Using spatially explicit call data of *Anhydrophryne ngongoniensis* to guide conservation actions

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Abstract

It's been barely 25 years since the Mistbelt Chirping Frog (*Anhydrophryne ngongoniensis*) was discovered. This secretive amphibian occurs only in the so-called mistbelt grasslands and montane forest patches of south-central KwaZulu-Natal, South Africa and is restricted to an area of occupancy of just 12 square kilometers. This species' habitat is severely fragmented due to afforestation and agriculture and only two of the remaining populations are formally protected. The species occurs mostly on fragmented grassland patches on forestry land, and any conservation strategies should include the management practices for the landowners. Updated density estimates and insight into habitat utilization are needed to proceed with conservation strategy for the species. Like many other frogs, this species is cryptic in its behaviour, making mark-recapture surveys prohibitively challenging. Audio transects have been used previously, but are dependent on surveyor's' experience, hindering standardization. Using automated recorders, in a spatially explicit array with GPS synchronization, one can confidently estimate the density of calling males and reveal the estimated locations of calling males, thus providing insight into their occupancy. We surveyed nine historic sites and detected the species at five of the sites in either isolated grassland patches or indigenous Afromontane forest. We successfully employed the spatially explicit catch recapture (SECR) method at three of the sites using Wildlife Acoustics™ Song Meters with extended microphones in an array. Audio data was processed with Pamguard™ open-source software and analysis done in R using the *ascr* package. Density estimates of calling males were much higher for the sites than estimated with previous methods. The results also provided insight into calling behaviour and the distribution of the species, which appears to be clumped and localised within a breeding site. The data obtained will be used to update population estimates and guide conservation measures, especially pertaining to land management practices. Recommendations to land owners include the stringent management of road verges and Afromontane forest patches. Even though density estimates were higher using SECR compared to transects, we recommend that the species retain its Endangered listing since the occupation within a breeding site is very limited.

Keywords: *Anhydrophryne ngongoniensis*, call data, density estimate, forestry conservation, land management, Mistbelt Chirping Frog, passive acoustic monitoring, Song Meter, spatially explicit catch recapture

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“The burden of conserving biodiversity will fall increasingly on sectors such as agriculture, forestry, mining and land-use planning. In order for these sectors to play a constructive role in conservation, it is essential that biodiversity concerns be integrated or mainstreamed into their policies and practices.”

(Pierce *et al*., 2005).

Chapter 1: Introduction and Literature overview

1.1 The plight of the frog

South Africa is home to 125 species of frogs, among the highest species richness for anurans in the world. In fact, South Africa has the 27th greatest known species richness at a global level and fifth at the Afro-tropical biogeographical realm (Stuart *et al.*, 2008). While this is impressive from a biodiversity perspective, it is important to understand that we are dealing with often very low or unknown numbers of threatened species.

The planet is experiencing a high loss in biodiversity and amphibians are one of the most affected groups of vertebrates, with a third of species currently Red Listed by the IUCN (2016). This trend is very much represented in South Africa where close to 30% of anuran species are listed as Critically Endangered (7%), Endangered (12%) or Vulnerable (10%) (Measey, 2011). The main cause for the decline in amphibian numbers is habitat loss (Stuart *et al.*, 2008; Measey, 2011) and so the formal protection of areas with high biodiversity or threatened amphibians is important and should be a conservation priority. Many species could face extinction if drastic measures aren't taken to protect habitat – those areas affected by clear threats but also areas not under obvious threat (Veath *et al.*, 2004).

The fact that the majority of threatened amphibians in Africa have small distributions and coincide with areas of high endemism (Measey, 2011), renders conservation not only imperative, but also challenging. It is common for amphibians to be included in the analysis of biodiversity for conservation planning (Scott *et al.*, 1993; Armstrong, 2001). However, to create and implement effective conservation strategies, it is important to understand the basic biology and ecology of the amphibians (Schmidt *et al.*, 2002; Stuart *et al.*, 2004). So, if we are to understand and ultimately reverse their decline we need insight into population dynamics and estimating demographic parameters, especially declines in populations and other changes in community structure (Marsh, 2001; Dodd, 2003; Buckley and Beebee, 2004).

Many species with a formerly continuous spatial distribution are being turned into possible metapopulations by habitat fragmentation (Measey, 2011). The effective long-term viability of populations of many of these species within these areas, must be assessed (Drinkrow and Cherry, 1995) so that relevant management remedies may be implemented to prevent total extinction. If an isolated endemic species becomes locally extinct, it is likely gone forever since groups of a species confined to newly fragmented habitat do not necessarily function as a metapopulation, due to poor dispersal ability (Hanski and Gilpin, 1991).

The Critically Endangered and Endangered species in South Africa will likely retain their threat categories at a global level as they are all endemic species (plus all Vulnerable species, with the exception of *Breviceps macrops*, are endemics) (Measey, 2011). But where do we place our efforts?

Lists of priority species are an important tool for the effective allocation of scarce conservation resources (Isaac *et al*., 2012). Such lists typically comprise the Endangered and Critically Endangered categories of the IUCN Red List. However, the concept that species' contribution to phylogenetic diversity should also be considered, is becoming increasingly popular (Isaac *et al*., 2012). In other words, if a species diverged from their closest relatives several millions of years ago, they will be viewed as extra important to protect. Therefore, we now consider not only the threat of extinction for a species, but also the risk of losing a part of evolutionary history.

To this effect, the Zoological Society of London established the Edge of Existence programme through which they scored the world's mammals, amphibians, corals and birds according to how Evolutionarily Distinct and Globally Endangered (EDGE) they are. A list is compiled for the top 100 EDGE scores for each group of animals. This is in an effort to provide conservation support to animals that would normally receive little to no conservation action as they are rarely the charismatic species that attract resources and funds. The Mistbelt Chirping Frog (*Anhydrophryne ngongoniensis*) is currently number 100 on the EDGE amphibian list (www.edgeofexistence.com, 2017).

By prioritizing species with phylogenetic uniqueness, in addition to extinction risk status, the conservation covers not only the non-randomness of extinction (with respect to phylogenetic position), but also the fact that evolutionarily distinct species could have important ecological roles and that their loss would result in an over-proportional loss of evolutionary history (Isaac *et al*., 2012).

1.2 Case study for conservation: Mistbelt Chirping Frog

The Mistbelt Chirping Frog (*Anhydrophryne ngongoniensis*) has been known to science for barely 25 years (Bishop and Passmore, 1993). This secretive amphibian is found only in the high altitude mistbelt grasslands of south-central KwaZulu-Natal, South Africa. The species is restricted to a tiny area (AOO) of just 12 square kilometers (IUCN, 2016). And the prognosis for its future existence isn't good – surveys conducted on the species 10 years after its discovery suggest that two historical subpopulations may already be extinct (Harvey, 2007*). To put that into perspective there are currently only 12 small and fragmented subpopulations known from four locations (Bishop, 2004a; Harvey, 2007). Consequently, the species is listed as Endangered (IUCN, 2016), and it features in the Top 100 ZSL EDGE amphibian species. A recent population estimate (Harvey, 2007) puts the total number of individuals alive at the time at roughly 3000, but that was over 10 years ago.

We can attribute the decline of *A. ngongoniensis* to two main factors – afforestation and agriculture. In fact, 50% of all South African frog species are affected by agriculture and 40 out of 43 frog species in South Africa are impacted by invasive alien vegetation and afforestation. Invasive plants threaten many species in protected as well as disturbed areas (Measey, 2011).

At present *A. ngongoniensis* receives limited conservation attention, and since all but one of its known sites occur in unprotected areas, the species needs all the attention it can get. Conservation of any threatened species is subjected to knowledge of the species' distribution, biology, and behaviour before a meaningful assessment of direct threats and impacts can be made (Measey *et al*., 2011a). Updated population size data is a critical step for conservation decision-making.

The statistics suggest that *A. ngongoniensis* has lost half its habitat in just 50 years (Harrison *et al*., 2001; Minter *et al*., 2004). Consequently, their habitat is becoming increasingly fragmented, while all but one subpopulation occur in unprotected areas, making them vulnerable to alien plant infestation and inappropriate burning regimes (Measey, 2011). Harvey (2007) provided guidelines for management of habitat for the species to the two local forestry companies on whose property the species occurs, namely Merensky and Sappi Forestry products. These recommendations focused on burning regimes, alien plant control and forestry activities. Today we are unsure whether these recommendations are still in place or what the situation is at the few other sites where these Endangered frogs occur.

This project aims to determine the status on recommended management practices and to improve on the limited knowledge of the abundance by employing a new passive acoustic monitoring (PAM) survey method and a spatially explicit capture-recapture (SECR) model.

1.3 Passive Acoustic Monitoring (PAM) and Spatially Explicit Capture-Recapture (SECR)

In order to monitor the frogs, we need to find the best way to detect them first. While detection of any animal species is typically not perfect, this is particularly true for species that are difficult to observe visually, which is certainly the case for *A. ngongoniensis* as well as for many other anurans (MacKenzie *et al*., 2002; Smith *et al.,* 2006). Anuran abundance estimates were traditionally conducted using the capture-mark-recapture (CMR) methods (Nelson and Graves, 2004; Phan *et al.,* 2007), but this requires a huge amount of man-hours if the species is hard to locate though visual cues. Luckily, frogs have well developed vocal chords that allow them to produce unique sounds or calls. Acoustic monitoring methods are based on the species-specific advertisement calls made by anuran males during the breeding season (Pierce and Gutzwiller, 2004; Pellet and Schmidt, 2005). Since for almost all frog species, only males vocalize, it is possible to obtain an estimate of calling males only during an acoustic survey. Qualitative estimates of call density indexed on a categorical scale for frog populations are consistent with estimates obtained from traditional capture-recapture methods i.e. there is a significant linear relationship between numbers of individuals heard calling and the number of individuals captured using different survey techniques, such as CMR (Shirose *et al*., 1997; Grafe and Meuche, 2005). This then suggests that counting the number of calling males at a breeding site during peak breeding season can be used to estimate male population size and infer population trends (Geiger, 1995; Stevens *et al*., 2002; Buckley and Beebee, 2004), but it should form part of an integrated and intense monitoring programme (Shirose *et al*., 1997). Although, as Stevens and Paszkowski (2004) noted, not many researchers have made use of these methods possibly due to the inherent difficulties in counting calling males in ponds and wetlands and the interpretation of such data.

Even when using standardised methods, count data can be highly biased if not adjusted for detection probabilities (Grafe and Meuche, 2005). Surveys should ideally aim for high detection probabilities at peak calling periods (Pellet and Schmidt, 2005). Imperfect detection is highly likely for species that are small, have inconspicuous calls and cryptic behaviour (Bishop, 2004b). *Anhydrophryne ngongoniensis* has a fairly soft, insect-like call that does not carry far, and is itself very well camouflaged even when heard calling (Passmore 1993; du

Preez and Carruthers 2009). They are also very small, measuring at just 20mm. As such, it is often difficult to detect, particularly if sympatric species are chorusing loudly (Bishop, 2004a).

Auditory survey methods have evolved from using call indices for the number of frogs heard calling, to using passive acoustic monitoring, and now arrays of microphones from which biologists can monitor the position of free-living animals based on the sounds they produce (Mennill *et al*., 2012). Furthermore, the equipment required is relatively simple to use and commercially available. Processing of data does however involve very specific analytical methods.

While physical trapping is a common means of obtaining capture–recapture data, models that incorporate a spatial component are new. The spatially explicit component in the model solves the problem of trapping only animals located closest to traps (Borchers, 2012). And this extends to trapping of individuals by the sounds they make. Furthermore, a single microphone can detect more than one sound and more than one microphone can detect a single sound (Efford *et al*., 2009a). The essence of SECR lies in the probability of a call being detected by at least one microphone in an array. This can be modelled by combining detector-wise probabilities, each of which we assume to be a decreasing function of distance from the source (Borchers and Efford, 2008; Efford *et al*., 2009a). Through call surveys, data can be obtained rapidly to identify species, map distributions and estimate abundances using the newly developed methods for spatially explicit capture–recapture. (Efford *et al*., 2009a; Channing *et al*., 2011). The relationship between calling intensity and animal abundance is an important topic that needs further research (Royle and Link, 2005). The aim of this study is to add valuable information to an already growing database to determine effective monitoring methods for amphibians in South Africa, along with other institutions also currently working on this e.g. CapeNature, Stellenbosch University and Endangered Wildlife Trust Threatened Amphibian Programme. Call density remains a good proxy for frog density as the number of males calling or chorusing is the best-known determinant of mating success in many anuran species (Halliday and Tejedo, 1995; Crouch and Paton 2002; Pellet, Helfer and Yannic, 2007).

The microphone array technology utilizes the subtle differences in sound arrival times at each microphone to calculate an animal's position. The modern spatially explicit capture–recapture (SECR) methods (Efford, 2004; Borchers and Efford, 2008; Royle and Young, 2008; Royle *et al*., 2013) basically combines the spatial component of distance sampling and the temporal nature of capture– recapture approaches (Stevenson and Borchers, 2014). Mennill *et al*. (2012) found that location accuracy of the sound source was significantly higher when the microphones were closer together and when sounds originated from inside the area bounded by the array. Methods are now being refined - by using signal strength of each call arriving at each microphone, the results become more accurate. An acoustic detection is defined as occurring when a received signal strength exceeds a particular threshold (Efford *et al*., 2009a; Stevenson and Borchers, 2014). This makes it identifiable above background noise and allows one to discard anything below the threshold. Efford *et al*. (2009b) suggests the use of signal strength above binary data only as it greatly improves precision when the microphone array is small.

The method assumes that individuals detected at the same place are acoustically separable and that all individuals are vocal during the recording interval for an estimation of cue density to translate directly to an estimate of population density. If cues from different individuals cannot be distinguished, an estimate of the cue production rate (per capita rate of vocalization) is needed to convert cue density to population density (Buckland *et al*., 2001; Efford *et al*., 2009b; Dawson and Efford, 2009).

When surveying frogs, the time difference of arrival (TOA) of the same call at different detectors and the received signal strength (SS) at each detector provide data on animal location. Instead of using actual distances to animals, SECR makes use of the distances between microphones (those that detected the animal and those that didn't) to calculate a distance-based detection probability surface (Borchers *et al*., 2015). Using acoustic SS on a passive acoustic array to supplement the SECR data leads to substantial improvements in the precision of density estimates for small arrays (Efford *et al*., 2009a; Borchers, 2012).

However, statistical analysis of PAM is not straightforward because not all individuals are detected, and not all detected animals live within the perimeter of the array. Conversely, the passive monitoring, although possible for extended periods of time, does not need to be long to yield good results. (Efford *et al*., 2009a). When individuals can be detected (virtually) simultaneously on multiple detectors (e.g. by virtue of the same call being recorded at multiple microphones), then 'recaptures' (or, more accurately, redetections) occur at different points in space rather than across time, thus removing the need for multiple survey occasions (Stevenson and Borcher, 2014). In fact, precise and unbiased estimates can be obtained from detectors that allow detection of the same individual by more than one detector (microphone) on a single occasion (Borchers, 2012).

1.4 Conservation planning and practice

The available habitat for *A. ngongoniensis* is offered very little official protection. Apart from the protected area of Ngele Forest, it is in the remaining patches of once vast grassland areas that *A. ngongoniensis* is found. This fragmented habitat is mainly spread across the property of two forestry companies, Sappi and Merensky. The grassland patches are interspersed among soft wood pine plantations. Improved protection and maintenance of the remaining grassland habitat was identified as priorities for the species in a recent strategy for conservation research for South African frogs (Measey, 2011).

