

The use of a sniffer dog for amphibian conservation ecology

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Abstract

Amphibians are threatened by a variety of factors, of which habitat loss (due to urbanization) and infectious diseases are a few of the largest contributors. Urbanisation and land development is one of the biggest threats to wildlife populations. Species that are particularly vulnerable, for example, burrowing species, are not easily detected by conventional survey methods, due to their cryptic lifestyles. The Giant Bullfrog (*Pyxicephalus adspersus*) is one of two amphibian species that are listed by the South African National Environmental Management: Biodiversity Act 2004 under the category *Protected Species*. Because Bullfrogs are fossorial (aestivate for up to 11 months at a time) and active for only a few weeks of the year, an area can easily be misidentified as not having any Bullfrogs during conventional surveys. This misidentification can then lead to the approval of construction work within their habitat. Thus, there is a need to find a way to locate these frog species without harming them in the process. It seems, however that scent detection dogs can help with locating and therefore, also protecting these species. Due to their heightened sense of smell (between 1 000 to 10 000 times higher) dogs have the ability to detect more diluted scents than humans can. Because of this sensitivity, Canines have been used for a variety of scent detection jobs. Using dogs for research and conservation purposes is a fairly new practice and therefore requires its own techniques. We made use of a sniffer dog to detect a burrowing species using a method called operant conditioning. This type of conditioning reinforces a natural behaviour in the dog. The ability of the sniffer dog to detect the Giant Bullfrog scent in amphibian conservation was tested, as a precursor for using this technology later on. Clicker training, in combination with operant conditioning, was used to prepare the dog for the detection of frog scents. This setup required three phases: 1) familiarizing the dog with the target scent in exposed containers; 2) introducing the training platform with diluted target scent and disturbances; and 3) training in simulated natural environments. After training, the dog could locate and indicate on containers with live frogs inside, and achieved 100% accuracy on a 1:100,000 dilution of the scent she was trained on. The results for this project indicated that human and amphibian scent disturbances are not a major distraction for the sniffer dog. The dog was able to detect scents, above and below the surface, under a variety of conditions. Our sniffer dog was also able to detect wild buried Bullfrogs and track the scent of live frogs over a body of water (in their natural environment), but digging to confirm the presences of the

frogs was not always possible. Ultimately, the successful application of this research could result in a mutual beneficial collaboration between the scientific community and industry partners to the means of environmentally sustainable development. Additional work was also done to condition the dog to detect other critically endangered South African amphibian species and also train the dog to identify pathogens such as *Batrachochytrium dendrobatidis*(*Bd*). The sniffer dog was able to detect all of the *Bd* targets (cultures) in the experiments. Even though a minute amount of false indications were made during all plank experiments, most of the false indications could be explained by the position of the target and/or the setup of the experiment on the plank equipment. One of the factors that were applicable to our experiments with regards to missed indication was the way in which plank equipment was used. It could be concluded from our experiments that the two best methods for preservation of frog scent was a 1:1000 dilution and a swab that were both kept under the same conditions (4°C) only if the swab was diluted before each test. During this study we also looked at historical and spatial data (using geographic information system) and compared the results with sampled data. Comparing the datasets revealed a correlation between three condensed soil patterns and Bullfrog distribution. A vital component of this research project was informing and educating the public on amphibians and conservation. Mainstream- and Social media, along with scientific platforms, were used to convey the applicability of this study to conservation ecology. By using the sniffer dog, a lot of public interest and awareness was generated for the duration of this study.

Keywords: amphibians, conservation, operant conditioning, sniffer dogs, plank training, media.

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Declaration

I, Esther Elizabeth Matthew, declare that this dissertation is my own, unaided work, except where otherwise acknowledged. It is being submitted for the degree of M.Sc. to the North-West University, Potchefstroom. It has not been submitted for any degree or examination in any other university.

A handwritten signature in black ink, appearing to read 'Matthew', is positioned above a horizontal line.

EE. Matthew

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1. Literature review and introduction

1.1. Amphibian conservation

Global amphibian populations have shown a concerning decline over the past three decades (Andreone *et al.*, 2012; Gible *et al.*, 2008) which led to the extinction of some species resulting in amphibians being more threatened than birds or mammals (Beebee and Griffiths, 2005). In the 1980's, a decline in global amphibian research was observed (Wells, 2010), but in 1989, at the First World Congress of Herpetology, scientists and biologists shifted their focus to the conservation of amphibians as the decline of amphibian populations was still increasing (Alford and Richards, 1999; Beebee and Griffiths, 2005; Stuart *et al.*, 2004). The current world count for amphibian species stands at a staggering 7455 (Frost, 2015). However, this number is an underestimate, since new species are still being discovered. These new discoveries are partially due to previously unknown areas being explored, but also since molecular techniques assist in revealing cryptic species (Halliday, 2001; Köhler *et al.*, 2005).

Amphibians' susceptibility to chemical and biological agents are due to a variety of characteristics, including their highly permeable skin and their reliance on moist and aquatic habitats (Wells, 2010). Amphibians are threatened by a variety of factors of which habitat loss and infectious diseases are the main contributors (Becker *et al.*, 2007). Stream dwelling, post-metamorphic and adult frogs with restricted geographic environments and small clutch sizes were observed dying without a major associated change in the environment. Status and trends on underlying causes such as the complex interactions among some factors (e.g. climate changes, small-scale chemical exposure, reduced habitat, increased UV exposure and amplified susceptibility to opportunistic pathogens) have been examined to find a cause for these deaths (Alford *et al.*, 2001; Biek *et al.*, 2002; Stuart *et al.*, 2004). Other causes include environmental contaminants, introduction of invasive species and direct exploration (Beebee and Griffiths, 2005; McCallum, 2007; Houlahan *et al.*, 2000). For many years the loss of habitat has been known to have a significant impact on amphibians, but the above mentioned factors could, by means of interaction or combination, cause population declines by increasing the level of mortality (McCallum, 2007). The current top three reasons for amphibian declines that are directly associated to anthropogenic activities are: habitat destruction, exploitation and spreading of diseases.

Amphibian declines and the advancement in survey methods (and equipment) has resulted in an increase in the research regarding amphibian habitat use (Yetman and Ferguson, 2011a). Habitat transformation and destruction is happening at an increasing rate (Lannoo, 2005; Thomas *et al.*,

2014) which also leads to amphibians being killed, their habitat being lost and the restriction of the animal's movement, preventing them from reaching their breeding sites (Collins and Storfer, 2003). Habitat degradation and fragmentation has also been known to cause large-scale amphibian declines (Houlahan and Findlay, 2003). According to Houlahan and Findlay (2003), habitat destruction has also had a negative effect on the abundance of species, species richness and the amphibian community composition. Urbanization, therefore, creates an interference with the use of amphibians as indicators of ecological health and reliability of wetlands (Guzy *et al.*, 2012).

The direct effects of human exploitation on amphibians have also caused additional declines (Jensen and Camp, 2003) as it is not uncommon for amphibians (especially frogs and toad) to be eaten in some parts of the world (Warkentin *et al.*, 2009). Apart from consumption, amphibians have mostly been affected by overharvesting and global trade (Warkentin *et al.*, 2009). Another human impact on these animals is road traffic (Fahrig *et al.*, 1995). According to Fahrig *et al.* (1995) an increase in traffic worldwide led to a great negative effect on amphibian density. The biggest contribution to mortality by means of road kill is the slow speed of the amphibians' movement across the road and the lack of visibility (Puky, 2005).

The spread of diseases has also been associated with amphibian declines (Conradie *et al.*, 2011; Fisher *et al.*, 2009). Strong evidence suggest that the extreme decline in amphibians can be explained by the infectious waterborne disease *Batrachochytrium dendrobatidis* (*Bd*), also referred to as amphibian chytrid (Berger *et al.*, 1998; Blaustein *et al.*, 2005; Ouellet *et al.*, 2012). *Bd* is a virulent fungus that causes a skin disease called chytridiomycosis and has been identified as one of the major causes of global amphibian mortality (Fisher *et al.*, 2009). These and other persistent threats have resulted in amphibians being the most threatened class of vertebrates, with almost one third of all species currently threatened or endangered (Gascon *et al.*, 2012; McCallum, 2007). Amphibians play a diverse role in natural ecosystems, which means that amphibian declines can ultimately result in a change of conservation status of these species (Dodd, 2010; Murray *et al.*, 2009). A lot of researchers consider this phenomenon, which is suggestive of a universal decrease in worldwide biodiversity, to be a mass extinction occurrence (Anguloa and Andreone, 2012).

