved in sound extraction and the need to account for animal movement in the aSCR model. The distribution range of *A. lightfooti* is limited to the Cape Peninsula, and most of the range is in the protected Table Mountain National Park (Channing 2004). The Cape Peninsula was more accessible in this study compared to the areas occupied by the other members of the genus, which allowed for frequent sampling to take place over two years during the austral winter breeding season. Qualitative population monitoring has taken place for species that occur in areas conserved by Cape Nature for *A. villiersi, A. subvoce, A. rugosa,* and *A. landrossii,* but, until now, no attempt at population monitoring for *A. lightfooti* across its range has been made (A. Turner *pers. comm.*). Examining the feasibility of applying aSCR techniques to assess population density using aSCR for this species is relevant for learning about the application of aSCR for all the members of the endemic genus.

I aim to assess the use of aSCR for estimating population densities of *A. lightfooti,* which would be the first time that the aSCR model developed by Stevenson and Borchers (2017) is assessed on a large field-gathered data set. In addition, I aim to examine the calling behaviour of *A. lightfooti,* in terms of the distribution of calling males within a microsite, through the use of aSCR. This would be the first study attempting to quantitatively estimate the populations of *A. lightfooti* across their entire range using a technique that is still novel in its application to estimating amphibian populations. Outcomes from this study can guide researchers making use of aSCR and provide information about a threatened endemic species that is critical in conservation efforts.

Chapter 2

Assessing the use of Acoustic Spatial Capture-recapture to study populations of the Cape Peninsula moss frog *Arthroleptella lightfooti*

INTRODUCTION

Techniques that are used to study the natural world, in terms of both data collection and data analysis, are evolving as the knowledge and data on natural systems improve, technology becomes more advanced and tools become more accessible (Lahoz-Monfort and Tingley 2018; Blumstein *et al.* 2011; Pollock *et al.* 2002). When possible, techniques that provide qualitative measures of populations should be augmented with quantitative techniques, as this allows for rigorous statistical comparisons and error estimates. Assessing the reliability of emerging techniques used to obtain population estimates of animals is crucial, as management and conservation bodies will make decisions based on these estimates (Legg and Nagy 2006). In addition, meaningful inferences from reported values can only be made if measurements are accurate (Marques *et al.* 2013). For this reason, numerous studies are dedicated to assessing the reliability of different population estimation techniques applied to a whole range of taxa, for example: black bears using DNA-snares, deer densities with portable thermal imaging, and krill with acoustics (Bellemain *et al.* 2005; Gill *et al.* 1997; Hewitt and Demer 2000). Identification of factors that affect the reliability of population estimates can lead to more effective data collection and data analyses and can provide insight into improving estimation techniques.

Advances in population estimation are often characterized by maximizing animal detections while minimizing effort and cost. In acoustic studies on animal populations, automated recording system-based (ARS) approaches have become increasingly popular in a field that was mainly dominated by manual surveys (Dorcas *et al.* 2009; Digby *et al.* 2013; Hutto and Stutzman 2009; Towsey *et al.* 2014). Major advantages of ARS over manual surveys include: the non-invasive nature of passively recording calling animals; monitoring can take place in difficult terrain; experts are not required for deployment of equipment in the field; and a lot of information can be obtained in a short amount of time (Menhill *et al.* 2012; Measey *et al.* 2017; Crump and Houlahan 2017). However, the reliability of emerging techniques to process and analyse acoustic data is often unknown and hard to obtain if manual survey data is missing.

Acoustic Spatial Capture Recapture (aSCR) is a statistical method used to estimate the population densities of acoustically active animals (Efford *et al.* 2009b; Dawson and Efford 2009). Like other spatial capture-recapture (SCR) methods, it marries capture recapture and distance sampling techniques under a single model (Borchers *et al.* 2015; Borchers 2012). The aSCR technique requires data in the form of recorded animal calls across multiple microphones, which are equivalent to detections across detectors, in an “acoustic array” (“microphones” and “detectors” will be used interchangeably from hereon) (Dawson and Efford 2009; Blumstein *et al.* 2011). Using more than one microphone across which a call can be detected allows for inferences to be made about the potential locations of calling animals, which are used to create a detection probability surface (Stevenson *et al.* 2015). The probability detection surface allows for the estimation of a sampling area, which together with abundance data, yields calling animal density with associated error (Efford *et al.* 2009a; Borchers and Efford 2008; Borchers 2012). The use of aSCR addresses the long-standing problem of studying populations of animals that are acoustically active, but visually cryptic (Marques *et al.* 2013). It has the potential to revolutionize population studies on acoustically active animals whose populations are normally difficult, if not impossible, to quantitatively estimate using existing population sampling techniques. But first, the reliability of outputs from this technique need to be assessed before its application to large-scale acoustic field studies.

Arthroleptella lightfooti is an endemic moss frog that inhabits seepages across the Cape Peninsula, South Africa. Despite its IUCN status of “Near Threatened” due to threats of invasive vegetation and too frequent fire (SA-FRoG 2010), extensive population studies on *A. lightfooti* have not been conducted. The incredibly cryptic nature of the frog renders the use of traditional population methods that provide quantitative population estimates impractical. Males and females reach an average snout-vent length of 14 and 15 mm respectively and are highly elusive. While the males can easily be heard calling throughout the austral winter, finding a single specimen can take up to four person-hours (Stevenson *et al.* 2015). The call of the males is a three-pulsed chirp-like call consisting of a single note (Channing 2004). It can easily be identified in the field as belonging to *A. lightfooti* as other animals in the frog’s distribution range do not have similar calls. The simple structure of an *A. lightfooti* call makes this species an ideal study species for a bioacoustic study, because simple-structured calls are preferable to complex calls when it comes to call recognition and extraction from a soundscape. Applying aSCR to study *A. lightfooti* has benefits that are two-fold. Firstly, the seepages where the frogs occur are distributed across the Cape Peninsula and occur in variable forms of terrain, which allows for testing the feasibility and reliability of using aSCR across a heterogeneous landscape. Secondly, quantitative population estimates of this species across its whole range will be available for the first time.

A study by Measey *et al.* (2017) used aSCR to estimate population densities of *A. lightfooti* at three sites 300 m apart and obtained information about the change of densities for those populations across a single season. For the three sites across one calling season, it was found that aSCR worked well in terms of reliable density estimates and that it was suitable in terms of practical deployment in the field (Measey *et al.* 2017). However, to infer reliability of the technique when it is used on a larger scale, perhaps for future annual monitoring across the species’ range, aSCR must be applied to a much larger set of sites. This would also allow for investigation into the practical feasibility of carrying out aSCR data gathering across terrain of varying difficulty in terms of access and ease of travelling with heavy equipment. The assessment of the accuracy of estimates from aSCR on a large scale has, like other studies (Petit and Valiere 2006; Smart *et al.* 2003; Manly 1970; Efford *et al.* 2004), relied on simulations of estimates of calling animal densities, and was found to have a relatively high precision (Stevenson *et al.* 2015). However, it is unknown whether this precision will hold in the field.

In this study, I used aSCR to obtain densities for more than 80 sites all over the Cape Peninsula where *A. lightfooti* are calling. There were many variables that differed between sites, but I chose to examine variables that were directly provided to the aSCR analysis in order to see how differences in sites could be affecting the reliability of density estimates. These variables include the numbers of calls received across the array, the formation of the acoustic array at each sampling site in the field, and the different numbers of microphones across which calls are detected in the field. I hypothesized that there would be a range of ideal values for these variables that would coincide with reliable density estimates and aimed to find the upper and lower thresholds of these variables above and below which density estimates become unreliable.

MATERIALS AND METHODS

Study Species

Arthroleptella lightfooti (Boulenger 1910) is an anuran that belongs to the family Pyxicephalidae whose members are endemic to sub-Saharan Africa (Channing 2004). The genus *Arthroleptella* is endemic to south-western South Africa with *A. lightfooti* being endemic to the Cape Peninsula and not occurring in sympatry with other members of the genus (Channing 2004). *Arthroleptella lightfooti* is a directly developing terrestrial species and is associated with seepage areas where the ground is moist but not inundated. Males emit advertisement calls during the day that consist of three-pulsed chirps and which have a signature frequency of 3.75 kHz (Channing 2004; Appendix A.1).

Study sites

Generating sampling sites

This study relied on acoustic data from sites where frogs were present and calling. Therefore, generating completely random sampling sites across the Cape Peninsula would not have been ideal as a large portion of random sites would be located in urbanized lowland areas where the frogs do not occur. I therefore generated sampling sites by a) using a species distribution model (SDM) with known occurrence data to determine where the frogs were most likely to occur on the Cape Peninsula, and then b) stratifying the frog presence probabilities from the SDM to focus the main effort (larger portion of generated sampling sites) to areas with higher probabilities of frogs being present, and lastly c) using a sample selection method to ensure random variation between the variables across the sites.

a.) Species distribution modelling

I used Maximum Entropy Species Distribution Modelling (MaxEnt Version 3.3.3k) to predict the probability of *A. lightfooti* presence in any cell (at a resolution of 30 m^2) across the modelled landscape of the Cape Peninsula. Input data included *A. lightfooti* presence (n = 367, based on locality data from 1898 to 2016 obtained from various sources; Appendix A.2) and pseudo-absence data (n = 10^4, points randomly selected across the Cape Peninsula). True absences (n = 18, locations of known true absences within *A. lightfooti* distribution) were also included in the species distribution model by incorporating these points with the randomly selected pseudo-absence points. Fourteen environmental predictor variables (Appendix A.3) were also included as input data. A total of 100 replicates of each model were fitted and maximum bootstrap iterations were set to 500. The default Random seed setting was kept for the model run, where 80% of the occurrence records are used in model fitting while the remaining 20% are dedicated for model testing (per model replicate). I used the average model from the 100 replicates for the subsequent stratification step in site determination (AUC = 0.931±0.007; Appendix A. 4).

b.) Stratification of sites based on probability of frog occurrence

Stratification of sites was necessary to focus the main sampling effort to sites where frogs were present without entirely neglecting those areas with lower probability (Kalton and Anderson 1986; Gormley *et al.* 2011). Therefore, sites with a likely absence of frogs were of lower priority. Stratification was applied to the output of the MaxEnt distribution model in terms of grouping 30 m^2 cells into strata according to the size of the probability of *A. lightfooti* being present. The four strata consisted of the following probability ranges of frog presence: 0.6 to 1, 0.4 to 0.6, 0.2 to 0.4, and 0 to

0.2. Of the total number of samples sites generated (n = 270), 80% were selected from the first stratum, 10% from the second, 5% from the third and 5% from the fourth.

c.) Sample selection method

I used Conditioned Latin Hypercube sampling using the package "clhs" (Roudier 2011) in R (v3.3.1, R Core Team, 2016) to select the sites from the different strata (Appendix A.5). This ensured near-random sampling between sites by varying the following characteristics of the sites: aspect, elevation, solar radiation during July, slope, mean minimum temperature during July, vegetation type, Maximum Normalized Difference Vegetation Index (NDVI) of 2015, and most recent fire as of January 2016.

d.) Accessing generated sampling sites

The generated sampling sites were scattered across the whole species range on the Cape Peninsula. Sampling took place from June to October in 2016 and from May to October in 2017. Due to the diurnal nature of the frogs, sampling could only start once the sun had risen. Sampling times varied depending on the accessibility to sites (in terms of difficulty of the terrain and distance to sites from parking), and this also determined the number of sites that could be sampled per day. Sampling at the first site could start as early as 08:30 if minimal hiking was required and the sampling at the last sites tended to start at 16:00. I accessed generated sampling sites by walking via paths when possible and with the use of a Garmin GPS (Garmin GPSmap 62, Garmin International, U.S.A) that has an accuracy of ±5 m. If frogs were calling at the generated site, acoustic arrays could be set up (see next section: Experimental Method). If there were no calling frogs present upon reaching a generated site, a protocol of finding the next closest location of calling frogs was implemented. This involved walking in a spiral around the generated site, stopping every quarter to listen for calling frogs. The next closest location where frogs were calling was then selected for recording. However, if calling frogs where not found within 100 m (direct distance) of the generated sample site, the site was considered to have no frogs present, and a density value of 0 frogs.m^{-2} was recorded for the site generated. The site at which a recording took place was then referred to as a sampling site, instead of the generated sample site.

Experimental Method

Setting up the acoustic arrays

An acoustic array consisted of detectors in the form of microphones attached to a recorder via cables. The arrays I set up consisted of six omni-directional microphones (Audio-Technica AT8004 Handheld), each held above the ground by a 1 m wooden dowel, connected to a multi-channel portable recorder (I used both Tascam DR 680 and Tascam DR680 MK2 recorders, TEAC, Wiesbaden, Germany) using 10 m cables. The six microphones were arranged around the recorder where the frogs were calling. The distances were not fixed and could vary between arrays depending on the site and the perceived calling locations of the frogs. I tried to arrange the array in such a way that the estimated location of calling frogs was covered or partly covered when possible. Each microphone recorded onto one of six independent tracks at a sampling frequency of 48 kHz and a resolution of 24 bit depth (Measey *et al.* 2017). Calling frogs were recorded on the array of microphones for 45 minutes and the following information was recorded at each site: site number, names of the personnel recording, weather and the time at the start of the recording. The immediate area around the site (200 m) was vacated while recordings took place. The recordings were deposited in a database of the South African Earth Observation Network (SAEON) and are accessible upon request.

Post-recording data collection

When 45 minutes of frog calls were obtained, the acoustic recording was stopped. I then measured the distances between each of the microphones using a 30 m measuring tape and noted the measurements to the nearest centimetre. The position of each microphone was also captured using a Garmin GPS to an accuracy of ±5 m. Main features of the site such as height of vegetation, presence of running water, rocks, footpaths, estimated positions of calling frogs and how the array stood in relation to these features, were noted with sketches (Appendix A.6).

Spatial Capture-Recapture

Data Preparation for aSCR

Four data components were required as input for the aSCR analysis: the frog calls extracted from the recording, the array formation used in the field, subsamples from the recordings by the arrays to analyse, and an average call rate specific to *A. lightfooti*.

a.) Extracting frog calls from the recorded soundscape

The microphones in the array recorded the entire soundscape at a sampling site, which included the frog calls. The latter needed to be extracted from the rest of the soundscape in order to be used in further analyses. Specifically, the following information was needed: the start time of each frog call, the microphones on which a call was detected and the amplitude (or signal strength) of each call. An open source software PAMguard (v1.14.00 BETA; Gillepsie *et al.* 2008; www.pamguard.org), which was developed for bioacoustics data collection and analysis, was used to obtain the frog calls and necessary associated information. The function called "Click Detector" made use of call classification parameters that described the chirp-like call of male *A. lightfooti* to identify and extract all sounds that conformed to the provided parameters. The program ran in real-time and calls were extracted from one recording from an array at a time (Appendix A.7). Every time that a bird call was picked up as a frog call or excessive noise (e.g. stream noises or wind) masked frog calls, the time was noted down (Appendix A.8). These sections could not be used for data analyses.

b.) Preparing the array formation data for aSCR

An accurate representation of every unique acoustic array that was set up in the field at each sampling site was needed in preparation for running the aSCR models. This representation was required in the form of coordinates on a Cartesian plane and was obtained with the Broyden-Fletcher-Goldfarb-Shanno algorithm (Fletcher 1987), which used two different components of data collected in the field at each site. The first consisted of the measured distances between the pairs of microphones that make up each array. The other component consisted of Cartesian coordinates obtained by hand from the sketches of the array in the field, where microphone#1 in the sketch was the origin, and all the other microphones were given coordinates relative to it. The coordinates obtained by hand from the sketches acted as starting points for the algorithm and the paired distances provided more detailed information about the positions of microphones relative to each other. The algorithm would converge on an array formation that was reflective of the array set up in the field (Appendix A.9; Appendix A.10).

The array area, which is the area between the microphones of the array, was obtained from the array formation produced by the algorithm using a Minimum Polygon Convex Estimator in the package “adehabitatHR” (Calenge 2006). The array area acted as a proxy for the array formation in statistical analyses and is a pre-aSCR variable. Pre-aSCR variables refer to variables examined in the data analyses that are input variables for the aSCR models, while post-aSCR variables are variables that are outputs from the aSCR model.

c.) Selecting subsamples

Subsampling in terms of minutes from a recording on which to run the aSCR model was necessary, as the computational power and time needed to run an entire recording was unfeasible. The first ten minutes of a recording were not used in the data analyses. This was to take into account the fact that setting up the array caused disturbance to the frogs and affected the calling behaviour of male *A. lightfooti*. Ten minutes was ample time for normal calling behaviour to resume (pers. obs.). Ten appropriate subsamples of one-minute length each were therefore selected between minute ten and minute 41 (the last four minutes were also not generally selected due to the disturbance of returning to the array to turn off the recording). “Appropriate” subsamples were minutes of recordings containing calling frogs that were free from bird calls misidentified as frog calls in PAMguard and free from overexposure of noise that was most often due to wind. The selected subsamples were defined for the aSCR model in terms of a range of seconds, where minute 15 is the range 900 – 960 seconds. These will be referred to as “subsample time ranges” from here on.

d.) *Obtaining an average call rate for* A. lightfooti

The aSCR analysis requires an average rate of calls emitted per minute, or a “species frequency”, in a step that converts estimated density of calls to calling animal density. Independent recordings of one-minute in length of individual calling frogs (n=13) were conducted and were used to calculate a “species frequency”.

Analysis through aSCR in R

The output from aSCR is an estimation of call densities or calling animal densities (when a call rate is included) for each sampled site. I used the R package “ascr” (Stevenson and Borchers 2017) to obtain these variables: the estimated calling density of animals and the error associated with these call densities (Appendix A.11). The detected calls, array formation and subsample time ranges from the first three steps in the data preparation stage first needed to be collated using a function within the “ascr” package. For each subsample, a signal strength cut-off, which determines which calls in the capture history should be used in the aSCR analyses, was obtained. Error estimation in terms of standard error associated with density estimates were calculated via a separate parametric bootstrap procedure.

a.) Collating detected calls, array formation and selected subsample time ranges into “capture histories”

Using the components obtained in data preparation - the frog calls, the array formation and the subsample time ranges - allowed for “capture histories” to be constructed. Capture histories are required for an aSCR model to run. A capture history describes information about an emitted call in the form of which microphones did and did not detect the call, the signal strength of the call at the different microphones and the exact times that it was detected at the different microphones (Table 2.1). A capture history is generated by identifying which calls identified by PAMguard across the different microphones are actually the same call detected on various microphones. The function convert.pamguard was used to obtain capture histories for all calls from the sampled sites. Capture histories were constructed using the detections that occurred during the specific subsample minute ranges selected in the data preparation step, and were required in the convert.pamguard function.

To determine whether detections at different microphones belong to the same emitted call, the function convert.pamguard examined the time of arrival data associated with each detection. If two detections occurred on two different microphones separated by a measured distance, the detections were considered to be from the same call if the difference between their times of arrival was less than the quotient of the distance between the microphones divided by the standard speed of sound through air:

1. Let **d** be the distance between two microphones.
2. Let t_1 be the time of arrival of a call at microphone 1 and t_2 be the time of arrival at microphone 2.
3. Let **s** be the standard speed of sound in air 340m.s^{-1}.

The detections are considered to be from the same call if:

$$| t_1 - t_2 | \leq d/s$$

Three pre-aSCR variables used in the analyses to investigate their effect on estimate error were obtained from these capture histories: a) the average number of calls received by the array, or "received calls"; b) the standard deviation in received calls per minute for each site; and c) the combination of detectors across which calls were heard, or detector frequencies. The detector frequencies describe the different numbers of calls picked up across one or more detectors for a sampling site (Appendix A.12).

b.) Determining the signal strength cut-off

Signal strength is the amplitude (or "loudness") of a received call. Every detection of a call at a microphone has a signal strength associated with it: how loudly it was perceived at the microphone. The aSCR model assumes that there are no changes in detectability of a call, but in reality, background noise changes over a survey. This means that softer frog calls will, at times, be easily detected (when there is little background noise), and other times will not be detected (Efford *et al.* 2009a). These discrepancies in detectability of a certain range of calls introduces bias in the aSCR model. It is necessary to define a "signal strength cut-off" at which to accept or discard calls from the capture histories for use in the aSRC model (Stevenson *et al.* 2015). A signal strength cut-off must be high enough so that the calls with weaker signal strengths that are not consistently detected are not included in the aSCR model; increasing the signal strength cut-off limits model fitting to calls that are almost always detected when they occur. Signal strength cut-offs were also applied to the study of ovenbirds by Dawson and Efford (2009). For my study, the cut-off was set to the median value of signal strengths in the capture histories for each subsample of one minute, and was therefore re-determined for every subsample (Appendix A.13). Therefore, signal strength cut-offs could differ between the subsampled minutes. Calls above the median signal strength were assumed to be detected more consistently than those below the median, and the latter were considered non-detections after the cut-off was implemented.

c.) Running the aSCR model

The aSCR model was run on the capture histories from calls in the field to obtain density estimates of calling frogs at the sampling sites. The aSCR model used a likelihood approach defined by

Stevenson *et al.* (2015). The aSCR model was run for every subsample. The required arguments for the aSCR model function “fit.ascr” included the species frequency (which would be the same every time the aSCR model is run for *A. lightfooti*), the signal strength cut-off (specific to the subsample being run), and the capture history (specific to the subsample being run). The outputs from running the fit.ascr function that I was interested in were the estimated call density, estimated calling animal density, and the detection functions associated with each subsample (see next section: Detection functions). Another output, the estimated locations of calls relative to the array, will be discussed in Chapter 3. Density estimates were obtained for each subsample. To obtain the calling animal density estimate for a site, the average of the density estimates of the subsamples was obtained.

i.) Detection functions

The aSCR model produces detection functions. A detection function describes the probability of detecting a frog call, given that it is a certain distance from the microphone (Appendix A.14). The aSCR model uses the capture history information of a subsample (where, when and how loudly calls were detected) and derives a detection function. As all microphones are assumed to have the same detection strength, detection functions are the same for every microphone in an array. Representing the detection functions for each microphone in the array simultaneously can provide a three-dimensional representation of a detection function associated with the array as a whole (Appendix A.15).

The detection functions were the only post-aSCR variable examined in relation to the density error estimates. Specifically, I used the probability of a detection function at distance zero from a microphone, referred to as the starting detection probability.

d.) Failure to converge on density estimates from aSCR

Sometimes an aSCR model failed to converge on estimate values such as the estimated calling animal densities or call densities. If there was a failure to converge on density estimates, there were two ways to achieve convergence. If other subsamples from a site had converged, then their converged estimates were provided as starting values for the model to run from. Another method to achieve convergence was to rerun the aSCR model and watch the estimated values produced in each step of the optimisation algorithm. Any large, unfeasible jumps in estimated values were noted, and boundaries in terms of lowest and highest possible density estimates were implemented. The algorithms would then only search for estimates between these boundaries.

e.) Density error estimation

To meet the overarching objective of assessing this technique under varying sampling conditions, the reliability of the estimated density estimates had to be determined. Reliability could be examined when the degree of error associated with the density estimates was known. I chose to examine uncertainty in terms of the standard errors of the density estimates and how they relate to the density values.

A parametric bootstrap was used to determine standard errors of density estimates (Stevenson *et al.* 2015). Approximately 300 bootstrap iterations were found to be sufficient in reducing the between-simulation variability, or the relative Monte Carlo Error, to below 0.05 (Koehler *et al.* 2009). Standard errors for density estimates were calculated based on the standard deviations between the density estimates of the resamples. Since the aSCR model had been run for each subsample, bootstrap resampling took place for the fitted aSCR model of each subsample. Standard errors were therefore determined for estimates of subsamples. However, standard error was required for a site as a whole in order to compare across sampling sites.

To obtain a standard error for a site from the standard errors of its subsamples, a subsample-based modification of the bootstrap procedure described in Stevenson *et al.* (2015) was applied (Appendix A.16).

In the examination of the relationships between the standard errors of density estimates and the various pre-aSCR and post-aSCR variables (see the next section: Data Analyses), the standard errors were expressed as coefficients of variation (CV). The CV is represented as a percentage of the value it is associated with.

To assess the reliability of density estimates, I used the standard errors associated with the density estimates. The standard errors were calculated as coefficients of variation (CV), which is expressed as a percentage of the density value with which the standard error is associated. For example, the standard error in the following observation 10±2, would be expressed as a CV of 20%. I considered density estimates to be reliable if their CVs were less than 30%, as CVs above this value represented sites with estimates that were considered too inaccurate for population estimates (Gibbs *et al.* 1998; Eberhardt 1978; Kraft *et al.* 1995; Thomas 1996).

Data analyses

To examine whether aSCR models perform well with field collected data (as aSCR has been applied to simulated call data and has been found to function reliably; Stevenson *et al.* 2015), I tested the fundamental assumption of a perfect positive relationship between the number of calls received by an array and the resulting estimated calling densities: an increase in received should be reflected by an increase in calling densities obtained through aSCR. I examined the relationship between the calls received and the calling animal densities by log-transforming the variables (to obtain normally distributed residuals) and running a linear regression in R.

To understand if aSCR performed well across the entire range of received calls, I quantified the relationship between the average received calls at a sampling site and the CVs associated with the sampling sites using general linear models in R. Outliers were defined by the CV variables and were considered to be CVs with values above 100%.

Using a linear regression in R, I tested whether deviation in calls received per minute over a sampling period at a site (also presented as CVs: where the standard deviation value is expressed as a percentage of the average calls received value which it is associated with) affected the CVs of density estimates. I examined the relationship with and without the CV values from the density estimates that exceeded 100%.

I examined the relationship between the array areas and the CVs using a Mann-Whitney test, where the distribution of CVs below the median of array areas was compared to the distribution of CVs above the median of array areas. For this relationship and the remaining relationships that involved the use of CVs as a variable, the CVs above 100% were excluded.

I used linear regressions in R to determine the slope values of the lines of best fit for the detector frequencies of each sampling site, and these were used as proxies of the different combinations of detector frequencies across the sampling sites. I plotted the slopes against the CVs of estimated calling animal densities to discern how they were related.

To see whether the CVs of a sampling site were correlated with the starting detection probabilities of detection functions, I used Pearson's product-moment correlation in R.