The grassland biome of South Africa contains a significant amount of ecosystem services surface water supply, water flow regulation, carbon storage, soil accumulation, and soil retention (Egoh *et al*., 2009) and whilst this might not be the main driver for the conservation of these areas by the forestry companies, they do seem genuinely interested in helping to protect and conserve the remaining *A. ngongoniensis* populations as is evident in their co-operation with work done by James Harvey* in 2005, as well as through their enthusiasm to meet with our team and allow us access to their property.

The grasslands biome in South Africa has received a fair amount of recent conservation attention (Little and Theron, 2014; Boakye *et al*., 2014; Little *et al*., 2015). Since it contains the economic heartland of South Africa, and is home to most South Africans, it has come under a huge amount of development pressure that is not sustainable. A *Grassland Programme* (GP) was developed to meet national biodiversity conservation targets for, and to seek more sustainable development of, the biome (Jackelman *et al*., 2006). "The Grassland Programme is a bioregional program managed by the South African National Biodiversity Institute (SANBI). A dedicated Program Management Unit (PMU) appointed by SANBI is currently busy with the design and planning phase of the GP. The strategic approach for this design and planning phase is to mainstream biodiversity in production landscapes and sectors within the grassland biome." Plantation forestry was identified as one of the key production sectors targeted by the programme and securing permanently unplanted forestry land for biodiversity and ecosystem services was set as a priority. Three steps were identified for the conservation of these areas (Jackelman *et al*., 2006):

1. Identify biodiversity priority areas within the grasslands biome owned by forestry companies that are permanently unplanted and that they are willing to secure for biodiversity conservation purposes.
2. Unpack the legal requirements to realize the options to formally secure the conservation status of these sites.
3. Negotiate and reach agreement with the relevant company and conservation authority on which option to pursue for securing and managing this land as formal conservation areas.

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1.5 Study Objectives:

This study aims to improve the likelihood of survival of *Anhydrophryne ngongoniensis* through improved conservation management by achieving the following objectives.

1. Determine the population abundance and distribution at 5 historical sites.
2. Employ a new SECR method for monitoring the species passively.
3. Investigate spatial habitat utilization.
4. Establish a standardized model for the species to process call data obtained in the future.
5. Review and update land management recommendations.
6. Increase awareness of frogs and their ecological importance through environmental education.

1.6 Mentorships, conferences & training

1.6.1 Endangered Wildlife Trust

This MSc project is part of the Endangered Wildlife Trust's Threatened Amphibian Programme under co-supervision of Dr. Jeanne Tarrant.

1.6.2 ZSL EDGE Fellowship

Funding for the project was secured through a Fellowship with the Zoological Society of London's EDGE of Existence Programme (see www.edgeofexistence.org). The Fellowship kicked off with a 4-week Conservation Tools training course for the 8 selected fellows at Caño Palma Biological Station in Tortugeuro, Costa Rica in 2015. There we received intensive training in:

Principles of Conservation Biology, Project Planning, Project Management, Statistics, Ecological Monitoring, Social Science, Action Planning and Communicating Conservation. A certificate is attached (Appendix 1). Furthermore, guidance and support for the project, especially with the processing of data and statistical methods was provided by the conservation biologists and ecologist of the ZSL EDGE programme.

1.6.3 Amphibian Conservation Research Symposium

A poster on my work was presented at the 2016 Amphibian Conservation Research Symposium (Appendix 2).

1.6.4 Student Conference of Conservation Science

Through the EDGE Fellowship I was able to apply for and secure a bursary to attend the Student Conference of Conservation Science (SCCS) at Cambridge University in April 2016. I presented my work with a talk entitled "*Spatially-explicit call surveys for the mistbelt chirping frog*" (Appendix 3). Prior to the conference I attended a 3-day short course in R, presented by Prof. Will Cresswell from the University of St. Andrews as part of the SCCS (Appendix 4). The bursary also enabled me to attend a month-long internship at Zoological Society of London at the EDGE of Existence Programme office's, where I worked closely with conservation biologist, Dr. Claudia Gray on furthering my results.

1.6.5 Media coverage for the project

1. The ZSL EDGE blog offered great exposure for myself and my project with an introduction (Appendix 5) as well as a follow-up article when we found the frog (Appendix 6).
2. An article in issue 116 of FrogLog (Appendix 7).
3. A feature on the UK publication, the Guardian's website was shared over 1000 times on social media (Appendix 8).

Chapter 2: Materials & Methods

2.1 Study site selection

The distribution of *Anhydrophryne ngongoniensis* is grouped into two areas in southern KwaZulu-Natal (Figure 1). The northern distribution falls in the Ixopo area, mainly on the property of Sappi Forestry Products and includes the sites: Lynford, Roelton Dam and Qunu Falls. It consists of fragmented sloped grassland habitat dispersed amongst large pine plantations. The elevation in the northern area (1000–1300 masl) is slightly lower when compared to the southern distribution (1200–1650 masl). The sites in the southern range of the distribution occur closer to the town of Kokstad, mainly on the property of Merensky Forestry Products, except for the Ngele Forest site, which is a government Nature Reserve. Other sites in the south include the Mpur Forestry area, Poortjie and Franklin. The southern distribution also consists of sloped grassland patches amongst pine plantations but with pockets of indigenous forest present. We aimed to collect call data from sites that would reflect the range of distribution of the species.

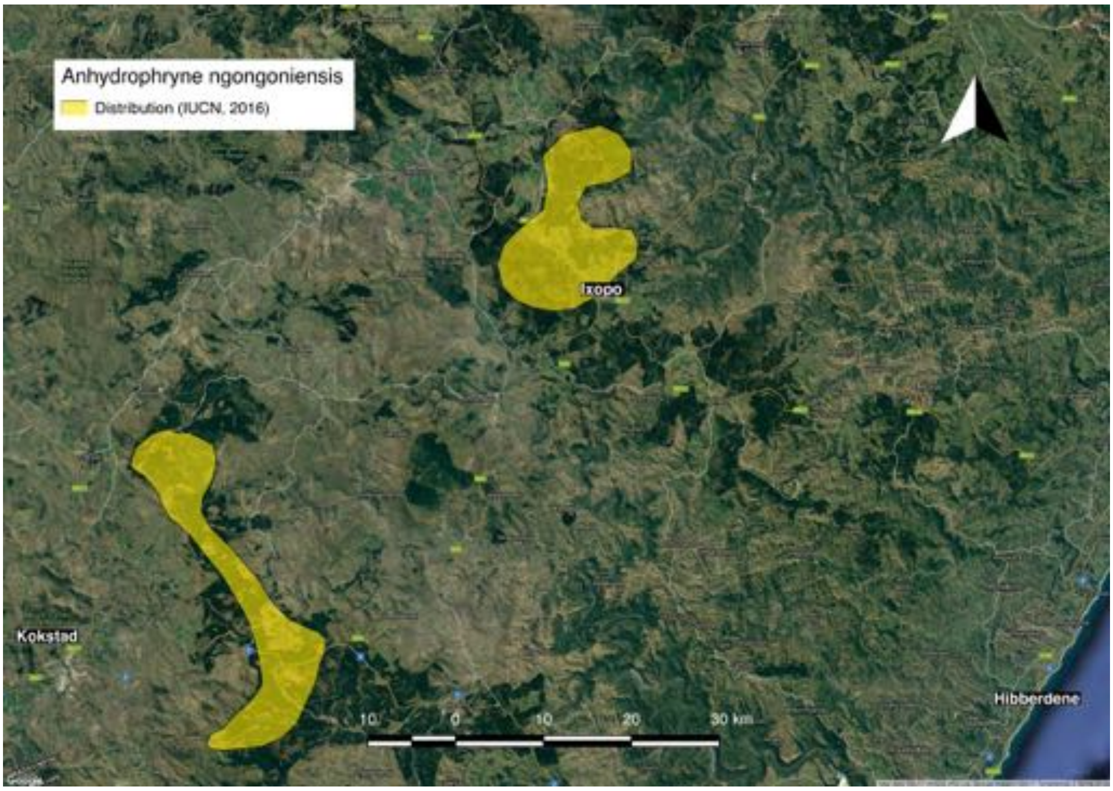

Figure 1. The interpreted distribution of *Anhydrophryne ngongoniensis* according to the IUCN Red List 2016.

We made use of the distribution records of Harvey (2007) to guide our efforts as the species was detected at multiple localities between 2004 and 2007. Nine sites were identified with potential for having *A. ngongoniensis* populations (Table 1). We visited these sites between 5

December 2015 and 2 March 2016. Variables including location (GPS coordinates), proximity to roads, vegetation type, temperature, wind, humidity and elevation were noted.

2.1.1 Poortjie Grassland

This site **(**Figure 2**)** consists of a vast Drakensberg Foothill Moist grassland (Mucina and Rutherford, 2006) around a medium sized wetland. The grassland is fairly level with slightly sloped areas leading to the wetland. The grassland is bisected by a dirt road and surrounded by dirt roads and pine plantation. A small dam is located at the bottom of the wetland. There is a small patch of American Bramble (*Rubus flagellaris*) encroaching on the grassland from the dirt road and cattle grazing takes place on a small scale but no further threats are obvious. The entire grassland and wetland area measures approximately 59 Ha while the patch of sloped grassland in which *A. ngongoniensis* is believed to occur is estimated at 7 Ha.

Table 1: A summary of the sites that were surveyed for *Anhydrophryne ngongoniensis* as part of this study.

Name	***Habitat type (Mucina and Rutherford, 2006)***	***Coordinates***	***Elevation (masl)***	***Grassland/Forest size***
Poortjie grassland	Drakensberg Foothill Moist Grassland (Gs10)	30.33278 S; 29.51472 E	1625	7 Ha
Poortjie forestry area	Midlands Mistbelt Grassland (Gs9) and Southern Mistbelt Forest (FOz3)	30.34686 S; 29.55260 E	1353	0.85 Ha
Mpur/Poortjie road verge	Drakensberg Foothill Moist Grassland (Gs10)	30.31809 S; 29.53083 E	1638	7 Ha
Lower Mpur Forest	Midlands Mistbelt Grassland (Gs9)	30.33201 S; 29.59759 E	1210	0.5 Ha
Franklin14 wetland	Drakensberg Foothill Moist Grassland (Gs10)	30.30076 S; 29.54979 E	1469	14 Ha
Ngele forest	Southern Mistbelt Forest (FOz3)	30.52873 S; 29.68539 E	1348	680 Ha
Qunu Falls	Southern Kwazulu-Natal Moist Grassland (Gs11)	30.01633 S; 30.0659 E	1150	15 Ha
Roelton Dam	Midlands Mistbelt Grassland (Gs9)	30.13188 S; 29.98666 E	1290	125 Ha
Lynford	Midlands Mistbelt Grassland (Gs9)	30.11643 S; 30.05071 E	1022	25 Ha

2.1.2 Poortjie Forestry Area

This site **(**Figure 3) consists of a small patch of Midlands Mistbelt grassland (Mucina and Rutherford, 2006) surrounded by pine plantation quadrants and remnants of Southern Mistbelt

Forest (Mucina and Rutherford, 2006). The grassland site is inaccessible due to a *R. flagellaris* infestation (Figure 4) and is estimated to be 0.85 Ha in size.

2.1.3 Mpur Road Verge

A narrow sloped Drakensberg Foothill Moist grassland (Mucina and Rutherford, 2006) between a dirt road and pine plantation quadrants (Figure 5). The grassland is not homogenous and various herbs and invasive weeds are present. The soil is exposed in some areas and a thick matt of pine needles is present where the grassland borders the plantation (Figure 6). The understory is thick in areas with mossy, moist microhabitat underneath (Figure 7). The entire continuous road verge is 7 Ha in size.

2.1.4 Lower Mpur Forest

This site (Figure 8) consists of small, heavily sloped Midlands Mistbelt grassland (Mucina and Rutherford, 2006) at the bottom of a small indigenous forest. There are plenty of invasive weeds, woody shrubs and other alien vegetation present, with a severe infestation of *R. flagellaris* (Figure 9). The site is surrounded by pine plantation and is about 0.5 Ha in size.

2.1.5 Franklin 14 Wetland

A large level Drakensberg Foothill Moist grassland (Mucina and Rutherford, 2006) adjacent to a large wetland (Figure 10). It is surrounded by pine plantation quadrants and service dirt roads. Grazing of livestock takes place in the grassland but invasive vegetation appears to be minimal. The size of the entire grassland area is approximately 14 Ha.

2.1.6 Ngele Forest

This large sloped Southern Mistbelt Forest (Mucina and Rutherford, 2006) (Figure 11) is bisected by a national carriage way (N2) and surrounded by pine plantation and grassland. There is minimal alien vegetation and a dense herbaceous understory with various ferns present (Figure 12). The canopy provides approximately 70% cover (Figure 13). The entire forest is 680 Ha in size, although *A. ngongoniensis* is restricted to small pockets only.


Figure 2. Poortjie wetland and adjacent grassland habitat in a typical mistbelt misty afternoon.


Figure 3. Poortjie pine plantation grassland behind a patch of indigenous forest (Poortjie Forest).


Figure 4. American Bramble infestation as can be seen from the road by Poortjie forestry area.


Figure 5. Mpur sloped grassland on road verge.


Figure 6. A thick matt of pine needles encroaching on the grassland road verge.


Figure 7. The matted dry grass understory reveals a moist, mossy microhabitat when parted.

Figure 8. The Midlands Mistbelt Grassland of lower Mpur forestry area.

Figure 9. American Bramble (*Rubus flagellaris*) infestation at lower Mpur.

Figure 10. The vast level grassland of Franklin14 with pine plantations in the background.

Figure 11. Ngele forest with the N2 carriageway in the foreground.

Figure 12. The lush understory supporting *Anhydrophryne ngongoniensis* in Ngele Forest.

Figure 13. The canopy of Ngele Forest letting through 30-40% sunlight.

2.1.7 Qunu Falls

A steep sloped Southern Kwazulu-Natal Moist Grassland (Mucina and Rutherford, 2006) leading to a small patch of indigenous forest (Figure 14). Dense patches of ferns are present in some areas with various herbs found throughout the grassland as well. There is a fair amount of cattle grazing taking place. The grassland and forest patches are approximately 15 Ha combined.

2.1.8 Roelton Dam This is a very large sloped Midlands Mistbelt Grassland (Mucina and Rutherford, 2006) with various foot slopes leading to a dam (Figure 15). Sporadic woody shrubs, palms and minimal alien encroachment is present. The biggest possible threat to the area is the mowing that takes place annually (Figure 16). The grassland is approximately 125 Ha.

2.1.9 Lynford

A large sloped Midlands Mistbelt Grassland (Mucina and Rutherford, 2006) surrounded by dirt roads and pine plantation with small forest patches at one end (Figure 17). There is a ditch with running water and reeds that runs through the grassland. Some soil disturbance is taking place due to erosion and what appears to be topsoil removal, plus there is large scale alien vegetation infestation occurring although some management of the problem seems to be in place. The grassland area is approximately 25 Ha in size.

2.2 Searching for frogs

Surveys started by actively searching for *A. ngongoniensis* within known sites from 17h00 until 19h30. If no frogs were heard calling by then, the survey was stopped. We used the roads as audio transects, stopping whenever calling was suspected. We also conducted a sweep search at historic sites by traversing the grassland patches and listening for calling males. All detected anuran species were noted, whether identified visually or audibly.