1.2. Typical amphibian survey methods

Research on amphibian conservation and ecology studies require a variety of surveys, because amphibians can move across terrestrial habitats and through water bodies (Carruthers and Du Preez, 2011) and are often camouflaged to blend in with their natural environment. Therefore, numerous survey methods exist for finding and obtaining information from these animals.

As most amphibians do not move around a lot during the survey period, area-based surveys are considered to be the best approach (Hill *et al.*, 2005). Terrestrial transect searches can be used to locate terrestrial frogs and toads, whereby defined units (for example: plots, quadrates or transects) are selected to randomly represent a larger area (Vences *et al.*, 2008). Amphibian samples are then taken from these smaller units by counting the animals that occur in each sample unit. This information is then used to determine the abundance and diversity of species (Dodd, 2010). Even though this type of survey method is inefficient and it can help to estimate the number of amphibians outside of their aquatic habitats, it can sometimes be time consuming (especially for large search areas).

Another survey method, egg searches, can be used for most amphibians, especially toads (Hill *et al.*, 2005). If egg strings and spawn clumps can be distinguished, reasonable estimations can be made about the presence of species. This method is good for small ponds and shows that breeding has occurred. Although this is a simple survey method, the number of amphibians cannot be determined by egg searches, for this requires identification expertise (Hill *et al.*, 2005).

Visual encounter surveys can be used during the breeding seasons, when amphibians exhibit cumulative behaviour around the ponds where they breed. Torch counts usually occur after nightfall and focus on observation, rather than collecting animals. With this method, abundance can be determined and breeding populations can be estimated (Hill *et al.*, 2005). This survey method is quick, non-invasive and recommended for use with all amphibians.

Other techniques include the capturing of animals via traps, drift fences and netting (Snodgrass *et al.*, 2000; Vences *et al.*, 2008; Doan, 2003). Pitfalls are the most common type of traps used because of its effectiveness on all types of amphibians. Pitfall traps are created when containers are placed in the ground. Drift fences are then used to direct animals to fall into the bucket (Yi *et al.*, 2012; Saska *et al.*, 2013). Even though it can be labour intensive to check the traps every morning, it is possible to catch the animals who are returning to the ponds (if the traps are used for an extended period) (Saska *et al.*, 2013). Sex ratio and population can be determined by pitfall traps. Netting is also a recommended capturing method for frogs, toads and tadpoles (Hill *et al.*, 2005). This method entails using basic nets to capture the animals and is particularly efficient for small ponds. Netting is also a quick and simple method for capturing amphibians, but cannot be used to detect all individuals like terrestrial breeders (Yi *et al.*, 2012).

Capture-mark-recapture sampling (Dodd, 2010) is one of the most common forms of surveying amphibians to determine population estimates. Mark-recapture sampling can be used on all amphibians. Amphibians can be marked with a variety of methods. The most common methods

used are toe-clippings, Visible Implant Elastomer (VIE) tags and pit tagging (Phillott *et al.*, 2007; Gibbons and Andrews, 2004). Mark-recapture sampling events can provide information to determine the survival and population structures of amphibians (Measey *et al.*, 2003). This method can also provide accurate results if (several) trapping events are efficient (Hill *et al.*, 2005).

Auditory (or acoustic) monitoring of amphibian populations has also become a popular survey method in recent years (Bridges and Dorcas, 2009; Vences *et al.*, 2008; Carruthers and Du Preez, 2011). For this method a recording device is used to document distinctive amphibian calls. After recordings are made they are analysed with sound wave software to determine the presence of different species (Lotz and Allen, 2007). Sound meters can be installed for extended periods, but amphibian calls are highly depended on season and time of day (or night).

The expertise on identifying and handling the animals is also required for most of these methods. Unfortunately, all these techniques can have inaccurate results, if a small number of animals are found (or heard). None of these methods, however, provide a way of locating any burrowing amphibian species during their inactive seasons. It is therefore vital to develop a novel, survey method that will enable the searcher to locate most amphibian species, including burrowing species. In this study, the natural scent released by amphibians was used to train a sniffer dog to help locate specific amphibians (e.g. the Giant Bullfrog).

1.3. Scent detection

1.3.1. Origin of scent

The idea surrounding sense of smell originated from the perception of the environmental molecules around us (de March and Golebiowski, 2014). A huge amount of information about the chemical composition of our environment is transmitted through odours (Manzini *et al.*, 2014). All of these odours consist of volatile molecules (Buck, 2005). If the structure of the volatile molecules is changed, they can be perceived as different odours. The perception of odours begins in the so-called olfactory receptors (Figure 1), which are sensory neurons residing in the olfactory epithelium that expresses G protein-coupled receptors (de March and Golebiowski, 2014; Golebiowski *et al.*, 2012). A signal transduction cascade is initiated from the binding of odour molecules to olfactory receptors (the binding converts olfactory stimulus into electrical signals). The signals are then broadcasted to the first relay centre in the olfactory pathway (the olfactory bulb) by means of the axons of the sensory neurons (Manzini *et al.*, 2014). The olfactory information is processed in the bulb and then transported to higher olfactory centres (cortex) passing through axons of mitral cells (Figure 1). The ability to smell helps organisms with point of reference in space, influences their

choice of sexual partners, warns of potential threats, regulates food intake and influences feelings and social behaviour in general (de March and Golebiowski, 2014). An estimated 10000 to 100000 distinct odours can be sensed by humans (Buck, 2005).

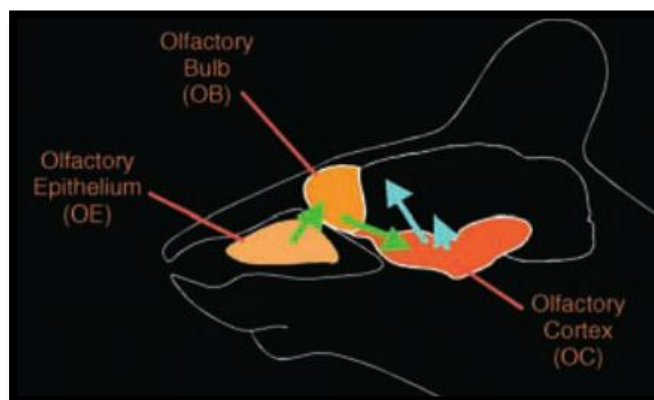


Figure 1: Odours are detected by olfactory sensory neurons in the olfactory epithelium and signals are relayed through the olfactory bulb to the olfactory cortex, and then sent to other parts of the brain (Buck, 2005).

1.3.2. Scent detection dogs

It has been recorded that dogs have a remarkable olfactory sensory system (Jezierski *et al.*, 2010; Lord, 2013). A dog's sense of smell is estimated to be 1,000 to 10,000 times more enhanced than a human's ability, depending on the breed (Craven *et al.*, 2007). It is also stated that the area in a dogs' brain dedicated to sense of smell is forty times larger than in the human brain. Therefore, with their extraordinary olfactory ability dogs are able to identify scents far more diluted than human beings can (Schoon, 1996; Lesniak *et al.*, 2008).

According to Johnen *et al.* (2013) dogs have been used for a variety of scent related jobs. These jobs include drug detection, explosives, forensics and hunting (Adamkiewicz *et al.*, 2013; Browne *et al.*, 2006; Hurt and Smith, 2009). Dogs are also used to screen for cancer (Walczak *et al.*, 2012) and to locate bigger wildlife (like bears) by means of detecting scat (Wasser *et al.*, 2004).

When it comes to human scents, dogs have the ability to detect bladder and prostate cancer by means of sniffing and indicating on the sample (Cornu *et al.*, 2011; Willis *et al.*, 2004). Using breath samples, dogs are also able to detect early stages of lung and breast cancer (McCulloch *et al.*, 2006; Walczak *et al.*, 2012). This ability is derived from dogs being able to pick up on small traces of toxins (volatile organic compounds) released by the tumours (McCulloch *et al.*, 2006). Dogs have

such remarkable scent abilities that they can even discriminate between identical twins (Pinc *et al.*, 2011).

In the case of locating bigger wildlife, the dogs are used to detect scat (Wasser *et al.*, 2004). Scat detection has in recent years been used to locate a variety of wildlife including foxes, tigers, wolves and moose (Smith *et al.*, 2003; Kerley, 2010; Wasser *et al.*, 2011). As in the USA, South Africa is also using dogs to locate bird carcasses on wind farms to determine the impact these farms have on populations of birds and bats (Paula *et al.*, 2011). Other related sniffer dog jobs in South Africa included: anti-poaching dogs, ivory sniffer dogs and conservation projects where dogs are being used to locate endangered and vulnerable species, like rabbits and tortoises (personal communication: Vicki Hudson).