In terms of the CVs of calling animal density estimates, I examined the values above 100% that I considered to be outliers. To understand why an outlier has a value so markedly different to others, the following procedure was followed: a) I examined raw data for signs of incorrect data entry, b) I compared the estimated location of detectors obtained through optimization methods with the sketch of the array configuration from the field, c) I reassessed notes taken during the PAMguard procedure for unusual observations (such as frog calls being heard on the recording but not being picked up in the program), and d) I assessed a subset of estimated call locations for anything unusual (such as the same call estimated to come from two different locations).

RESULTS

Of the 251 sites visited in 2016 and 2017, 149 sites had more than two frogs calling, while 97 either had either two frogs calling, less than two frogs calling, no frogs calling or were inaccessible. Five recordings failed due to technical errors. For example, a cable or microphone had become faulty. The majority of recordings took place between 10:55 and 13:55, with the earliest start of a recording being at 08:44 and the latest at 16:19. Due to the long computational time required for aSCR analyses, a subset of 85 sites were analysed and densities obtained. Ten subsamples of one minute could not always be recovered from each recording, typically if noise in the form of wind, birds or water dominated. Sampling sites had a mean of 8.4 subsamples. One of the recordings could not be used in some of the analyses as only one subsample was suitable to be run through aSCR and therefore only one subsample represented the sampling site.

Of the sites sampled, 26.2% had CVs above the maximum acceptable cut-off value of 30%. Values that were very far above the threshold were outliers that often masked relationships, and when these were removed to be analysed separately ($n = 6$), 20.5% of the remaining samples had CVs greater than 30%. The inclusion of the CVs above 100% greatly obscured any relationships in the tests. Unless stated otherwise, the six estimates with CVs larger than 100% were removed from the analyses.

The assumption of a relationship between the calls received and the density estimates was found to hold true with a significant positive relationship ($ß = 1.06$, $p < 0.001$; Figure 2.1). Residual plots revealed that there were three sampling sites that deviated from this trend. About 49% of the variance in calling densities could be explained by the received calls ($R^2 = 0.4876$).

There was a negative exponential relationship between the average calls received per minute and the CVs: as the average number of calls received by the array per minute increased, the CV sizes decreased (Figure 2.2). A threshold of 111 call.min^{-1} was found: calls received across the array below this threshold had CVs that exceeded the 30% reliability threshold, while most calls above this (91% of calls above 111 calls.min^{-1}) had CVs in the acceptable CV range. Given that the call rate obtained from independent recordings of *A. lightfooti* was about 20 calls.min^{-1}, a threshold of 111 calls.min^{-1} represented approximately five or less calling frogs.

The standard deviations of calls received in terms of coefficients of variance had a significant negative relationship with the CVs of density estimates ($\beta = -0.31$, $p < 0.001$; Figure 2.3). When the outliers were included in the linear regression, the relationship still had a significant negative trend ($\beta = -2.249$, $p < 0.05$).

The Mann Whitney U test revealed that the distributions of CVs above and below the median array area of 75 m^2 were not significantly different to one another (W=463, $p > 0.05$), but the CVs below an area of 75 m^2 do include more instances of being above at least 50% (Figure 2.4).

In the relationship between the slope values (the proxy for detector frequencies) and the CVs of densities, the slope values associated with CVs above the 30% threshold had slopes that were between -233 and 36 (Figure 2.5). This range of slopes represented best fit lines that were not very steep in comparison to other range values (with the range minimum being -1105). Of the CVs between the range of -233 and 60, 34% represented unreliable estimates.

The CVs and the starting probabilities of detection functions were not significantly correlated (R^2 = 0.169, df = 76, $p > 0.1$): CVs less than 30% do not appear to be associated with any particular starting probabilities of detection functions (Figure 2.6).

Five of the six outliers had only one frog calling (Table 2.2).

Table 2.1. The capture histories shown for a few seconds from a subsample of a sampling site: a) the binary data shows which detectors did and did detect the specific chirp; b) the time of arrival (s) of the detections of a call at each microphone; c) the signal strength information associated with each detection at each microphone.

a)

Cues	**Detectors**					
Presence/absence	**1**	**2**	**3**	**4**	**5**	**6**
1	1	0	1	1	0	0
2	0	0	0	0	0	1
3	0	1	1	1	1	1
4	0	0	0	0	1	0
5	0	0	1	1	1	1
6	0	0	1	0	0	1
…						

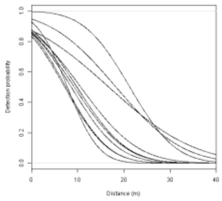
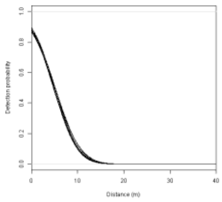
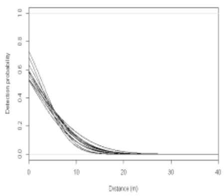
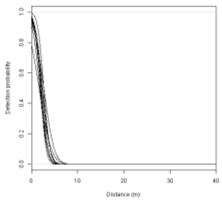
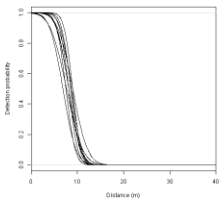
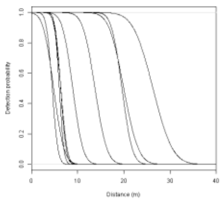
b)

Signal Strength						
1	167.2	0	166.21	169.38	0	0
2	0	0	0	0	0	164.76
3	0	162.59	164.86	165.97	180.72	170.56
4	0	0	0	0	177.96	0
5	0	0	164.71	164.04	177.27	164.61
6	0	0	164.87	0	0	164.76
…						

c)

Time of Arrival (s)						
1	1020.02	0	1020.01	1020.01	0	0
2	0	0	0	0	0	1020.04
3	0	1020.25	1020.24	1020.23	1020.22	1020.23
4	0	0	0	0	1020.64	0
5	0	0	1020.66	1020.65	1020.66	1020.65
6	0	0	1020.68	0	0	1020.67

Table 2.2. A summary of the density estimates that had CVs greater than 100%. The most common factor that led to these sites being outliers is that 2 or less frogs were calling at these sites (which was determined in the field and could be reassessed by listening to the recording and viewing it in PAMguard), but other factors such as incorrect data entry (for example, where the Estimated Detector Locations do not match the sketch of the array in the field) could have also led to these sites being outliers. The Estimated Calling Locations are an output from aSCR that visually displays where frogs could be calling from in relation to the acoustic array, and for some outliers, the estimated location of calling frogs did not match where the frogs were approximated to be calling from in the field.

Outlier	**CV Calling Animal Density (%SE)**	**Number of frogs likely calling**	**Estimated Detector Locations**	**Detection functions**	**Estimated Calling Locations**
1	2243.75135	2 or less	Match sketch		Uncertain calling locations
2	629.4633678	1	Match sketch		Anti-intuitive calling locations
3	172.4085443	1	Match sketch		Normal calling locations
4	168.4247536	more than 3	Match sketch		Normal calling locations
5	118.8866714	2 or less	Match sketch		Normal calling locations
6	113.6573165	1	Does not match sketch		Anti-intuitive calling locations

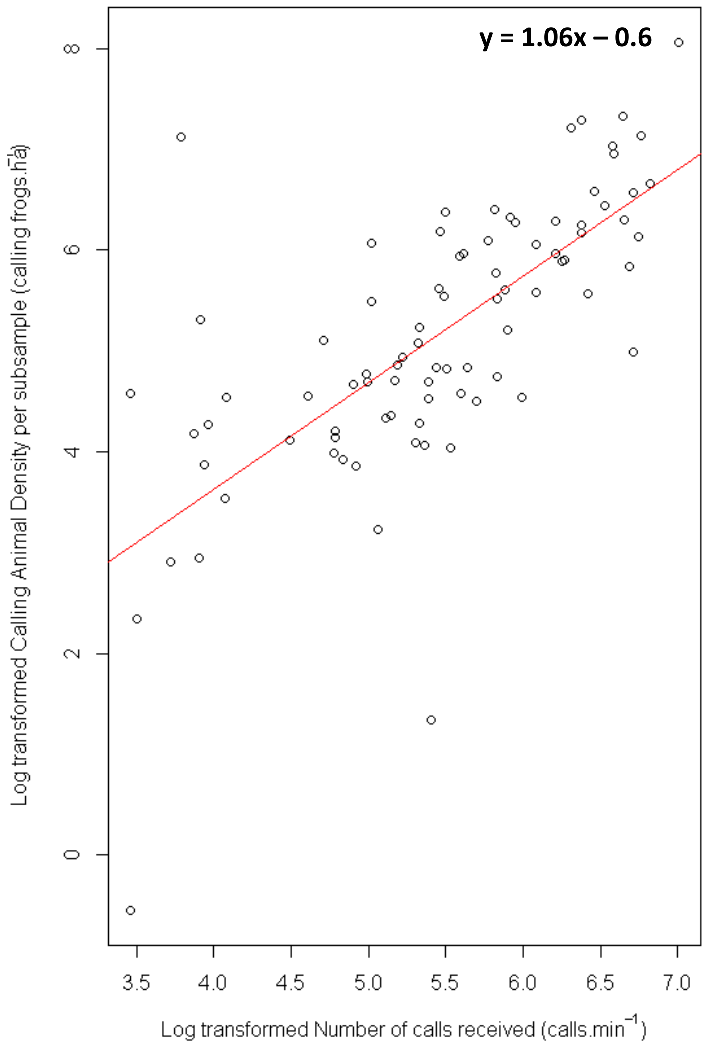


Figure 2.1. Log-transformation of the Calling Animal Density and the Number of Calls Received by the array reveals a significant positive linear relationship.

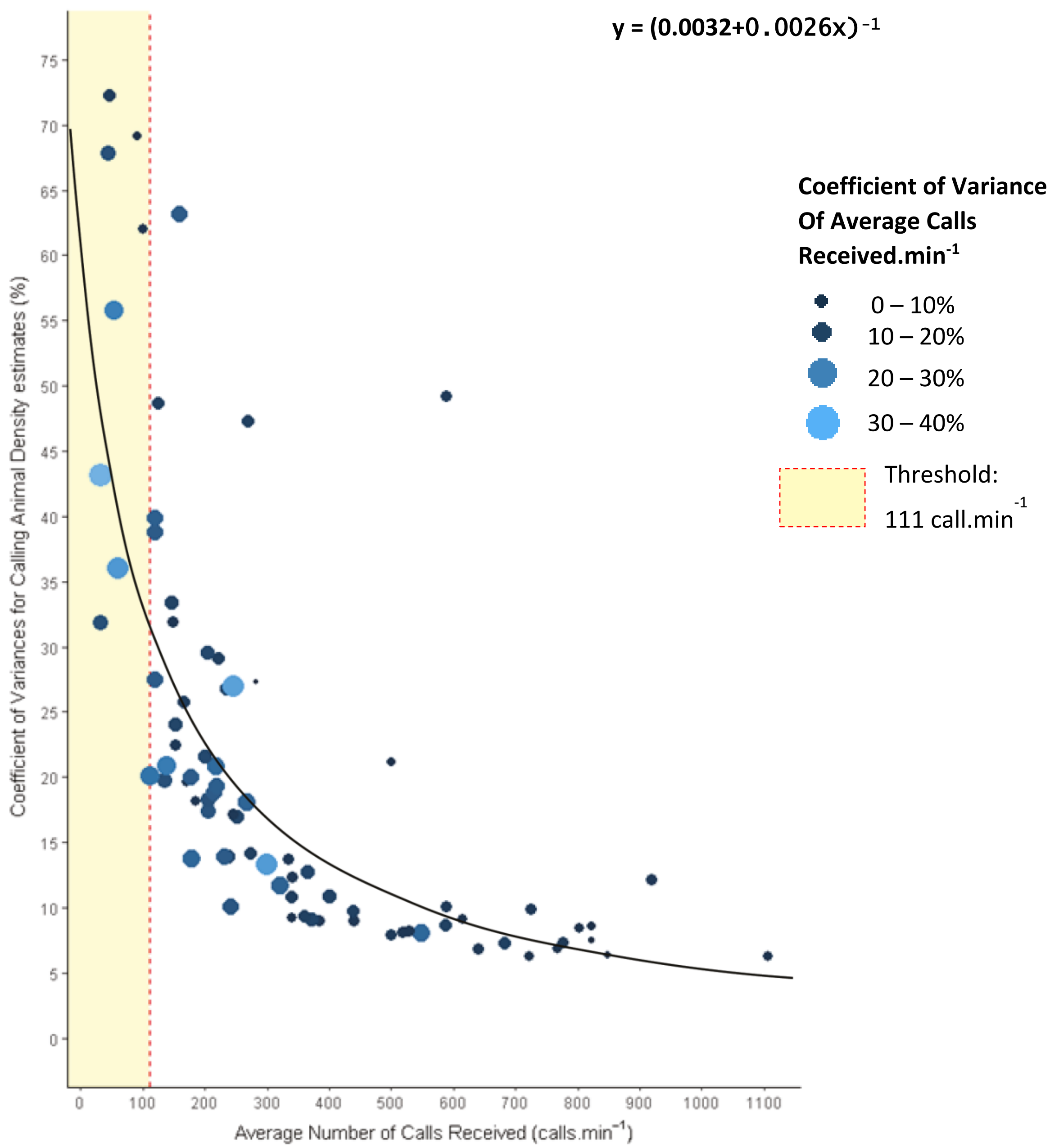


Figure 2.2. The coefficients of variances (CVs) of calling animal density estimates, determined from the standard errors of the estimates, decreased as more calls were received per minute across an acoustic array (n=78 after removal of extreme outliers that obscured the relationship). When calls were less than 111 call.min^{-1}, the CVs of the calling animal density estimates were above 30%, which is considered too high for the estimates to be trustworthy. However, above this threshold, 91% of the observations had CVs below 30%. There is more variation in the number of calls received, indicated by the size and colour of the observations, when fewer calls were received per minute.

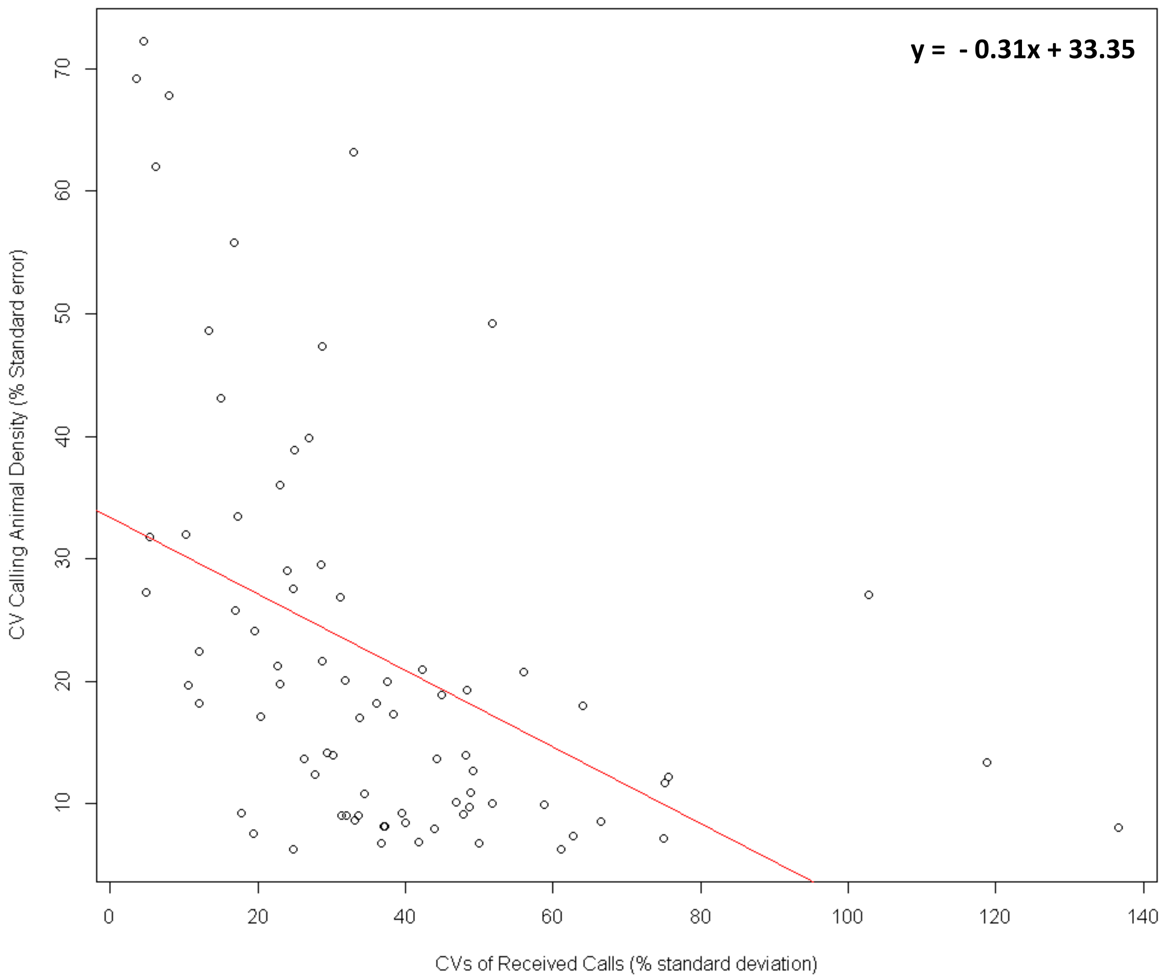


Figure 2.3. The standard deviations of the average received calls, presented as coefficients of variance (CVs) here, had a significant negative linear relationship with the CVs of calling animal density estimates.

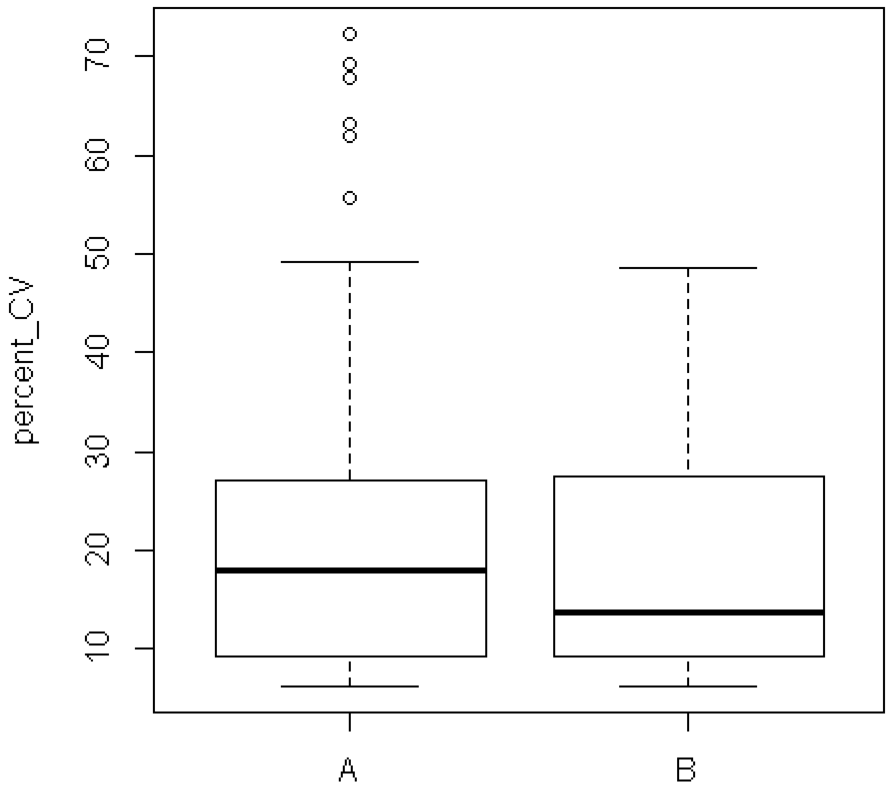


Figure 2.4. The distribution of CVs below the median of 75 m^2 (A) and above the 75 m^2 (B). There is no significant difference but it is worth noting that the upper values in the CVs at smaller array areas are much higher than those at larger array areas.

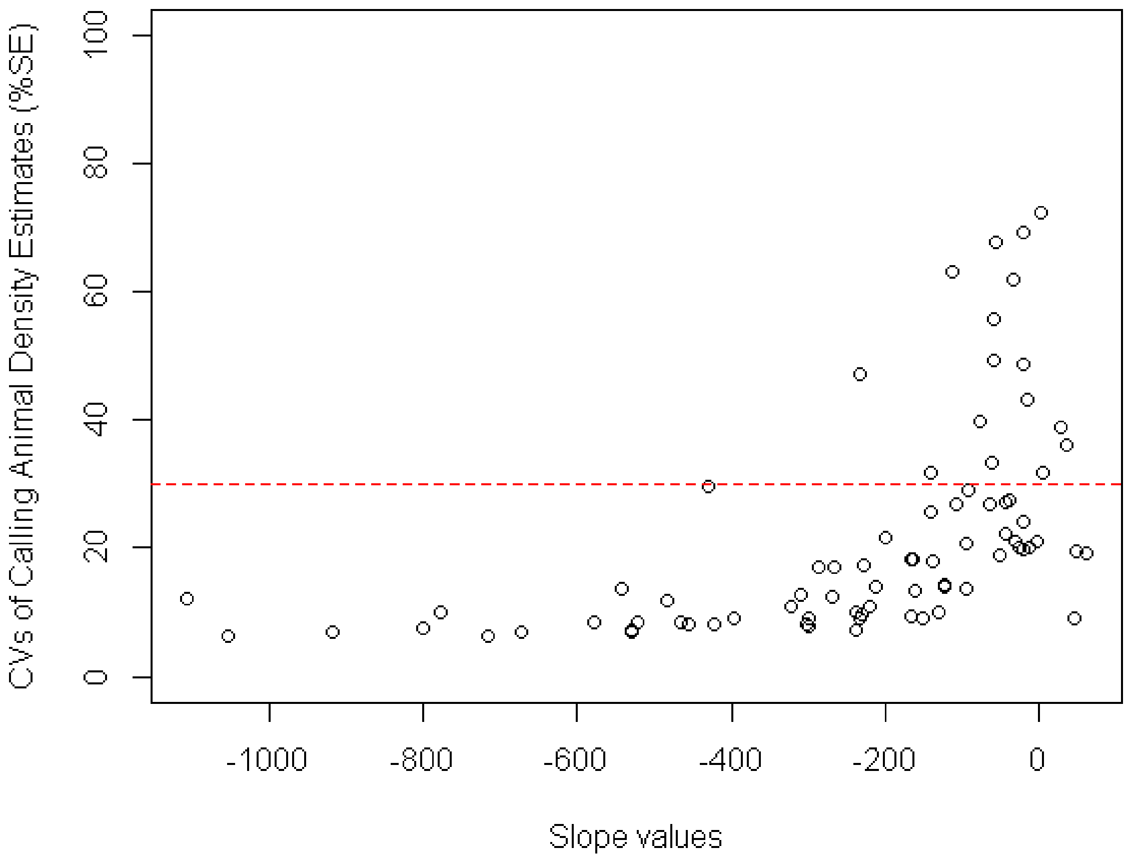


Figure 2.5. More variation in the CVs of calling animal density estimates was present when slopes tended towards zero. Unreliable estimates represented by CVs above threshold of 30% were present in the slope range of -235 to 40; they represent 34% of the values in that range.

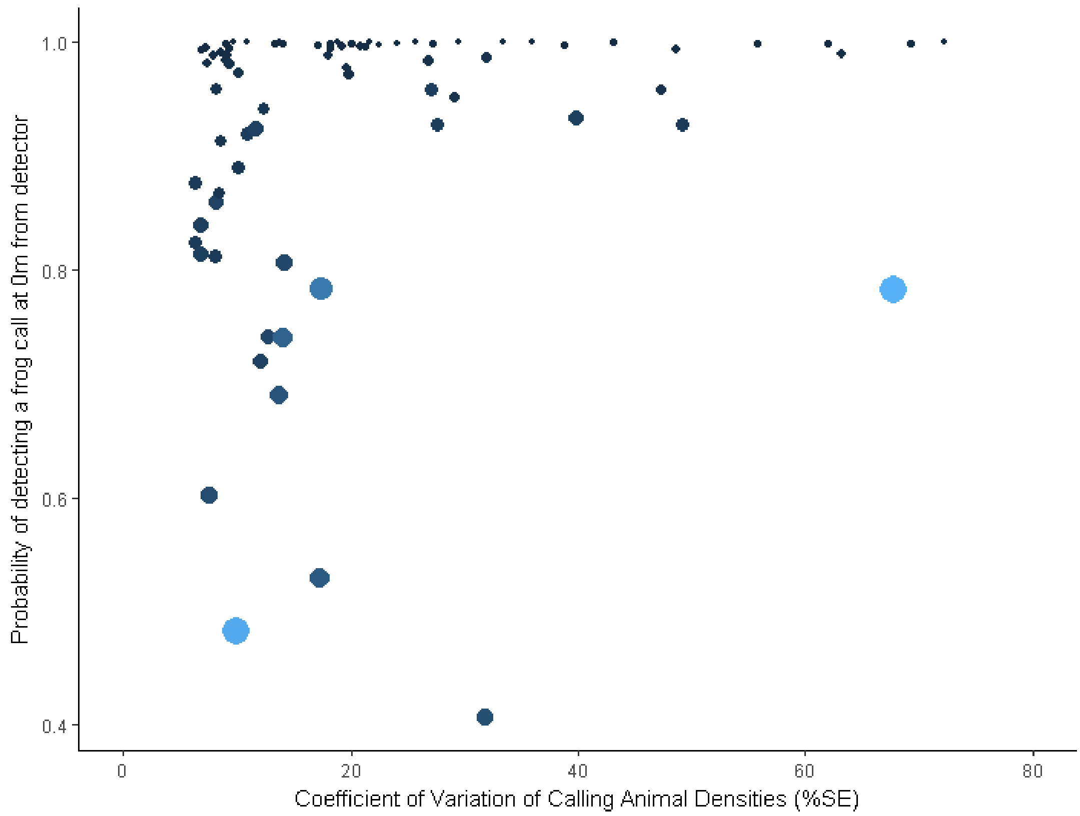


Figure 2.6. There is no clear relationship between the coefficient of variation of the calling animal density estimates (based on the standard error associated with these estimates) and the probability of detecting a frog call at 0 m. The size and colour of the points represent the standard deviations of the detection probabilities at zero meters as CVs, but contrary to expectation, there is no discernible evidence that larger variation amongst detection probabilities of subsamples affected the error associated with the density estimate of a site.