Figure 14. The sloped Southern Kwazulu-Natal Moist Grassland of Qunu Falls.

Figure 15. The vast grasslands of Roelton Dam with the dam visible at the far end.

Figure 16. The mowed winter grasslands at Roelton Dam.

Figure 17. The Lynford site with pine plantations in the background.

Figure 18. The SM3 in the field with extension cable on the right.

Figure 19. The extended microphone.

2.3 Passive Acoustic monitoring: Equipment and survey design

For the array setup we used three Wildlife Acoustics Song Meters (model: SM3-GPS; Wildlife Acoustics Inc., Concord, MA, USA). The SM3 bioacoustics recorders come with two omnidirectional microphones attached. Each recorder had one of the standard microphones bypassed and replaced with an omnidirectional weatherproof microphone (model: SMM-A1) on an extension cable of 10 m to extend the active distance of the recorders. The song meters were placed in arrays with either 2 song meters (4 microphones) or 3 song meters (6 microphones) per array, depending on the habitat and predicted area of occupancy of the target species. Recorders and microphones were attached to 1,5 m steel droppers that were placed in the ground. The equipment was attached with cable ties approximately 0,5 m above the ground (Figures 18 and 19). The song meters are battery operated and record onto SD memory cards.

All recorders in each array were synchronized via a specialized GPS (model: SM3-Garmin; Wildlife Acoustics Inc., Concord, MA, USA) that plugs into each recorder and synchronizes clocks to one millisecond. Once the array was set up on site, the exact GPS point of each microphone was determined with a Garmin GPSMAP64 but the information is also stored via the Song Meter in the metadata of the recordings. The exact distances between all microphones were measured in millimetres using a Bosch laser range finder. These exact measurements are imperative for calculating the exact position of calling individuals using the call's time of arrival and signal strength (Stevenson and Borchers, 2014).

One survey was conducted per known site of *A. ngongoniensis*. The array was only set up if at least five males were heard calling, except for sites where it was deemed safe to leave the recorders out overnight. A total of four arrays were done across the nine sites (Table 2). Audio recordings took place after sunset. Subsets of the recordings that were used for population studies were sampled between 18:30 and 21:00. At three sites, we recorded through the night (12+ hours) to determine the peak in calling behaviour for the species. Once the array was set up, all observers would leave the site during the recording period so as not to disturb the animals or influence calling behaviour.

Table 2: A summary of the arrays that were set up indicating the number of audio channels per array as well as the initial number of calling males detected by observers.

Name	*Males heard calling*	*Audio data collected*	*Channels*
Poortjie grassland	5 to 10	One hour of audio data	6
Lower Mpur Forest	0	12 + hours of audio data, left overnight	6
Poortjie forestry area	3	12 + hours of audio data, left overnight	2
Franklin14 wetland	0	none	0
Mpur/Poortjie road verge	10 to 20	One hour of audio data	4
Ngele forest	40+	12 + hours of audio data, left overnight	6
Qunu Falls	1	none	0
Roelton Dam	0	none	0
Lynford	0	none	0

2.4 Software and data processing (PAM)

2.4.1 Acoustic pre-processing

Once the song meters were retrieved, the sound files were downloaded in .wav format. Some pre-processing of the sound files was required: The open-source software, Audacity (see www.audacityteam.org), was used to create synchronised multi-channel subsets from the field recordings, with each microphone assigned to a channel. Multi-channel 16-bit wav files were created per array.

The start times for the processed array recordings were as follows:

- Mpur/Franklin Road verge 19h42
- Poortjie Grassland 18h27
- Ngele Forest 19h25
- Lower Mpur Forest/Grassland 18h40

For successful arrays (where the species was detected), the array was reconstructed virtually in drawing software (Adobe Indesign®) to obtain Cartesian coordinates of the microphones relative to a selected microphone, as required by PAMGUARD (Figure 20a-c).

2.4.2 Passive acoustic Monitoring (PAM) analysis

Pamguard™ is an open-source software package used for the acoustic detection and localization of animals. It was designed for real-time operation in the field to detect cetaceans, but can equally well analyse archived data from stored files of many other sound-producing animals (Gillespie *et al*., 2008; see www.pamguard.org). Pamguard™ was used to identify calls of *A. ngongoniensis* using the Ishmael Spectogram Correlation module, which was set to detect calls in the predetermined

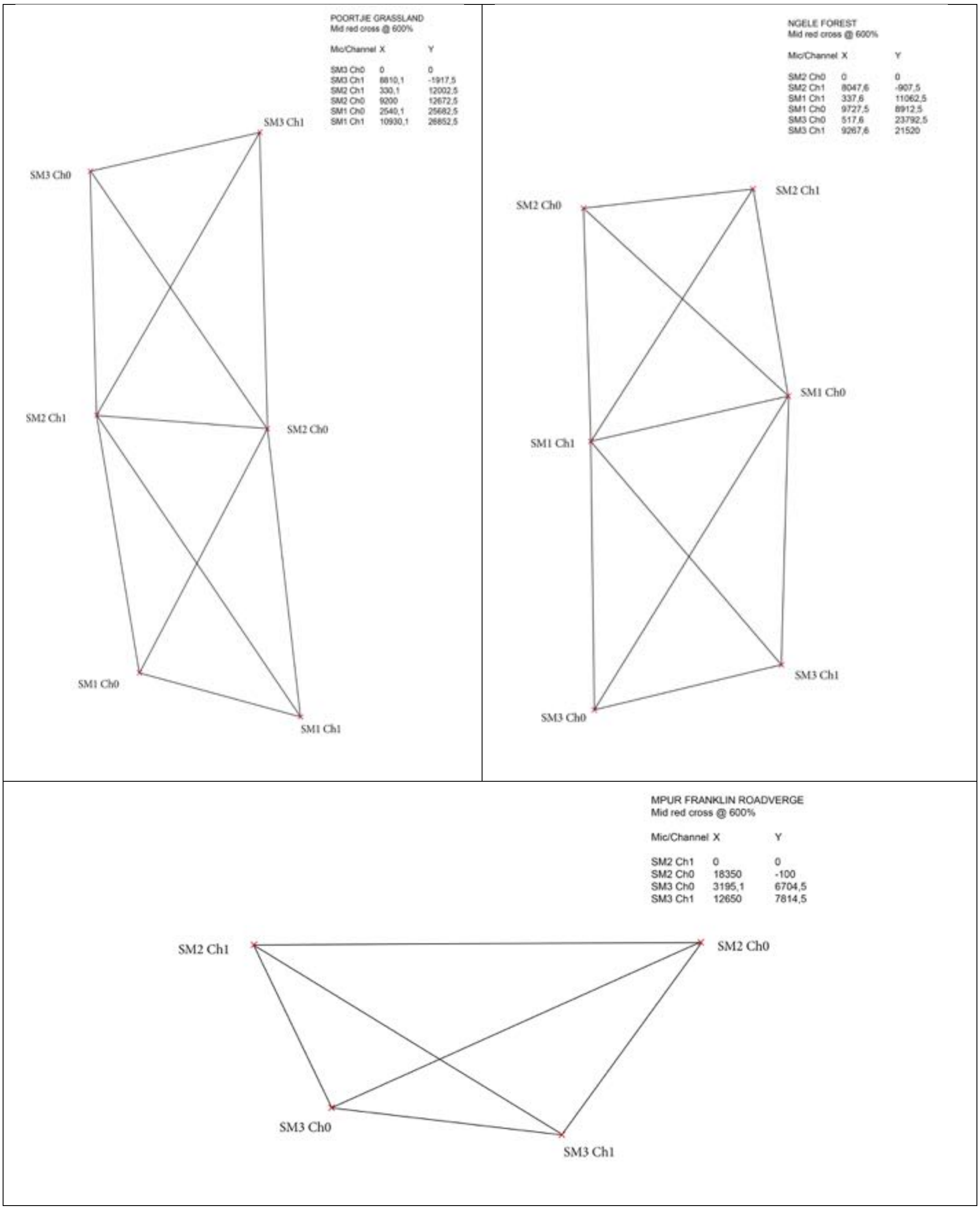

Figure 20a-c. The reconstructed arrays to obtain Cartesian coordinates. The names and channels assigned to each microphone is also indicated here.

frequency and decibel range. Furthermore, detections were only recorded above a threshold of 0.1 units; this cancelled out the background noise but ensured maximum amount of calls detected. Peak height (loudness) of the calls was used as signal strength (SS), since signal strength can be any measurable signal attribute that decreases with distance (Efford *et al.*, 2009a). The exact time of arrival (TOA) was also recorded for each detection. The data was written to a SQLiteStudio database via database module in Pamguard™. The Ishmael Spectogram Correlation data table is then exported as a .csv file that can be processed using the statistics software, R.

2.5 Statistical analysis (SECR)

The Spatially Explicit Capture-Recapture (SECR) parameter estimates were obtained using R. Stevenson and Borchers (2014) divided the method into 3 steps:

1. An acoustic SECR survey from which call density is estimated.
2. Estimation of the average call rate allowing for conversion of the call density estimate into a calling animal density estimate. The call rate was calculated from 6 males' calling data collected at times independent from the array recordings.
3. A parametric bootstrap procedure for variance estimation (standard error and confidence intervals) using parameter estimates from Step 1 and Step 2.

For the results of Step 1 and Step 3, the R package, *ascr* was used (Stevenson and Borchers, 2014; see https://github.com/b-steve/ascr). This software uses numerical maximization of the log of the simplified likelihood to provide parameter estimates. An AD Model Builder generates a call to an executable for optimisation and then numerical integration is used to estimate marginalisation over call locations (Stevenson and Borchers, 2014). This method makes the following important assumptions:

1. Sounds are detected by more than one microphone
2. Individuals all emit calls with the same strength
3. Signal propagation occurs uniformly across all directions, i.e. sound energy declines uniformly in all directions
4. The SECR model assumes that individual calls are identifiable, i.e. it's known if two detections at different microphones are of the same ca§ll or not. (Dawson and Efford, 2009; Stevenson and Borchers, 2014).

The R script is attached in Appendix 9.

Any uncertainties associated with the density estimators decrease as survey length and number increase (Stevenson and Borchers, 2014), which is why as many samples as was viable was processed for each site and samples were as long as possible for the processing thereof to be manageable by the computer. Furthermore, with small arrays such as these, estimation of the density of sound sources is substantially improved by modelling signal strength (Efford *et al*., 2009a). And we used signal strength and time of arrival.

The passive acoustic monitoring method is suited to the species, due to its cryptic behaviour, small size, sometimes difficult terrain and the universal benefit of substantially saving on man hours and fieldwork costs. The sites are easily accessible, by the forestry companies' environmental managers and any conservation agency that works with them. Added to that, the monitoring can be easily replicated and extended over long periods. Furthermore, as recommended by Measey (2011), the method delivers quantitative results with confidence intervals. This means that future results can be directly compared to the results found herein. All of this bodes well for sustainable long-term monitoring of the species.

To calculate the density estimate, the statistics software R makes use of the following parameters:
- The 6 call rates as calculated above.
- The location of the microphones.
- Which microphones a call was detected at.
- The time of arrival of the detected call at each microphone.
- The signal strength (calling peak) of the detected call.

The results from R are provided in the following parameters: *esa*, *Dc* and *Dc*, where *esa* is estimated sampling area, *Da* is calling males per hectare and *Dc* is calls per hectare per second. The bootstrap method was run (n=100) on all samples to provide standard error for each parameter. It is important to note that this is density of calling males only, and not of the entire population.

Chapter 3: Results

3.1 Surveys: *Anhydrophryne ngongoniensis*

Surveys were conducted between 17h00 and 19h30 with at least two hours spent at each site during surveying. Of the nine historic sites that were surveyed, *A. ngongoniensis* was detected at five sites (Figure 21). These included four grassland sites: Poortjie Grassland, Poortjie Forestry Area, Mpur Road Verge and Qunu Falls, and one indigenous forest site, Ngele Forest. No detections occurred at Lynford grassland, Roelton Dam grassland or Lower Mpur Forest, all of which had previously been recorded as positive sites. No additional populations were discovered, however a previously unknown locality was discovered as a result of a sighting of an individual along Mpur Road Verge (see 3.1.3).

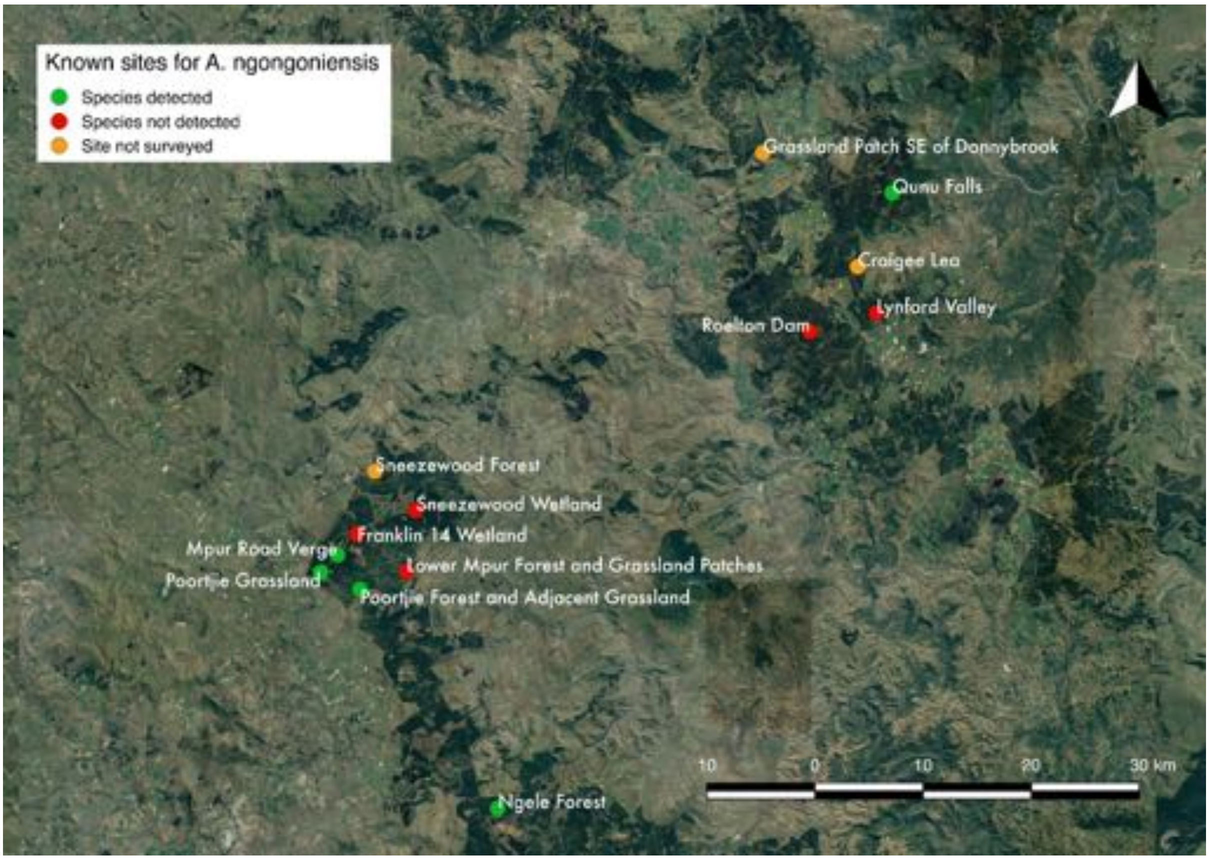

Figure 21. All known sites of *Anhydrophryne ngongoniensis* within the northern and southern distribution of the species. The species was detected at five sites (green), not detected at five sites (red) and three of the historical sites were not visited during this study (orange).