In recent years, there has been an increase in popularity when it comes to the use of conservation dogs for ecological surveys (Reed *et al.*, 2011). The area surveyed and the efficiency of a survey can be greatly improved by using working sniffer dogs (Reed *et al.*, 2011). As dogs have been used for the detection of a variety of human and biological scents (Browne *et al.*, 2006) there is a high likelihood that dogs will be able to detect amphibian scent as well.

1.3.3. Operant conditioning

Training dogs for scent detection typically requires a method called operant conditioning (Jezierski *et al.*, 2010; Batt *et al.*, 2008). Operant conditioning is a training mechanism by which consequences of an initially spontaneous behaviour, may reinforce or inhibit recurrence of that behaviour (Blackman, 1974). Positive reinforcement (rewarding for correct behaviour) and negative reinforcement (no reward) is widely regarded as the best way to train a dog (Blackman, 1974; Geller, 2008; Hiby *et al.*, 2004). It is also common to use reward-based clicker training, where the “click” sound is used to reinforce the dog's behaviour (as it is followed by a reward) as part of operant conditioning (Cornu *et al.*, 2011; Yoon *et al.*, 2000; Fjellanger *et al.*, 2002). Verbal and physical punishment is not recommended as it does not improve the trainability (or obedience) of the dog and may result in other unwanted behavioural problems (Hiby *et al.*, 2004).

There is also a lot of other factors that determine the success of a dog training program (Batt *et al.*, 2008). Some of these factors include the sudden appearance of something or someone, the dog's temperament, disobedience and other distractions (e.g. noises) (Batt *et al.*, 2008; Bennett and Rohlf, 2007). Owner - dog companion interaction is a vital element for dog training, because a lot of behavioural issues can be prevented through shared activities by the owner (trainer/handler) and

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the dog (Bennett and Rohlf, 2007). The dog's accuracy is also influenced by the handlers ability to pick up on behavioural changes and the dog's olfactory (scent detection) capability (Concha *et al.*, 2014).

There are, however, no significant differences to scent abilities between breeds, sexes or the specialities of the dogs (Adamkiewicz *et al.*, 2013; Rooney and Bradshaw, 2004). Williams and Johnston (2002) claim that sniffer dogs have the ability to detect up to ten different scents in a set search scenario. They concluded that the detection performance of the dogs did not weaken whenever a new scent was added (after refreshment training). It seems that in some cases over time the addition of new scent resulted in more efficient conditioning, as less trails were required to achieve conditioning (Williams and Johnston, 2002).

Even though multiple scent detection is possible, it is important to realize the underlying cognitive concepts and learning paradigms that the scent detection dogs are exposed to (Lit and Crawford, 2006). According to Lit and Crawford (2006), cross-training a dog (e.g. using the same dog to locate live-humans and cadavers) is not recommended, as this results in confusion. Therefore, if scents are too contradicting (or cause a conflict of interest) it can lead the dog to indicate when no target scent is present, which in turn results in the inability to deploy the dog in a search situation.

Studies have shown that animals can obtain information by means of communicative cues they receive from humans (Lit *et al.*, 2011). This phenomenon was first observed in the early twentieth century and named the *Clever Hans effect* (Pfungst, 1911) . This effect was seen with a horse that was believed to be able to complete mental tasks. It was later revealed that horses can receive visual cues from humans and they can also pick up on subtle changes in body language (McKinley and Sambrook, 2000). Further research indicated that The *Clever Hans effect* can also create problems when conditioning sniffer dogs, if the handler has knowledge about the position of the target (Lit *et al.*, 2011; Miklösi *et al.*, 1998).

1.4. Concealment and hibernation in fogs

The majority of South African amphibians have developed complex protective mechanisms to keep them safe during their inactive seasons (Poynton, 1964). These mechanisms included concealment, hibernation and aestivation. In most cases, rainfall seems to be the dictating factor of when amphibian fauna emerge. For this study, the focus was on two such species, the Giant Bullfrog (that aestivates for the larger part of the year), and the Amathole toad (that is concealed during the day). Both of these species are conservation concerns, albeit for different reasons.

1.4.1. Giant Bullfrog

An adult Giant Bullfrog (*Pyxicephalus adspersus*) has an maximum body mass of over 1 kilogram and a body length of up to 245 mm, which makes it southern Africa's largest amphibian species (Du Preez and Carruthers, 2009). Sexual dimorphism is displayed by males growing much larger than the females (Balinsky and Balinsky, 1954). They are usually dark olive green with short white dorsal ridges on their back (Figure 2).



Figure 2: An adult male Giant Bullfrog (*Pyxicephalus adspersus*).

Bullfrogs use temporary water bodies as breeding sites and only breed if the conditions are ideal (Poynton, 1964; Van Wyk *et al.*, 1992). They usually emerge from the ground after consecutive days of at least 20 mm of rainfall during the summer months in southern Africa.

During the breeding season eggs are laid in shallow water and during tadpole development, an adult male bullfrog stays behind to watch over the tadpoles until completion of metamorphoses (Balinsky and Balinsky, 1954; Cook *et al.*, 2001). All the other adult bullfrogs soon retract to their fossorial burrows a few days following the breeding event. It is suspected that the guarding behaviour of the male bullfrogs is a form of parental care. Bullfrogs are said to be very aggressive and this behaviour is crucial for the survival of the tadpoles (Cook *et al.*, 2001).

In the active season Bullfrogs consume large amounts of food. They are carnivorous (eating almost any prey it smaller than themselves) and even display cannibalism during juvenile development (Conradie *et al.*, 2010; Yetman and Ferguson, 2011b). Bullfrogs hibernate/aestivate for most of the year. During this time they burrow down an average of 1 m, and become enclosed (apart from the two nostrils) by a thick layer of moulted skin, while their metabolism slows down and they go into

semi-dormant state (Du Preez and Carruthers, 2009; Loveridge and Withers, 1981; Van Wyk *et al.*, 1992). According to Minter *et al.* (2004) the Bullfrogs are widely distributed in South Africa, occurring in all provinces, but most commonly in the Northern and central parts of the country (Figures 3 and 4).

In 2004 the Atlas and Red Data Book of the Frogs of South Africa, Lesotho and Swaziland had classified the Bullfrog as *Near Threatened* due to the loss of habitat (Minter *et al.* 2004), but this status is currently being revised. Other threats include: road kills, the indiscriminate use of pesticides and pet trade. Bullfrogs are also often killed and consumed by humans (Okeyo, 2004).

According to the IUCN's (International Union for Conservation of Nature) official list, the Giant Bullfrog is listed under the category *Least Concern*, since it has a wider distribution outside the borders of South Africa (Conradie *et al.*, 2010; Van Aardt and Weber, 2010). However, in the Gauteng province its numbers have declined due to the severe degradation of its habitat (Thomas *et al.*, 2014). Given these circumstances, Giant Bullfrogs are one of two amphibian species that are listed by the National Environmental Management: Biodiversity Act 2004 (Act 10 of 2004) as *Protected Species*, thus requiring national protection. Because of their fossorial existence, an area can easily be misidentified as not having any Bullfrogs during a single-event biodiversity inventory survey (Yetman and Ferguson, 2011a).



Figure 3: Global distribution range of the Giant Bullfrog. Its range is fairly extensive, but limited to southern and East Africa (IUCN Red List, 2015).