DISCUSSION

I found that the Acoustic Spatial Capture-recapture (aSCR) model provides reliable density estimates across a large range of call abundances (111 to 1200 calls.min^{-1}), and that this method for obtaining reliable quantitative estimates could augment or replace methods that obtain qualitative data on calling frogs (Sargent 2000; Crouch and Paton 2002). No upper threshold in terms of received calls resulting in poor CVs was found, but I found that that there is large variation in the error of density estimates when five or fewer frogs were calling, indicating that aSCR should not be used when densities are this low. However, obtaining density estimates for such a low number of frogs can be easily accomplished by a human observer provided an effective sampling area is defined. Population monitoring can be expensive (Sousa-Lima *et al.* 2013; Gill *et al.* 1997) and knowing this threshold of frog calls under which aSCR is not ideal is conducive to minimizing expenditure of effort, time and expenses in data collection and analyses. I found that there is a near perfect positive relationship between calls received and calling animal density estimates, suggesting that aSCR functions as expected across a heterogeneous environment and under variable sampling conditions. These insights on the optimal use of aSCR provides a fundamental framework on which to base future data collection and data analysis for monitoring of this species.

Population studies that use aSCR to conduct field-based data collection will need to assess the reliability in their estimates and can use the low call density threshold found here as a starting point above and below which to test reliability of estimates for their study species. The lack of an upper threshold in terms of received calls and reliability of estimates indicates that the aSCR model either successfully managed to distinguish between frog calls at very high densities, or capture histories were assembled erroneously without affecting the CV of estimates. The latter case is possible if detections from different calls being emitted very close to each other were grouped as coming from the same call. This would be evident if a plateau is observed in a plot of density estimates of all the sampling sites. In my data, density estimates steadily increased as received calls increased, but no plateau was reached. This plateau may be exposed with the addition of more density estimates from more sampled sites with even higher densities of calling frogs; essentially where the difference in the time of arrival of calls at a pair of microphones is less than the distance separating the pair of detectors divided by the speed of sound. This potential underestimation of higher densities, or lack of an upper threshold, should not be dismissed, as revealing its cause could have implications for the use of aSCR with animals that call in high abundances.

My results suggest that the way in which detectors are placed in relation to calling animals may impact the errors associated with density estimates. Currently, there is no standard way for an array to be laid out (Measey *et al.* 2017), enabling flexibility of placing the array in the field. The combination of detector frequencies associated with densities that had lower CVs had steep negative slopes of the lines of best fit. This means that CVs were minimized when most calls were heard across one microphone, and fewer were heard across two, three, four, five and six microphones. If the microphones surround a patch of calling frogs and the array is not very large, the frog calls are likely to all be picked up across six microphones. This was shown to yield high estimate error as the detection surface generated from this shows perfect detection, which falsely suggests that frogs can be detected at any distance from the microphones. Setting up an array in the field needs to minimize the possibility of all calls being picked up by all detectors. Therefore, it is important to have an irregular-shaped array that does not encase the entire area within the array where frogs are suspected to be calling from (Kidney *et al.* 2016; Measey *et al.* 2017) as this will ensure that not all calls are heard on all six microphones. Setting up an array in the field therefore requires an understanding of the aSCR technique, as the array will have to extend in such a way that some microphones are amongst the calling frogs, but that others are far enough to not pick up all the calls. In cases where there are discrete patches of calling animals, the array would need to be set up in such a way that some microphones are close to one patch, while other microphones are close to a different patch, given that the distance between patches does not largely exceed the lengths of the cables connecting the microphones to the recorder. It may also be necessary in the analyses to manually apply a “mask” over the area where frogs were definitely calling, in order to exclude “listening” to another patch.

Most of the outlier sites, with CVs above 100%, had only one or two frogs calling. It is not clear why the single sampling site where there were more than two frogs calling is an outlier, as detector locations, detection functions and calling locations appear fine. One of the sites was assigned an array configuration by the algorithm that did not match the sketch of the array produced in the field. For that site, a measurement error in terms of one or more of the distances measured between the microphones in the field may have occurred.

My study shows that the use of aSCR for studying amphibians is feasible, and aSCR could therefore be integrated into existing studies on amphibian populations. In the wake of a decrease in amphibian populations worldwide (Houlahan *et al.* 2000; Stuart *et al.* 2004; Grant *et al.* 2016), funding has been granted to either establish or support volunteer or non-volunteer-based bodies to monitor frogs, such as the North American Amphibian Monitoring Program (NA-AMP), Environment Canada, Frogwatch USA, Amphibian Research and monitoring Initiative (ARMNI; de Solla *et al.* 2005). The monitoring techniques they employ are largely qualitative (Sargent 2000; Crouch and Paton 2002). Such qualitative techniques are also employed for some amphibians in South Africa. For example, the other species in the *Arthroleptella* genus are regularly monitored through chorus-rank methods (A. Turner *pers. comm.*). However, with the aSCR model proven to produce reliable density estimates of *A.lightfooti,* the possibility exists of modifying the call recognition and extraction process for other anurans and applying the technique to obtain quantitative data. Since getting to calling sites of the other species in the genus is an investment already accounted for, the remaining costs would be a once-off purchase of appropriate equipment. Initially, both qualitative and quantitative techniques should be employed as processing the acoustic data to obtain density estimates does take time, whereas chorus-rank monitoring provides instant results. However, quantitative data over time can be used to reliably infer population dynamics, create more accurate predictive models, and make more informed decisions concerning management, and the benefits of this outweighs the costs.

The aSCR technique can be used in a cross-disciplinary framework to assist in evaluating impacts on amphibians in an increasingly human dominated environment. Conjoining population information with physiological (Hayes *et al.* 2010; Salinas *et al.* 2017; Zhelev *et al.* 2018) or behavioural (Caorsi *et al.* 2017; Lukanov *et al.* 2014) information would provide a more accurate representation of an amphibian’s state in terms of population densities in a changing world. For example, Zhelev *et al.* (2018) found that members of a frog species, *Pelophylax ridibundus,* from rice fields suffer significantly more stress than the members in a natural river. Examining whether stress is associated with population estimates would be the next step in a conservation setting. Predictive models could use these inputs from multiple disciplines to produce potential survival scenarios for species, which would be valuable input in management efforts. Yet, study animals need not be limited to anurans, but can extend to other acoustically active fauna such as birds, mammals, or insects. With the right sound extraction methods, aSCR could be applied to more than one calling taxon recorded in a soundscape. Multiple data gathering for species monitoring could take place simultaneously.

Improvements of this study should be taken into consideration in future research related to assessing the applicability of aSCR for studying populations of calling taxa. For example, the relationships between more variables that affect the reliability of aSCR can be assessed. One variable in the aSCR process that I did not consider in my analyses, but that could be contributing towards poor CVs when few animals are calling and in detector frequencies where calls were heard across one and across six microphones, is the selection of a signal strength threshold. It is important to note that detector frequencies were obtained from capture histories that had not gone through the signal strength cut-off step and contain detections that were omitted after the threshold was applied. At already low calling abundance, a signal strength threshold set to the median of signal strengths of all detections will result in an even smaller capture history on which the aSCR model has to run. Similarly, in the detector frequency where the majority of detections are split between being heard on one microphone and being heard across all microphones, those that are heard on one may have been omitted by the signal strength threshold (as they would have been frog calls picked up far outside the array). Therefore, at sites with these characteristics (low abundance of calls and detector frequencies as described above), the signal strength threshold needs to be "manually" lowered from the median. However, when working with analysing large datasets, stopping at every subsample to analyse characteristics before continuing is too time-consuming, and means to automate an analysis of abundance of calls and their distribution on detectors needs to be implemented. The proportion of density estimates with high CVs could potentially be significantly lowered.

The sound extraction from the soundscape step could also be improved with more expertise on the use of the sound extraction programme PAMguard. The parameters under which a call was identified as a moss frog often allowed segments of bird calls and trickles of water to be classified as frog calls. To avoid this misclassification, more detailed parameters could be implemented. Acoustic arrays that were set up amongst frogs but close to roads often had massive disturbance in the form of cars. From the call extraction, it was not possible to understand if the frogs were perturbed by the vehicles and stopped calling or whether the sound of the passing car masked the calls being picked up. While I suspect that it is the latter case, this can only be proven with a better understanding of implementing the software to function at its optimum.

Improving knowledge on the biology of the animal to which density methods are applied can be essential for accurate estimates and conservation efforts (Witmer 2005; Dorcas *et al.* 2009). For example, call rates were found to be more variable at low densities compared to high densities. Perhaps call rates can be more inconsistent when fewer males are calling and competition is limited, as the female may easily be able to distinguish between the locations of calling males. While at higher densities, call rates may have to be more consistent in order to direct females to the calling location in the midst of numerous other calls being emitted. Variability at low calling abundances could be taken into account and adjusted for in the aSCR model which could potentially minimize the error associated with density estimates from small densities.

Conclusion

The aSCR method is an effective density estimation tool for calling animals, and for the understudied and highly cryptic *A. lightfooti* populations*,* it functions well across a whole range of call densities. Knowledge on the optimal use of aSCR to estimate *A. lightfooti* populations has improved with the finding that aSCR should not be used when only a few (1 to 5) of frogs are calling. Further assessments of the technique should focus on means to automate the selection of an ideal signal strength threshold. The question of an upper threshold in the relationship between received calls and CVs can still be addressed with more sampling or simulations where "saturation" in terms of calls per unit area has taken place. The finding that aSCR is practical to apply in the field and functions well in terms of reliability can direct sampling efforts that rely solely on qualitative data to include the use of aSCR.

CHAPTER 3

A bird's eye view on flirting frogs: studying calling behaviour of *Arthroleptella lightfooti* using Acoustic Spatial Capture-recapture and drone imagery

INTRODUCTION

An awareness of spatial distributions of animals within conservation areas is important for adequate management (May 1986). While many studies focus on the distribution of populations of a species (MacArthur 1972; Phillips *et al.* 2006; Guisan and Zimmerman 2000), the distribution of members within a population in a highly specific habitat (referred to as microdistribution from hereon), can provide insights into their behaviour (Loertscher *et al.* 1995; Jenkins 1969). The behaviour of animals in response to biotic and abiotic factors in their environment influences intraspecific distribution patterns. Human-induced changes to a microhabitat can therefore influence the behaviour of animals within a microhabitat. If a human-induced alteration of a habitat (a removal or addition of a feature) causes the microdistribution of a species to change in such a way that there are negative fitness consequences for the species, or a cascade of negative effects for the rest of the community, wildlife managers must seek practical means to restore and maintain the microcommunity in a functional state. Knowledge of the factors that influence microdistributions of species is becoming increasingly important as conservation areas become smaller, more fragmented, more frequently visited by an increasing number of people, or more isolated within an anthropogenic-dominated matrix (Atmar and Patterson 1993). However, a simple cognisance of distributions is not enough for the implementation of effective management: accurate knowledge based on evidence is required for informed management decisions (Pullin *et al.* 2004). Therefore, research needs to be invested in techniques that can be used to study distributions of animals at microsites and this is particularly important in protected areas where endemism across taxa is high.

The Cape Peninsula is an area of high conservation value as it is part of a biodiversity-rich area known as the Greater Cape Floristic Region (Myers *et al.* 2000; Rebelo *et al.* 2011a; Born *et al.* 2007). There are 307 endemic species on the Cape Peninsula and 332 plant and animal species listed as threatened on the IUCN Red List (Rebelo *et al.* 2011b). Approximately 24 000 ha of the Cape Peninsula is conserved within the Table Mountain National Park (TMNP), which runs the length of the Cape Peninsula and includes 80% the Table Mountain Chain (Helme and Trinder-Smith 1996). It gained the status of a World Heritage Site due the exceptional biodiversity it contains (van Wilgen 2012). The northern half of the park is largely surrounded by the ever-growing metropolis that is Cape Town, and it includes areas of both public and privately managed land (Ralston and Richardson 2004). Human disturbance in TMNP is evident by the presence of habitat alterations such as roads, footpaths, and the occasional buildings such as huts (Ralston and Richardson 2004).

During the rainy, austral winter season, water is abundant in the Cape Peninsula in the form of streams and seepage areas (Le Maitre *et al.* 1996). These seepages form microhabitats that are transitional zones between terrestrial and aquatic environments and can be rich in terms of species diversity (Williams and Dodd 1978). However, these microhabitats can be altered by human presence through establishment of footpaths (Alston and Richardson 2006) as many footpaths can run close to or through seepage areas and streams. Deviations in the microdistributions of indicator species at these microsites can be reflective of an alteration of the microsites due to human interference. In 2008, TMNP was the most visited park on the continent with 3.5 million visitors

(Ferreira 2011; Sinclair-Smith 2009 cited in Rebelo *et al.* 2011a), and Donaldson (2017) estimated an annual increase in visitors to 3.6 million in 2016.

Unique species for terrestrial-aquatic transitional zones include water-dependent fauna such as frogs (Gibbs 1998). Frogs make use of the areas along streams because these areas provide a sheltered, wet microhabitat that can be climatically more stable compared to the environment immediately outside (Picker 1996). Amphibian diversity in TMNP is rich compared to the rest of South Africa (Colville *et al.* 2014; Turner and Channing 2008; Rebelo *et al.* 2011a). There are low- and high-altitude amphibian species and both categories are highly threatened by anthropogenically induced change (Mokhatla *et al.* 2015). Frogs often react to a habitat change by dispersing to other suitable habitats (Blaustein *et al.* 2010). Many amphibians have poor dispersal capabilities (Sinsch 1998; Smith and Green 2006; Blaustein *et al.* 1994), but there are various factors that compound the challenge of dispersal for the amphibians of TMNP. Most of the Cape Peninsula is surrounded by the Atlantic Ocean, and the rest is in close contact with the metropolis. The lowland amphibian species are therefore mostly confined to the margins and fragmented lowland areas of TMNP due to the surrounding environment either being human dominated or rising steeply to form mountains with unsuitable habitats. The high-altitude amphibian species are limited in their distribution possibilities by their specific habitat requirements found at the higher altitudes on the mountain, and these montane habitats are often separated by lowland areas with unsuitable habitat (Tolley *et al.* 2010).

The only endemic vertebrates on the Cape Peninsula are amphibian species: The Table Mountain ghost frog *Heleophryne rosei,* Rose's Mountain toadlet *Capensibufo rosei,* the Flat dainty frog *Cacosternum platys,* and the Cape Peninsula moss frog *Arthroleptella lightfooti* (Picker and Samways 1996; Channing *et al.* 2017; Channing *et al.* 2013; Channing 2004). The members of the genus *Arthroleptella* are endemic to south-western South Africa (Minter *et al.* 2004), and the Cape Peninsula moss frog, *Arthroleptella lightfooti,* which can only be found in mossy seepages on the Cape Peninsula (Channing 2004), is currently assessed as Near Threatened by the IUCN (SA-FRoG & IUCN 2017). The specific habitat, in combination with the distribution limitations associated with high altitude amphibians on the Cape Peninsula, renders change to their habitat potentially threatening to the survival of the species. Using this species to examine the effect of human-induced disturbance on the transitional zones on the mountain is therefore valuable – not only for understanding the future of *A. lightfooti* in the light of anthropogenic disturbances but the effects of disturbances of microhabitats on high altitude amphibians in general. However, determining the use of microhabitats by studying the locations of *A. lightfooti* within the microhabitats is particularly cumbersome as the species is visually cryptic and very small, with males and females having mean snout-vent lengths of 14 and 15 mm, respectively. The males call during the austral winter, but even with calls revealing the presence of the frogs, it can take up to four person-hours to locate a single individual (Stevenson *et al.* 2015). Thankfully, the emergence of new techniques to study acoustically active animals provides potential to study these frogs within their microhabitats.

Acoustic Spatial Capture-recapture (aSCR) is a statistical method used to estimate densities of acoustically active animals and which provides information on their estimated calling location (Efford *et al.* 2009a; Marques *et al.* 2013; Stevenson *et al.* 2015). The aSCR method makes use of multiple microphones attached to a recorder, known as an "acoustic array", to detect the calls emitted by animals in the field (Borchers 2012). The microphones act as detectors of known locations upon which an animal call can be detected, and the same call can be detected across multiple microphones (Efford *et al.* 2009b). Knowing which microphones (also referred to as detectors) did and did not detect a call is informative about the location of the emitted call, and probabilities of an estimated calling location can be constructed from this information (Efford *et al.* 2009b). Additional information about calls at microphones include the time of arrival of calls at the different microphones and the signal strength, or how loudly the call was detected, of the calls on microphones which detected them (Borchers 2012; Stevenson *et al.* 2015). Incorporating auxiliary information from the calls into the estimated call location can narrow down the probability of where the call was emitted. One important feature of the aSCR technique is the determination of an estimated sampling area based on the information of calls received across the different microphones (Efford *et al.* 2009b). Frogs are assumed to be calling in a homogenous distribution in the estimated sampling area obtained by aSCR. However, frogs are often limited in their distribution by the shape of the habitable seep; some seeps are long and slim (typically on the side of a slope), while other seeps can cover a large flat area. Obtaining the locations of each calling individual relative to the array of microphones provides a means to effectively compare the distribution of calling *A. lightfooti* across topographically different microsites.

In this study, I aimed to examine the distribution of calling *A. lightfooti* males within their microhabitats using aSCR. I hypothesized that the presence of natural features such as rocks and streams, as well as anthropogenic features such as footpaths running through a microhabitat, will influence the distribution of calling frogs. I hypothesized that males may call farther away from a footpath as calling close to a footpath could mean regular disruptions (in the form of passing humans) compared to other positions within the microsite.

MATERIALS AND METHODS

Study Species

Arthroleptella lightfooti (Boulenger 1910; Anura: Pyxicephalidae) is a moss frog endemic to the Cape Peninsula (Channing 2004). It is a terrestrial species: these frogs do not rely on or inhabit standing or flowing water for any stages of their life-cycle (Altig and McDiarmid 2007). Instead, they inhabit seepage areas that are often characterized by moist, moss-covered soil. Breeding takes place during the austral winter, which is when males become vocally active and emit chirp-like advertisement calls to attract females during the day (Channing 2004).

Study Sites

Permission was granted by South African National Parks (SANParks) to sample at 10 sites (Appendix B.1) across the Cape Peninsula where the use of a small drone (DJI Phantom Standard 3, Dà-Jiāng Innovations Science and Technology Co., 2015) was deemed acceptable: two sites at southern Table Mountain National Park (TMNP), three sites at central TMNP, and five sites at northern TMNP. The agreement of taking aerial photographs included that a SANParks ranger be present during the proceedings. Since calling behaviour was assessed, *A. lightfooti* had to be present and calling at these sites. Sites were chosen in order to cover a range of features within and across sample sites: presence of standing water, rocks, tall vegetation (above one meter), short vegetation (below one meter), bare ground, and evident seepage patches present.

Experimental Method

Setting up the acoustic arrays

At each sampling site, an acoustic array that consists of a Tascam recorder (Tascam, TEAC, Wiesbaden, Germany) from which six omni-directional microphones (Audio-Technica AT8004 Handheld) extend was placed amidst the calling frogs. Recording the frogs’ calls took place for 45 minutes and sampling sites were visited near the end of the calling season in September 2017 (see Experimental Method in Chapter 2). Acoustic arrays could only be put up when there was no rain or excessive wind. Measurements of the distances between each pair of microphones to the nearest centimetre were obtained using a 30 m tape measure. The positions of the microphones relative to prominent features at the site were captured with a sketch, together with the locations where frogs were estimated to be calling using human ears.

Aerial imaging

The mapping tool feature of the mobile application Pix4Dcapture (v.4.1, Pix4D Capture, 2017) was used to create a flight plan for the drone at each site on a tablet (iOs 8.1, Apple Inc., 2014), which was termed a "mission". In the application, a rectangular polygon (about 100x100m) was drawn over the site at which the array was set up and this shape represented the area that the drone had to cover: it would traverse from one side to the other while taking photographs. Aerial photographs were taken with the Phantom 3 Standard built-in camera of 12 mega-pixels at a height of 20 m. Sufficient overlap between the pictures was required for successful stitching of the photos to take place. The frequency of photographs that was needed to obtain a fully stitched image of the area was automatically calculated into the flight plan. The drone could then be launched and the mission could be started and completed autonomously.

Spatial Capture-Recapture

The acoustic data was processed with Acoustic Spatial Capture-recapture models (aSCR) using the package "ascr" (Stevenson and Borchers 2017) in R (v3.3.1, R Core Team, 2016). The information on calls received was fed to the aSCR model after the frog calls were extracted from the surrounding soundscape using an open source software called PAMguard (v1.14.00 BETA; Gillepsie *et al.* 2008; www.pamguard.org). Another essential input for the aSCR model was the array formation, which was obtained using the sketch of the positions of microphones in the field and the distances measured between the microphone pairs. The data preparation and analysis are described in detail in Chapter 2. Due to the computational time and effort required to process the acoustic data, ten subsamples of one minute were chosen to be run through the aSCR model. Selected samples needed to be free of bird calls that could be misidentified as frog calls and other major acoustic interference (for example, gusts of wind or a passing helicopter) that could affect the aSCR model output.

For every subsample, the estimated locations of calling animals were produced in the form of a plot containing an aerial view of the array formation and the estimated locations of frogs in relation to this (Appendix B.2). The *fit.ascr* function in the ascr package requires that an area greater than a potential estimated sampling area be provided in order to plot the array and, after running the aSCR model, the estimated sampling area. This larger area will be referred to as a "habitat mask". The habitat mask is a discretized continuous habitat, where the points that it is discretized into consist of potential points where frogs could be calling (Appendix B.3; Efford 2017). A function called *locations* uses the aSCR model output to calculate how likely it is that a call originated from a particular point on the mask (Appendix B.4).

The most representative subsample from a site in terms of the estimated calling locations of animals was chosen to be examined in conjunction with the drone imagery. This was a subsample where there was the most agreement on estimated calling locations of animals (i.e. where there was one dot from the mask representing the location of an individual, instead of a cluster or streak of dots; Appendix B.5), and they tended to have the smallest errors associated with the density estimates.

Overlaying the aerial image with the aSCR output

The aerial photographs were stitched together using the Pix4Dmapper programme (v3.3.29, Pix4D, 2017). I used Photoshop CS6 (v13.0, Adobe Systems Inc., 2012) to import the stitched drone image and the output from aSCR. These were matched up and the drone image was overlain with the aSCR output. The estimated sampling area was obtained from the detection function associated with the chosen subsample. The detection function describes the probability of detecting a frog call, given that it is a certain distance from a microphone. Using the function *show.detfun* in the ascr package provided the border of an estimated sampling area relative to the microphones in the array based on the detection function determined from the capture histories of the selected subsample (Appendix B.6). This, too, was matched up with the drone image using exact locations of microphones in both images, and the estimated calling locations. Lastly, I constructed a 5x5m grid in Photoshop and placed this over the images.

Data Analyses

Each of the 5x5 m grid cells within the estimated sampling area was coded according to an alphabetical x-axis and a numerical y-axis. Then, each grid cell was examined for the presence and/or absence of the following features which were each coded as binary variables per sample square: estimated calling locations of *A. lightfooti,* standing water, bare ground, rock, footpath, vegetation below one meter, vegetation above one meter and wet/seepage ground. Seepage ground consists of moist ground that is not inundated with water. Of the coded features from each grid cell, the response variable was the presence of *A. lightfooti* (when there was an estimated calling location within a cell), while the predictor variables were the presence/absence of standing water, bare ground, rock, footpath, vegetation below one meter, vegetation above one meter and wet/seepage ground. A binomial logistic regression was run in R to determine the relationship between the presence/absence of *A. lightfooti* and the presences/absences of the predictor variables. When running logistic regressions with small datasets, it is possible for a phenomenon known as "perfect separation" to occur, where the response variable separates the predictor variable completely (i.e. when the predictor variable is present, then the response variable is always present, and when the predictor variable is absent, the response variable is *always* absent). Perfect separation can yield models that are extremely inaccurate (Heinze and Schemper 2002; Lesaffre and Albert 1989). If perfect separation occurred, I used a form of penalized regression in the package "glmnet" (Friedman *et al.* 2010) to examine the relationships between the response and predictor variables.

RESULTS

Obtaining aerial images at designated sites could only take place when the weather was viable (no rain or wind) and when a ranger was available for supervision. Coinciding these two events reduced potential site visits to only five. Acoustic arrays were erected at these five different sites and aerial image data were collected successfully for three of these sites, which will be referred to as Site 1, Site 2 and Site 3 (Table 3.1). Site 1 was characterized by a linear seepage, no footpaths and mostly low vegetation (Figure 3.1). The seep at Site 2 was more extensive compared to Site 1 and vegetation within the estimated sampling area (ESA) of Site 2 was mostly tall and interspersed with numerous large boulders and rivulets of near-still water (Figure 3.2). Site 3 had a footpath running through it which also branched off and which was present in about 43% of the area covered by the ESA (Figure 3.3). There was a mix of tall and short vegetation and the seepage was fairly narrow compared to Site 2.

The estimated calling locations of frogs from Site 3 that were overlaid on the aerial image did not reflect the locations where frogs were noted to be calling from in the field (Figure 3.4). The standard error associated with the density estimate as a coefficient of variation is 18.2% for Site 3. The detector frequency, or combination of microphones across which calls were heard, has the pattern associated with reliable density estimates where most calls were heard on one microphone and fewer calls were detected across all six microphones (see Chapter 2 for details). The detection function, which described the probability of picking up a frog call as a function of distance from the microphones follows an intuitive trend: it starts at a probability of one at distance zero from the microphones and decreases as the distance from the microphone increases. I continue to use this site in subsequent analyses as it is possible that more individuals started calling when I left the acoustic array during its recording period of 45 minutes.

Running separate logistic regressions for each site revealed errors of perfect separation. When running each site with penalized regressions there was no significant relationship between any of the predictor variables and the presence of frogs for Site 1, Site 2 or Site 3. Coalescing all the cells from the three sites into a larger sample set solved the problem of perfect separation. Running a logistic regression on this larger dataset revealed a significant relationship between the presence of water and the absence of *A.lightfooti* as well as the presence of seepy ground and the presence of *A. lightfooti* (β = -2.81, $p < 0.05$; and β = 2.09, $p < 0.05$ respectively). The odds of calling frogs being present in an area of 5 x 5 m^2 with a seepage present compared to one with no seepage is 708.15%, while the odds of calling frogs not being present in an area without water to one with water is 93.99%.