3.1.1 Poortjie Grassland

Approximately five *A. ngongoniensis* males were heard calling from a small patch of sloped grassland adjacent to the wetland and bordering a dirt road. The rest of the grassland (approximately 7 Ha) was traversed but no other individuals were heard. A six-channel array was set up in the centre of the patch where the frogs were heard calling, which delivered an hour's worth of audio data. Upon analysis of the data in PAMGUARD, it became apparent that the call of *Strongylopus grayii* is on a similar frequency to *A. ngongoniensis*. Consequently, the Spectrogram Correlation settings in the software identified both species as *A. ngongoniensis*. Due to this interference, a subset of audio that did not contain any *S. grayii* calls had to be selected manually to calculate the density estimate for *A. ngongoniensis*. This resulted in a subset of one minute of audio data being used, which only contained 18 calls. The subpopulation that we detected was confined to the sloped section within the area. Even though the rest of the grassland seemed suitable habitat for the species, it was absent elsewhere including on the slope on the opposite side of the dirt road. With no individuals (dead or alive) spotted on the road or outside of the sloped patch, we can hypothesise that the subpopulation is localised within the grassland.

3.1.2 Poortjie Forestry Area

Upon the initial site inspection, two to three males were heard calling at a distance estaimted at 30 m at 16h00 from within the pine plantation quadrant. The actual site where the species was heard was not visible from within the forestry quadrant and it was inaccessible due to an American Bramble (*Rubus flagellaris*) infestation. This made it impossible to erect a microphone array near the calling males. To confirm that the species was present and to get an idea of how many individuals may be calling, we installed one song meter and left it to record through the night for 12 hours. *Anhydrophryne ngongoniensis* males were recorded calling between 16h00 and 19h30. It is estimated that there were no more than 10 males calling at the site. An array would give a better idea of actual density and where the frogs were calling from.

3.1.3 Mpur Road Verge

This narrow sloped grassland had what seemed like a few lone individuals calling throughout the grassland, detected audibly from the dirt road. In an effort to locate a calling male, three experienced observers searched a patch of approximately four square metres for more than 15 minutes before the individual was found amongst pine needles under a fallen pine tree (Figure 22 and 23) from the adjacent plantation. This bodes for how well the species is camouflaged and just how cryptic their behaviour is. Even when heard calling, it was difficult to locate the frog through triangulation and combing through undergrowth. This is yet another reason why passive acoustic monitoring is a suitable method of observation for the species. On the southwest end of the grassland, wedged between the dirt service road and the plantation quadrant, we heard a few calling males. Upon further inspection, the sloped patch of grassland appeared to host a good subpopulation of *A. ngongoniensis.* An array with four microphones was set up and 60 minutes of audio data recorded. We heard two males calling from the other side of the road so it is plausible that individuals move across the dirt roads.

Figure 22. The patch of sloped grassland on the Mpur Road Verge where a calling male was found under a fallen pine tree, on grass covered in pine needles.

Figure 23. The calling Mistbelt Chirping Frog (*Anhydrophryne ngongoniensis*) that was found after 15 minutes of searching and triangulation by three experienced observers.

3.1.4 Lower Mpur Forest

No *A. ngongoniensis* were heard calling upon first visiting this site on 5 December between 17h30 and 18h30. Lower Mpur Forest was considered to be in a safe area so we left the song meters in an array overnight to investigate whether the species would call at some point during the night, but no individuals were detected in the 12-hour audio recording.

3.1.5 Franklin 14 Wetland

This large grassland was traversed by the team of five observers for two hours with no *A. ngongoniensis* being detected. No array was set up and no audio data collected. The grassland has no sloping areas, but the species was detected here previously by Harvey (March 2005).

3.1.6 Ngele Forest

This steep sloped indigenous forest hosts a large and seemingly stable population of *A. ngongoniensis.* Ngele Forest was by far the most densely populated of the surveyed sites. An array was set up and left to record for 12 hours throughout the night to a) determine the population density and b) establish peak calling times during the night. Interestingly, the frogs seemed concentrated at a specific elevation interval which appeared to be associated with vegetation composition. Below and above this interval almost no frogs were heard calling. But even along this elevation the frogs appeared to be calling from pockets within the forest. Calling was at its peak between 19h00 and 20h00 with only a handful of sporadic calls between 20h00 and 04h00. But at 04h00 calling commenced again. A cicada calling near the array rendered it impossible to analyse call data after 04h15 as it overpowered the frequency range in the audio files where the *A. ngongoniensis* calls would be.

Other anuran species detected in the forest were the Plaintive Rain Frog (*Breviceps verrucosus*) and the Bushveld Rain Frog (*Breviceps adspersus*).

3.1.7 Qunu Falls

Although this site seems like the perfect habitat of sloped grassland and adjacent indigenous forest, only one calling male was detected. The survey was done at the end of February and it is possible that it was simply too late in the season to detect calling males. A follow-up survey is recommended.

3.1.8 Roelton Dam

This grassland is very large and is believed to host a healthy population of *A. ngongoniensis* however no calling males were detected when the site was visited in February. Again, this might have been after the peak breeding season for the species. No array was erected. A follow-up survey is recommended. This site has been mowed in sections every year for over a decade by a local farmer. According to the landowners, this has now been stopped, but the impact that mowing would have on *A. ngongoniensis* is unclear.

3.1.9 Lynford

This site consists of a large grassland (approximately 25 Ha) adjacent to indigenous forest patches and pine plantation with a drainage ditch across the grassland. There is an infestation by various invasive species of vegetation and some topsoil removal appears to be taking place. No calling males were detected at this site. A follow-up survey is recommended.

3.2 The Call of *Anhydrophryne ngongoniensis*

3.2.1 Call Structure

The call of *A. ngongoniensis* was identifiable from up to 40 m in the grassland areas and 35 m in forested areas. But when other frogs and insects were chorusing it was more difficult to distinguish the cryptic insect-like call of *A. ngongoniensis*. The *A. ngongoniensis* call has a signature frequency of 4.3-4.8 kHz with the dominant frequency at 4.5 kHz (Bishop and Passmore, 1993) (Figure 24) and a call duration of 55 ms with 8-10 pulses (Figure 25).

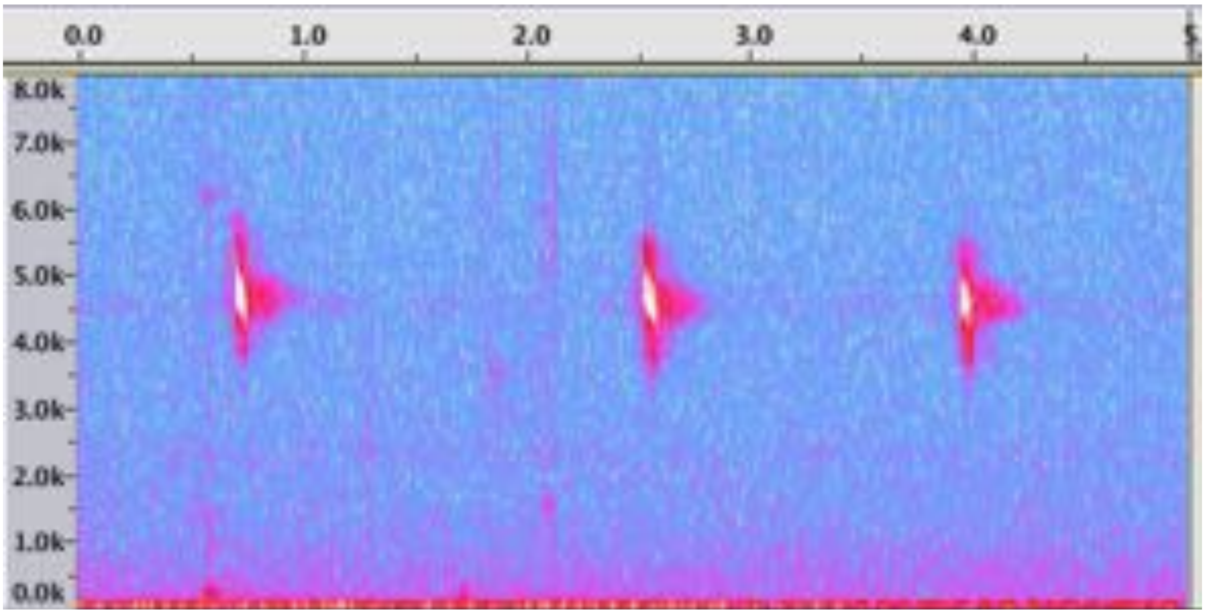

Figure 24. The spectrogram of three chirps of a calling male *Anhydrophryne ngongoniensis*. The frequency is between 4300 Hz and 4800 Hz with a midpoint at 4500 Hz.

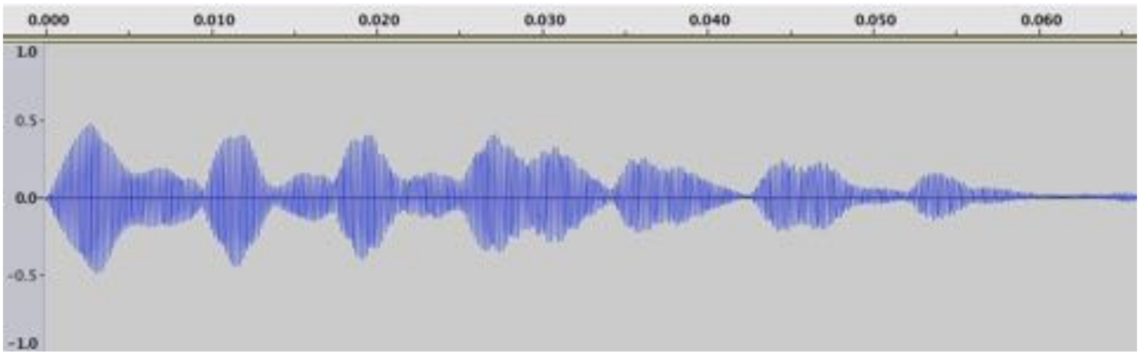

Figure 25. The oscillogram of a single chirp of *Anhydrophryne ngongoniensis.* A single chirp lasts 55 ms and consists of 8-10 pulses.

3.2.2 Call rate

With data independent from the audio surveys, six calling *A. ngongoniensis* individuals were used to obtain the following average call rates (calls per unit time):

3.071429

3.444444

4.111111

4.615385

4.8125

4.615385

This gives a mean call rate of x = 4.11216 calls ($\sigma = 0.64883$), which is in line with the literature that estimates the call rate of *A. ngongoniensis* as three to four with up to 7 (Bishop, 2004a).

3.3 Density analysis

3.3.1 Mpur Road Verge calling male density

The audio data provided a 58-minute usable recording. This was broken into 11 x 5-minute samples for processing through PAMGUARD (see www.pamguard.org) and R (see www.r-project.org) (Table 3). There was one outlier, "Mpur Road Verge H" in which the call density estimate was much higher. This could be due to unknown interference on the microphones or other species of frog, insect or bird calling.

A mean density estimate of calling males for the site is 114 males per hectare ($\sigma = 11.24183$). A single call was plotted in R using confidence interval contours indicating where the individual is likely calling with 0.16 to 0.95 confidence represented by the contours (Figure 26). The same call is plotted as a dot showing where the calling individual is with 0.95 confidence (Figure 27). The red crosses indicate the microphones. For a single call, the encircled crosses indicate at which microphones the call was detected. Multiple calls can also be plotted (Figure 28), however the microphones where the calls were detected is only representative of the first single call.

Table 3: Results from Mpur Road Verge array after PAMGUARD and analysis in R with the package *ascr*.

Sample Name	***Length of sample (min)***	***Number of calls detected***	***Da (calling males per Ha)***	***Standard Error***	***esa (estimated sampling area Ha)***	***Standard Error***
Mpur Road Verge A	5	281	107.590345	14.2582	0.635200	0.0139
Mpur Road Verge B	5	305	116.54672	16.1432	0.63647	0.0119
Mpur Road Verge C	5	250	95.930122	14.8186	0.633815	0.0128
Mpur Road Verge D	5	254	138.171555	27.9032	0.447088	0.0593
Mpur Road Verge E	5	261	110.699239	17.7652	0.573421	0.0341
Mpur Road Verge F	5	268	111.54508	19.4410	0.58434	0.0304
Mpur Road Verge G	5	309	127.458743	17.6470	0.589612	0.0259
Mpur Road Verge H	5	281	211.774560	39.6591	0.322708	0.0499
Mpur Road Verge I	5	260	103.649596	15.8584	0.610075	0.0239
Mpur Road Verge J	5	287	114.423331	17.7036	0.610021	0.0247
Mpur Road Verge K	5	287	114.269091	18.0909	0.610845	0.0200

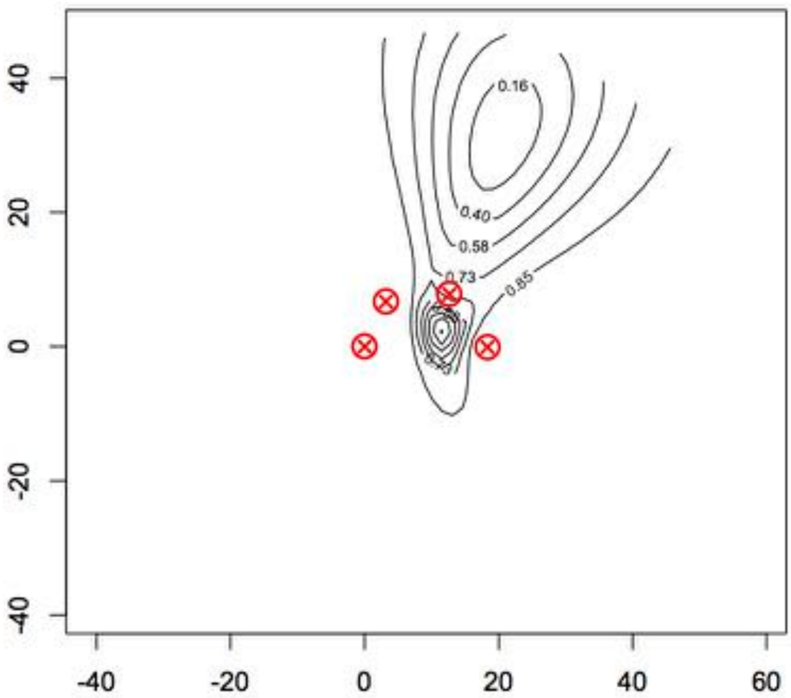

Figure 26. The microphone array at Mpur Franklin showing confidence contours for the most likely location of a calling individual at 0.95 confidence in the centre of the densest concentric contours. The microphones are represented by the red crosses and this call was detected by all four microphones as is indicated by the circles around the red crosses.