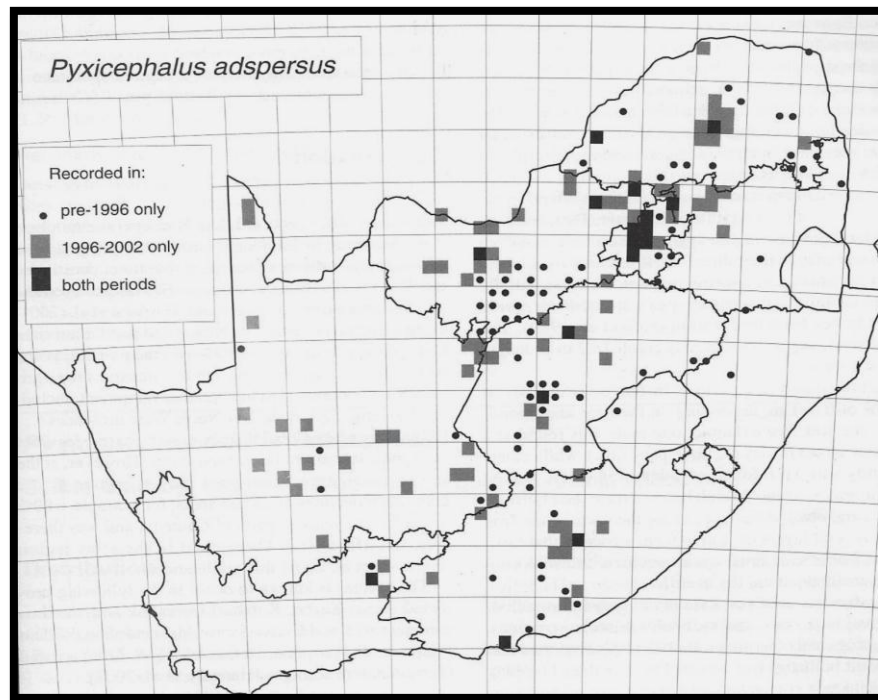


Figure 4: Detailed distribution map of Giant Bullfrog in South Africa (Minter *et al.*, 2004).

1.4.2. Amathole toad

The *Critically Endangered* Amathole toad, *Vandijkophrynus amatolicus*, is one of South Africa's rarest frogs (Tarrant and Cunningham, 2011). This cryptic toad is relatively small (up to 40 mm) and is usually described as olive or dark grey of colour with a light vertebral line and may sometimes have irregular dark markings (Figure 5) (Du Preez and Carruthers, 2009; Hewitt, 1925). It is restricted to the Montane grasslands of the Winterberg and Amathole mountains, and is listed as *Critically Endangered* based on its limited distribution and rarity (IUCN Red List, 2015).



Figure 5: An adult female Amathole toad (*Vandijkophrynus amatolicus*) in its natural environment.

Photo: W. Conradie.

The Amathole toad breeds in temporary pools and seepages, in upland grasslands, during heavy rains, between September and January. Having disappeared for 13 years, between 1998 and 2011, the species made it onto the IUCN's *Lost Frog* search campaign list in 2010. Despite a concerted search effort in August of that year (and in years prior), the toad was not found and rumours of its extinction emerged (Conradie and Tarrant, 2011). Then, in 2011, a single female was discovered along with a small puddle of eggs and tadpoles (Tarrant and Cunningham, 2011). A single male was located at a new location (in 2012) and three Amathole toads were found during the breeding season in 2013 (personal communication: Jeanne Tarrant).

On the 8th of October 2015, Jeanne Tarrant, manager of the EWT's Threatened Amphibian Programme teamed up with Christine Coppinger from EWT's of the Source-to-Sea Programme, as well as Werner Conradie of Bayworld (in Port Elizabeth) to test out new survey techniques at one of the known Amathole sites. They discovered two adult females (under logs) and a few pools filled with tadpoles. These discoveries brought the total sightings of the Amathole toad, during the last four years, to seven adults (personal communication: Jeanne Tarrant).

Possibly South Africa's rarest amphibian species, this toad has only been seen 25 times since its discovery in 1925 (Hewitt, 1925). Compared to historical sightings of large numbers of the species gathering to breed, the Amathole toad remains elusive and warrants novel search methods to maximise detection probability.

1.5. Public interested in amphibian conservation

The collection of ecological information is not the only important component for an effective biological conservation initiative. An often overlooked important component, is public involvement and adherence to protection programs, because awareness, knowledge and perception play a fundamental role in the community's willingness to protect species (Smith and Sutherland, 2014; Vincenot *et al.*, 2015). Thus, to ensure successful conservation intervention it is vital for scientists to engage with the general public through public- and social media regarding contemporary conservation issues (Papworth *et al.*, 2015). Social media engines, such as Facebook, created new ways to accelerate the communications between the online public and conservation scientists. Although this can be an effective way of spreading information the rate of dispersal can be influenced by a variety of factors (Papworth *et al.*, 2015). Some of these factors include the emotional tone set by the post, length of the article (and length of title) and if the post involves

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charismatic animals and interesting pictures (Papworth *et al.*, 2015). This is evident by the fact that the support for wildlife conservation is directly linked to public attitude towards the subject (Vincenot *et al.*, 2015). Warner *et al.* (2014) concluded that social media could only lead to action-taking if there is a shared philosophy between the value of action-taking and the information provided by the environmental educator.

According to Archer (1997) human beings have a strong mental attachment to pets, like dogs and cats. It is believed that some human owners get greater pleasure out of an interaction with a dog than with another human being. Evidence suggests that there is a much bigger domestic relationship and social compatibility between dogs and humans than humans and any other animal (Morey, 2006). Due to this social bond, it is reasoned that dogs can be used (via social and public media) to create awareness for other causes like amphibian conservation research.

1.6. Problem statement and hypothesis

Habitat destruction is a threat to wildlife populations, and there is an urgent need to prevent the further loss of populations due to the developments on natural land. Species that are particularly vulnerable are those with cryptic lifestyles (such as burrowing frogs) that are not easily detected with conventional survey methods. However, when one harnesses the natural ability of dogs to detect scented objects in their environment and combine it with wildlife monitoring methods, the potential exists to detect the presence of particular animal species within an area that has, for example, been allocated for development. This is an important step in mitigating the effects of habitat loss of protected species. This study hypothesises that the use of sniffer dogs can vastly improve the ability to detect fossorial frog species such as the Giant Bullfrogs.

This study will therefore test the training methods of a sniffer dog, the dog's ability to detect amphibian scent, and the possibilities of creating public interest with this type of work. Tests will also be done on the ability of a sniffer dog to: 1) detect the scent of Giant Bullfrog scent in a simulated environment; and 2) detect the Giant Bullfrogs in their natural habitat. Furthermore, this research will answer fundamental questions concerning the ecology of the Giant Bullfrog with regards to the micro habitat requirements (e.g. soil structure, soil moisture) for aestivation and how this relates to habitat availability or population distribution.

1.7. Aim and objectives

The aim of this study was to harness the natural ability of dogs to detect scent and using this as a wildlife monitoring method in the natural environment. This method is reasoned to allow the

detection of species for research and conservation purposes (Reed *et al.*, 2011; Wasser *et al.*, 2004; Yetman and Ferguson, 2011a). The following objectives were set for this study:

Objective 1. Reinforce target detection in a sniffer dog. The method used for training is called operant conditioning (Blackman, 1974; Jezierski *et al.*, 2010).

Objective 2. Test the sniffer dog's ability to detect Bullfrog scent and finding the species in the wild for research and conservation purposes.

Objective 3. To determine the best method to preserve scent over time. For future scent detection work and to determine how to stock up on scent if it is not readily available.

Objective 4. Demonstrate that a sniffer dog can be used to investigate certain habitat requirements for Giant Bullfrogs.

Objective 5. To determine if the sniffer dog can help locate other endangered species.

Objective 6. To establish if media can be used for educational purposes and creating awareness. Because human-dog relationship are one of the strongest bonds between species (Bennett and Rohlf, 2007), this study will also try to establish whether more awareness could be created for amphibian research and conservation through the interaction with a sniffer dog.

1.8. Chapter division

The thesis is introduced with a summary (**Abstract**). **Literature review and introduction** (Chapter 1) is followed by the **Material and Methods** (Chapter 2) that contains that contains a terminology table, which provides terms used during the dog training phases of this study, and all of the materials and methods used during each component of the study. The **Results** (Chapter 3) are all represented in one chapter and the main components can roughly be divided in: the role of plank training, sniffer dog experiments, preservation of frog scent, Bullfrog habitat utilization, detection of *Bd* and engaging with the public. The combined results and their implications are considered in the **Discussion and conclusion** (Chapter 4). References for all chapters follow (Bibliography- Chapter 5) and additional information is added in the **Appendices** (Chapter 6).

2. Material and Methods

2.1. Terminology

Condition training uses certain terms that have a specific meaning in the context of animal training. Realizing that this study contains components that differ slightly from the more common sniffer dog research and training techniques, an outline of the common terms are given as well as a unique set of terms relevant to dog training, as used in this study (Table 1).

Table 1: Terminology related to conditioning of a sniffer dog.