Table 3.1. The three sites in Table Mountain National Park (TMNP) at which acoustic data for the aSCR model were recorded and aerial images obtained.

Site	Section of TMNP	Coordinates	
		Latitude	Longitude
1	Northern TMNP	-33.9727	18.39776
2	Central TMNP	-34.0607	18.38455
3	Northern TMNP	-34.9666	18.40676

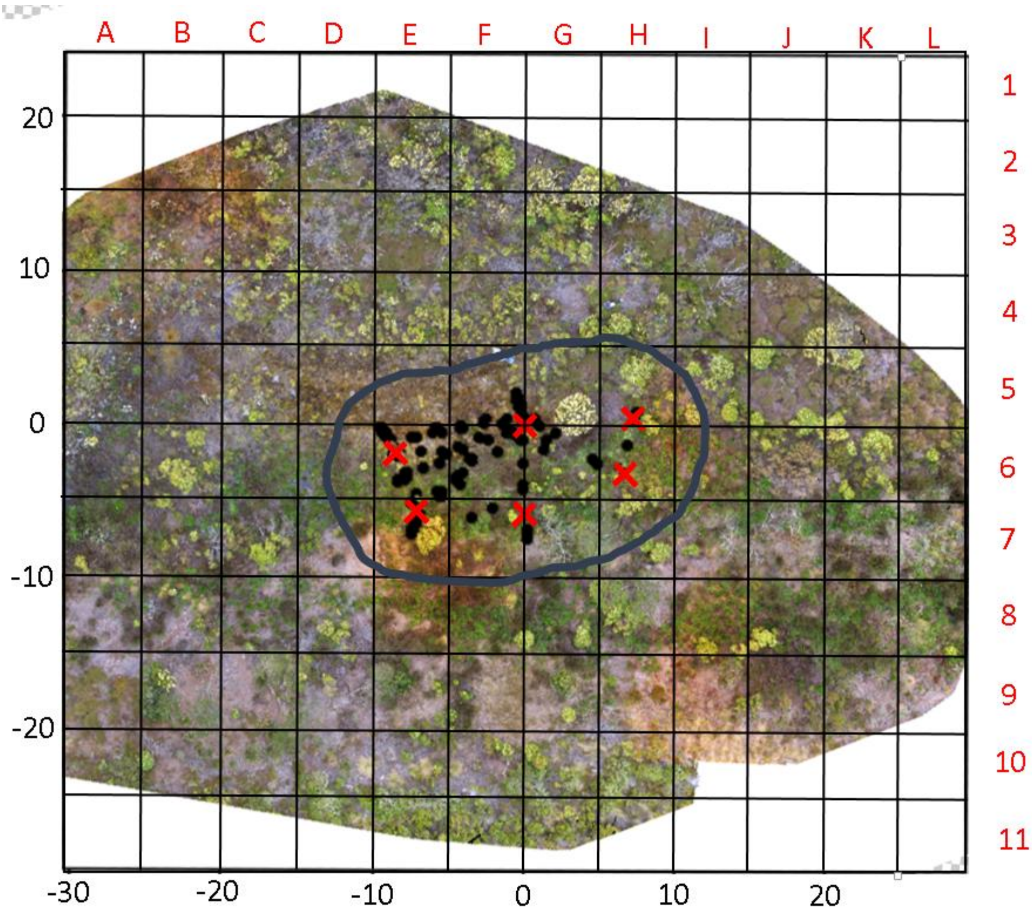


Figure 3.1: Site 1 where acoustic arrays were put up and aerial images obtained. The red crosses represent the positions of the microphones in the acoustic array. The black dots are the estimated calling locations of frogs. The shape surrounding the array and calling frogs marks the edge of the estimated sampling area associated with that particular array as was determined by the aSCR model.

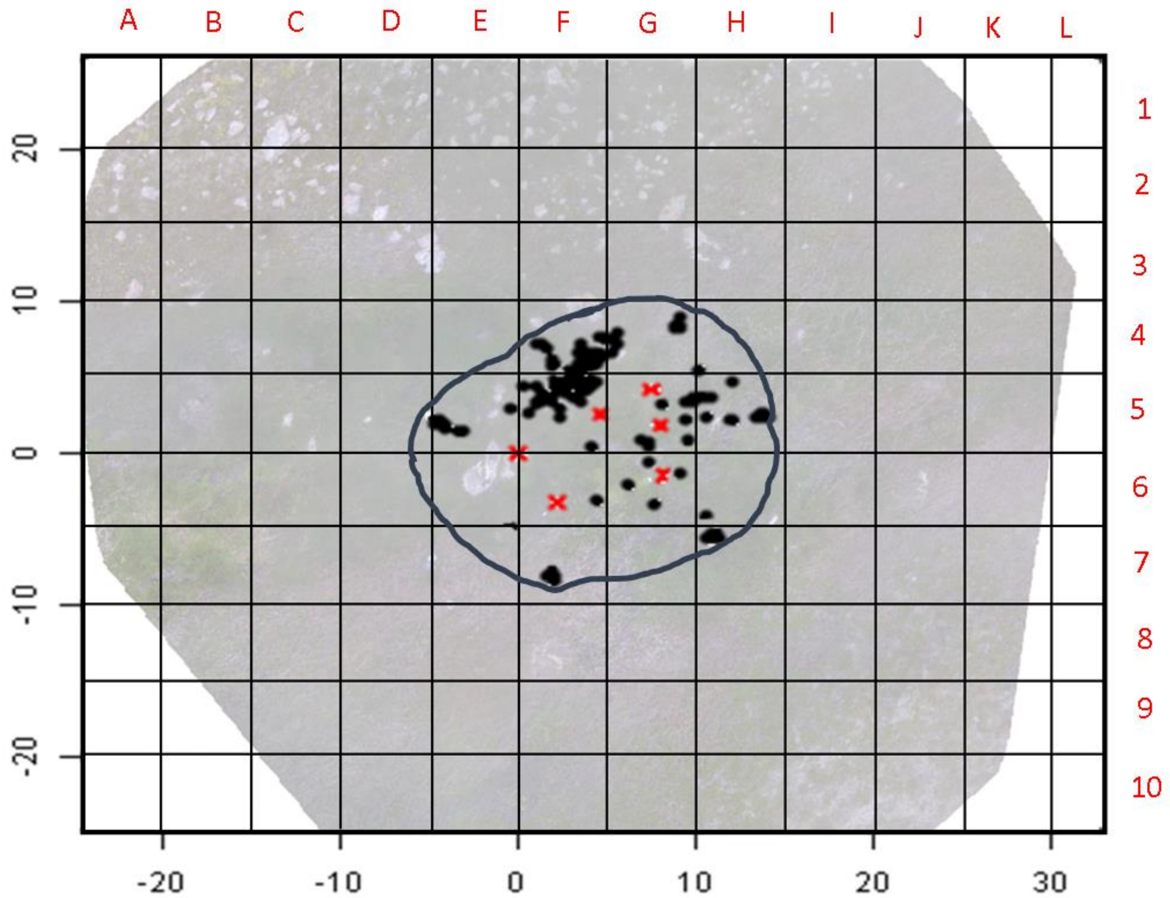


Figure 3.2: Site 2 where acoustic arrays were put up and aerial images obtained. Images were obtained on a cloudy day. The red crosses represent the positions of the microphones in the acoustic array. The black dots are the estimated calling locations of frogs. The shape surrounding the array and calling frogs marks the edge of the estimated sampling area associated with that particular array as was determined by the aSCR model.

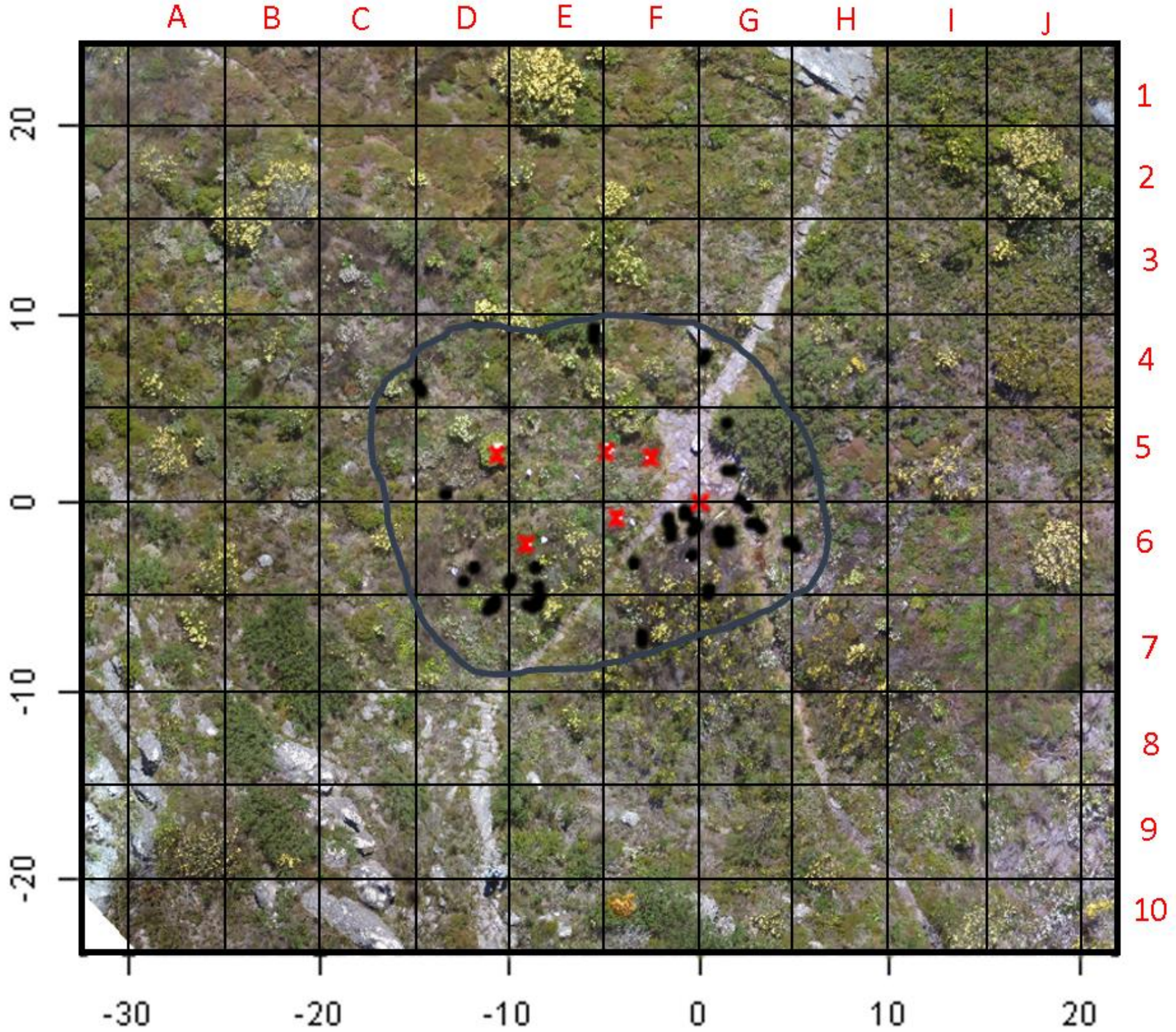


Figure 3.3: Site 3 where acoustic arrays were put up and aerial images obtained. The red crosses represent the positions of the microphones in the acoustic array. The black dots are the estimated calling locations of frogs. The shape surrounding the array and calling frogs marks the edge of the estimated sampling area associated with that particular array as was determined by the aSCR model.

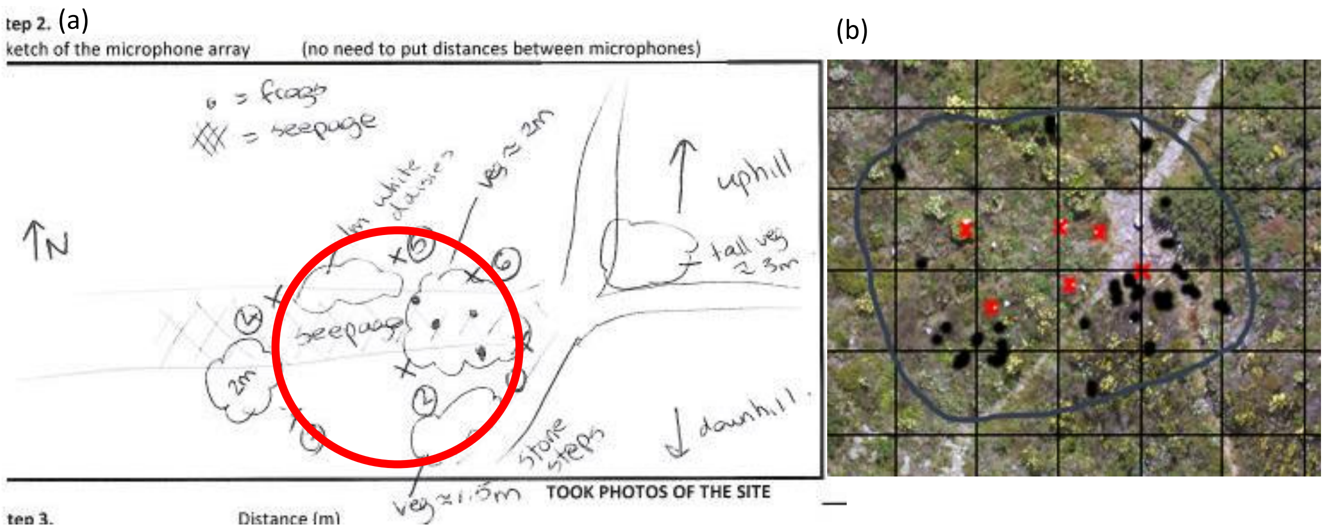


Figure 3.4. The comparison of a) the estimated calling locations recorded on a sketch in the field (where the red circle highlights where frogs were estimated to be calling from using human ears) and b) the estimated calling locations produced by the aSCR model, indicated with black dots. The red crosses represent the positions of microphones in the array. Frogs were heard on only one side of the footpath in the field, but were estimated to be calling from all around the footpaths by the aSCR model.

DISCUSSION

I found that the presence of calling *Arthroleptella lightfooti* males was significantly associated with the presence of wet, peaty seepage ground. There was also a significant lack in the presence of calling frogs where standing or near-still water was present. Many anurans are not restricted to water for all their life stages and are not adapted (morphologically or behaviourally) to thrive in water. For example, some species brood eggs in pouches on the female instead of water (Del Pino *et al.* 1975) or lay eggs in foam nests above water where development takes place until the tadpoles drop into water (Heyers and Rand 1977). Other frogs do not rely on immersion in water for any of their life stages and are either true direct developing terrestrial species where a fully formed adult hatches from the egg, such as *Eleutherodactylus coqui* (Callery *et al.* 2001), or the frogs have a tadpole stage that develops in moist conditions outside water, such as certain *Adenomera* species where tadpoles develop in foam underground (Kokubum and Giaretta 2005). In the latter case, the tadpoles tend to be endotrophic, or non-feeding, and reach metamorphosis relatively quickly (Wake 1980; Del Pino *et al.* 2004; Dawood and Stam 2006). Standing water is not required for *A. lightfooti* eggs as they are laid in wet tussocks of vegetation. When the larvae hatch, they remain in these moist sites without feeding until metamorphosis is complete after 10 to 14 days (Morgan *et al.* 1989). The morphology of an adult *A. lightfooti* is not well adapted for the locomotory requirements for a life stage in water, which could explain the dissociation between water and the presence of calling males. In addition, when *A. lighfooti* tadpoles are placed in water, they do not attempt to swim (Rose 1950), which has also been observed with other direct developing species (Wake 1980). For terrestrial species, moisture in the environment is essential to avoid desiccation at all of the life stages (Mitchell and Gatten 2002). Seepage sites offer the moist conditions necessary for the successful development of all life stages of *A. lightfooti.* For males calling from these suitable oviposition sites, the possibility of mating success may be higher compared to males advertising from less suitable sites as is the case for the American Bullfrog *Lithobates catesbeiana* and Green frog *L. clamitrans* (Wells 2007). In this study, I suggest that the seepage areas and the inundated areas in the microhabitats of *A. lightfooti* influence the calling behaviour of the male frogs in terms of calling spatial distributions within a microsite. Conserving or properly managing these microsites, especially maintaining the seepage areas and managing inundated water, is beneficial for the persistence of this threatened, endemic species.

While the Cape Peninsula is an area of high conservation importance due to its impressive assemblages of endemic species, it also has recreational, economic and spiritual value for humans (Picker and Samways 1995; Cowling *et al.* 1996). The precarious position of a growing metropolis surrounding the isolated and protected Table Mountain National Park has resulted in research efforts that are relevant to the management and conservation of a range of taxa that inhabit various different habitats. Knowledge of the presence of habitat through the calling distributions of *A. lightfooti* can now be added to the database of studies that guide conservation, which includes research on cave-dwelling invertebrates (Sharratt *et al.* 2000), mammals such as Cape clawless otters and baboons (Okes 2017; Lewis and O'Riain 2017), birds such as peregrine falcons (Jenkins and Ben 1998), and other studies on endemic amphibians such as Rose's mountain toadlet *Capensibufo rosei* (Cressey *et al.* 2015; Edwards et al 2017).

While the presence of footpaths was not significantly linked to the presence or absence of *A. lightfooti* in this study, only one site was examined with this feature and there was uncertainty surrounding the estimated call locations for that particular site. More sampling sites need to be visited and aerial imagery and calling locations obtained to make conclusions based on quantitative analyses about footpaths. Based on personal observation, I suspect that a greater sampling effort will reveal that footpaths do affect microsite use by the moss frog. In addition, *A. lightfooti* occurs in sympatry with *C. rosei,* for which management action in terms of diverting hiking traffic that affected breeding sites has taken place. At sites in Table Mountain National Park (TMNP) where seepages and footpaths interact, seepages cause footpaths to get muddy, which results in visitors walking into the surrounding vegetation (and stepping on tussocks of grass where *A. lightfooti* would be calling from) to avoid the mud. In addition, footpaths have been shown to erode and widen (Lance *et al.* 1989), which affects the vegetation on either side of the path and could minimize the microsites available to *A. lightfooti* from which to call, mate and lay eggs. A management effort can include the barring of access to walking trails that run through delicate seepage areas during the Cape Peninsula moss frog breeding season. Alternatively, paths that cross seepage areas could be re-routed.

Due to the cryptic nature of *A. lightfooti*, research on this endemic species has been sparse. The most comprehensive formal research was conducted by Dr Andrew Turner for his Doctoral thesis at the University of Western Cape on the phylogeography and speciation of frogs in the genus *Arthroleptella* (Turner 2010). The habitat preference for the different species of *Arthroleptella* was discussed, but the distribution of calling males at microsites was not addressed. Nor do the current published studies involving *A. lightfooti* and aSCR address the application of aSCR to study calling behaviour in terms of distribution of calling frogs (Stevenson *et al.* 2015; Borchers *et al.* 2015; Measey *et al.* 2017). Therefore, this study adds to the knowledge of this cryptic species by quantifying the significant relationship between calling locations and the seepage spots in a microhabitat. This investigation is not only valuable for quantifying a relationship observed in the field but also for testing the scope of the use of aSCR in terms of the estimated calling location output. It provides a foundation for future studies that make use of outputs from aSCR to investigate animal behaviour.

The estimated calling location output from aSCR can be applied to study distribution behaviour of other cryptic anurans, including other members of the genus *Arthroleptella*. Differences between the species have been investigated through the classification of calls, morphological measurements, and through examination of the percentages of differences between mitochondrial and nuclear DNA (Turner *et al.* 2004; Turner and Channing 2008; Channing *et al.* 1994). Behavioural differences in terms of the use of microhabitats have not been examined. As the characteristics of *Arthroleptella* are very similar, one hypothesis could be that the distribution of calling males within the moist habitats occupied by the different species are similar. For Critically Endangered species such as *A. rugosa* and *A. subvoce,* which only occupy areas of 14 and 0.06 km^2 respectively (IUCN 2017; Turner *et al.* 2004; Turner and Channing 2008), the relationship between environmental factors and the distribution of calling males can provide insights into land management that maximizes persistence of the species by encouraging environments that offer suitable microsites. It is possible that the estimated call location output can be applied on larger arrays to locate calling individuals of very rare anuran species, such as the Critically Endangered *Heleophryne rosei* which, like *A. lightfooti,* is highly visually cryptic but vocally active. *Heleophryne rosei* is endemic to Table Mountain (Boycott and de Villiers 1986) and using aSCR would not only provide opportunities to locate and study this rare frog but also to identify the use of the environment by breeding males. This would be particularly interesting for *H. rosei* as adults have been found to travel great distances from the ponds that contain tadpoles (Boycott and de Villiers 1986), and the use of aSCR could reveal relationships with environmental factors that explain this. Other applications of aSCR and the estimated call output are not only useful in conservation studies but can also be applied to important research concerning food security. Bioacoustics is already being applied in the pest industry to test for the presence of pest insects in container crops or stored goods (Mankin 2011;

Mankin *et al.* 2011). Being able to locate (and not only determine presence or absence) a calling pest can facilitate eradication.

This study has been useful in establishing a protocol to examine the calling locations of acoustic animals using aSCR and aerial imagery. Due to the small sample size to which this protocol has been applied, I suggest that a repetition of this study with more sample sites be conducted in order to affirm the relationships found here. In particular, an investigation of the lack of presence of frogs around still water, as was found in this study, would provide valuable insight on this species' natural history, because wet peaty ground often surrounds water and the lack of frogs could be due to other factors affecting calling distribution. Since the site with footpaths in this study yielded results that did not align with the ground-truthing of the estimated call locations, further investigation is also necessary. Data gathering of features at the site could also be more detailed in successive studies, where the species of plants and even the location of other calls in the soundscape could be taken into account in the analyses of calling frog locations relating to environmental features. The use of drones in National Parks and the rules and administration that surrounds it, especially in the context of research, is a relatively new development, and until it becomes commonplace to request a permit for research, obtaining permission will remain a lengthy procedure.

For the aSCR model, the frogs are assumed to be distributed independently according to homogenous Poisson point process (Stevenson *et al.* 2015). From observations in the field over the last two years, this is not commonly the case, although I suspect that an approximate uniform distribution of frogs could be possible at sites with barely any slope and seepages that are spread (in contrast to thin seepages down slopes). From the estimated calling locations at sites from this chapter, the frogs are also not distributed uniformly. Allowing for density estimates (with accompanying call location estimates) from heterogeneous calling densities within the estimated sampling area of an array could improve the precision of the estimated call locations. In addition, I was limited to the use of six microphones due to the number of ports for microphones on the recorder. However, the use of more microphones might provide more accurate probabilities of calling locations for frogs, as the aSCR model would have more information from detections to run on. However, a maximum number of detectors required for accurate use of aSCR may exist, above which the addition of more microphones would be superfluous. This would need to be determined with experimentation. This study of calling locations could be improved with more knowledge on the behaviour of *A. lightfooti* in terms of the rate of emitted calls under different circumstances. From Chapter 2, I expect that the calling rate is density dependent, with males calling more consistently at higher calling densities. Advertising males in some frog species exhibit a different behaviour when calling alone compared to when calling en masse (Ryan 1985). Formally testing this hypothesis is necessary to improve not only the understanding of the behaviour of the species but it can aid in improving the aSCR model that assumes a constant call rate.

This study on determining the spatial distribution on calling *A. lightfooti* within a microhabitat using aSCR and aerial imagery is a starting point from which the distributions of cryptic, but acoustically active taxa can be studied. For *A. lightfooti*, more sample sites need to be examined to define reliable relationships between features in the microhabitat and the presence of calling males. However, the protocol described and applied in this chapter is a base from which many studies can stem, and contributions from its use to management and conservation could prove invaluable.

Chapter 4

General Conclusion

The use of aSCR was found to be appropriate across a large range of calling abundances. A lower threshold of 111 calls.min^{-1} was found for the average number of calls from male *Arthroleptella lightfooti* received by an array below which the density estimates obtained by aSCR were considered unreliable. The standard errors were represented as coefficients of variances (CVs), and these CVs were considered to be unreliable if they exceeded 30%. In terms of calling frogs, this threshold value represents about five frogs calling. Therefore, when five or fewer frogs are calling at a sampling site, the use of aSCR is not ideal for the estimation of calling animal densities. This finding will significantly improve the speed of data collection, because arrays would not be put up where less than five frogs are calling. If two frogs are calling very near each other and almost at the same time, it may be difficult to discern whether it is one or two individuals calling. At more homogenous distributions of calling frogs, it is fairly easy to distinguish between less than five calling individuals. However, as arrays would not be put at such low calling abundances, an alternative protocol needs to be employed to estimate density. If frogs can be confidently distinguished from one another, the number of calling frogs for 10 x 1 minute over a period of 45 minutes can be identified by the observer. This will be comparable to the protocol that involves setting up arrays for aSCR, where ten subsamples of one minute are used in the aSCR model to estimate calling animal density at a sampling site. The mean of these samples will represent the mean calling frog abundance. However, if there is uncertainty concerning the number of calling frogs, the number of calls received could be recorded for 10 x 1 minutes instead. However, a sampling area will need to be determined, and a set protocol to keep this constant will need to be established. It is possible to take mean of the estimated sampling areas from aSCR and to apply that as a sampling area when few frogs are calling.

The presence of *A. lightfooti* males was significantly related to the presence of wet, seepy areas within the habitat delineated by estimated sampling areas obtained from aSCR. While this has been observed in the field, this is the first time the relationship is quantitatively explored. The frog eggs and tadpoles require the moisture provided by the seepage areas to avoid desiccation and to develop normally. Attracting females to these seepage spots for mating and egg laying increases the potential of the offspring to survive. In addition, there was a relationship between the presence of calling *A. lighftfooti* males, and the absence of standing water. Unlike some other members of the family Pyxicephalidae, *A. lightfooti* did not evolve to inhabit water. Adults do not have the morphological features required by life stages in water (large, webbed feet for example), and tadpoles are also direct developing and do not require water. However, while these quantitative relationships are reflective of the behaviour observed in the field, these relationships are based on a small sample size of data (n=3) as the larger, intended sampling could not take place. I recommend a repetition of the sampling process of putting up acoustic arrays and obtaining aerial images, however, at a much larger scale, with adequate variation between sites to avoid bias. The use of more microphones in the array should also be explored to see if more accurate results in terms of calling locations of frogs could be obtained. Furthermore, experiments on the array formation on the same population may reveal more guidance as to the ideal manner in which to put up an array at a calling site.