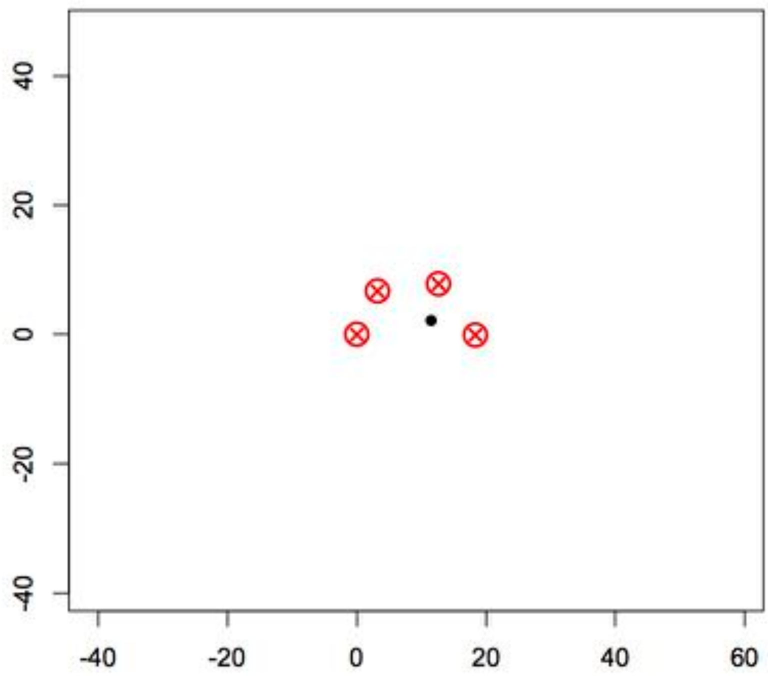

Figure 27. The microphone array at Mpur Franklin showing a dot for the location of a calling individual with 0.95 confidence that was detected at all four microphones.

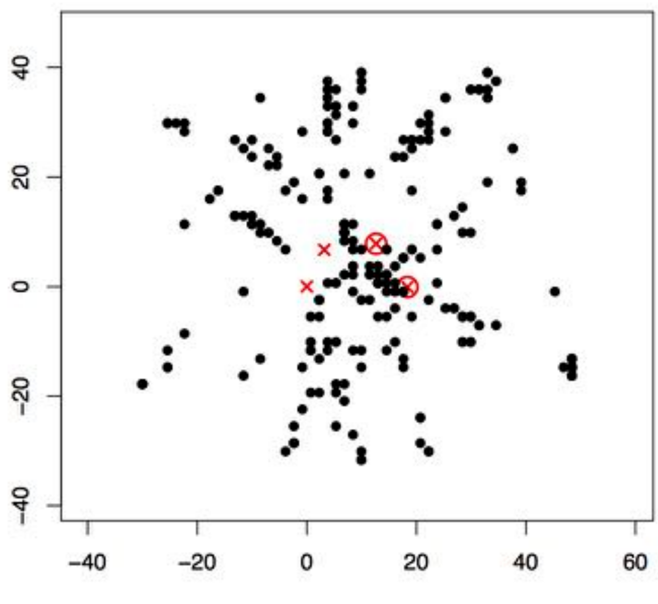

Figure 28. The microphone array at Mpur Road Verge with all 309 detections from a five-minute sample (G) plotted. The red crosses indicate the microphones and the circled ones are the microphones at which the first call was detected at.

3.3.2 Poortjie Grassland calling male density

The single subset of audio data provided a density estimate of 19.561403 calling males per Ha. The standard error is big (27.7) but during the survey it was estimated that no more than five males were heard calling, so when this density estimate is extrapolated to the estimated sampling area (esa) of 0.223795 Ha, it provides an estimated 4 calling males. The location of calls is illustrated in Figure 29.

Table 4: Results from Poortjie Grassland array after PAMGUARD and analysis in R with the package *ascr*.

Sample Name	***Length of sample (min)***	***Number of calls detected***	***Da (calling males per Ha)***	***Standard Error***	***esa (estimated sampling area Ha)***	***Standard Error***
Poortjie Grassland A	1	18	19.561403	27.7017	0.223795	0.0632

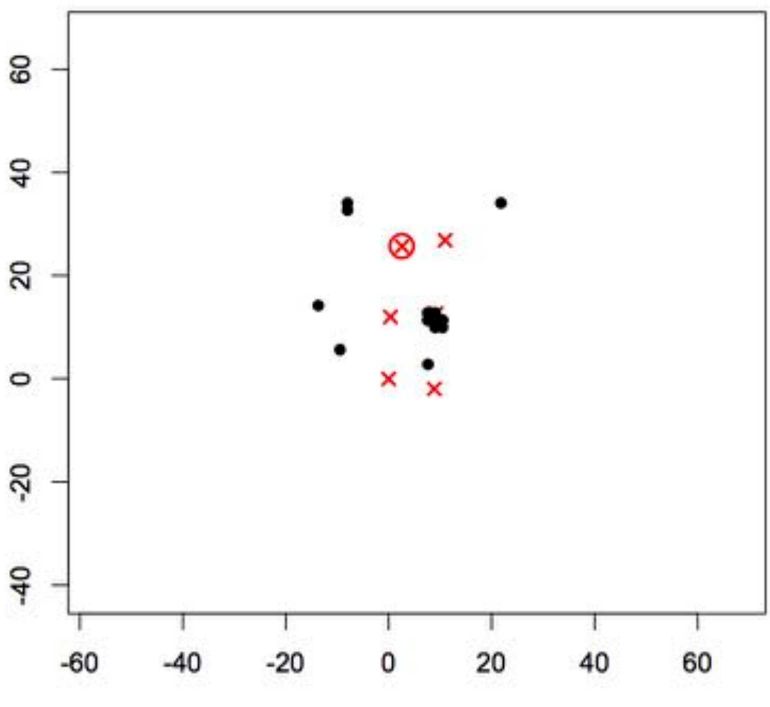

Figure 29. The array at Poortjie Grassland with all calls plotted. The red crosses represent the microphones of the array. The encircled microphone indicates that the first call was only detected at that one microphone.

3.3.3 Ngele Forest calling male density

The first hour of recording was used for the density estimates and was broken into 12 x 5-minute samples. The density estimate drops substantially between the start of the survey and an hour into the survey. This is indicative of the peak calling period (17h30 to 19h30). The mean density estimate for the duration of the survey is 129.9697 calling males per Ha (σ = 192.56725) but the highest density estimate was obtained at the beginning of the survey at 610.41 calling males per Ha. Subsamples H through J are unreliable due to large standard error. This is likely because of the small sample size with very few males calling at the time. The location of calling males is illustrated in Figure 30.

Table 5: Results from Ngele Forest array after PAMGUARD and analysis in R with the package *ascr*.

Sample Name	***Length of sample (min)***	***Number of calls detected***	***Da (calling males per Ha)***	***Standard Error***	***esa (estimated sampling area Ha)***	***Standard Error***
Ngele Forest A	5	1178	610.41	65.4847	0.46935	0.0184
Ngele Forest B	5	992	371.96	42.2664	0.60286	0.0203
Ngele Forest C	5	601	240.5669	33.4793	0.6076	0.0299
Ngele Forest D	5	345	155.235474	26.9982	0.540513	0.0502
Ngele Forest E	5	71	26.060059	7.5394	0.662614	0.0617
Ngele Forest F	5	9	2.930749	1.9037	0.746864	0.1552
Ngele Forest G	5	2	2.930749	1.9939	0.746864	0.1196
Ngele Forest H	5	14	4.8933851	409.0821	0.6958190	0.1614
Ngele Forest I	5	14	4.8933851	409.0821	0.6958190	0.1614
Ngele Forest J	5	14	4.8933851	409.0821	0.6958190	0.1614
Ngele Forest K	5	14	4.8933851	3.1943	0.6958190	0.1779
Ngele Forest L	5	14	4.8933851	3.1943	0.6958190	0.1779

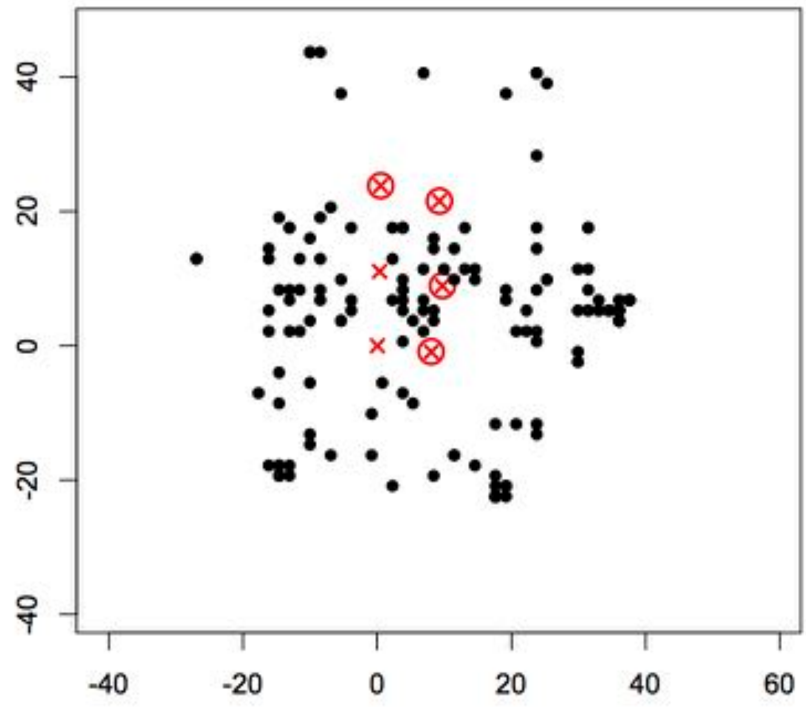

Figure 30. The microphone array at Ngele forest with the first 200 calls plotted. The red crosses represent the microphones of the array. The encircled microphones indicate at which microphones the first call was detected.

3.4 Habitat Utilisation

3.4.1 Mpur Road Verge

When the calls are overlaid onto satellite imagery of the site (Figure 31), we can see that the distribution is clumped. Some of the calls are close to the road as well as into the pine plantation with minimal calls towards the rest of the grassland verge. We can also see individuals on the opposite side of the dirt road. Calls that are scattered in a straight line could be due to the signal strength at which an individual is calling not being consistent. However, it could also imply that the individual was moving during the recording time. Likewise, a cluster of calls could indicate minimal movement of an individual male displaying territorial calling behaviour. With all calls plotted, however, it becomes difficult to distinguish individuals or tell calls apart. When we plot subsamples separately (Figure 32a-c), we can better see the movement and estimated density.

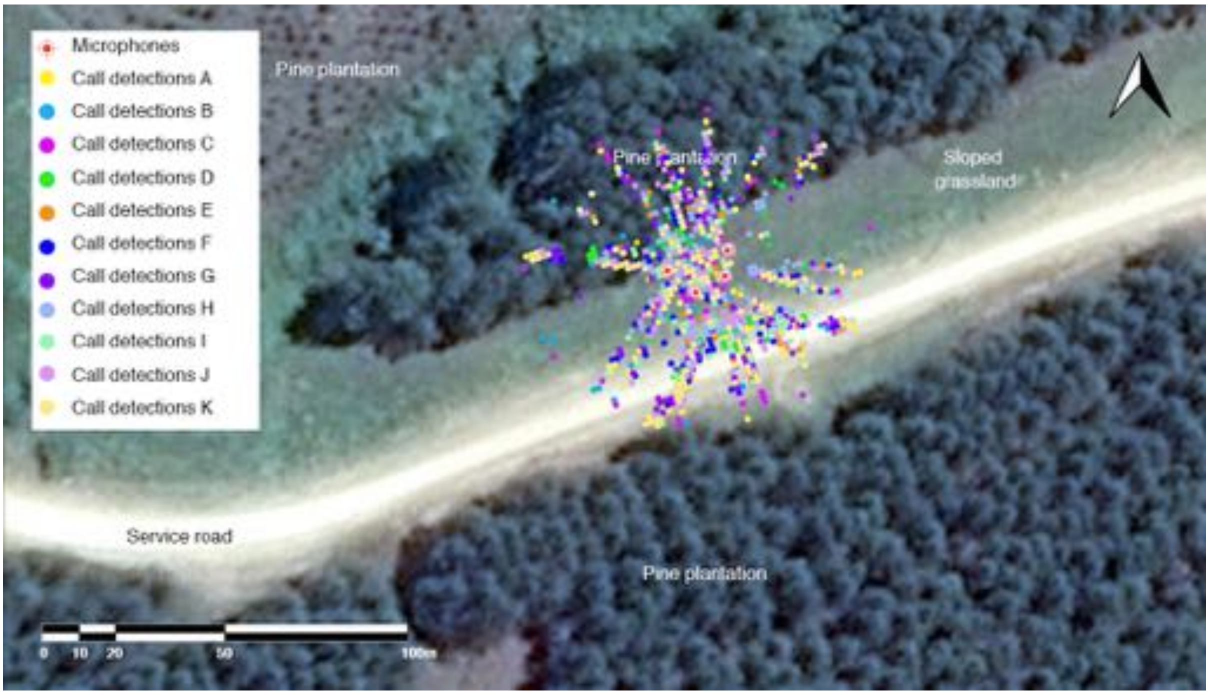

Figure 31. All calls of *A. ngongoniensis* detected at Mpur Road Verge combined from each five-minute subsample to show detections for the entire survey.

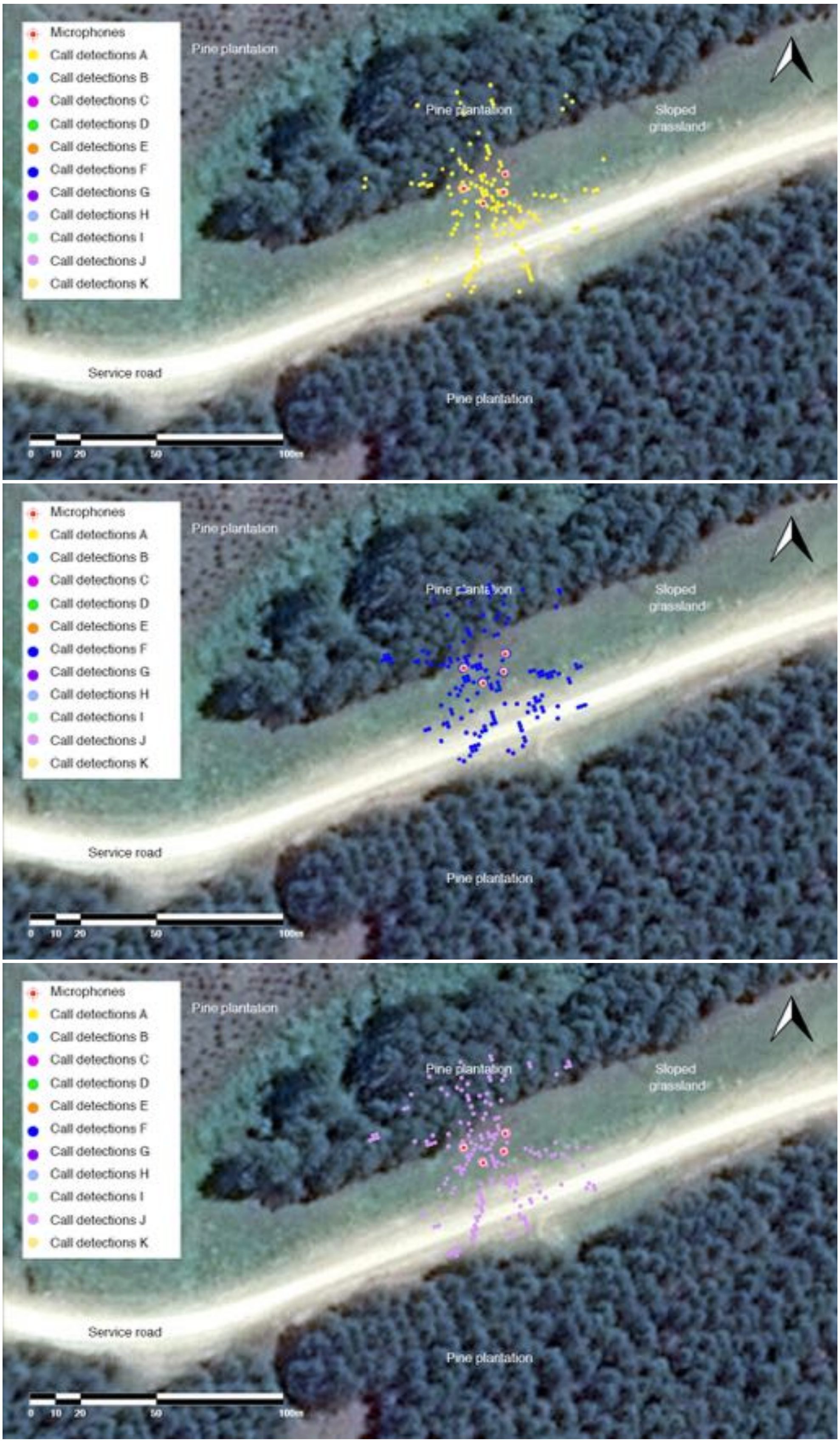

Figure 32a-c. Calls of *A. ngongoniensis* detected at Mpur Road Verge over time. Subsample A (top) yielded a density estimate of 107 calling males per hectare from 281 detections. Subsample F (middle) yielded a density estimate of 111 calling males per hectare from 268 detections and subsample J (bottom) yielded a density estimate of 114 calling males per hectare from 287 detections.