Term	Description
Clicker	A manually operated device used immediately after a correct response by a dog, producing a short “click” sound. After a “click” the dog is rewarded, e.g. by a treat or toy. This method is used to reinforce a very specific behaviour and is a faster way to <u>reinforce</u> the dog for the right behaviour.
Indication	An operant conditioned response of the dog (sitting, pawing or lying down) at a <u>positive target</u> .
Targets	Defined as all the possible locations where samples are hidden. This includes negative and <u>positive targets</u> .
Positive target	A target is a target location containing the <u>scent sample</u> the dog is being trained on (for example frog scent). This is the required target the dog should indicate on.
Negative target	A target is a location containing scent that does not match the positive scent. These can also be seen as <u>disturbances</u> .
Reward	In this study, a reward refers to a treat (dried meat) or a ball which the dog receives after indicating on the positive target
Praise	Key words spoken by the handler which indicate that the dog's response was correct. Praise is usually combined with a reward for additional reinforcement.
Reinforcement	Positive reinforcement (part of operant conditioning) entails rewarding the required behaviour; while presenting no reward to decrease unwanted behaviour such as false indications.
Disturbance	Can be anything that is seen as a distraction or obstacle for the dog during training or testing. These include other scent samples, weather conditions and the presences of other humans or animals.
Scent sample	This is any sample containing a certain scent. Scent from an animal (e.g. frog) collected by either swabbing the animal or by placing absorbent material inside the animal's enclosure for a

	period of time that would allow the scent to be absorbed.
Scent dilution	A scent sample that has been diluted in distilled water. Different dilutions are produced by mixing the sample with various volumes of water.
Miss (MI)	A lack of indication on a positive target for whichever reason.
False indication (FI)	Refers to an indication made by the dog on a negative target.
Plank	A training aid which directs the dog to multiple targets and usually contains holes below which containers with scent are placed.
Run	One run is a session where the dog examines the 10 targets on the plank, back and forth (2 chances to indicate on positive target). The number of positive targets in a run may vary according to the type of test.
Chances	The number of opportunities for the sniffer dog to indicated on a target. This can be used to evaluate the sniffer fog for negative and positive targets.

2.2. Materials

This study involved the acquisition, upkeep and training of a sniffer dog which is the primary research tool used to address the research problem. Additional materials required for conditioning training are rather simplistic and include the following:

- equipment – GPS, video camera, digital camera, wooden planks
- consumables – plastic containers (Figure 6a), stainless steel shakers, plastic tubs, laboratory glassware, insulation tape, permanent markers, cotton swabs and wipes, 15ml centrifuge tubes
- husbandry and training aids – dog food, dog treats, clickers (Figure 6b), leads and tennis balls
- live frogs – disturbances (for example other amphibians) and live food for animals

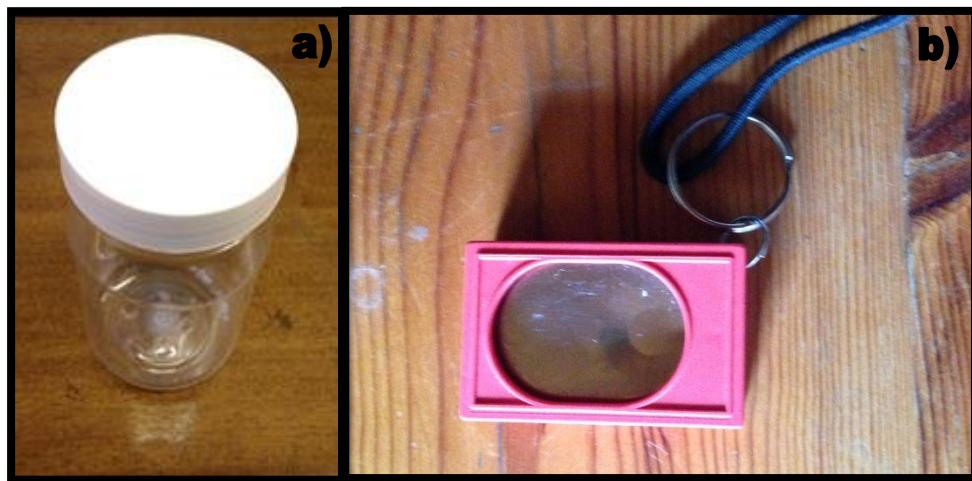


Figure 6: Example of a) a plastic container used to house target scents and disturbances, and b) clicker device used during reinforcement training of dog.

2.3. Obtaining the right sniffer dog

It is important to select the right dog for the specific role it needs to fulfil. Although all dogs have a great sense of smell (much greater than that of humans), the abilities of the dog is associated with the breed and other relevant features. Selecting the right breed is based on three basic features: 1) sense of smell; 2) drive, with the ability to learn (working pedigree is a preference); and 3) compatibility with the type of job and trainer (Rooney and Bradshaw, 2004; Willis, 1995). It is also recommended to get the dog while it is still a small puppy, as many behavioural obstacles can be prevented or easily overcome.

According to research (Willis, 1995; Schoon, 1996; Lit and Crawford, 2006), German shepherd dogs and Belgian shepherd dogs (Belgium Malinois) are most commonly used scent related jobs and are therefore regarded as the dogs with the best noses. German shepherd, French poodle and Border collies are seen as the most intelligent breeds (Jayson, 2009; Rooney and Bradshaw, 2004).

Based on this above mentioned information one would expect that a German Shepherd would be the best breed to use, however, additional research indicated that German Shepherd puppies are very expensive, sometimes difficult to train, and often have a lot of health concerns (like hip and elbow dysplasia). According to international experts in South Africa, quality German Shepherd dogs are also not readily available (³personal communication: W. Wragg).

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After carefully considering all of the requirements for suitable dog breeds, a Border Collie was chosen for this study. Border Collies are more affordable, extremely easy to train (highly intelligent), have a good sense of smell and the fact that South Africa is known to have good quality Border Collie dogs, they are more readily available than some other commonly used breeds (Jayson, 2009; Pilley and Reid, 2011). Border Collies are known for having high levels of energy and physical stamina, which makes them perfect for working in the field (Dale, 2015). This breed excels at search and rescue, obedience, agility and scent detection given the right training circumstances (Lit and Crawford, 2006; Rooney and Bradshaw, 2004). They are usually extremely ball-driven; a characteristic that is vital for training with a toy-based reward. The Border Collie used in this study, Jessie (Figure 7), was obtained as a puppy from a pedigree of working dogs on a farm close to Bloemfontein, Free State province.



Figure 7: The sniffer dog used during this study – a female border collie named Jessie.

2.4. Husbandry and care

2.4.1. Border collie dog

It is very important to take good care of your sniffer dog in order to maximize its potential (Berns *et al.*, 2015; Clark and Boyer, 1993). A healthy and happy dog is also more willing to please his/her trainer. The most important components for husbandry and care can be summarized as follow:

- **Nutrition:** It is extremely important that a working dog gets all the possible nutritional value out of his/her food. Montego dog food (classic puppy) was used for the dog in this study. This dog

food contains 26.5% protein, 12% fat and 1.3% calcium. The protein helps to build and maintain muscle tissue while the fat provides extra energy. The calcium helps to build strong bones. The sniffer dog was fed 300g of dog food every evening (around 17:30). Occasionally, meats and bones were added to the dog's diet as additional nutrients.

- Health care: Keeping your dog healthy is a vital component in this line of work. If a dog feels ill or injured he/she might not be able to work at all. It is important to prevent injuries from occurring and to monitor them when they do occur. The dog used in this study acquired all the necessary vaccinations (including Rabies and Parvovirus) at three, six and 18 months. Furthermore, the dog was also given Bravecto tablets, which is used for treatment and prevention against tick and flea bites (for up to 3months). This treatment was necessary due to the long hours spend working in the field. Bone and muscle injuries were also minimized by conditioning the dog with a proper exercise regime.
- Exercise: Not only does exercise help to prevent muscle injuries, it also provides the dog with the stamina it needs to work for longer periods of time, and in more physically draining conditions. The sniffer dog used in this study enjoyed a variety of exercises which included: swimming, playing fetch (Figure 8b), playing Frisbee, going for daily walks and even clearing obstacles on agility courses.
- Discipline: This is one probably of the most important components of canine care. Without discipline it is very hard to establish a trainer-dog-relationship. Discipline allows the trainer to effectively communicate with the dog and control the dog during handling. In this study the dog was trained on obedience (Figure 8a) from the age of 3 months, with basic commands like sit, down and roll-over (Marshall-Pescini *et al.*, 2008). Over the duration of the project the dog was periodically trained to perform more than 20 commands ("tricks"), as well as learning to master most of the agility course's equipment. Depending on the difficulty level the dog was able to learn a new command within a two hour period. The new command was then reinforced for a week (in-between older commands) to allow the dog to register the command in her long-term memory.
- Affection: Last but not least the dog should always be rewarded with affection after training or work. Each dog's personality is different and thus the type of affection they require may differ significantly, but affection is also the start of a trusting and loyal relationship between the trainer

(owner) and the dog (Andics *et al.*, 2014). The dog used in this study was rewarded with a game of fetch after a hard day's work.