The results from this study can be used to inform monitoring plans that could be developed, for the first time, to examine the populations of *A. lightfooti* over time. Quantitative monitoring through aSCR can be applied to all the species of the moss frog genus, as they are all threatened and inhabit restricted ranges, and are all too small to monitor with traditional quantitative techniques. The only major adaptation between monitoring different species would be in extracting the calls from the soundscape using PAMguard and using different call classifications suited to each species. Until individual call recognition can take place (where an individual is recognized by the signatures of his specific call), then the call rate per minute for each of the different species needs to be determined. The aSCR model can be used to study populations recovering from fire, which will provide information on one of the major threats to the genus.

These frogs are species that are endemic to the Greater Cape Floristic Region (GCFR) (Tolley *et al.* 2014). As the ecological role of *A. lightfooti* and other members in the genus have not been studied, their failure to survive may have unforeseen ecological consequences. Studying and conserving their populations may therefore benefit the fynbos ecosystem in ways that we are not aware. As the GCFR is host to endemic flora and fauna and the biodiversity is threated by habitat loss and invasive alien species (Allsopp *et al.* 2014), it is necessary to conserve and maintain ecosystems in a functional state. The aSCR technique is an addition to a host of techniques that can help achieve this purpose.

REFERENCES

Allsopp, N., Colville, J.F., and Verboom, G.A. 2014. *Fynbos: Ecology, Evolution, and Conservation of a Megadiverse Region.* Oxford University Press, United Kingdom.

Alston, K.P., and Richardson, D.M. 2006. The roles of habitat features, disturbance, and distance from putative source populations in structuring alien invasions at the urban/wildland interface on the Cape Peninsula, South Africa. *Biological Conservation* **132:** 183-198.

Altig, R., and McDiarmid, R.W. 2007. Morphological diversity and evolution of egg and clutch structure in amphibians. *Herpetological Monographs* **21:** 1-32.

Atmar, W., and Patterson, D. 1993. The measure of order and disorder in the distribution of species in fragmented habitat. *Oecologia* **96:** 373-382.

Bellemain, E., Swenson, J.E., Tallmon, D., Brunberg, S., and Taberlet, P. 2005. Estimating population size of elusive animals with DNA from hunter-collect feces: four methods for brown bears. *Conservation Biology* **19:** 150-161.

Blaustein, A.R., Wake, D.B., and Sousa, W.P. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* **8:** 60-71.

Blaustein, A.R., Walls, S.C., Bancroft, B.A., Lawler, J.J., Searle, C.L., and Gervasi, S.S. 2010. Direct and indirect effects of climate change on amphibian populations. *Diversity* **2:** 281-313.

Blumstein, D.T., Mennill, D.J., Clemins, P., Girod, L., Yao, K., Patricelli, G., Deppe, J.L., Krakaur, A.H., Clark, C., Cortopassi, K.A., Hanser, S.F., McCowan, B., Ali, A.M., and Kirschel, A.N.G. 2011. Acoustic monitoring in terrestrial environments using microphone arrays: applications, technological considerations and prospectus. Journal of Applied Ecology **48**: 758-767.

Borchers, D. 2012. A non-technical overview of spatially explicit capture-recapture models. Journal of Ornithology **152**: 435-444.

Borchers, D.L., and Efford, M.G. 2008. Spatially Explicit Maximum Likelihood Methods for Capture-Recapture Recapture Studies. Biometrics **64**: 377-385.

Borchers, D.L., and Marques, T.A. 2017. From distances sampling to spatial capture-recapture. *AStA Advances in Statistcal Analysis* **101:** 475-494.

Borchers, D.L., Stevenons, B.C., Kidney, D., Thomas, L., and Marques, T.A. 2015. A unifying model for capture-recapture and distance sampling surveys of wildlife populations. *Journal of the American Statistical Association* **110:** 195-204.

Born, J., Linder, H.P., and Desmet, P. 2007. The Greater Cape Floristic Region. *Journal of Biogeography* **34:** 147-162.

Boycott, R.C., and de Villiers, A.L. 1986. The status of *Heleophryne rosei* Hewitt (Anura: Leptodactylidae) on Table Mountain and recommendations for its conservation. *South African Journal of Wildlife Research* **16:** 129-134.

Buckland, S.T., Anderson, D.R., Burnham, K.P., and Laake, J.L. 1993. *Distance Sampling: Estimating abundance of biological populations.* Chapman and Hall, India.

Burnham, K.P., Anderson, D.R., and Laake, J. 1980. Estimation of Density from Line Transect Sampling of Biological Populations. *Wildlife monographs* **72:** 3-202.

Calenge, C. 2006. The package adehabitat for the R software: a tool for the

analysis of space and habitat use by animals. Ecological Modelling, 197, 516-519

Callery, E.M., Fang, H., and Elinson, R.P. 2001. Frogs without polliwogs: evolution of anuran direct development. *BioEssays* **23:** 233-241.

Caorsi, V.Z., Both, C., Cechin, S., Antunes, R., and Borges-Martins, M. 2017. Effects of traffic noise on the calling behaviour of two Neotropical hylid frogs. PLOS ONE: https://doi.org/10.1371/journal.pone.0183342

Channing, A. 2004. Arthroleptella lightfooti. In: Minter, L. R. *Atlas and red data book of the frogs of South Africa, Lesotho, and Swaziland* (pp. 214-215). Smithsonian Institution, Washington, D.C.

Channing, A., Hendricks, D., and Dawood, A. 1994. Description of a new moss frog from the south-western Cape (Anura: Ranidae: *Arthroleptella). South African Journal of Zoology* **29:** 240-243.

Channing, A., Measey, J.G., Villiers, A.L., and Tollay, K.A. 2017. Taxonomy of the *Capensibufo rosei* group (Anura: Bufonidae) from South Africa. *Zootaxa* **4232:** 282-292.

Channing, A., Schmitz, A., Burger, M., and Kielgast, J. 2013. A molecular phylogeny of African Dainty Frogs, with the description of four new species (Anura: Pyxicephalida: *Cacosternum*). *Zootaxa* **5:** 518-550.

Cincotta, R.P., Wisnewski, J., and Engelman, R. 2000. Human population in the biodiversity hotspots. *Nature* **404:** 990-992.

Coville, J.F., Potts, A.J., Bradshaw, P.L., Measey, J.G., Snijman, D., Picker , M.D., Proches, S., Bowie, R.C.K., and Manning, J.C. 2014. Floristic and faunal Cape biochoria: do they exist? In: Allsopp, N., Colville, J.F., and Verboom, A.G. *Fynbos ecology, evolution, and conservation of a megadiverse region* (pp. 73-92). Oxford University Press, New York, U.S.A.

Cowling, R.M., MacDonald, I.A.W., and Simmons, M.T. 1996. The Cape Peninsula, South Africa: physiographical, biological, and historical background to an extraordinary hot-spot of biodiversity. *Biodiversity and Conservation* **5:** 527-550.

Cressey, E.R., Measey, G.J., and Tolley, K.A. 2015. Fading out of view: the enigmatic decline of Rose's mountain toad *Capensibufo rosei*. *Oryx* **49:** 521-528.

Crouch, W.B., and Paton, P.W.C. 2002. Assessing the use of call surveys to monitor breeding anurans in Rhode Island. Journal of Herptelogy **36**: 185-192.

Crump, P.S., and Houlahan, J. 2017. Designing better frog call recognition models. Ecology and Evolution **7**: 3087-3099.

De Solla, S.R., Shirose, L.J., Fernie, K.J., Barrett, G.C., Brousseau, C.S., and Bishop, C.A. 2005. Effect of sampling effort and species detectability on volunteer based anuran monitoring programs. Biological Conservation **121**: 585-594.

Dawood, A., and Stam, E.M. 2006. The taxonomic status of the monotypic frog genus *Anhydrophryne* Hewitt from South Africa: a molecular perspective. *South African Journal of Science* **102:** 249-253.

Dawson, D.K. and Efford, M.G. 2009. Bird Population Density Estimated from Acoustic Signals. Journal of Applied Ecology **46**: 1201-1209.

Del Pino, E.M., Avila, M., Perez, O.D., Benitez, M., Alarcon, I., Noboa, V., and Moya, I.M. 2004. Development of the dendrobatid frog *Colostethus machalilla. The International Journal of Developmental Biology* **48:** 663-670.

Digby, A., Towsey, M., Bell, B.D., and Teal, P.D. 2013. A practical comparison of manual and autonomous methods for acoustic monitoring. Methods in Ecology and Evolution **4:** 675-683.

Donaldson, R. 2017. Managing national parks: counting visitors to the open-access urban national park of Table Mountain. *Journal of Public Administration* **52:** 74-88.

Dorcas, M.E., Rice, S.T., Walls, S.C., and Barichivich, W.J. 2009. “Auditory monitoring of anuran populations: Chapter 16”. Amphibian ecology and conservation: a handbook of techniques, Ed. Dodd, K.C. New York: Oxford University Press Inc., 281-298.

Eberhardt, L.L. 1978. Appraising variability in population studies. *The Journal of Wildlife Management* **42:** 207-238.

Edwards, S., Tolley, K.A., and Measey, J. 2017. Habitat characteristics influence the breeding of Rose’s dwarf mountain toadlet *Capensibufo rosei* (Anura: Bufonidae). *Herpetological Journal* **27:** 287-298.

Efford, M.G. 2004. Density Estimation in Live-trapping Studies. *Oikos* **106:** 598-610.

Efford, M.G. 2017. Habitat masks in the package secr. University of Otago. Accessed 8 February 2018 at: http://www.otago.ac.nz/density/pdfs/secr-habitatmasks.pdf.

Efford, M.G., Borchers, D.L., and Byrom. A.E. 2009a. Density estimation by spatially explicit capture-recapture: likelihood-based methods. In: Thompson, D.L., Cooch, E.G., Conroy, M.J. *Modeling demographic processes in marked populations.* Spring, New York: 255-269

Efford, M.G., Dawson, D.K., and Borchers, D.L.2009b. Population density estimated from locations of individuals on a passive detector array. *Ecology* **90**: 2676-2682.

Efford, M.G., Dawson, D.K., and Robbins, C.S. 2004. DENSITY: software for analysing capture-recapture data from passive detector arrays. *Animal Biodiversity and Conservation* **27**.1: 217-228.

Efford, M.G., Warburton, B., Coleman, M.C., and Barker, R.J. 2005. A field test of two methods for density estimation. *Wildlife Society Bulletin* **33:** 731-738.

Ferreira, S.L. 2011. Balancing people and park: towards a symbiotic relationship between Cape Town and Table Mountain National Park. *Current Issues in Tourism* **14**: 275-293.

Fletcher, R. 1987. Practical methods of optimization (2nd ed.). John Wiley & Sons, New York.

Friedman, J., Hastie, T., and Tibshirani, R. 2010. Regularization paths for generalized linear models via coordinate descent. *Journal of Statistical Software* **33:** 1-22.

Gibbs, J.P. 1998. Distribution of woodland amphibians along a forest fragmentation gradient. *Landscape Ecology* **13:** 263-268.

Gibbs, J.P., Droege, S., and Eagle, P. 1998. Monitoring populations of plants and animals. *BioScience* **48:** 935-940.

Gill, R.M.A., Thomas, M.L., and Stocker, D. 1997. The use of portable thermal imaging for estimating deer population density in forest habitats. Journal of Applied Ecology **34**: 1273-1286.

Gillepsie, D., Gordon, J., McHugh, R., McLaren, D., Mellinger, D., Redmond, P., Deng, X.Y. 2008. PAMGUARD: Semiautomated, open source software for real-time acoustic detection and localisation of ceteaceans. Proceedings of the Institute of Acoustics.

Gormley, A.M., Forsyth, D.M., Griffioen, P., Lindeman, M., Ramsey, D.S.L., Scroggie, M.P., and Woodford, L. 2011. Using presence-only and presence-absence data to estimate the current and potential distributions of established invasive species. *Journal of Applied Ecology* **48:** 25-34.

Grant, E. H. C, Miller, D.A.W., Schmidt, B.R., Adams, M.J, Amburgey, S.M., Thierry Chambert, Sam S. Cruickshank, Robert N. Fisher, David M. Green, Blake R. Hossack, Pieter T. J. Johnson, Maxwell B. Joseph, Tracy A. G. Rittenhouse, Maureen E. Ryan, J. Hardin Waddle, Susan C. Walls, Larissa L. Bailey, Gary M. Fellers, Thomas A. Gorman, Andrew M. Ray, David S. Pilliod, Steven J. Price, Daniel Saenz, Walt Sadinski & Erin Muths. 2016. Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. Nature Reports **6**: 10.1038/srep25625

Guisan, A., and Zimmerman, N.E. 2000. Predictive habitat distribution models in ecology. *Ecological Modelling* **135:** 147-186.

Hayes, T.B., Falso, P., Gallipeau, S., and Stice, M. 2010. The cause of global amphibian declines: a developmental endocrinologist's perspective. *The Journal of Experimental Biology* **213**: 921-933.

Heinze, G., and Schemper, M. 2002. A solution to the problem of separation in logistic regression. *Statistics in Medicine* **21:** 2409-2419.

Helme, N.A. and Trinder-Smith, T.H. 2006. The endemic flora of the Cape Peninsula, South Africa. *South African Journal of Botany* **72:** 205-210.

Hewitt, R.P., and Demer, D.A. 2000. The use of acoustics sampling to estimate the dispersion and abundance of euphausidds, with an emphasis on Antarctic krill, Euphausia superba. *Fisheries Research* **47**: 215-229.

Heyers, R., and Rand, S. 1977. Foam nest construction in the leptodactylid frogs *Leptodactylus pentadactylus* and *Physalaemus pustulosus* (Amphibia, Anura, Leptodactylidae). *Journal of Herpetology* **11:** 225-228.

Holdren, J.P., and Ehrlich, P.R. 1974. Human population and the global environment: population growth, rising per capita material consumption, and disruptive technologies have made civilization a global ecological force. *American Scientist* **62:** 282-292.

Houlahan, J.E., Findlay, C.S., Schmidt, B.R., Meyer, A.H., and Kuzmin, S.L. 2000. Quantitative evidence for global amphibian population declines. *Nature* **404**: 752-755.

IUCN . 2017. *The IUCN Red List of Threatened Species.* Version 2017-3. <http://iucnredlist.org>.

Jenkins, T.M. 1969. Social structure, position choice and microdistribution of two trout species (*Salmo trutta* and *Salmo gairdneri)* resident in mountain streams. *Animal Behaviour Monographs* **2:** 57-123.

Jenkins, A.R., and Benn, G.A. 1998. Home range size and habitat requirements of peregrine falcons on the Cape Peninsula, South Africa. *Journal of Raptor Research* **32:** 90-97.

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

Kalton, G., and Anderson, D.W. 1986. Sampling Rare Populations. *Journal of the Royal Statistical Society* **149:** 65-82.

Keeley, J.E., Fotheringham, C.J., and Morais, M. 1999. Reexamining Fire Suppression Impacts on Brushland Fire Regimes. *Science* **284:** 1829-1832.

Kery, M., Gardner, B., Stoeckle, T., Weber, D., and Royle, J.A. 2011. Use of spatial capture-recapture modelling and DNA data to estimate densities of elusive animals. *Conservation Biology* **25:** 356-364.

Kidney, D., Rawson, B.M., Borchers, D.L., Stevenson, B.C., Marques, T.A., and Thomas, L. 2016. An efficient acoustic density estimation method with human detectors applied to gibbons in Cambodia. PLOS ONE 55: e0155066. https://doi.org/10.1371/journal.pone.0155066.

Koehler, E., Brown, E., and Haneuse, S.J.P.A. 2009. On the assessment of Monte Carlo error in simulation-based statistical analyses. *The American Statistician* **63:** 155-162.

Kokubum, M. N. C., Garietta, A.A. 2005. Reproductive ecology and behaviour of a species of *Adenomera* (Anura, Leptodactylinae) with endotrophic tadpoles: systematic implications. *Journal of Natural History* **39:** 1745-1758.

Kraft, K.M., Johnson, D.H., Samuelson, J.M., and Allen, S.H. 1995. Using Known Populations of Pronghorn to Evaluate Sampling Plans and Estimators. *The Journal of Wildlife Management* **59:** 129-137.

Kraaij, T., and van Wilgen, B.W. 2014. Drivers, ecology, and management of fire in fynbos. In: Allsopp, N., Colville, J.F., and Verboom, A.G. *Fynbos ecology, evolution, and conservation of a megadiverse region* (pp. 47-72). Oxford University Press, New York, U.S.A.

Lahoz-Monfort, J.J., and Tingley, 2018. The technology revolution: improving species detection and monitoring using new tools and statistical methods. In: . Legge, S., Lindenmayer, D.B., Robinson, N.M., Scheele, B.C., Southwell, D.M., and Wintle, B.A. *Monitoring threatened species and ecological communities* (pp 303-313). EdCSIRO Publishing.

Lance, A.N., Baugh, I.D., and Love, J.A. 1989. Continued footpath widening in the Cairngorm Mountains, Scotland. *Biological Conservation* **49:** 201-214.

Legg, C.J., and Nagy, L. 2006. Why most conservation monitoring is, but need not be, a waste of time. *Journal of Environmental Management* **78:** 194-199.

Le Maitre, D.C., Van Wilgen, B.W., Chapman, R.A., and McKelly, D.H. 1996. Invasive Plants and Water Resources in the Western Cape Province, South Africa: Modelling the Consequences of a Lack of Management. *British Ecological Society* **33:** 161-172.

Lesaffre, E., and Albert, A. 1989. Partial separation in logistic discrimination. *Journal of Royal Statistical Society* **51:** 109-116.

Lewis, M.C., and O'Riain, M.J. 2017. Foraging profile, activity budget and spatial ecology of exclusively natural-foraging Chacma baboons (*Papio ursinus)* on the Cape Peninsula, South Africa. *International Journal of Primatology* **38:** 751-779.

Loertscher, M., Erhardt, A., and Zettel, J. 1995. Microdistribution of butterflies in a mosaic-like habitat: the role of nectar sources. *Ecography* **18:** 15-26.

Lukanov, S., Simeonovska-Nikolova, D., and Tzankov. 2014. Effects of traffic noise on the locomotion activity and vocalization of the Marsh Frog, Pelophylax ridibundus. *North-Western Journal of Zoology* **10**: 359-364.

MacArthur, R.H. 1972. *Geographical Ecology: Patterns in the Distribution of Species.* United Kingdom, Princeton University Press.

Mankin, R.W. 2011. Applications of acoustics in insect pest management. *CAB Reviews* **7:** 1-7.

Mantin, R.W., Hagstrum, D.W., Smith, M.T., Roda, A.L., and Kairo, M.T.K. 2011. Perspective and Promise: a century of insect acoustic detection and monitoring. *American Entomologist* **57:** 30-44.

Manly, B. F. J. 1970. A simulation study of animal population using the capture-recapture method. *Journal of Applied Ecology* **7:** 13-39.

Manning, J., and Goldblatt, P. 2012. *Plants of the Greater Cape Floristic Region. 1: The Core Cape Flora.* Strelitzia 29. South African National Biodiversity Institute, Pretoria.

Marques, T.A., Thomas, L., Martin, S.W., Mellinger, D.K., Ward, J.A., Moretti, D.J., Harris, D., and Tyack, P.L. 2013. Estimating animal population density using passive acoustics. *Biological Reviews* **88**: 287-309.

Marques, T.A., Thomas, L., Martin, S.W., Mellinger, D.K., Jarvis, S., Morrissey, R.P., Ciminello, C., and DiMarzio, N. 2012. Spatially explicit capture-recapture methods to estimate minke whale density from data collected at bottom-mounted hydrophones. *Journal of Ornithology* **152:** S445 – S455.

May, R.M. 1986. The search for patterns in the balance of nature: advances and retreats. *Ecology* **67:** 1115-1126.

Measey, G.J., Stevenson, B.C., Scott, T., Altwegg, R. and Borchers, D.L., 2017. Counting chirps: acoustic monitoring of cryptic frogs. *Journal of Applied Ecology* **54:** 894-902.

Menhill, D.J., Battison, M., Wilson, D.R., Foote, J.R., and Doucet, S.M. 2012. Field test of an affordable, portable, wireless microphones array for spatial monitoring of animal ecology and behaviour. *Methods in Ecology and Evolution* **3:** 704-7012.

Minter, L.R. 2004. *Atlas and red data book of frogs of South Africa, Lesotho, and Swaziland.* Smithsonian Institution, Washington, D.C.

Mitchell, N.J., and Gatten, R.E. 2002. Low tolerance of embryonic desiccation in the terrestrial nesting frog *Bryobatrachus nimbus* (Anura: Myobatrachinae). *Copeia* **2:** 364-373.

Mokhatla, M.M., Rodder, D., and Measey, J. 2015. Assessing the effects of climate change on distributions of Cape Floristic Region amphibians. *South African Journal of Science* **111:** 1-7.

Morgan, B.E., Passmore, N.I., and Fabian, B.C. 1989. Metamorphosis in the frog *Arthroleptella lighfooti* (Anura, Ranidae) with emphasis on neuro-endocrine mechanisms. In: Bruton, M. N. *Alternative Life-History Styles of Animals* (pp.347-370). Kluwer Academic Publishers, Dordrecht.

Myers, N., Mittermele, R.A., Mittermeler, C., da Fonseca, G.A.B., and Kent, J. 2000. Biodiversity hotspots for conservation priority. *Nature* **403:** 853-858.

Okes, N.C. 2017. Conservation ecology of the Cape clawless otter, *Aonyx capensis,* in an urban environment. *Doctoral thesis*, University of Cape Town: http://hdl.handle.net/11427/27353.

Petit, E., and Valiere, N. 2006. Estimating population size with noninvasive capture-mark-recapture data. *Conservation Biology* **20**: 1062-1073.

Picker, M.D., and Samways, M.J. 1996. Faunal diversity and endemicity of the Cape Peninsula, South Africa – a first assessment. *Biodiversity and Conservation* **5:** 591-606.

Phillips, S.J., Anderson, R.P., and Schapire, R.E. 2006. Maximum entropy modelling of species geographic distributions. *Ecological Modelling* **2006:** 231-259.

Pollock, K.H., Nichols, J.S., Brownie, C., and Hines, J.E. 1990. *Wildlife Monographs* **107:** 3-97.

Pollock, K.H., Nichols, J.D., Simons, T.R., Farnsworth, G.L., Bailey, L.L., and Sauer, J.R. 2002. Large scale wildlife monitoring studies: statistical methods for design and analysis. *Environmetrics* **13:** 105-119.

Pullin, A.S., Knight, T.M., Stone, D.A., and Charman, K. 2004. Do conservation managers use scientific evidence to support their decision-making? *Biological Conservation* **119:** 245-252.

Ralston, S., and Richardson, D. 2004. The role of legislation in the management of invasive alien plants: human dimensions affecting the implementation of legal instruments on the Cape Peninsula, South Africa. *Master's Thesis,* University of Cape Town.

Rebelo, A.G., Holmes, P.M., Dorse, C., and Wood, J. 2011a. Impacts of urbanization in a biodiversity hotspot: Conservation challenges in Metropolitan Cape Town. *South African Journal of Botany* **77:** 20-35.

Rebelo, T.G., Freitag, S., Cheney, C., and McGeoch, M.A. 2011b. Prioritising species of special concern for monitoring Table Mountain National Park: The challenge of a species-rich, threatened ecosystem. *Koedoe* **53.2:** 158-171.

Rose, W. 1950. *The Reptiles and Amphibians of Southern Africa.* Maskew Miller, Cape Town.

Roudier, P. 2011. Clhs: a R package for conditioned Latin hypercube sampling.

Rouget, M., Richardson, D.M., Cowling, R.M., Lloyd, J.W., and Lombard, A.T. 2003. Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Florist Region, South Africa. *Biological Conservation* **112:** 63-85.

Rouget, M., Barnett, M., Cowling, R.M., Cumming, T., Daniels, F., Hoffman, M.T., Manuel, J., Nel, J., Parker, A., Raimondo, D., and Rebelo, T. 2014. Conserving the Cape Floristic Region. In: Allsopp, N., Colville, J.F., and Verboom, A.G. *Fynbos ecology, evolution, and conservation of a megadiverse region* (pp. 321-336). Oxford University Press, New York, U.S.A.

Royle, A., Karanth, K.U., Gopalaswamy, A.M., and Kumar, N.S. 2009. Bayesian inference in camera trapping studies for a class of spatial capture-recapture models. *Ecology* **90:** 2322-3244.

Ryan, M.J. 1985. *The Túngara Frog: A study in sexual selection and communication.* The University of Chicago Press, United State of America.

Salinas, Z.A., Baraquet, M., Grenat, P.R., Martino, A.K., and Salas, N. E. 2017. Morphology and size of blood cells of Rhinella arenarum (Hensel, 1867) as environmental health assessment in disturbed aquatic ecosystem from central Argentina. Environmental Science and Pollution Research 24: 24907-24915.

Sargent, L.G. 2000. Frog and toad population monitoring in Michigan. Journal of Iowa Academy of Science **107**: 195-199.

Seber, G.A.F. 1986. A Review of Estimating Animal Abundance. *Biometrics* **42:** 267-292.

Sharratt, N.J., Picker, M.D., and Samways, M.J. 2000. The invertebrate fauna of the sandstone caves of the Cape Peninsula (South Africa): patterns of endemism and conservation priorities. *Biodiversity and Conservation* **9:** 107-143.

Sinclair-Smith, K. 2009. The expansion of urban Cape Town. Unpublished report. Metropolitan Spatial Planning Branch, City of Cape Town.

Sinsch, U. 1998. Migration and orientation in anuran amphibians. *Ethology, Ecology and Evolution* **2**: 65-79.

Skalski, J.R., and Douglas, S.R. 1992. *Techniques for wildlife investigations: design and analysis of capture data.* Academic Press, USA.