3.4.2 Poortjie Grassland

The calls detected at this site appears to represent only five to six individuals. It can clearly be seen how the one individual is moving around (Figure 33). Since the species was heard nowhere else in the grassland or wetland we can assume a clumped distribution here as well. No individuals were heard calling near the road.

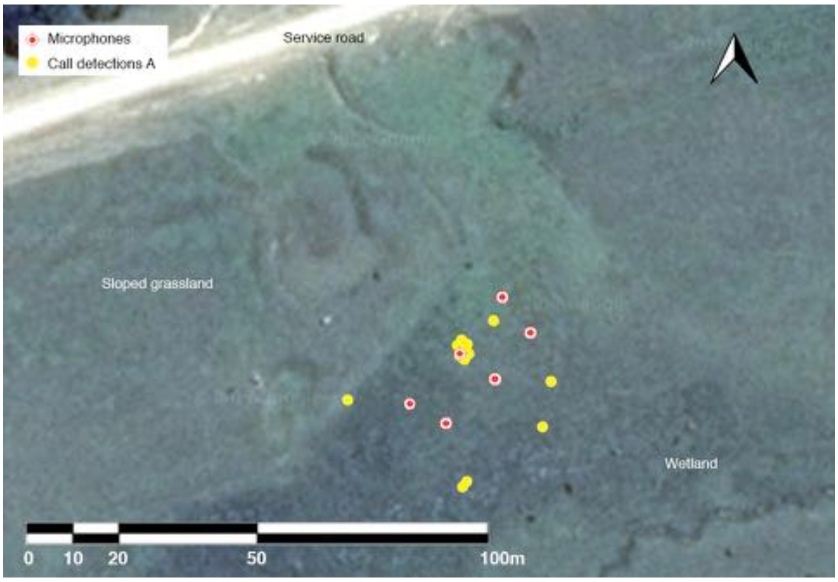

Figure 33. All calls of *A. ngongoniensis* from the usable one-minute sample at Poortjie Grassland, which yielded a density estimate of 19 calling males per hectare from 18 detections.

3.4.3 Ngele Forest

Anhydrophryne ngongoniensis seemed to be found along a specific elevation at this site. The entire forest was not surveyed but it would appear that calls are not found homogenously throughout the forest. The distribution appears to be slightly more uniform than in grassland sites (Figure 34), but is still considered to be clumped. It is worth noting again that the statistical software allows the microphones to only detect calls within the set buffer of 40m.

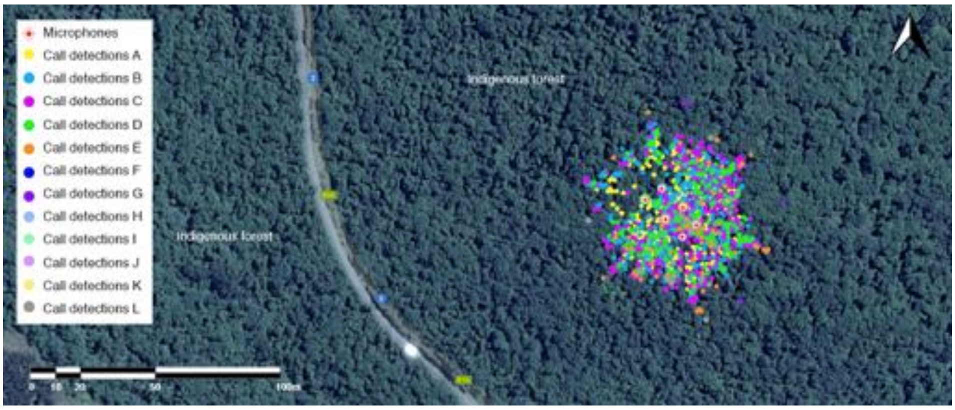

Figure 34. Calls of *A. ngongoniensis* detected at the Ngele Forest array combined for all subsamples to show calls detected for the entire survey.

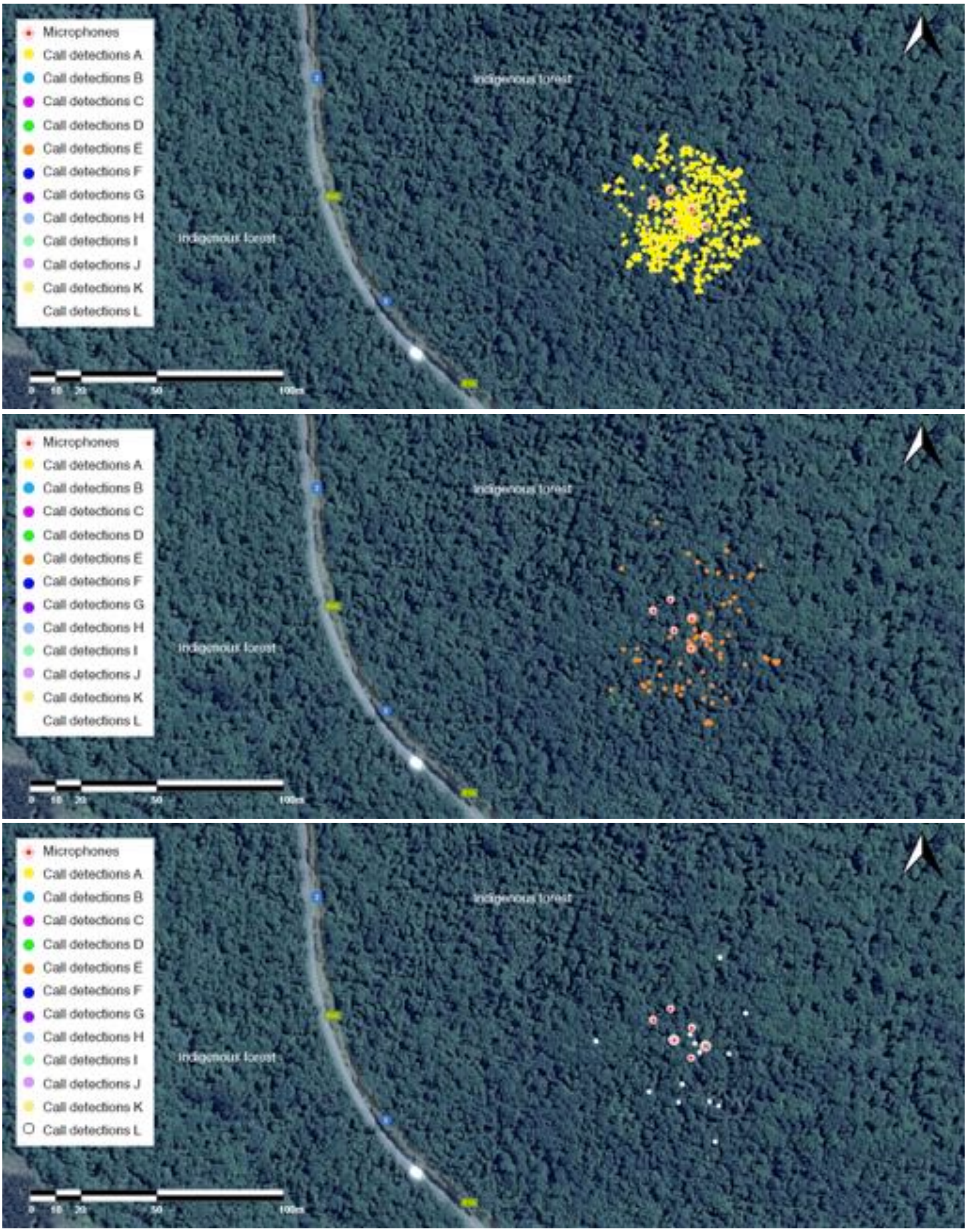

Figure 35a-c. Calls of *A. ngongoniensis* detected at the Ngele Forest over time. Subsample A (top), which yielded a density estimate of 610 calling males per hectare from a total of 1178 detections. Subsample E (middle) yielded a density estimate of 26 calling males per hectare from 71 detections and subsample L (bottom) yielded a density estimate of only 4 calling males per hectare from a total of 14 detections.

We can see once again that the calls are hard to distinguish from one another when all plotted together. But when the calls are separated by subsample, the maps (Figure 35a-c) clearly show the drop in calling behaviour and density estimates with time.

3.5 Other species

Interestingly, *A. ngongoniensis* does not occur exclusively in this habitat, and other sympatric anuran species detected include Bronze Caco (*Cacosternum nanum*), Plaintive Rain Frog (*Breviceps verrucosus*), Bushveld Rain Frog (*Breviceps adspersus*), the Endangered Long-toed Tree Frog (*Leptopelis xenodactylus*), Raucous Toad (*Sclerophrys capensis*), Delaland's River Frog (*Amietia delalandii*) and Clicking Stream Frog (*Strongylopus grayii*).
S. grayii was consistently detected co-occurring with *A. ngongoniensis* at all the grassland sites. *Cacasternum nanum* was detected at most of the grassland sites as well but not amongst *A. ngongoniensis*, rather on the edges of the arrays. *Amietia delalandii* were associated with the streams draining the wetlands. *Breviceps verrucosus* was the only other anuran species detected in the indigenous forest site (Ngele Forest) but they did not occur amongst *A. ngongoniensis*.

Chapter 4: Discussion

4.1 Value of Passive Acoustic Monitoring (PAM) as a conservation tool for *Anhydrophryne ngongoniensis*

Because of the cryptic nature of this species, auditory cues are vital for its detection. It is challenging to find an actual individual of this species. Even after hearing a call, determining an approximate location and triangulating the call to find the individual, you can still be unsuccessful. Only once during this study, was an individual of *A. ngongoniensis* seen, and even that was after a long search by experienced observers, triangulating the call and searching through the undergrowth. Multiple sites were surveyed where no visual detections were made. Therefore, the efficacy of Passive Acoustic Monitoring (PAM) is evident in the fact that we could survey frogs that we could not see. PAM has been successfully employed for this study and proves to be an efficient tool for monitoring *A. ngongoniensis*. Not only could we determine the best time of day for surveys but we also obtained density estimates through a standardised and repeatable method. All surveys should be done during the peak breeding period to ensure best use of man hours and for yielding accurate results, however it is not clear from literature or previous studies exactly when this is or if the species is a prolonged or explosive breeder.
In terms of calling behaviour within a 24-hour period, our PAM data suggests that *A. ngongoniensis* calls between 15h00 and 20h00 depending on weather conditions, but that 18h30 to 19h30 appears to be the peak calling period during the breeding season, even though the species has been observed calling throughout the day during humid and misty conditions (Bishop and Passmore, 1993).

PAM was also useful in confirming species presence/absence. At two sites the recorders were erected during the day when no males were calling and left overnight. At the one site (Lower Mpur Forest and grassland patches) no detections were made by the recorders throughout the night, while at the other site (Poortjie forest and adjacent grassland) the species was detected by the recorders and software, confirming its presence. This is useful before arrays are set up to determine density, due to the time and effort involved in setting up a microphone array. Besides the skill required to set up the equipment and process the data, one other disadvantage of the method is that, for *A. ngongoniensis* at least, there was interference from other animal vocalisations (in this case insects and other frog species) that call on the same frequency as *A. ngongoniensis* rendering some of the call data unusable.

The passive acoustic monitoring enabled an array set-up that could be used to not only estimate calling male density but also provide insight into spatial habitat utilization. Setting up an array is not particularly cumbersome, but exact measurements between song meters and GPS synchronization of all song meters is paramount. If employed and interpreted correctly the method provides an even deeper understanding of the breeding behaviour (calling and movement patterns) of *A. ngongoniensis*. This could be valuable to the conservation of the species as it can give clear direction to land managers about sensitive habitat the frogs are using for breeding related activities.

4.2 Status of *Anhydrophryne ngongoniensis*

4.2.1 Distribution

The current or perceived distribution range for southern *A. ngongoniensis* populations as determined by this study was similar to what is known from historic records (Harvey, 2007). Out of the nine historic sites that were surveyed, *A. ngongoniensis* was detected at five sites in 2016/2017 (Figure 13). We cannot confirm that the distribution of *A. ngongoniensis* has shrunk based on historic distribution records. Although we detected the species at only one of the northern sites, this data is based on only one survey that took place at the end of the season. Weather conditions are known to affect the calling behaviour of various frog species (Dyson *et al*., 1999; Hazell *et al*., 2004; Botts *et al*., 2011), but it was not particularly windy or cold during the survey. Even though the species had been detected before during this time of year it was towards the end of the known breeding season. If we account for annual fluctuation in season it is plausible that the frogs were not breeding at the time of the survey. According to the Weather Service of South Africa, the country experienced an all-time low rainfall in 2015 (over 200mm below annual average) which could have influenced frog breeding activity and success (Touchon and Warkentin, 2009). It is unclear if *A. ngongoniensis* are locally extinct or if external factors influenced the calling behaviour at Lower Mpur Forest, Roelton Dam and Lynford Valley, and follow up monitoring is recommended. At one site, however (Lower Mpur Forest), the species was not detected even though this survey was done concurrent to others in the same area where the species was detected. Seasonal fluctuation is thus likely not at play here. It may well be that the species is locally extinct at this site.

The presence of *A. ngongoniensis* in indigenous afromontane forest patches bodes well for the species as they are likely to be less vulnerable to disturbance and habitat degradation in these forests, compared to grasslands since burning and mowing is less of a threat. These forest patches also seem to be less vulnerable to alien infestation. Formal protection is provided at Ngele Forest as it is owned by the Department of Water Affairs and Forestry and is an official Nature Reserve. As for other forested areas, there is no formal protection. Although there are currently no plans to clear these areas to make way for plantations, there is not long-term guarantees for protection. Fortunately, these areas are not burnt or subjected to pesticides, but they are vulnerable to infestation by invasive vegetation such as Bramble (*Rubus spp*).