Figure 8: Our sniffer dog engaging in a) obedience training and b) a physical exercise activity.

The training of the sniffer dog was done by the author (EE Matthew), who is also the owner. The methods used for basic obedience training of the dog were based on techniques used by Ceaser Millan also known as *The Dog Whisperer*, and dog behaviourist Tamar Geller. Milan believes that dogs should be trained using exercise, discipline and affection, in that order (Millan and Peltier, 2007). While Geller, the author of *The gentle way to train your dog* believes that dog behaviour can be explained through looking at the way wolves interact with each other. She also comments on ways to correct unwanted behaviour without using aggressive behaviour (Geller, 2008).

2.4.2. Amphibians

Twelve Giant Bullfrogs, *Pyxicephalus adspersus*, were kept to insure that fresh scent was always available to work with. The two adult frogs where rescued from residential properties in Potchefstroom and the 10 juveniles were collected as tadpoles at one of our Bullfrog sites (Ventersdorp, North-West province). Some of these amphibian were also used in experimental trials. The amphibians used for these trials were always protected by a container or other barrier to prevent injuries.

The Bullfrogs were kept in enclosures that consisted of a sandy soil substrate, with a water bath and were fed live crickets three times a week. To collect the scents from the Bullfrogs, the skins of the Bullfrogs were wiped with a cotton swab on the surface, which was then used to train the dog. Overall, the handling of the amphibians was kept to a minimum, except when taking skin swabs. Gloves were always worn when handling frogs to prevent the contamination of scents.

During fieldwork, other species were collected that were used as distractions (scents) during training (Permit number: OP4374/2915). These species included *Breviceps adspersus*, *Amieta queckettii*, *Amietophrynus maculatus* and *Xenopus leavis*. Two of these species were collected in Ndumu Game reserve (*A. maculatus* and *B. adspersus*) the other species were collected in Potchefstroom, in the North-West Province. Two animals per species were kept in captivity for the duration of the study. They were kept separately (except for metamorphs that were grouped in pairs) in glass aquariums which contained a thick layer of sandy soil, water baths, rocks and PVC tubes. The tubes were used by the amphibians as shelters or burrows. These amphibians were also fed with live crickets three times a week.

2.5. Scent detection training

The mechanism employed to train the sniffer dog is referred to as operant conditioning (Blackman, 1974). The dog was challenged with various reinforcement protocols – procedures that deliver a reinforcer to an organism according to some well-defined rule. Positive reinforcement was used during which behaviour is followed by a stimulus that is rewarding, consequently increasing the frequency of that behaviour.

The first step was to introduce the dog to the discipline of scent detection. For this, a piece of dried meat (in a container) was used as a target. Then more containers were added for the dog to search for the target (up to five freestanding containers were used). Tests were repeated several times until the dog could consistently indicate on the correct target and a specific mode of identification was established. Next, black tea (non-diluted) was used as a target scent which was then later diluted (from 100% to 0.1%) to make the dog accustomed to weaker scent signals and also increasing the dog's sensitivity. The tea used in this case was *Five Roses African blend*. The tea bag was placed in 1 litre of boiling water for 5 minutes and then left to cool down for at least 20 minutes (this was referred to as the 100% tea solution).

In addition, diluting the tea forced the dog to focus on the scent rather than the colour of the target (as a visual cue). After great success with the detection of the tea targets the tea was replaced with the scent of Bullfrogs. Some initial tests also included the use of live Bullfrogs in containers. The purpose of these tests was to further reinforce the specific scent. For all of the scent detection experiments data sheets were always used to help evaluate the detection success of the sniffer dog (APPENDIX A).

2.5.1. Plank Training (Dilutions)

After the initial training phases (dried meat and tea) the dog was taught that Bullfrog scent was the required target scent, and that she would be rewarded for indicating on this scent. A wooden plank structure was introduced to further restrict the dog's focus on visual cues (Figure 9 and 10). Two of these planks were placed end-to-end (against a wall) to create a 10-target setup for tests (APPENDIX H). For experimental purposes the holes were numbered 1 to 10 starting from the right hand side. Therefore, one run was performed by navigating the sniffer dog across numbers 1 to 10 and back from 10 to 1.

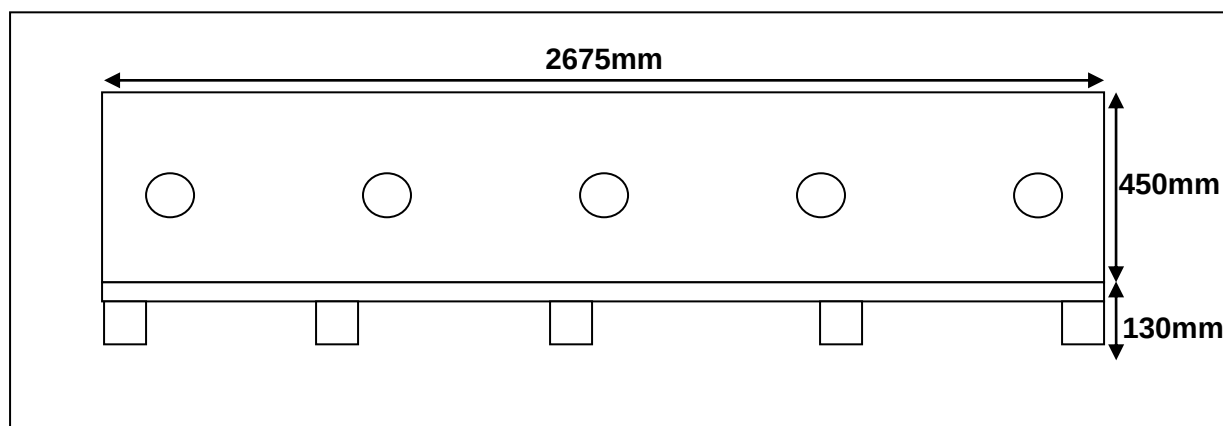


Figure 9: Wooden plank structure measurement and design. The holes (for containers) are spaced 530 mm apart, while the holes on the sides are 75 mm from the edge of the plank.

It is important that as the conditioning progresses, the challenges become more difficult in order to prevent the dog from losing interest in the task. We continued operant conditioning of the sniffer dog by employing a training plank with a series of diluted, frog scents. Dilutions were made from swabs taken from the skin of a live frog, which was then diluted in water. A 1:1 dilution was made from one such swab mixed in 1ml of water for 1 min. We trained with the following dilutions 1:1, 1:10, 1:100, 1:1 000, 1:10 000 and 1:100 000. This method allows for repetitive conditioning while varying the position (location of the positive target) and concentration of the target scent together with gradual introduction of various disturbances in non-target containers. The trainer and assistants' scent was placed on all the equipment and containers to prevent the dog from indicating on human scent. Even when moving the positive target, all of the negative targets were also touched or moved. For variation and to assure that the dog does not pick up on human scent, gloves and 70% Ethanol was used for all of the experiments conducted on the wooden plank structure, while the human scent was used as a distraction during the other experiments.