Smart, J.C.R., Ward, A.I., and White, P.C.L. 2004. Monitoring woodland deer populations in the UK: an imprecise science*. Mammal Review* **34**: 99-114.

Smith, M.A., and Green, D.M. 2006. Sex, isolation and fidelity: unbiased long-distance dispersal in a terrestrial amphibian. *Ecography* **29:** 649-658.

South African Frog Re-assessment Group (SA-FRoG), IUCN SSC Amphibian Specialist Group. 2010. Arthroleptella lightfooti. The IUCN Red List of Threatened Species 2010:

Sousa-Lima, R.S., Norris, T.F., Oswald, J.N., and Fernandes, D.P. 2013. A review and inventory of fixed autonomous recorders for passive acoustic monitoring of marine mammals. *Aquatic Mammals* **39**: 23-53.

Stevenson, B.C., and Borchers, D. 2017. ascr: Acoustic Spatial Capture-recapture in AD Model Builder. R package version 2.0.3. https://github.com/b-steve/ascr

Stevenson, B.C,. Borchers, D.L., Altwegg, D., Swift, R.J., Gillespie, D.M., Measey, J.G. 2015. A general framework for animal density estimation from acoustic detections across a fixed microphone array. Methods in Ecology and Evolution **1**: 38-48.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., and Waller, R.W. Status and trends of amphibian declines and extinctions worldwide. *Science* **306**: 1783-1786.

Thomas, D. 1996. Needed: less counting of caribou and more ecology. *The Seventh North American Caribou Conference:* Thunder Bay, Ontario, Canada.

Tolley, K.A., Bowie, R.C.K., Measey, J.G., Price, B.W., and Forest, F. 2014. The shifting landscape of genes since the Pliocene: terrestrial phylogeography in the Greater Cape Floristic Region. In: *Fynbos: ecology, evolution and conservation of a megadiverse region.* Oxford University Press, USA.

Tolley, K.A., De Villiers, A.L., Cherry, M.I., and Measey, J.G. 2010. Isolation and high genetic diversity in dwarf mountain toads (*Capensibufo)* from South Africa. *Biological Journal of Linnean Society* **100:** 822-834.

Towsey, M., Jason, W., Williamson, I., and Roe, P. 2014. The use of acoustic indices to determine avian species richness in audio-recordings of the environment. Ecological Informatics **21**: 110-119.

Turner, A.A. 2010. Phylogeography and speciation in the genus Arthroleptella (PhD thesis). University of the Western Cape.

Turner, A.A., and Channing, A. 2008. A new species of *Arthroleptella* Hewitt, 1926 (Anura: Pyxicephalidae) from the Klein Swartberg Mountain, Caledon, South Africa. *African Journal of Herpetology* **57**: 1-12.

Turner, A.A., and Channing, A. 2017. Three new species of *Arthroleptella* Hewitt, 1926 (Anura: Pyxicephalidae) from the Cape Fold Mountains, South Africa. *African Journal of Herpetology* **66:** 53-78.

Turner, A.A., and de Villiers, A.L. 2007. Amphibians. In: *Western Cape Province State of Biodiversity.* (pp.36-54). CapeNature Scientific Services, Cape Town.

Turner, A.A., De Villiers, A.L., Dawood, A., and Channing, A. 2004. A new species of *Arthroleptella* Hewitt, 1926 (Anura: Ranidae) from the Groot Winterhoek Mountains of the Western Cape Province, South Africa. *African Journal of Herpetology* **53:** 1-12.

Turner, A.A., and De Villiers, A.L. 2007. Western Cape Province State of Biodiversity. Cape Nature Scientific Services. *Amphibians*: 36-54.

Van der Meijden, A., Crottini, A., Tarrant, J., Turner, A., and Vences, M. 2011. Multo-locus phylogeny and evolution of reproductive modes in the Pyxicephalidae, an African endemic clade of frogs. *African Journal of Herpetology* **60:** 1-12.

Van Wilgen, B. W. 2012. Evidence, Perceptions, and Trade-offs Associated with Invasive Alien Plant Control in the Table Mountain National Park, South Africa. *Ecology and Society* **17**: 23.

Wake, M.H. 1980. The reproductive biology of *Nectophrynoides malcolmi* (Amphibia: Bufonidae), with comments on the evolution of reproductive modes in the genus *Nectophrynoides*. *Copeia* **2:** 193-209.

Wells, K.D. 2007. *The Ecology and Behaviour of Amphibians.* The University of Chicago Press, United States of America.

Williams, J.D., and Dodd, C.K. Jr. 1978. Importance of wetlands to endangered and threatened species. *Wetland Functions and Values: The State of Our Understanding. American Water Resrources*: 565-575.

Wilson, J.R., Gaertner, M., Griffiths, C.L., Kotze, I., Le Maitre, D.C., Marr, S.M., Picker. M.D., Spear, D., Stafford, L., Richardson, D.M., van Wilgen, B.W., and Wannenburgh, A. 2012. Biological invasions in the Cape Floristic Region: history, current patterns, impacts, and management challenges. In: Allsopp, N., Colville, J.F., and Verboom, A.G. *Fynbos ecology, evolution, and conservation of a megadiverse region* (pp. 273-298). Oxford University Press, New York, U.S.A.

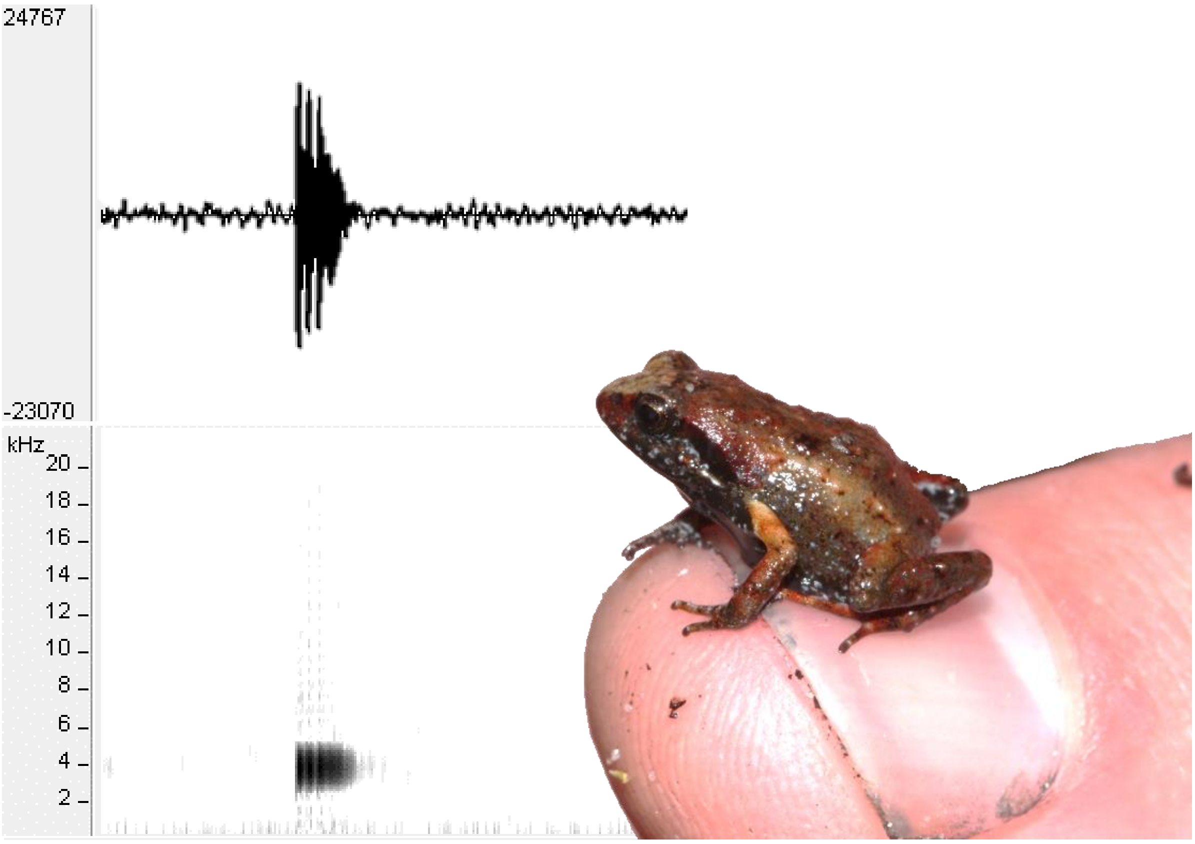
Witmer, G.W. 2005. Wildlife population monitoring: some practical considerations. Wildlife Research **32**: 259-263.

Zhelev, A., Tsonev, S., Georgieva, K., and Amaudove, D. 2018. Health status of Pelophylax ridibundus (Amphibia: Ranidae) in a rice paddy ecosystem in Southern Bulgaria and its importance in assessing environmental state: haematological paramters. Environmental Science and Pollution Research: https://doi.org/10.1007/s11356-017-1109-5

APPENDICES

Appendix A

A.1. A waveform diagram(top) and spectrogram diagram (bottom) showing a typical chirp from an *Arthroleptella lightfooti* male, as well as a picture of a male *A. lightfooti* that emits these calls. The three pulses are contained in this specific call of length 0.066s.



A.2. Presence data for *A.lightfooti* from various sources used in the MaxENT model. A compilation of presence data was provided by Alexander D. Rebelo that included data from the Animal Demography Unit (ADU), CapeNature, the Iziko South African Museum, the KwaZulu-Natal (KZN) Museum, the South African Institute for Aquatic Biodiversity (SAIAB), iSpot and his own observations in the field. John Measey and Francois Becker also contributed all the localities at which they had noted the presence of *A. lightfooti.* For the generation of sites for the second field season in 2017, calling locations of *A. lightfooti* noted during 2016 en route to sampling sites were included in the presence data, as well as the sites that had *A. lightfooti* calling during the 2016 sampling period.

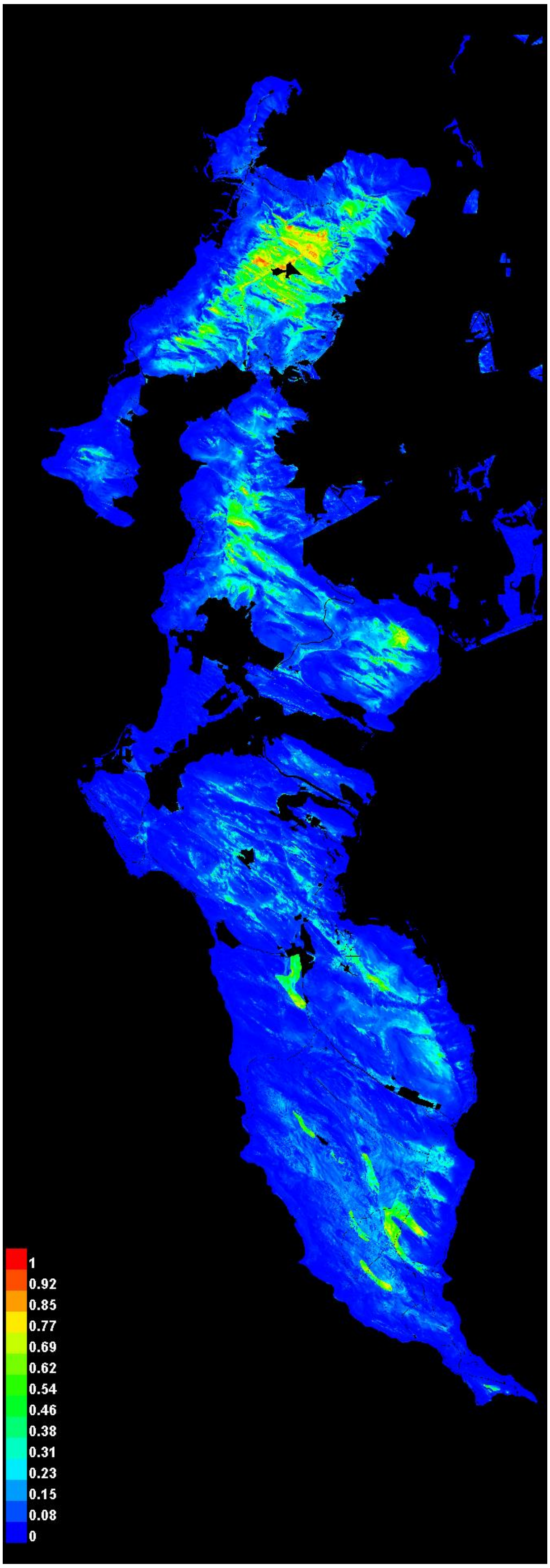
Source	Contribution to Presence data (%)
ADU	3.04
CapeNature	14.23
Iziko musuem	3.98
KZN museum	2.66
SAIAB	1.71
iSpot	0.19
A. D. Rebelo	9.3
J. Measey	1.34
F. Becker	48
en route 2016 sites	7.58
2016 field sites	7.97

A.3. A subset of the 14 variables used in the MaxEnt modelling for species distribution of *Arthroleptella lightfooti.* Data can be requested from Jasper Slingsby at SAEON. The variables s1 and s2 are coordinates from the Cape Peninsula, and the 14 environmental variables are coded by letters:

A = veg_subtypes, B = veg_communities_cowling, C=vegtypes, D= topographicPositionIndex30m, E =Tmin_jul_mean, F=Tmax_jan_mean, G= slope, H= roughness, I= northsouth, J= mount, K= jul_solar_radiation , L = jan_solar_radiation, M= elevation, N= eastwest

s1	s2	A	B	C	D	E	F	G	H	I	J	K	L	M	N
268965	6246105	5	80	7	-4.14942	10.00803	28.80365	0.005732	0.216441	0.002963	0.005732	3381.167	8990.167	13.67377	0.999996
268995	6246105	5	80	7	-2.63893	9.997221	28.75642	0.005517	0.355451	0.254967	0.005335	3384.083	8990.917	13.47367	0.96695
269025	6246105	5	80	7	-1.66171	10.02592	28.8059	0.004884	0.347664	-0.13453	0.00484	3369.833	8988.417	13.13908	0.99091
269055	6246105	5	80	7	-0.95412	10.11593	29.00138	0.00626	0.338573	-0.99281	0.000749	3346.083	8983.667	12.99561	0.119716
269085	6246105	5	80	7	-1.22191	10.1785	29.14991	0.011468	0.509325	-0.7463	-0.00763	3337.917	8981.917	13.22756	-0.66561
269115	6246105	5	80	7	-1.06081	10.21281	29.23412	0.011929	0.508483	-0.30239	-0.01137	3358.333	8986	13.67809	-0.95318
269145	6246105	5	80	7	-0.33286	10.26461	29.35528	0.005512	0.527918	0.638042	-0.00424	3381	8990.5	14.28348	-0.77
269175	6246105	5	80	7	0.965699	10.28874	29.38732	0.005021	0.167621	0.828764	-0.00281	3365.917	8987.333	13.76269	-0.5596
269205	6246105	5	80	7	1.747086	10.2051	29.16924	0.014854	0.583167	-0.57125	-0.01219	3313.417	8976.583	13.76033	-0.82078
269235	6246105	5	80	7	2.855944	10.20891	29.15438	0.03114	1.305217	-0.83792	-0.017	3264.167	8966.583	14.43189	-0.5458

A.4. The species distribution model of *A.lightfooti* generated through MaxEnt with absence and pseudo-absence data (AUC = 0.931±0.007).

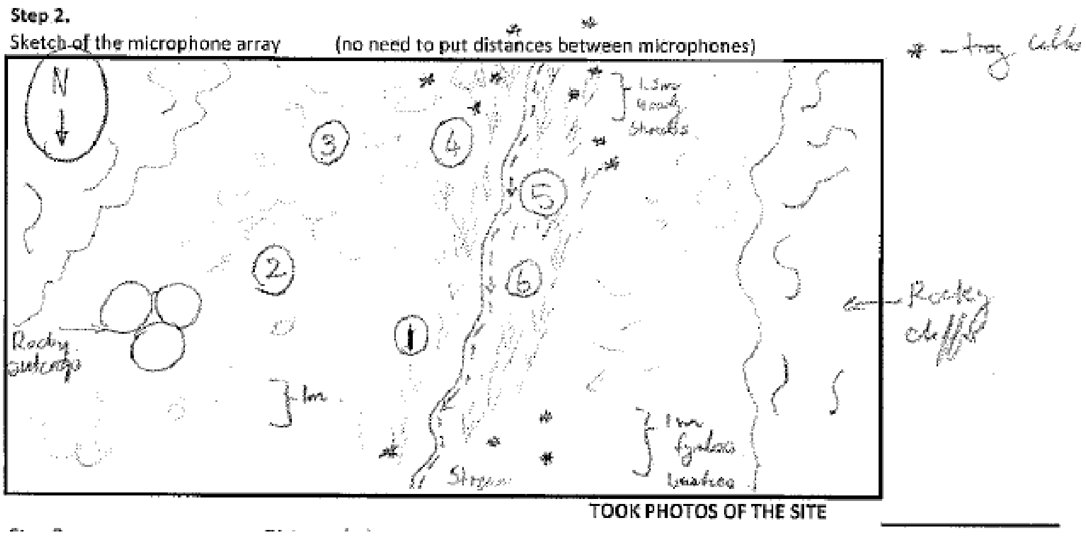


A.5. Conditioned Latin Hypercube sampling was applied to stratification of potential sites form which to choose sampling sites based on the probabilities of *A. lightfooti* being present. These probabilities were obtained using a Maxent model. The following code explains the stratification and site selection and was used to accomplish both.

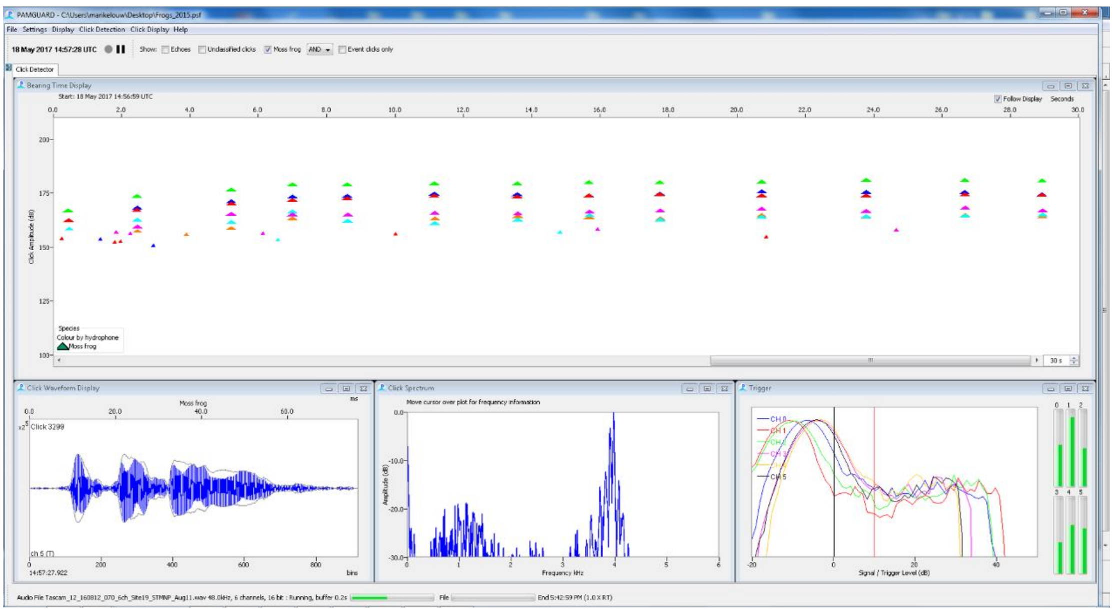
```
# STRATIFIED MEANS TO OBTAIN 200 SAMPLING SITES
#rows selected based on data that was sorted with Alightfooti
probabilities from smallest to largest.

#80% of 200 is 160. That means 160 sites will be randomly
selected using the package clhs from rows which contain
probabilities of frogs calling that lie between 0.6 and 1.

#10% of 200 is 20. That means 20 sites will be randomly
selected using the package clhs from rows which contain
probabilities of frogs calling that lie between 0.4 and 0.6.

#5% of 200 is 10. That means 10 sites will be randomly selected
using the package clhs from rows which contain probabilities of
frogs calling that lie between 0.2 and 0.4.

#5% of 45 is 10. That means 10 sites will be randomly selected
using the package clhs from rows which contain probabilities of
frogs calling that lie between 0 and 0.2.

# Creating subsets based on the probabilities of A.lightfooti
being present
sub1<-subset(pot.sites_with_fire,alight_may8<0.9 &
alight_may8>=0.6)
sub2<-subset(pot.sites_with_fire,alight_may8<0.6 &
alight_may8>=0.4)
sub3<-subset(pot.sites_with_fire,alight_may8<0.4 &
alight_may8>=0.2)
sub4<-subset(pot.sites_with_fire, alight_may8<0.2 &
alight_may8>=0)

nrow(sub1) # selecting 160 sites from 4924 potential sites
nrow(sub2) # selecting 20 sites from 10007 potential sites
nrow(sub3) # selecting 10 sites from 28675 potential sites
nrow(sub4) # selecting 10 sites from 296073 potential sites

#The following columns used: (pot.sites[,c(3,4,5,6,7,8,9,10)]-->
using aspect30m, elevation30m, jul_solar_radiation30m,
slope30m,
# Tmin_jul_mean30m, vegtypes, Max_NDVI_2015_Landsat8,
Most_Recent_Fire_as_of_Jan2016,.

#Selecting 160 sites:
result1 <- clhs(sub1[,c(1,2,3,4,5,6,7,8,9,10)], size=192,
simple=F) # selecting the columns/layers you want to include in
the CLHS run. size = 192 means that you want 192 sites selected
from your input file through CLHS sampling.
                        #Check out "??clhs" for more arguments
that you can add in the sampling if necessary.
dim(sub1[,c(1,2,3,4,5,6,7,8,9,10)]) # @@@ here you can see
the new dimensions of the subset that you passed to clhs
str(result1) #FINE

#Selecting 20 sites:
result2 <- clhs(sub2[,c(1,2,3,4,5,6,7,8,9,10)], size=24, simple=F)
dim(sub2[,c(1,2,3,4,5,6,7,8,9,10)]) # @@@ here you can see
the new dimensions of the subset that you passed to clhs
str(result2) #FINE

#Selecting 10a sites:
result3 <- clhs(sub3[,c(1,2,3,4,5,6,7,8,9,10)], size=12, simple=F)
dim(sub3[,c(1,2,3,4,5,6,7,8,9,10)]) # @@@ here you can see
the new dimensions of the subset that you passed to clhs
str(result3) #FINE

#Selecting 10b sites:
result4 <- clhs(sub4[,c(1,2,3,4,5,6,7,8,9,10)], size=12, simple=F)
dim(sub4[,c(1,2,3,4,5,6,7,8,9,10)]) # @@@ here you can see
the new dimensions of the subset that you passed to clhs
str(result4) #FINE

#Plotting the selected sites and saving them to a .csv
sub1$number<-c(1:length(sub1[,1])) #essentially numbering all
the rows. Gives each row a number, from 1 to the last row.
selected_sites1<-data.frame(lat=sub1$y[sub1$number %in%
result1$index_samples],long=sub1$x[sub1$number %in%
result1$index_samples]) # result1$index_samples -> gives
which rows are selected for clhs for subset1. Give lat and long
for ANY rows that were selected.
plot(pot.sites_with_fire$x,pot.sites_with_fire$y,pch=16,cex=0.
5)
#points(sub1$x,sub1$y,pch=16, col="red")
points(selected_sites1$long,selected_sites1$lat,pch=16,
col="yellow", cex=0.8) #plots the points selected by clhs onto
the background of your working area.
write.csv(selected_sites1,
"C://SelectinSites2017_postJHB//May8_new_maxent//Trial7_1
92_May9.csv")

sub2$number<-c(1:length(sub2[,1]))
selected_sites2<-data.frame(lat=sub2$y[sub2$number %in%
result2$index_samples],long=sub2$x[sub2$number %in%
result2$index_samples])
plot(pot.sites_with_fire$x,pot.sites_with_fire$y,pch=16,cex=0.
5)
#points(sub2$x,sub2$y,pch=16, col="red")
points(selected_sites2$long,selected_sites2$lat,pch=16,
col="yellow",cex=0.8)
write.csv(selected_sites2,
"C://SelectinSites2017_postJHB//May8_new_maxent//Trial7_2
4_May9.csv")

sub3$number<-c(1:length(sub3[,1]))
selected_sites3<-data.frame(lat=sub3$y[sub3$number %in%
result3$index_samples],long=sub3$x[sub3$number %in%
result3$index_samples])
plot(pot.sites_with_fire$x,pot.sites_with_fire$y,pch=16,cex=0.
5)
#points(sub3$x,sub3$y,pch=16, col="red")
points(selected_sites3$long,selected_sites3$lat,pch=16,
col="yellow",cex=0.8)
write.csv(selected_sites3,
"C://SelectinSites2017_postJHB//May8_new_maxent//Trial7_1
2a_May9.csv")

sub4$number<-c(1:length(sub4[,1]))
selected_sites4<-data.frame(lat=sub4$y[sub4$number %in%
result4$index_samples],long=sub4$x[sub4$number %in%
result4$index_samples])
```

```
plot(pot.sites_with_fire$x,pot.sites_with_fire$y,pch=16,
cex=0.5)
#points(sub4$x,sub4$y,pch=16, col="red")
points(selected_sites4$long,selected_sites4$lat,pch=16,
col="yellow", cex=0.8)
write.csv(selected_sites4,
"C://SelectinSites2017_postJHB//May8_new_maxent//Trial7_1
2b_May9.csv")

save.image("selectin_sites_postjhb_May9_Trial7.Rdata")
```

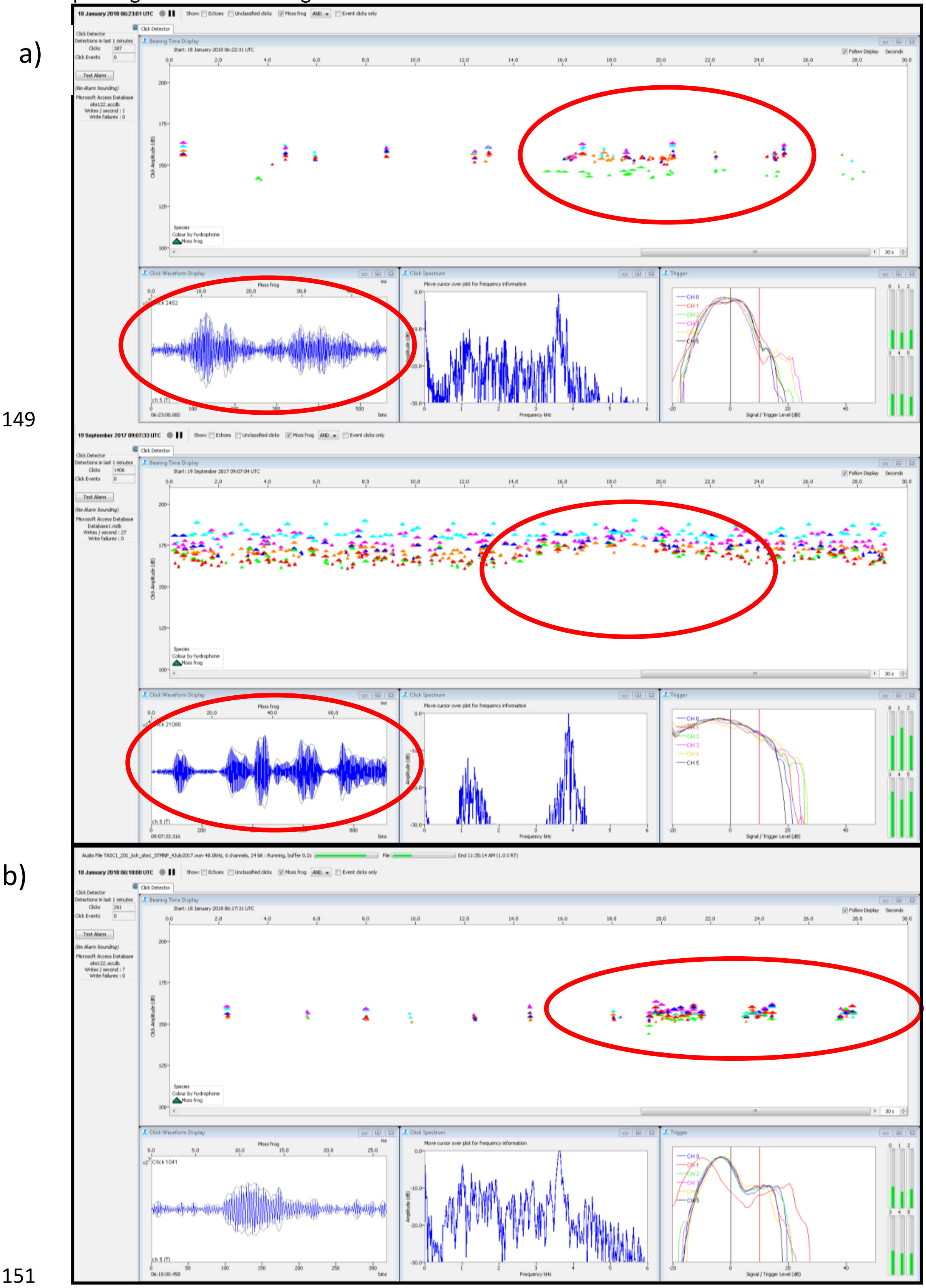
A.6. Obligatory sketch made at each site where an acoustic array was put up. The microphones were always placed clockwise with the sketcher standing at microphone one. North was always indicated on the sketch. Notable features were drawn. Positions where frogs are thought to be recording were also sketched.