4.2.2 Density estimates

Density estimates were obtained for sites where an array was erected and adequate sound data was collected. Previous density estimates (Harvey, 2007) were derived from walking audio transects and the counting of actual calls. These density estimates were much lower than the density estimates obtained through passive acoustic monitoring during this study. However, the historic data does not mention the area of occupancy to which the density estimates were extrapolated to arrive at a population estimate.

At Poortjie grassland the previous study estimated density of calling males at 1.125 per Ha while this study yielded a density estimate of 19.56 calling males per Ha. The standard error on this sample was high (27.7) so this result is questionable. Mpur Road Verge yielded a previous density of 1.875 calling males per Ha versus 114 for this study. But with an estimated occupied area of only 0.25 Ha this translates to less than 30 calling males for the site. For Ngele Forest the previous calling male density estimate was 1.222 males per Ha compared to 610.41 males per Ha during the peak calling period for this study. Furthermore, the density estimate at this site varied immensely across time. During the peak calling period the density was estimated at 610 males per Ha and it dropped to 2.931 males per Ha only 25 minutes later. This shows just how much calling behaviour can vary during a single survey and iterates the importance of prolonged recordings and multiple surveys.

Accurate interpretation of call density estimates requires an analysis of call signal strength, since this is known to vary in individuals. Our method assumed that individuals emit calls at the same strength consistently. Therefore, if the software counted calls from the same individual as separate individuals due to a variance in signal strength of the calls, the resulting population density would have been overestimated. It is quite possible for and individual's calls to vary in strength depending on the number of competing males surrounding a calling individual, as well as the frog's level of fatigue (Kohler *et al*., 2017). Nonetheless the realised area of occupancy within a site appears to be very small (approximately 0.25 Ha in grassland sites). Subsequently population numbers are relatively low.

Our data indicates that habitat type affects calling male density. The calling male densities at the sites that occur in grassland habitat (114 calling males per Ha for Mpur Road Verge and 19 for Poortjie Grassland) are significantly lower than that of the indigenous forest, (up to 610 calling males per Ha for Ngele Forest). Although there are elevational differences among grassland and forest sites (approximately 300m) that may affect abundance, a more plausible explanation could be that the forest microhabitat is more suitable to their terrestrial mode of breeding. In terms of resilience to disturbances, forest populations are inherently more protected from burning, mowing and grazing to which grassland populations are intermittently exposed. It could well be that *A. ngongoniensis* is more of a forest species than grassland species.

4.2.3 Spatial habitat utilisation

From the maps (Figure 31-35) we can deduct that *A. ngongoniensis* does not occupy a site homogenously. Even when large areas of habitat may seem suitable, the species displays a clumped distribution and are very much localised within the grassland sites. These pockets are on sloped areas but otherwise don't appear much different to the surrounding habitat. We can postulate that the populations are inherently small and therefore concentrate in areas to maintain a viable population size by having access to mates, while a sloped landscape provides better drainage for terrestrial nests than the flat valley bottoms that are more prone to flooding. That said, the species is found close to service roads, on both sides of dirt roads, and right up against the pine plantations, so some resilience to less than perceived ideal conditions seems evident, for example the calling male located among the pine needles. We could conclude that it is more likely to find the species near service roads and plantations in remnant grassland pockets, rather than larger areas of suitable habitat but isolated from other populations due to surrounding plantation.

4.3 Implications for Conservation

There are three factors considered influential in a species' commonness or rarity (Rabinowitz, 1981): geographic range, habitat specificity and local abundance size. *Anhydrophryne. ngongoniensis* surely fits the description of a rare species. Its geographic range is very much restricted. Its habitat tolerance appears to be narrow and habitat requirements are very specific. And finally, the local population sizes and abundance are evidently small (Harvey, 2007). *Anhydrophryne ngongoniensis* has been identified as being inadequately protected (Armstrong, 2010) and in view of an extremely restricted and fragmented distribution, priority should be given to the conservation and management of the remaining habitat, itself classified as Endangered, of this species before it becomes extinct. The majority of the known *A. ngongoniensis* populations occur on private forestry land. Thus, private landowners play a crucial role in the conservation of this species. While the landowners (Merensky and Sappi) appear to cooperate with species conservation initiatives, they need assistance, clear guidance and practical plans to bridge the gap between research and implementation of conservation plans. Two-thirds of conservation assessments published in peer-reviewed journals fail to deliver conservation action, since most researchers don't plan for implementation (Knight *et al*., 2008). It is important to work with the decision makers of the forestry companies and to motivate for the use of research to back up decision-making (Pressey, 1999; Pressey *et al.*, 2011, Prendergast *et al*., 1999). "The burden of conserving biodiversity will fall increasingly on sectors such as agriculture, forestry, mining and land-use planning. In order for these sectors to play a constructive role in conservation, it is essential that biodiversity concerns be integrated or mainstreamed into their policies and practices." (Pierce *et al.,* 2005).

As it stands, conservation assessment remains the main focus of conservation planning, often at the expense of other stages that could be more important for implementing effective conservation action (Knight *et al*., 2008). If we as scientists want industry, government and their consultants and decision makers to implement and act on our recommendations, it is crucial that we provide target-driven conservation planning products that are easy to use and implement (Pierce *et al*., 2005).

We must enable the forestry companies to follow through on recommendations for land management and long-term monitoring. If the research community can help industry see the benefits to the implementation of conservation plans, then there is better motivation to do so (e.g. focus on ecosystem services, biodiversity status or good publicity). Research indicates that actions taken to conserve biodiversity in South Africa will also protect certain ecosystem services (Egoh *et al*., 2009) and that can be leveraged for a company's public profile. There is real opportunity for these companies to leverage their biodiversity status as other threatened species that occur in these areas include: The Long Toed Tree Frog (*Leptopelis xenodactylus*), the Blue Swallow (*Hirundo atrocaerulea*) and the Grey Crowned Crane (*Balearica regulorum*) to name a few. One site, Roelton Dam, is in the process of being formally proclaimed a Nature Reserve through the Biodiversity Stewardship Programme, facilitated by Birdlife.

While the grasslands where *A. ngongoniensis* is found are not vast stretches of land, the patches are important for conservation. Research has shown that species richness and biodiversity health will increase with patch size but decrease with patch isolation (Parris and Lindenmayer,

2004). Thus the larger the protected areas and the closer together they remain, the more advantageous it becomes for population viability of a species. For another EDGE amphibian and terrestrial breeder, the Giant Barred River Frogs (*Mixophyes iteratus*) of Australia, Lemckert and Brassil (2000) recommended a 30 m buffer zone along streams to protect breeding habitat from logging activities. However, the breeding habitat of *A. ngongoniensis* is within the grassland patches, often just on road verges that are barely 30 metres wide. One study also emphasized how the biota inhabiting retained patches of native forest within plantation landscapes change when adjacent stands of Pine are clear-felled (Lindenmayer *et al*., 2009). These case studies serve as examples of how important sound land management practices are.
Capacity building is another important element in the implementation of conservation plans as it educates and encourages conservation. This goes hand in hand with creating awareness at all levels of age, gender and background. We need to raise awareness within South Africa's population all the way from educating the public and encouraging citizen scientists, through to producing and supporting academic scholars and conservation professionals (Tolley *et al*., 2011).

We therefore make the following recommendations to the forestry companies and conservation agencies, regarding management and research interventions specific to maintaining populations of *A. ngongoniensis* in order of priority:

4.3.1 Land Management Practices

1. The sites with intact populations and higher density be used as model sites to provide guidelines for habitat management of other known as well as potential sites.
   ACTION: Survey known sites and note microhabitat composition, level of infestation by invasive vegetation and distance to nearest sub-population of *A. ngongoniensis*.

2. Since density is higher in forested areas, it would be good practice for the landowners to pay attention to the maintenance and upkeep of any indigenous forest on their land. The growth of indigenous forest and even replanting thereof should be a priority.
   ACTION: Additional surveys of the indigenous forest on forestry land for *A. ngongoniensis*. Clear all invasive vegetation from the forests.

3. We recommend that mowing be stopped at Roelton Dam as this is not a natural process and could affect the understory of the grassland, which is important habitat for *A. ngongoniensis.* Burning in block and alternating seems a fair practice.

ACTION: Update land management plans.

4.3.2 Monitoring

1. While PAM is valuable as a tool for collecting baseline data and establishing the status of the species, it is perhaps too onerous and expensive for long-term monitoring. We therefore recommend that audio transects (driving in a vehicle) be used to determine absence/presence of the species at known and potential sites.
   ACTION: For one week in December, surveyors should drive around all known and potential sites between 17h30 and 19h30 to listen for the calls of *A. ngongoniensis*. Note approximate number of calling males and georeference sites where males were heard calling.

2. Arrays should be erected on rotation at sites where populations are present to compare density estimates and determine fluctuation in the population. The software settings for PAMGUARD and R are already in place after this project and can be run on any Windows PC with processing power of 3GHz and up.
   ACTION: Set up array at Ngele Forest and Mpur Road Verge for another round of data collection in the beginning of December and estimate density of calling males using the same method as in this study to enable a comparison.

3. The only site that has had long-term formal protection is the Ngele Forest. But it could be coincidental that this has the highest calling male density. However, due to its protected status, it would be beneficial to conduct long term surveys at Ngele Forest:
   ACTION: Place recorders along the elevation to cover a larger forest area and determine distribution of the species in the forest. Long-term data can reveal the peak breeding period in forest. The grassland above the forest should also be surveyed for presence of the species.

4. We recommend that an acoustic array is erected at Poortjie Forest, although this would require prior clearance of the invasive bramble that is preventing access to the forest patch and grassland. Ongoing clearing of invasive vegetation is important on all road verges, the forest and grassland areas around Poortjie, the lower Mpur forest and grasslands and the grasslands at Lynford.
   ACTION: Clear invasive infestation at above sites and erect array at Poortjie to establish male calling density.

4.3.3 Research

1. To understand the relationship between the various sub-populations and how this will affect the conservation status of *A. ngongoniensis*, we recommend that a study of the population genetics be conducted on the species.
   ACTION: Procure funding and student bursary as a first step.

2. To understand the higher density in Ngele Forest, we recommend a study that compares breeding success (breeding frequency, clutch size, hatching rates) between grassland and forest habitat.
   ACTION: Procure funding and student bursary as a first step.

3. As part of the EDGE Fellowship, a Survival Blueprint for the species will be compiled. This will include recommendations and actions for environmental education, stakeholder workshops with landowners and capacity building through the training of monitoring teams for long-term monitoring of the species. Planning of a workshop for the education of colleagues on the processing of audio data is already underway.
   ACTION: Complete survival blueprint and distribute.

We believe that these recommendations are achievable and realistic, and that they will go a long way in increasing the chances of survival of *A. ngongoniensis* in the long-term. A stakeholder workshop can be used as an opportunity to discuss these recommendations and to come to an agreement on which parties will assume responsibility for each action as well as assigning a timeline to the actions.

Chapter 5: Conclusion

South Africa is home to 125 species of frogs but close to 30% of these species are threatened (Measey, 2011). The main cause for a sharp decline in frog numbers is habitat loss (Stuart *et al*., 2008), which also leads to fragmentation of habitat, turning many species into possible metapopulations (Measey, 2011). A case in point is *Anhydrophryne ngongoniensis*. Even though the species has been known to science for 25 years and its status is of noteworthy conservation concern (Bishop and Passmore 1993), the species remains poorly studied. The only previous population estimate (Harvey, 2007) put the total number of individuals alive at roughly 3000 individuals, but these data were collected over 10 years ago and therefore this species was in need of a reassessment, especially when considering its Endangered status (IUCN, 2016). We can attribute the decline of *A. ngongoniensis* to two main factors – afforestation and agriculture, both linked to the growing of commercial pine plantations. *Anhydrophryne ngongoniensis* is also evolutionary distinct, as its family (Pyxicephalidae) diverged 70 million years ago. We thus not only face the threat of extinction for a species, but also the risk of losing a part of evolutionary history. *Anhydrophryne ngongoniensis* is consequently on the EDGE amphibian list (www.edgeofexistence.com, 2015). Conservation of any threatened species is subject to knowledge of the species' distribution, biology, and behaviour (Measey *et al*., 2011b) and so, up-to-date population and distribution data are critical for conservation decision-making. The aims of this study were to determine the status of previously recommended management practices (Harvey, 2007) and to improve on the limited knowledge of the abundance by employing a new passive acoustic monitoring (PAM) survey method and a spatially explicit capture-recapture (SECR) model.

Anhydrophryne ngongoniensis is a difficult species to detect even when heard calling, because it has a soft, insect-like call that does not carry far, and its small size (20mm) and cryptic colouration implies that they are well camouflaged (Passmore 1993; du Preez and Carruthers 2009). These characteristics all serve as motivations to employ PAM. The PAM method we employed consisted of a microphone array in which all recorders were synchronised via GPS. The technology utilizes the subtle differences in sound arrival times at each microphone to calculate an animal's position and it also models for signal strength of each call for further accuracy. We aimed to collect call data from sites that would reflect the range of distribution of the species. Heterogeneity of the sites and detectability of populations allowed a total of four arrays to be conducted across the nine historic sites. The presence of *A. ngongoniensis* was confirmed at five sites and density estimates were calculated for three of these sites. The monitoring can be easily replicated and extended over long periods and as recommended by Measey (2011), the method delivers quantitative results with confidence intervals. This means that future population assessment results can be directly compared to the results found herein. It became evident from the spatial distribution data that the species has a clumped distribution.

PAM has been successfully employed in this study and proves to be an efficient tool for monitoring *A. ngongoniensis*. Not only could we determine the best time of day for surveys but we also obtained density estimates through a standardised and repeatable method. PAM was also useful in confirming species presence/absence. The distribution range for southern *A. ngongoniensis* populations was similar to what is known from historic records and the presence of *A. ngongoniensis* in indigenous afromontane forest patches bodes well for the species as these are likely to be less vulnerable to disturbance and habitat degradation in these forests, compared to grasslands due to…..

Auditory cues from calling anuran males is an effective and practical way for detecting species presence but can also be used for more in-depth monitoring and surveying through Passive Acoustic Monitoring. Such auditory monitoring can provide important ecological data, such as density estimates, population estimates and spatial habitat utilisation (Ref). All of these are important factors in providing a sound conservation strategy for any species, and have been employed successfully for *A. ngongoniensis*. There has been very little research done on the biology of this species. Apart from its original description, a handful of presence/absence surveys and one population estimate – not much information exists on the life history of *A. ngongoniensis*. Improving our understanding on the ideal habitat for the species requires further research into their breeding biology. Thus studying thriving populations may provide insight into their habitat requirements.

As far as application for monitoring of this species goes, PAM is a straightforward and user-friendly method. Furthermore, the method provided solid baseline data on which future studies can build, because its standardized methodology makes it highly repeatable. PAM enabled us to not only estimate calling male density but also gain insight into spatial habitat utilization. If employed and interpreted correctly the method provides a deeper understanding of the breeding behaviour (calling and movement patterns) of *A. ngongoniensis*. Density estimates obtained in this study are much higher than the previous study (Harvey, 2007) where density estimates were derived from walking audio transects and the counting of actual calls. Accurate interpretation of call density estimates requires an analysis of call signal strength, since this is known to vary in individuals. Our method assumed that individuals emit calls at the same strength consistently. It is possible that software counted calls from the same individual as separate individuals resulting in an overestimation of population density. Nonetheless, the realised area of occupancy within a site appears to be approximately 0.25 Ha in grassland sites, resulting in low numbers of the frog. It is clear that sites are not occupied homogenously. We believe populations are inherently small and therefore concentrated in areas to maintain a viable population size, while a sloped landscape provides better drainage for terrestrial nests than the flat valley bottoms that are more prone to flooding.