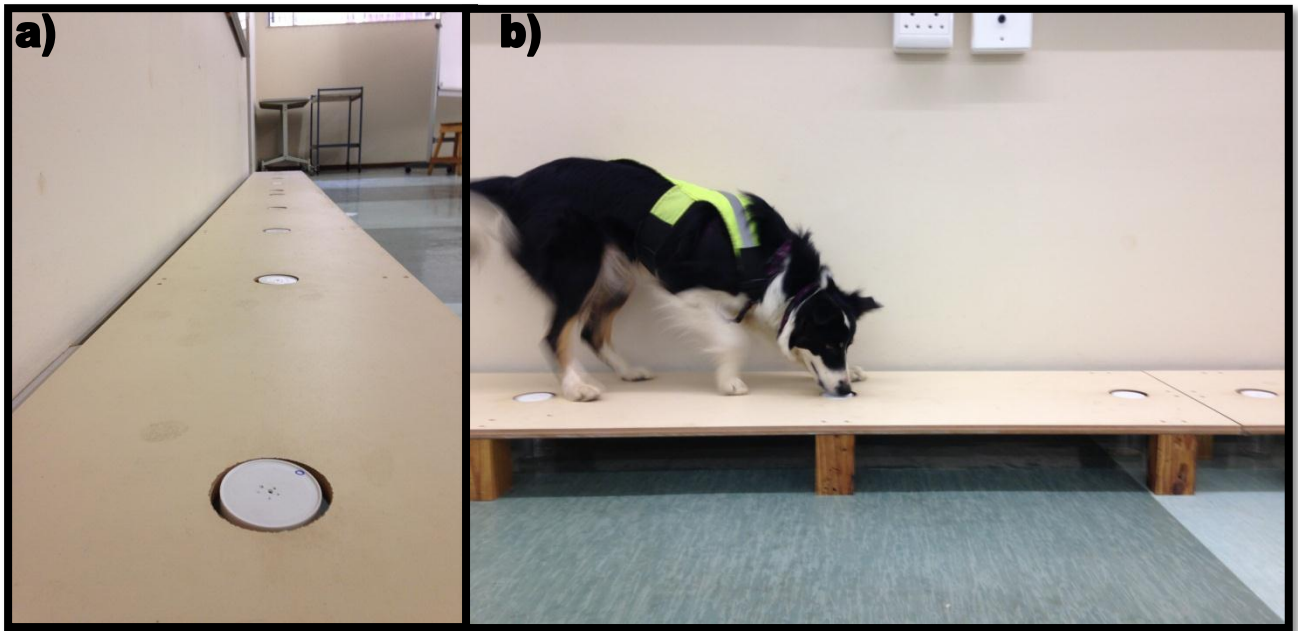


Figure 10: Wooden training planks indicating a) containers with breathable lids, and b) how the planks are used to train the dog.

2.5.2. Disturbances and outdoor training

For the training phase of this study it was extremely important to gradually add a variety of disturbances or distractions to the training tests (Gazit *et al.*, 2005). Anything that might cause the dog to lose focus or indicate on the wrong target can be seen as a disturbance. Disturbances can be either other people, animals, items like soil, other amphibian species or change in the training environment. Any other scent can also be distracting (even the scent of a reward item). To compensate for all of these variables, different types of tests were conducted under a variety of conditions to insure that our sniffer dog could still perform in the presence of a natural disturbance. Therefore, outdoor training was also performed on a regular basis. Outdoor training entails working in a semi-natural environment where the scent was randomly placed on the grass or ground (no visual cues provided). During these tests, negative targets were also placed in the same search area as the positive targets.

During most of the plank tests and outdoor training, a 1:1,000 dilution was used as it was reasoned that the scent would be "stronger" in nature (live Bullfrogs) than the weakest dilution used for the experimental setup. Since it is unclear how much of the scent reaches the surface (from buried frogs), a 1:1 000 dilution was used, because it is diluted enough to make it challenging to detect, without being too diluted.

During all of the training and test phases each test was gradually made more realistic, according to the natural habitat of the frog (including use of diluted scents and burying live frogs in a field). When the outcome of training-tests indicated that the dog reached reasonable accuracy (at least 70% accuracy), more challenging tests were administrated. The difficulty of a test was increased by further diluting the scent, adding disturbances or working at new locations. Different types of scent were also used to train the dog on (e.g. dilutions, dried skin samples and live frogs).

To assure that the dog did not pick up on human scent during outdoor tests, the handler (and/or assistant) walked inside the areas where targets were placed to distribute their scent evenly in the search area. This means that the dog could not track the handler's (and/or assistant's) movements to locate the target. During plank and outdoor training sessions, volunteers positioned the target scents, creating a double blind test. A double blind test is a search where both the dog and the handler do not know where the positive target is placed. This type of tests insured that the *Clever Hans effect* did not play a role during the conditioning of the dog.

After a certain period the food reward was replaced by a toy reward in some cases (mostly during outside training where enough space was available) as the sniffer dog preferred a toy for a reward.

2.5.3. Matching scent detection training

Matching scent detection is when the dog is provided with a sample scent before the search starts (Schoon, 1997). During plank training (with dilutions) and outdoor training, a scent was always provided at the start of the test/training. Even though the sniffer dog was mainly focussed on finding Bullfrogs (and recognizing the specific scent), sample scents were also used before each search. This was done in order to help the dog to realize that a target scent was provided and to make it easier for the dog to find other scents in future research.

The dog was also regularly trained to detect other random household items from a sample scent. These household items included: teabags, matches, toothpicks and plastic caps. Using this method improves the dog's ability to match scents.

2.5.4. Depth training

Because the focal species is a burrowing frog, it was important to do training with targets that are buried underground. Three different types of experimental setups were used for this: 1) short-term tests in artificial environments, 2) short-term tests in natural environments, and 3) long-term tests in natural environments.

Artificial environment: short-term tests

For this method small frogs were placed in breathable containers in a sandbox, around 50 cm under loose building sand (Figure 11 and APPENDIX H). At first juvenile Bullfrogs were placed in the sandbox and later a second species of burrowing frog (*Breviceps adspersus*) was placed in the box as a disturbance. When training tests proved that the dog was able to locate the target frog scent, the tests were made more complicated for the dog (e.g. training in a natural environment).



Figure11: The sandbox used for initial depth training. A ramp was placed against the sandbox to make it easier for the sniffer dog to reach the surface.

Natural environment: short-term tests

For this method shallow holes (approximately 30 cm deep) were dug in a former agricultural field, where live adult frogs were also placed in containers and covered with loose soil (Figure 12). A total of eight holes were dug in the field of which two of the holes contained bullfrogs, while the others served as negative targets (empty containers). The tests on the farm provided a lot of additional disturbances (cattle, horses and sheep). During the setup, volunteers positioned the frogs and the empty container in the holes, making it a blind search. The volunteers also walked across their own tracks to prevent human scent tracking.

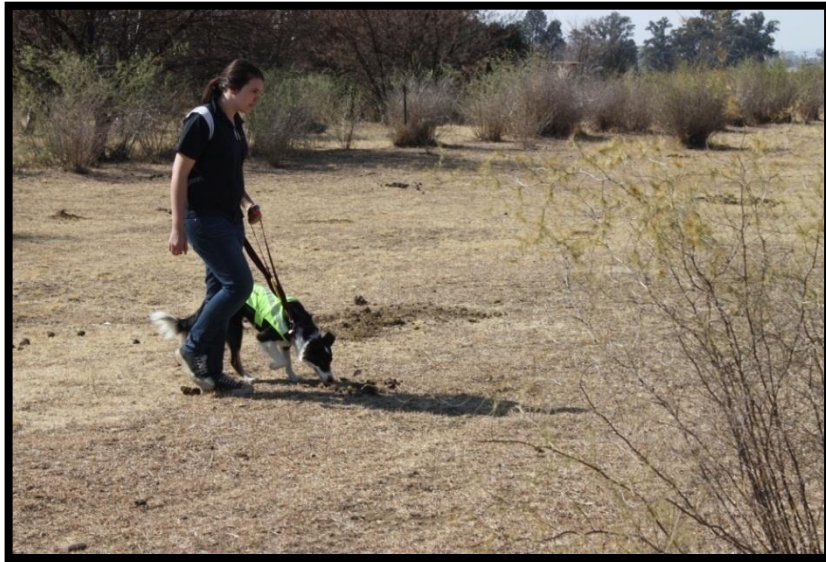


Figure 12: Short-term depth training in an abandoned field – natural environment.

Natural environment: long-term tests

Lastly, clean medical gauze (material) was placed, for 24 hours, in the same clean enclosure as the frogs. The material was then removed from the enclosure and put in the breathable containers. The containers were then buried underground at different depths (30 cm, 60 cm and 90 cm) for a month before administrating the tests with the sniffer dog (Figure 13).



Figure 13: Long term depth training in natural environment – Ekorehab training facility.

2.5.5. Dog scent tracking

A valuable aspect of sniffer dog training is scent tracking. Even though the Bullfrogs functioned as stationary targets, and their scent paths are faded or might even be gone by the time we visit a site, we still trained the dog to track scents over short distances. On most occasions a track of liquid Bullfrog scent was placed over a distance of 10-30m, and a stronger scent was placed at the end of the track.

In other instances, a cotton wipe, dipped in Bullfrog scent and tied to the end of a rope, was dragged over the entire track distance. This exercise was done to help the sniffer dog to focus and locate the strongest source of the scent. This theoretically means that at a site, the dog would be more accurate and indicate closer to the target after initially picking up on the scent. To avoid the dog tracking human scent, the handler placed scent throughout the test area.