A.7. In PAMguard, the following modules were used to display frog captures in real-time: a.) Bearing Time Display; moss frog calls get displayed as a triangle symbol along a graph of Click Amplitude (dB) vs Time (seconds). Different coloured triangles represent the different detectors at which the call was picked up; b.) Click Waveform Display; the waveform of a moss frog call is represented; c.) Click Spectrum; the power spectrum of each detected call d.) Trigger display; the amplitude of the signal at different detectors. To the right of the window are bars reflecting the soundscape from each channel and is an easy way to tell if a microphone was not working during the recording.



A.8. (a) Misidentifications of bird calls as frog calls in PAMguard. The top window and bottom window show different species of birds that are detected as frog calls across multiple microphones. The calls are picked up in the Bearing Time window display as highly grouped frog calls, but the waveform display does not show the typical waveform diagram for *A. lightfooti.* (b) The effect of a passing car on a recording. Wind has a similar effect.



A.9. The functions used in obtaining the estimated locations of detectors written by David Borchers.

```
# Functions for estimation of microphone locations

nm2m=function(nm) return(nm*1852)

latlon2m=function(lat,lon,origin=1)
{
 latm=nm2m(lat*60)
 lonm=nm2m(lon*60)*cos(lat*pi/180)
 latm=latm-latm[origin]
 lonm=lonm-lonm[origin]
 return(data.frame(x=lonm,y=latm))
}

#negloglik=function(par,odl,loc0=c(0,0)){
negloglik=function(par,odl){
 sigma=exp(par[length(par)])
 xy=par[-length(par)]
 ndet=length(xy)/2
 locs=matrix(xy,nrow=ndet)
 # locs=rbind(loc0,locs)
 ds=as.matrix(dist(locs))
 ds=as.vector(ds[lower.tri(ds)])
 lik=dnorm(odl,mean=ds,sd=ds*sigma)
 return(-sum(log(lik)))
}

center=function(xdat,on=1){
 xdat[,"x"]=xdat[,"x"]-xdat[on,"x"]
 xdat[,"y"]=xdat[,"y"]-xdat[on,"y"]
 return(xdat)
}

startVest=function(estloc,starts){
 xlim=range(estloc[,1],starts[,"x"])
 ylim=range(estloc[,2],starts[,"x"])
 plot(estloc,pch=as.character(1:dim(estloc)[1]),xlab="X
(m)",ylab="Y (m)",xlim=xlim,ylim=ylim)
 points(estloc,cex=2.5)

points(starts[,c(2,3)],pch=as.character(starts[,"mic"]),col="red")
 title(main="Estimated Locations (circled)",sub="(Start locations
red)",col.sub="red")
}

compare.dists=function(loc,dst,label=TRUE,...){
 ds=as.matrix(dist(loc,upper=TRUE))
 dl=as.vector(ds[lower.tri(ds)])
 dd=data.frame(obs=dst,calc=dl)
 dlim=range(dd,dl)
 dnames=rep("",length(dl)); for(i in 1:length(dl))
dnames[i]=paste("(",od[i,1],";",od[i,2],")",sep="")
 if(label) {
  plot(dd,xlim=dlim,ylim=dlim,pch="",xlab="Measured Distance
(m)",ylab="Estimated Distance (m)")
  text(dd,labels=dnames,cex=0.75)
 }else plot(dd,xlim=dlim,ylim=dlim,...)
 # title("Estimated vs Measured distances")
 lines(c(0,max(dd)),c(0,max(dd)),lty=2)
 diffs=dd$obs-dd$calc
 if(label) {
  plot(diffs*100,pch="",ylab="Differences (cm)")
  text(1:15,diffs*100,labels=dnames)
 } else plot(diffs,...)
 title("Differences")
 lines(c(0,length(diffs)+1),c(0,0),lty=2)
 return(list(pair=dnames,difference=diffs))
}


#negloglik.ns=function(par,od,sigma=0.025){
# # like negloglik, but with fixed sigma
# xy=par
# ndet=length(xy)/2
# locs=matrix(xy,nrow=ndet)
## locs=rbind(loc0,locs)
# ds=as.matrix(dist(locs))
# ds=as.vector(ds[lower.tri(ds)])
# lik=dnorm(od,mean=ds,sd=sigma)
# return(-sum(log(lik)))
#}
#
#negloglik2=function(par,od,gloc{
##--------------------------------------------------------------------
## par is c(x,y,sigma.o,sigma.g)
##
##--------------------------------------------------------------------
# npar=length(par)
# sigma.o=exp(par[npar-1])
# sigma.g=exp(par[npar])
# xy=par[-c(npar-1,npar)]
# ndet=length(xy)/2
# locs=matrix(xy,nrow=ndet)
# locs=rbind(loc0,locs)
# ds=as.matrix(dist(locs))
# ds=as.vector(ds[lower.tri(ds)])
# lik.o=dnorm(od,mean=ds,sd=sigma.o)
# lik.g.x=dnorm(gloc$x,mean=locs[,1],sd=sigma.g)
# lik.g.y=dnorm(gloc$y,mean=locs[,2],sd=sigma.g)
# return(-sum(log(lik.o))-sum(log(lik.g.x))-sum(log(lik.g.y)))
#}
#
adhoc.fix=function(starts,dst){
 # do an ad-hoc.fix/check of distances
 ds=as.matrix(dist(loc,upper=TRUE))
 dl=as.vector(ds[lower.tri(ds)])
 dd=data.frame(obs=dst,calc=dl)
 dfit=lm(calc~obs-1,data=dd) # fit zero-intercept regression of
dists calculated from coordinates on measured distances
 slope=dfit$coefficients
 starts[,c(2,3)]=starts[,c(2,3)]/slope # ad hoc fix
 return(starts)
}

est.locs=function(starts,sigma,od,control.fit,center.on=1,metho
d="BFGS") {
 sigma=0.1;theta=log(sigma)
 par=c(starts[,"x"],starts[,"y"],theta)

fit=optim(par,negloglik,od=od,control=control.fit,method=meth
od)
 sigmaest=exp(fit$par[length(fit$par)])
 xyest=fit$par[-length(fit$par)]
 estloc=matrix(xyest,nrow=length(xyest)/2)
 colnames(estloc)=c("x","y")
 estloc=center(estloc,on=center.on)


return(list(estloc=estloc,multiplicative.sigma=sigmaest,starts=st
arts,start.sigma=sigma,measuredist=od))
}
```

A.10. This script was written by David Borchers and makes use of the functions stipulated in A.9. This script is combines the microphone pair distance information and the Cartesian positions of microphones in an array relative to each other to estimate the formation of the array.

```
#-------------------------------------------------------------------------------
--
# Code for estimating microphone locations given distances
between them and initial
# guesses of locations.
#
# Uses locestfuns.r
#-------------------------------------------------------------------------------
--

# Name of file where locestfuns.r and data are located

working.dir="C://Users//marikelouw//Documents//Records_20
17//42_2017"
site.number=42

# distance data must have 3 cols: mic1, mic2, dist: they can be
named anyhow but
# 1st will be taken as microphone number
# 2nd as 2nd microphone number,
# 3rd as distance between them,
# 1st col numbers change slower than 2nd col numbers and are
in increasing order

dist.file.name="Dist42_2017.csv" # file of measured distances
between microphones

# data must have 3 cols: mic number, x-coord, y-coord: they
can be named anyhow but
# 1st will be taken as microphone number
# 2nd as x-coord of microphone,
# 3rd as y-coord of microphone,
# 1st col numbers must be in increasing order

start.loc.file.name="Guess42_2017.csv" # file of guesstimated
locations

# Set the Working Directory

setwd(working.dir)

# Source the functions to estimate locations

source("locestfuns.r") # get functions to estimate locations

# get observed (measured) distances between microphones

od=as.matrix(read.csv(dist.file.name,header=FALSE))
odl=od[,3] # measurement column
n=length(odl)

# read starting values for locations

starts=as.matrix(read.csv(start.loc.file.name,header=FALSE))
colnames(starts)=c("mic","x","y")
plot(starts[,c(2,3)],pch=as.character(starts[,1]),col="red",main=
"Original Microphone Location Estimations", ylim=c(-20, 12))

# calculate and look at differences between estimated and
measured distances

sdistd=compare.dists(starts[,c(2,3)],odl)

# ad-hoc fix if estimated and measured distances seem wrong
by contant multiplier
#starts=adhoc.fix(starts,odl)

## estimate, with estimated sigma
# starting value for sigma

sigma=0.1

# (see help(optim) for this)

control.fit=list(trace=5)

# "center.onâ?? is number of mic in start.loc.file.name to make
the origin; this should be equal to 1

est=est.locs(starts,sigma,odl,control.fit,center.on=1)

par(mfrow=c(1,2))

# calculate and look at differences between estimated and
measured distances

distd=compare.dists(est$estloc,est$measuredist)

# plot final vs start locations

startVest(est$estloc,est$starts)

# Write estimated Locations to CSV file

write.csv(est$estloc,file =
paste0("estlocs",site.number,".csv"),row.names=FALSE)

# End Script
```

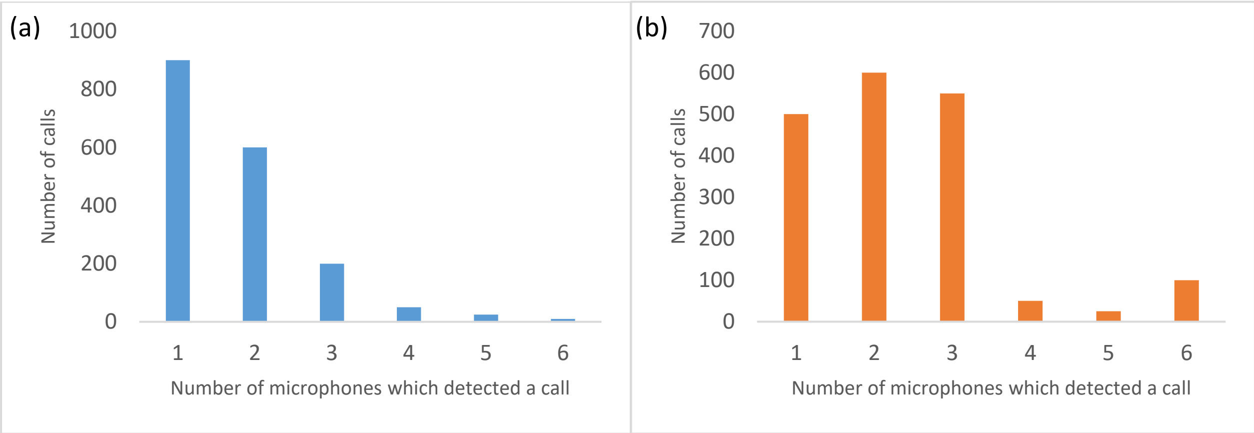
A.11. A shortened version of the R script termed "Masterscript" contains the data preparation steps and the ascr model function for obtaining calling density estimates from the converted recordings. This version contains the ascr modelling step for only one subsample, but the full version contains 10 subsample sections (loop functions could not be used due to the nature of sometimes needing to edit every subsample separately).

```
# Ben Stevenon's aSCR package cannot be downloaded through
install.package() function. Run the script found at the following
URL:
# https://raw.githubusercontent.com/b-
steve/ascr/master/inst/scripts/install.r

library(ascr)
library(parallel)
library(secr)
library(data.table)

#Defining a function needed soon
detfn <- function(fit, d){
 detfn <- fit$args$detfn
 pars <- get.par(fit, pars = fit$detpars, cutoff = fit$fit.types["ss"],
        as.list = TRUE)
 if (any(names(pars) == "sigma.b0.ss")){
  if (pars$sigma.b0.ss != 0){
   warning("Detection probabilities only calculated for the
average emitted cue strength.")
  }
 }
 if (any(names(pars) == "b2.ss")){
  if (pars$b2.ss != 0){
   warning("Detection probabilities only calculated for a
detector directly in line with the direction of the cue.")
  }
 }
 ascr:::calc.detfn(d, detfn = detfn, pars = pars, ss.link =
fit$args$ss.opts$ss.link)
}

# set path, if you're on windows use c-drive pathway
dat.dir="C://Site##"
setwd(dat.dir)

#Press "CTRL + F" and replace the "XX" with the site number
you are working with. The "XX" appears something like 34 times
in
#this script and the SITE NUMBER is needed in it's place.

site.number= XX

# As you are likely to only work with sites recorded in 2017, use
Rep.number =2. Those from 2016 were rep.number=1.
rep.number=2

#FOR THE INITIAL RUN, set n.boot=0. Only once you are sure a
site is ready to run, then you can set it at 300 (for a start)
n.boot=300# number of bootstrap iteration. Always start at 300
for the first proper run. The MCE afterwards will tell you
whether this is enough. MCE needs to be below 0.05.
cores_use=4 # number of cores to be used in bootstrap

#Most of the timeranges will be unique for each site (not have
excessive wind/bird calls). There need to be at least 10
subsamples.
#Replace the following numbers with the selected minutes that
you listened to and determined where wind and bird free.
#These will be the subsamples run for each site

a1 <-16
b1 <-17
c1 <-18
d1 <-19
e1 <-20
f1 <-21
g1<-22
h1<-23
i1<-24
j1<-25

a2 <- c(a1*60,a1*60+60)
b2 <- c(b1*60,b1*60+60)
c2 <- c(c1*60,c1*60+60)
d2 <- c(d1*60,d1*60+60)
e2 <- c(e1*60,e1*60+60)
f2 <- c(f1*60,f1*60+60)
g2<- c(g1*60,g1*60+60)
h2<- c(h1*60,h1*60+60)
i2<-c(i1*60,i1*60+60)
j2<-c(j1*60,j1*60+60)

subsample.number.1 = 1
time.range1=a2

subsample.number.2 = 2
time.range2=b2

subsample.number.3 = 3
time.range3=c2

subsample.number.4 = 4
time.range4=d2

subsample.number.5 = 5
time.range5=e2

subsample.number.6 = 6
time.range6=f2

subsample.number.7 = 7
time.range7=g2

subsample.number.8 = 8
time.range8=h2

subsample.number.9 = 9
time.range9=i2

subsample.number.10 = 10
time.range10=j2

# Call on your CSV files (CDC_S#_R2.csv and estlocs#.csv
# Data_dets is the pamguard output file (CDC...)
# Data_mics is the microphone locations file (estlocs..)

data_dets <-
fread(paste0("CDC_S",site.number,"_R",rep.number,".csv"),
data.table=FALSE)
data_mics <-
as.matrix(fread(paste0("estlocs",site.number,".csv"),
data.table=FALSE))

# Call rate per minute
# Must be taken independently to site recordings
```

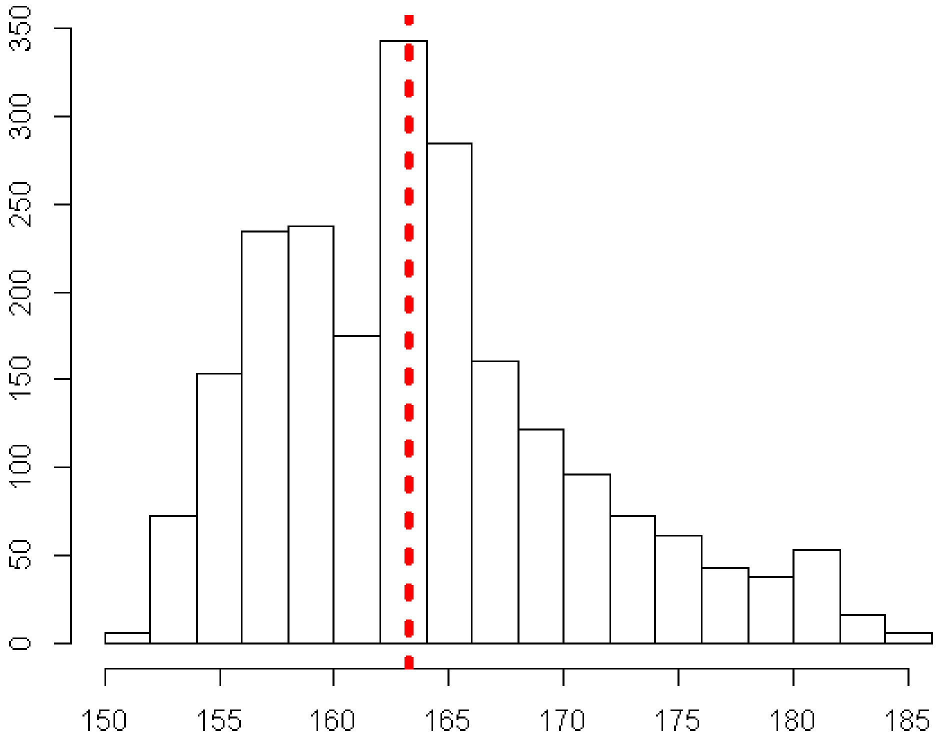
```
# I have determined this call rate from individual recordings.
You need not change anything here.
species_freqs <- c(10, 17, 13, 9, 13, 20, 10, 11, 27, 14, 32, 24,
11) #number of cues emitted by each independently sampled
animal per unit time
freq_length = 1  #length of survey in time units matching what
you used for the call rates above. This 1 means 1 minute.

buffer <- 100 #buffer according to GPS accuracy. Effectively
how large you "zoom" into the array. Change this to 50 and run
the next three lines of code to see how it changes.
data_mask2 <- create.mask(data_mics, buffer)

#These red crosses are the microphones and this is what the
array looked like
plot(data_mask2, pch = ".")
points(data_mics, pch = 16, col = "red")

################################ SUB SAMPLE 1
################################

#Set capture history
#This takes the CDC file, uses TOA to create a capture history
(see Ben's thesis), and sees how this is related to the
microphone array (estlocs file)
data_capt1<-convert.pamguard(dets = data_dets, mics =
data.frame(data_mics),
            time.range = time.range1, sound.speed = 330,
new.allocation = TRUE)

data_capt1.ss <- data_capt1[c("bincapt", "ss")] #this shows
which mics picked up calls and how LOUD the calls were picked
up at those mics
data_capt1.toa <- data_capt1[c("bincapt", "toa")] #this shows
which mics picked up calls and WHEN the calls were picked up
at those mics.
data_capt1.simple <- data_capt1["bincapt"] #this simply shows
which mics picked up calls (binary form)

data_ssmax <- max(data_capt1$ss) #find out max ss in sample

#Write a CSV which has the data of how many calls were heard
on each mic.
datacapt1_simple<-as.data.frame(data_capt1.simple)
write.table(datacapt1_simple, file="SiteXX_calls_on_mics.csv",
col.names = FALSE, row.names = TRUE, sep=",", append=TRUE)

# A signal strength cut-off is needed in the aSCR model. See
Ben's thesis about signal strength cut-offs.
hist(data_capt1$ss[data_capt1$ss > 0])
threshold_cutoff=median(data_capt1$ss[data_capt1$ss > 0])

#This sets some very large boundaries for the model to run so
that it does not take enormous computational time.
data_boundsSSTOA <- list(D = c(0, 100000), b0.ss = c(120,
data_ssmax),
          b1.ss = c(0, 30), sigma.ss = c(0, 30), sigma.toa = c(0,
0.01))

#If a particular subsample is having trouble converging, but a
previous subsample has converged, then you can run the
# variable to which the fit.ascr was done with (data_fit.sstoa_#)
and you will obtain the following:

# Coefficients:
#  D    b0.ss    b1.ss   sigma.ss  sigma.toa   mu.freqs
# 1.389287e+04 1.808717e+02 1.656697e+00 7.986281e+00
8.030577e-03 1.623077e+01

#Use the b1.ss, sigma.ss, and sigma.toa to fill in the next line
(this will provide "starting values" for the model so that it can
hopefully converge)

#sv0 <- list(b0.ss = 120, b1.ss = 1, sigma.ss = 12) #base on
subsample 9
#Make sure to take the comment away if you specified sv in the
code below:

data_fit.sstoa_1 <- fit.ascr(capt=data_capt1, mask =
data_mask2, traps = data_mics,
           cue.rates = species_freqs, survey.length = freq_length,
           ss.opts = list(cutoff = threshold_cutoff), bounds =
data_boundsSSTOA,
           trace=TRUE) #sv=sv0)

#The code above will give the you density estimate of density
of calls (Dc) and density of calling animals (Da). Type
summary(data_fit.sstoa1) to see the density values.

#Some code if you don't want to include signal strength in the
aSCR modelling. DOn't worry about this for now.
# If not using signal strength and time of arrival (TOA) ONLY
# data_fit.sstoa_1 <- fit.ascr(capt=data_capt1.toa, detfn = "hn",
mask = data_mask2, traps = data_mics,
#            bounds = data_boundsSSTOA,
#            trace=TRUE) #sv=sv0)

subsample<-subsample.number.1
ESA <- coef(data_fit.sstoa_1, pars = "esa")
Animals_Per_Hectare_Da <- coef(data_fit.sstoa_1, pars = "Da")
# Da is animal density which is animal per hectre.
Calls_per_hectare<-coef(data_fit.sstoa_1, pars ="D")
SiteArea <- attr(data_mask2, "area")
Site <- site.number
Rep <- rep.number
data_TOTALCALL <- coef(data_fit.sstoa_1, pars = "D") *
coef(data_fit.sstoa_1, pars = "esa")
detfn_yintercept1<- detfn(data_fit.sstoa_1,0)

data_DF_1 = data.frame(Site, subsample, Rep, SiteArea, ESA,
Animals_Per_Hectare_Da, Calls_per_hectare, data_TOTALCALL,
detfn_yintercept1)

write.table(data_DF_1, file = "SiteXX.csv", row.names=FALSE,
na="", append=TRUE, col.names = TRUE, sep=",")

#I alwways create this image for the first subsample run, but it's
not necessary to run this code for the next subsamples as they
will all be the same.
png(file =
paste0("S",site.number,"R",rep.number,"SR",subsample.numbe
r.1,"SurveyArea_1.png"),
  width = 800, height = 800, units = "px", pointsize = 12)
data_SurveyArea <- show.survey(data_fit.sstoa_1)
dev.off()

#Run the following code to see what the detection function for
this subsample looks like. See Ben's thesis and Measey et al.
2017 for examples of good detection functions.
#When we Skype, I can also show you examples.

# png(file =
paste0("S",site.number,"R",rep.number,"SR",subsample.numbe
r.1,"DetFun_1.png"),
#   width = 861, height = 800, units = "px", pointsize = 12)
# data_DetFun <- show.detfn(data_fit.sstoa_1, xlim = c(0, 40))
# dev.off()
#
```

```
# png(file = paste0("S",site.number,"R",rep.number,"SR",subsample.number.1,"DetSurf_1.png"),
#   width = 861, height = 800, units = "px", pointsize = 12)
# data_DetSurf <- show.detsurf(data_fit.sstoa_1)
# dev.off()
#
# png(file = paste0("S",site.number,"R",rep.number,"SR",subsample.number.1,"IndivCallLoc23_1.png"),
#   width = 861, height = 800, units = "px", pointsize = 12)
# data_IndivCallLoc <- locations(data_fit.sstoa_1,id = 23,levels = c(0.50,0.9,0.95), plot.estlocs = TRUE, add=TRUE,
plot(data_mask2, col='gray', xlim = range(data_mask2[,1]),
#                                        ylim = range(data_mask2[,2])))
# dev.off()

#This will only work once you define a n.boot number (normally 300). This is what will take up most of your computational power.
data_boot_1.fit <- boot.ascr(fit = data_fit.sstoa_1, N = n.boot, prog = TRUE, n.cores = cores_use, infotypes = NULL)

boot1sum<-summary(data_boot_1.fit)
boot1sumsum<-as.data.frame(boot1sum)
names(boot1sumsum)

#want to add D
D_calls<-boot1sumsum["D","coefs"]
#want to add D_StdErr
D_StdErr<-boot1sumsum["D","coefs.se"]
#want to add Da
Da_calls<-boot1sumsum["b0.ss","derived"]
#want to add Da_StdErr
Da_StdErr<-boot1sumsum["b0.ss","derived.se"]
#want to add esa
esa<-boot1sumsum["D","derived"]
#want to add esa_stdErr
esa_stdErr<-boot1sumsum["D","derived.se"]

mce1<-ascr:::get.mce(data_boot_1.fit, estimate = "se")["D"]/stdEr(data_boot_1.fit)["D"]
CI1<-confint(data_boot_1.fit, level=0.95)
CI1r2.5<-CI1["D","2.5 %"]
CI1r97.5<-CI1["D","97.5 %"]

subsample<-subsample.number.1

boot1_total_summary<-data.frame(Site, Rep, subsample, D_calls, D_StdErr, Da_calls, Da_StdErr, esa, esa_stdErr, CI1r2.5, CI1r97.5, mce1)
write.table(boot1_total_summary, file = "SiteXX_bootstrap_summaries.csv", row.names=FALSE, na="", append=TRUE, col.names = TRUE, sep=",")

png(filename="SiteXX_300boots_alldetfuns.png")
data_DetFun <- show.detfn(data_fit.sstoa_1, xlim = c(0, 40))
data_DetFun <- show.detfn(data_fit.sstoa_2, xlim = c(0, 40), add=TRUE)
data_DetFun <- show.detfn(data_fit.sstoa_3, xlim = c(0, 40), add=TRUE)
data_DetFun <- show.detfn(data_fit.sstoa_4, xlim = c(0, 40), add=TRUE)
data_DetFun <- show.detfn(data_fit.sstoa_5, xlim = c(0, 40), add=TRUE)
data_DetFun <- show.detfn(data_fit.sstoa_6, xlim = c(0, 40), add=TRUE)
data_DetFun <- show.detfn(data_fit.sstoa_7, xlim = c(0, 40), add=TRUE)
data_DetFun <- show.detfn(data_fit.sstoa_8, xlim = c(0, 40), add=TRUE)
data_DetFun <- show.detfn(data_fit.sstoa_9, xlim = c(0, 40), add=TRUE)
data_DetFun <- show.detfn(data_fit.sstoa_10, xlim = c(0, 40), add=TRUE)
dev.off()


p <-c(
 nrow(datacapt1_simple),
 nrow(datacapt2_simple),
 nrow(datacapt3_simple),
 nrow(datacapt4_simple),
 nrow(datacapt5_simple),
 nrow(datacapt6_simple),
 nrow(datacapt7_simple),
 nrow(datacapt8_simple),
 nrow(datacapt9_simple),
 nrow(datacapt10_simple)
)
s<-sum(p)
q <-mean(p)
t<-sd(p)
summary6<-data.frame(p,s,q,t)
write.table(summary6, file="SiteXX_2016_2017_calls_on_mic_info.csv", row.names=FALSE, na="", append=TRUE, col.names = FALSE, sep=",")
summary6


dint<-c(
 detfn(data_fit.sstoa_1,30),
 detfn(data_fit.sstoa_2,30),
 detfn(data_fit.sstoa_3,30),
 detfn(data_fit.sstoa_4,30),
 detfn(data_fit.sstoa_5,30),
 detfn(data_fit.sstoa_6,30),
 detfn(data_fit.sstoa_7,30),
 detfn(data_fit.sstoa_8,30),
 detfn(data_fit.sstoa_9,30),
 detfn(data_fit.sstoa_10,30)
 )

dintmean<-mean(dint)
dintsd<-sd(dint)
dint_summary<-data.frame(dint,dintmean,dintsd)
write.table(dint_summary, file="SiteXX_detectionfunction_summary.csv", row.names=FALSE, na="", append=TRUE, col.names = FALSE, sep=",")
dint_summary


save.image(file = "SiteXX_300boots_updatedAug2017.RData")

# png(filename="Site17_subsample_5_call_estimations.png")
# plot.mask_5 <- create.mask(data_mics, buffer=20, spacing=0.25)
# locations(data_fit.sstoa_5, 1:nrow(data_fit.sstoa_5$args$capt$bincapt), mask=plot.mask_5, plot.estlocs = TRUE, plot.contours = FALSE)
# dev.off()
```