The calling male densities at the sites that occur in grassland habitat are significantly lower than that of the indigenous forest. We postulate that the forest microhabitat is more suitable to their terrestrial mode of breeding. In terms of resilience to disturbances, forest populations are inherently more protected from burning, mowing and grazing to which grassland populations are intermittently exposed. During this study it was unfortunate that detections were not made at all of the sites visited, emphasizing the need for repeated surveys that are required to determine density estimates for sites where the species could be present but weren't detected. Timing of some surveys after the breeding season had already concluded was likely the cause of the lowered detection rate. Another limitation compared to other survey methods is that the recording equipment is expensive, especially since more than one recorder is required for a microphone array setup. In addition, while the set-up of an array is straightforward, the data preparation for processing is a cumbersome and intensive process. Setting up an array is not particularly cumbersome, but exact measurements between song meters and GPS synchronization of all song meters is paramount.

Acoustic monitoring methods are based on the species-specific advertisement calls made by anuran males during the breeding season (Pierce and Gutzwiller, 2004; Pellet and Schmidt, 2005). Since for almost all frog species, only males vocalize, it is possible to obtain an estimate of calling males only during an acoustic survey. As far as the data obtained in this study goes, it may have been limited by the mas that was created for each array – with literature stating that the call can be heard at 35-40m. When the mask was increased in size to 100 m, calls were still picked up further afield (Figure 24), which could mean that the microphones are more sensitive and can survey a much larger area than originally anticipated. Another disadvantage of the method is that, for *A. ngongoniensis* at least, there was interference from other animal vocalisations (in this case insects and other frog species) that call on the same frequency as *A. ngongoniensis* rendering some of the call data unusable.

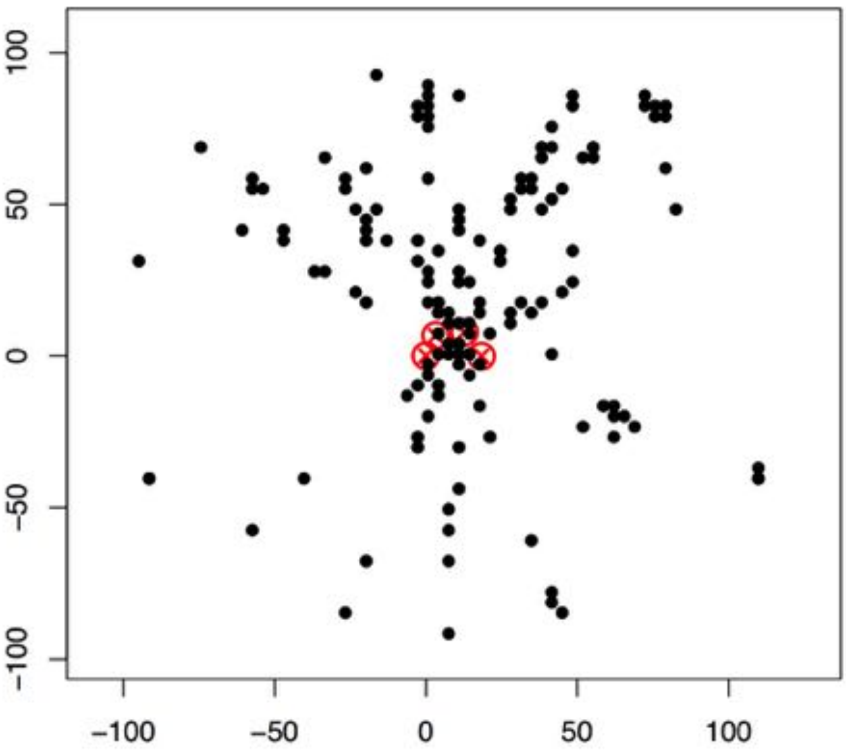

Figure 36. The microphone array at Mpur Road Verge with the mask extended to 100m showing calls detected beyond the initial masked area of 40m. The red crosses indicate the microphones.

This study will add valuable information to an already growing database to determine effective monitoring methods for amphibians in South Africa. With the baseline data gathered in this study, future research will be useful in furthering the conservation of the species as set out in the recommendations (see 4.3.1 – 4.3.3). However, there are solid recommendations for its conservation that can be implemented right now. If these guidelines are implemented, the chances of survival for the species will hopefully increase. That said, the population size is small and subpopulations are isolated, which does not bode well for the species, even if the population size is bigger than previously thought. We therefore recommend the species retains its Endangered listing.

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Appendix 1. Certificate of completion of Conservation Tools training course at Caño Palma Biological Station, Costa Rica.


EDGE of Existence

Conservation Tools Course

Held at Caño Palma Biological Station, Costa Rica
13th January - 10th February 2015

This certificate is awarded to

Mea Trenor

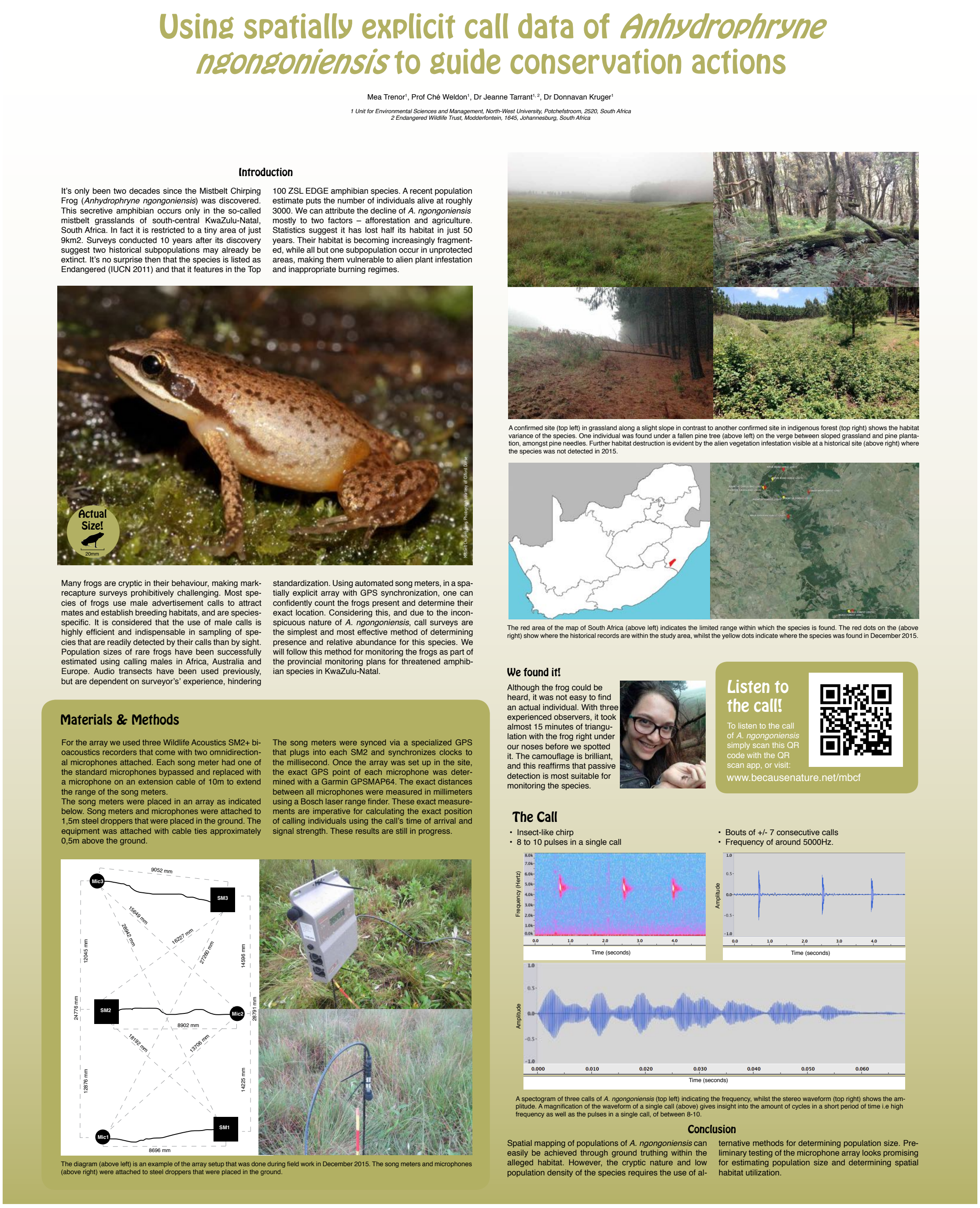
for successfully completing modules in Principles of Conservation Biology, Project Planning, Project Management, Statistics, Ecological Monitoring, Social Science, Action Planning and Communicating Conservation

EDGE

ZSL

LIVING CONSERVATION

Appendix 2. Poster presented at the Amphibian Conservation Research Symposium, North-West University.



Appendix 3. Certificate of attendance and presentation at the Student Conference of Conservation Science, Cambridge UK.

UNIVERSITY OF CAMBRIDGE

This is to certify that

Mea Trenor

has attended the Student Conference on Conservation Science, 22-24 March 2016,

at the University of Cambridge, Downing Street, Cambridge, UK

and presented a talk entitled

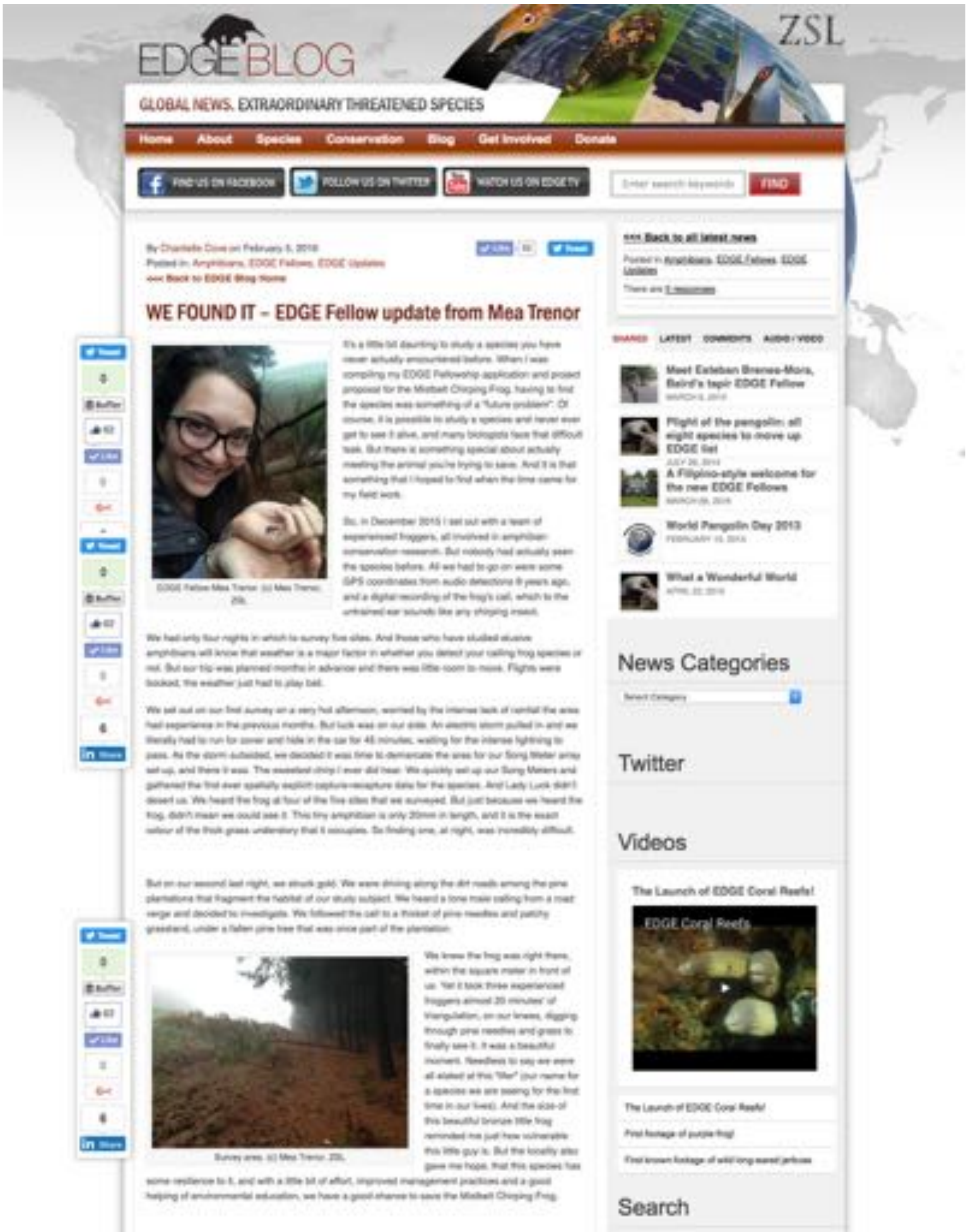
"Spatially-explicit call surveys for the mistbelt chirping frog"

Administrator SPGreen   Date: 22 March 2016

Appendix 4. Certificate of completion of a short course in R at the University of Cambridge.

Appendix 5. Blog post introducing the project published on the ZSL EDGE blog.

Appendix 6. Blog post on finding the Mistbelt Chirping Frog, published on the ZSL EDGE blog.



Appendix 7. Article published in the International publication, FrogLog.

An Amphibian on the EDGE: Saving South Africa's Mistbelt Chirping Frog

Appendix 8. Popular media article published online in the Guardian, UK.


theguardian

'I wasn't contributing anything to saving our beautiful planet': from consumer magazine editor to frog conservationist

theguardian

Appendix 9. R code accompanying the *ascr* package.

```
#load the package
library(ascr)

#load your data
setwd ("C:/Users/name/Documents/MBCF/FINAL SM DATA/Ngele Forest")
getwd ()
data <- read.csv ("Ngele Forest A.csv")
data

data$amplitude_original <- data$amplitude
data$amplitude <- data$PeakHeight

mics <- read.csv ("Ngele mic coords.csv")
mics

mics2 <- mics[,3:4]
mics2

convert.pamguard
names (data)

data2 <- convert.pamguard(data, mics2)
data2

mask <- create.mask(traps = mics2, buffer = 40)
mask

freqs <- read.csv ("MBCF call rate.csv")
freqs

freqs2 <- as.numeric(freqs[1,2:7])
freqs2

fit <- fit.ascr(capt = data2, traps = mics2, mask = mask,
cue.rates = freqs2, survey.length=1,ss.opts = list(cutoff = 0.1))

summary(fit)


# Carrying out bootstrap procedure to give std error.
boot.fit <- boot.ascr(fit = fit, N = 1000)
boot.fit_1000 <- boot.fit
summary(boot.fit_1000)

# plot the location of the first call
locations(fit, 1)

# plot one call
locations(fit,1, density = F, levels = c(0.95))

# plot dots instead of contours for 300 calls as example
for(i in 2:300){
+ locations(fit,i, density = F, add = T, plot.estlocs = T,
plot.contours = F)}
```