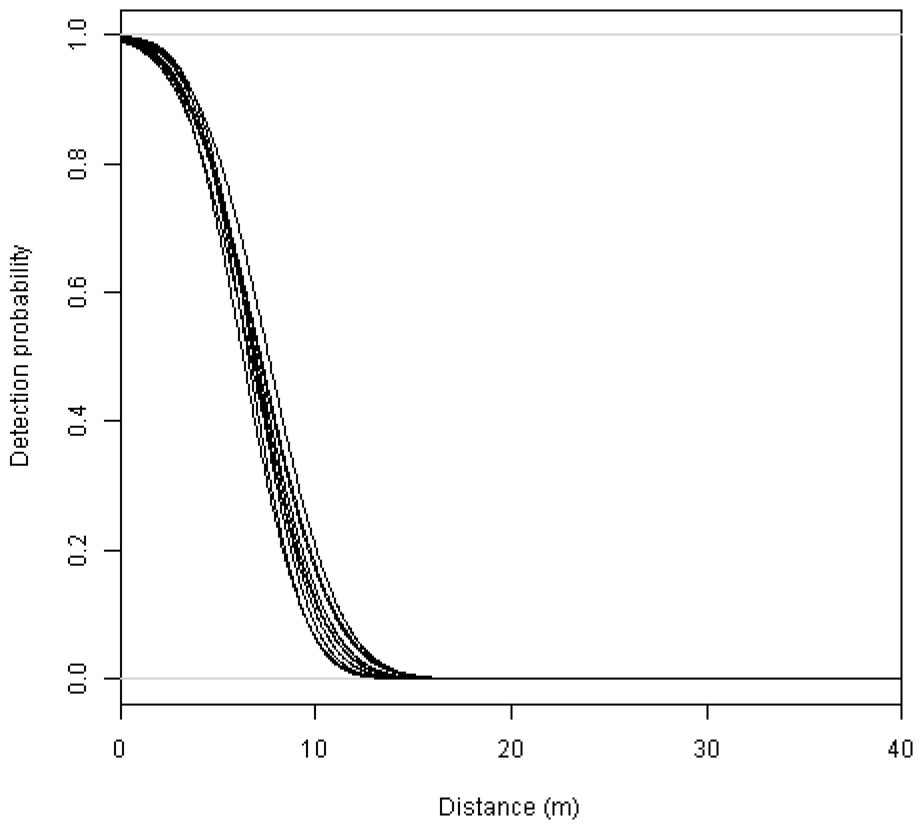
A.12. The detector frequencies refer to the different number of microphones which picked up a frog call. In the example (a), most calls were only picked on one microphone while very few calls were picked up across all six microphones. While (b) shows a different result for a sampling site where most calls could be heard across two microphones and very few across five.



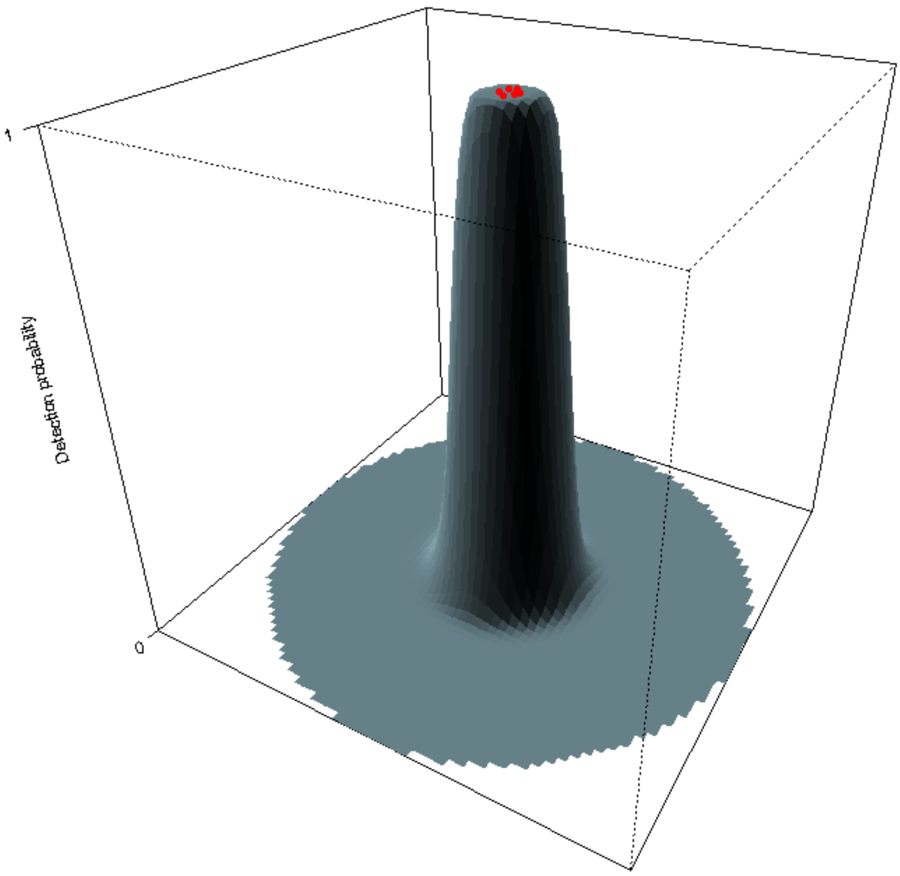
A.13. A frequency plot of the signal strengths received during a subsample of one minute of recorded frog calls. The signal strength cut-off was selected as the median value of signal strengths received (red dashed line, in this case 163.27), as calls above this were assumed to be received with more consistency which leads to improved call density estimates.



A.14. Ten detection functions from 10 subsamples from a single site. Detection functions describe the probability of detecting a frog call given that it is a certain distance away from the microphone detector.



A.15. A detection probability surface for a specific array (each array will have a different detection surface) obtained from the detection functions associated with that array. As the distance from the detectors (red dots) increase, the probability of detecting a frog call decreases.

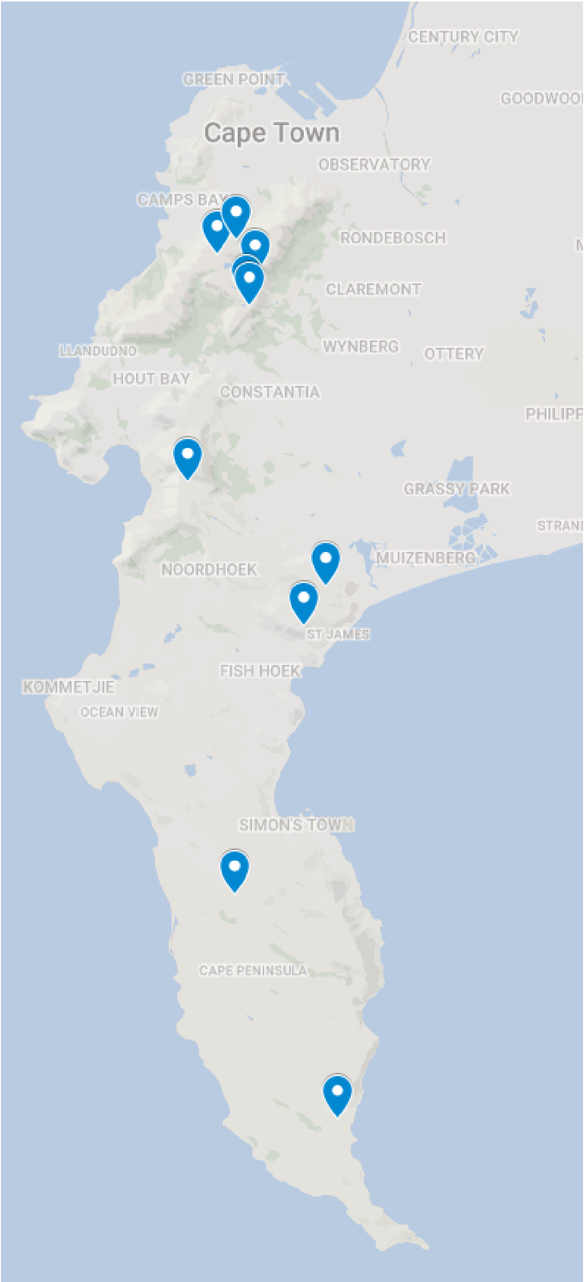


A. 16. The subsample-based modification of the bootstrap procedure described in Stevenson *et al.* 2015. It contains a function written by B. Stevenson that calculates the mean of the subsample standard errors. As some sites have different numbers of subsamples used in the density estimation, this cannot be looped for every site but has to be done manually for every site. In addition, every parameter of interest needs to be specific and the function rerun to obtain the averaged standard error (parameters are calling animal density, call density and estimated sampling area density). If extreme outliers were shown by the generated boxplot, a “ceiling value” would need to be implemented to exclude those outliers in the process of averaging across standard errors.

```
library(ascr)
library(plyr)
## Arguments:
## (1) Provide all bootstrapped model fits.
## (2) par: Parameter for which to calculate standard error.
## (3) plot: TRUE/FALSE, indicating whether or not to draw
boxplot.
## (4) ceiling: An upper limit; values above this are discarded.
subsample.se <- function(..., par = "Da", plot = TRUE, ceiling =
NULL){
 boot.list <- list(...)
 n.fits <- length(boot.list)
 FUN <- function(x, par){
  x$boot$boots[, par]
 }
 mean.pars <- apply(t(laply(boot.list, FUN, par = par)), 1, mean)
 if (!is.null(ceiling)){
  mean.pars <- mean.pars[mean.pars <= ceiling]
 }
 if (plot){
  boxplot(mean.pars)
 }
 sd(mean.pars, na.rm = TRUE)
}
#z is Da error
#k is Dc error
## Running the function and making boxplot.
k<-subsample.se(
 data_boot_1.fit,  data_boot_2.fit, data_boot_3.fit,
data_boot_4.fit, data_boot_5.fit, data_boot_6.fit,
data_boot_7.fit, data_boot_8.fit, data_boot_9.fit,
data_boot_10.fit, plot = TRUE, par ="Da", ceiling=NULL)
k #this is Da st.err
z #This is Dc st.err
g #This is ESA st.err

## If there are hectic outliers, set the ceiling value
appropriately.
```

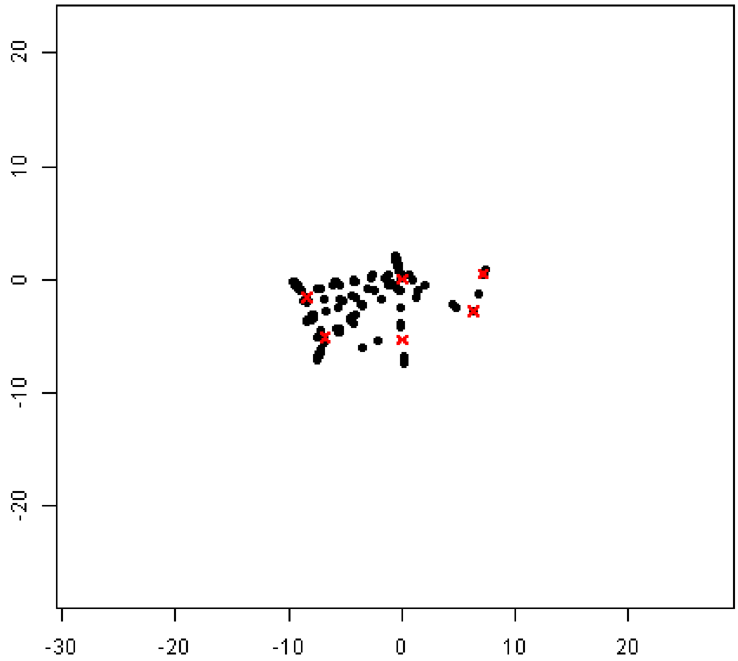
Appendix B

B.1. The 10 selected sample sites at which I set up arrays for the aSCR analysis and obtained aerial photographs with the drone.

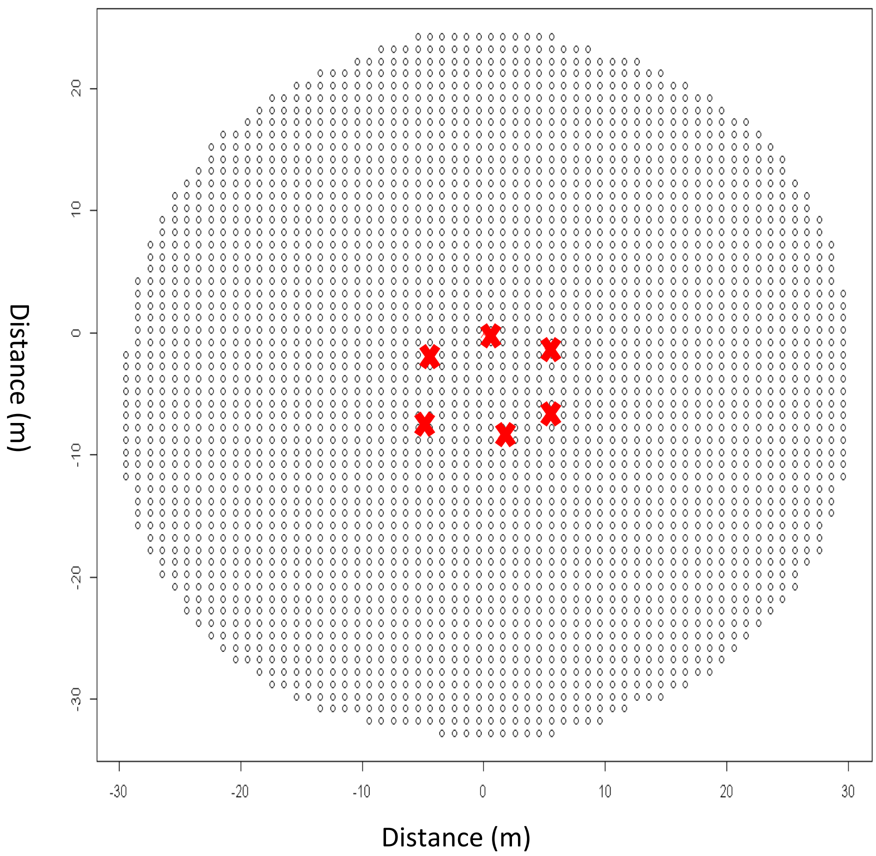


Site	**Latitude**	**Longitude**
Site 1	-33.9924	18.41902
Site 2	-34.101	18.44868
Site 3	-33.9799	18.41606
Site 4	-33.9896	18.41167
Site 5	-34.1165	18.43819
Site 6	-34.2203	18.40642
Site 7	-34.3072	18.45403
Site 8	-33.9727	18.39776
Site 9	-34.0607	18.38455
Site 10	-33.9666	18.40676

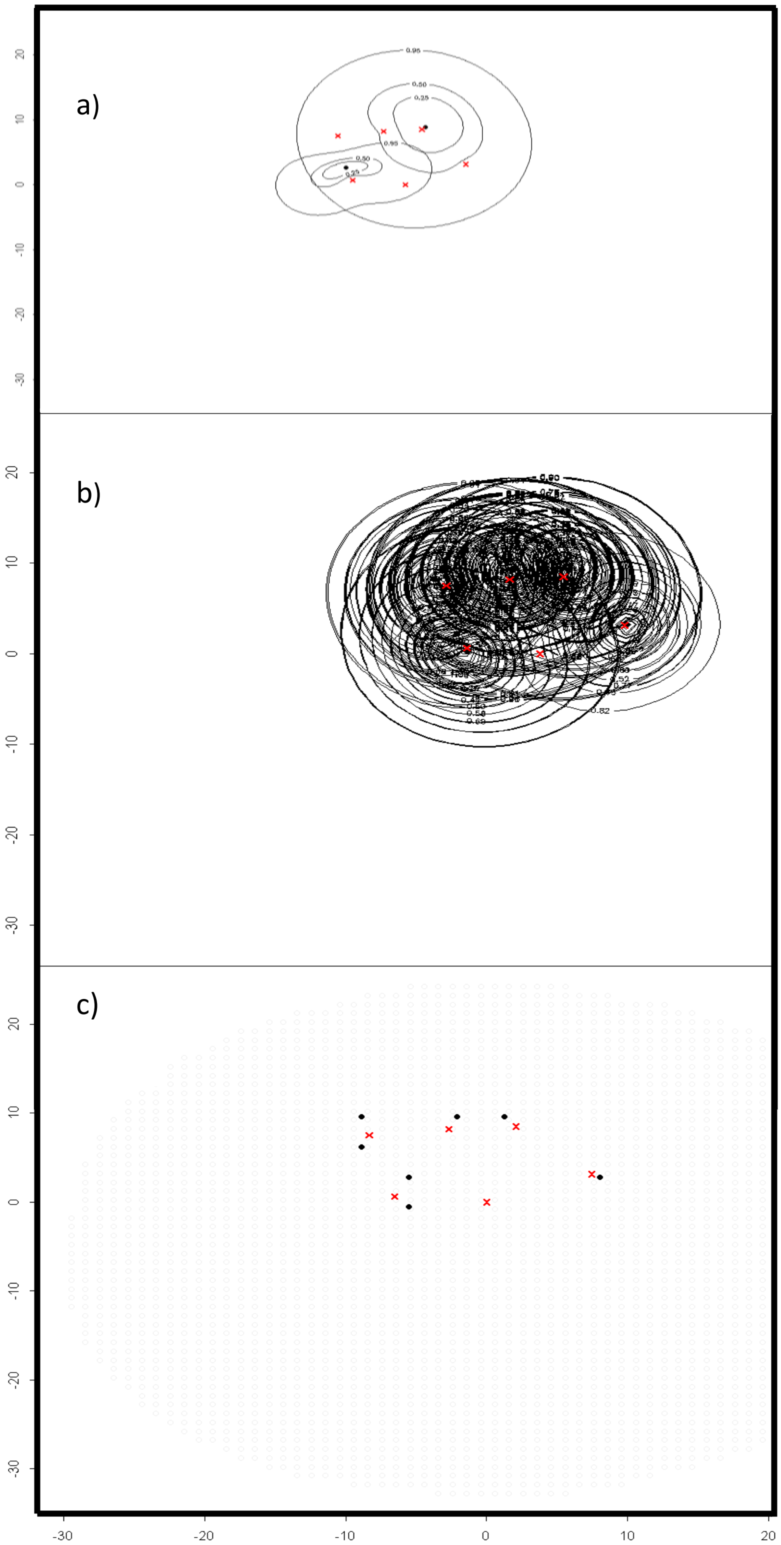
B.2. The estimated calling locations of frogs (as black dots) plotted in relation to the array where the position of microphones are represented as red crosses. Microphone one is situated at the origin.



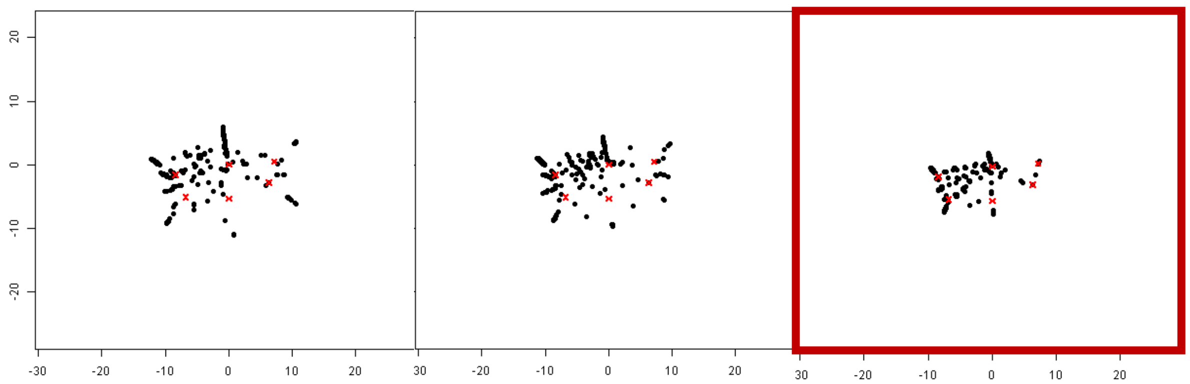
B.3. The habitat mask in spatial capture recapture analyses is an area surrounding the acoustic array that is divided into discrete points. For illustration, the number of points of which the habitat mask is composed in this picture have been reduced and outlined in grey. In the function *locations* from the "ascr"package, these points can be darkened to be dots, which represent the most likely calling location of a frog as determined by the aSCR model. The habitat mask must be larger than the estimated sampling areas.



B.4. a) A calling location is described by contours which represent different probabilities that a call was emitted within the contour. An example of contours associated with two different calls are shown. The outer contour represents the probability that an individual is located within the contour with a probability of 0.95, the middle contour represents a probability of 0.50 while the innermost contour is a probability of 0.25. b) When these probability contours are represented for each estimated calling location, the plot gets too messy to be informative, which is why c) the dots are used to represent the most likely location from which a frog was calling (based on the probability contours).



B.5. The most representative subsample (boxed in red) is the subsample with the smallest error associated with the density estimate, and also has call locations that "agree" on the most likely position of calling frog (i.e. they are preferably not smudged).



B.6. a) The detection function associated with one microphone can be applied to all microphones in an array to provide b) a three-dimensional detection probability surface where the red crosses at the top of the cone represent the microphones. c) From this, the estimated area that the array cover can be obtained.