2.6. Operant experimentation

During the experiments, a food stimulus was given to the dog when it was engaged in target behaviour (e.g. sitting, laying down or scratching at the desired target). Concurrently, negative reinforcement was employed when the dog engaged in non-target behaviour, resulting in a decrease in that behaviour. Clicker training was also used as part the reward of operant experiments (Cornu *et al.*, 2011). It is important to note that for the return run the actual position of the positive target on the plank did not change, but the position relative to the start of the plank (dog's perspective) was a mirror image to the forward run and can therefore be considered to be in a different position.

The dog's ability was tested to detect diluted frog scent (as mentioned in 1.5). Initially it was important to determine what the dog's sensitivity towards the scent was. Bullfrog scent was also used for this experiment. A variety of dilutions ranging from 1:1 to 1:100 000 was used in these experiments. As 1:100 000 is already extremely diluted, the decision was made not to continue with the experiment beyond this dilution. Each experimental test was done with 30 possible positive indications (per dilution). This accounts for a total of 180 positive targets out of 1800 possible targets.

Another test was done to determine what the best way is to preserve the scent of amphibians. Scent from the sample animal, the Giant Bullfrog, was used and to test four different ways to preserve the scent. Two 1:1 000 samples were made by diluting a skin swab in 1 litre of water. Each dilution was, kept in either a refrigerator (4°C) or a freezer (20°C). Two more skin swabs were made at the start of the experiment; both of them were stored in a refrigerator till the time of use. At

the beginning of each test, one of the swabs was used directly, while the other one was diluted in 10 ml of water (diluted swab) 10 minutes before the start of the test.

For another experiment, live amphibians were placed in containers as disturbances for the sniffer dog. The species used for this experiment, included a semi-aquatic frog (*Amieta quecketti*), a terrestrial toad (*Amietophrynus maculatus*) and an aquatic frog (*Xenopus leavis*). The same experiment was repeated with diluted scents (1:500) of each species. The aim of the experiment was to determine if the sniffer dog could remain focussed on a specific frog species in the presence of other amphibians. The preferred target during these trials remained the scent of *P. adspersus* (Giant Bullfrog). The animals were only kept in these containers for a short period of time and every trial of this experiment was repeated at least 20 times.



Figure 14: Live amphibians in breathable containers as used during sniffer dog testing.

2.7. Statistical analyses of results

The statistical analyses of the results were conducted using STATSTICA (StatSoft, 2016). Dr. Suria Ellis from the Specialist statistical consultant services of the North- West University assisted in the analyses of the randomized tests and evaluating the reasons for false and missed indications.

2.8. Locating Bullfrogs and assessing soil preference

2.8.1. Sniffer dog surveys

After successfully completing all of the tests and training, with the addition of a variety of distractions and disturbances, field searches were conducted. Searches were carried out at known Bullfrog sites in central South Africa. The site names were awarded to each site with regards to the closest town/city. Table 2 provides the names and GPS coordinates of the five Bullfrog sites where fieldwork was conducted. The location of these study sites are also mapped in Figure 15.

Table 2: Site details where fieldwork was conducted.

Site Name	Province	GPS coordinates	
		Latitude	Longitude
Bloemfontein	Free State	28°49'19.3"S	26°07'40.4"E
Diepsloot	Gauteng	25°56'23.59"S	28°01'21.88"E
Heidelberg	Gauteng	26°27'53" S	28°25'48" E
Ventersdorp	North-West	26°10'20.3" S	26°43'32.5" E
Viljoenskroon	Free State	27°12'53" S	26°58'12" E

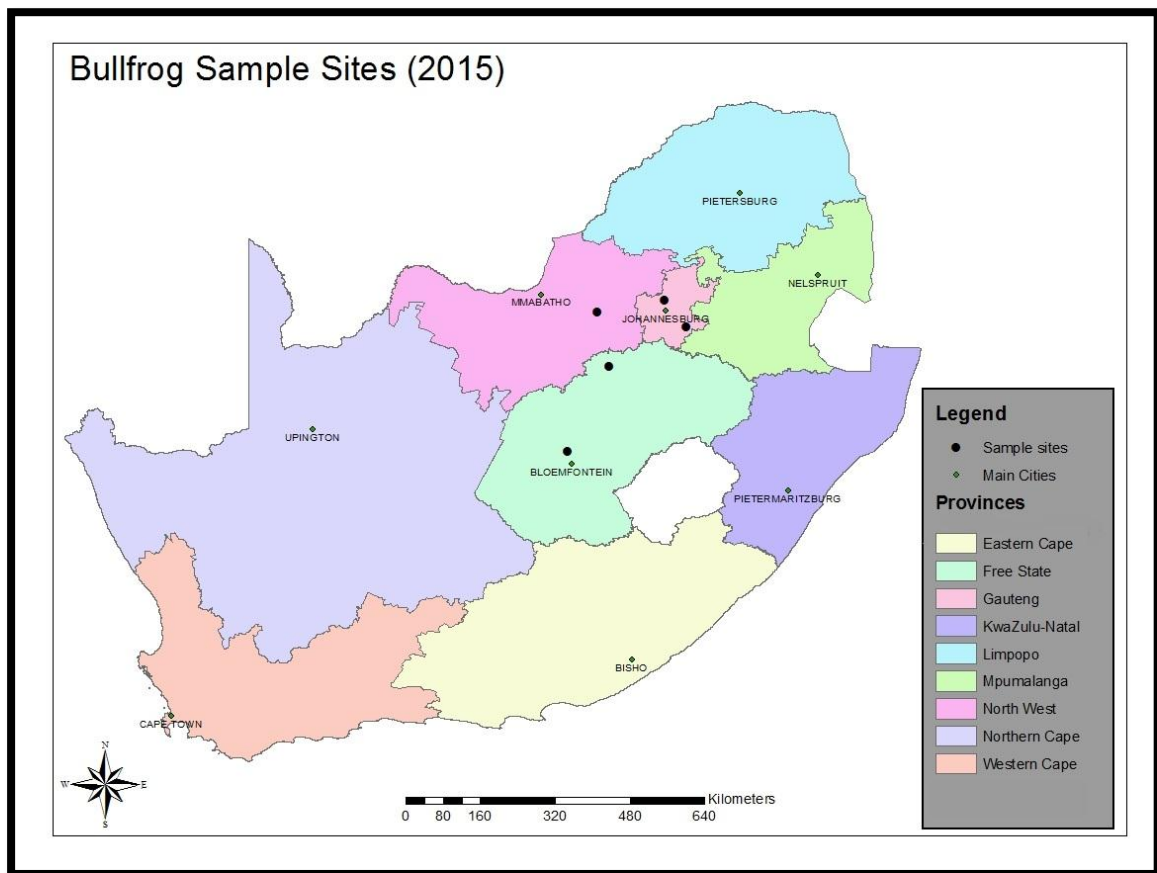


Figure 15: Map of South Africa indicating locations and distribution of bullfrog field sites.

The dog was kept on a 10 m leash to control the dog's speed and thoroughness. A sample scent was always presented to the dog, before a search, to make sure it knew what to search for. The area around a known breeding site was systematically scanned by the dog and handler. The area was scanned along transects that radiated from the dried up pan at each site. Because Bullfrogs are known to move between 200 and 1000 m away from their breeding site (Yetman and Ferguson, 2011a), a 100 m transect was scanned on four side of the pan starting at a distance of 400 m away from the pan. The transect consisted of a band of 20 meters, which was equal to the distance that the dog could move away from the handler (10 m on each side). A search period of one hour was performed at a time to maintain efficiency. The sniffer dog was provided with a 10 minute rest period in between searches, in which case water was always provided (Adams and Johnson, 1994). This process of work-rest was repeated approximately four times at each location, depending on the conditions (e.g. such as weather or rough terrain).

2.8.2. Collection and analysing soil samples

To determine if Bullfrogs have specific soil preferences, soil samples were taken at the four field sites. A hand auger (Figure 16) was used to collect soil samples on three locations, at a maximum depth of 1.2 m, on a 100m transect. When a restricting layer (such as rock) was encountered, the depths of the samples were shallower. The position of the observation and sampling points was determined by GPS coordinates taken during Bullfrog surveys..



Figure 16: Example of a hand auger used to collect soil samples
(<https://neseattle.myturn.com/library/inventory/show/4339>).

Four soil observations were made and classified (Soil Classification Working Group) according to the Taxonomic System for South Africa (Macvicar, 1991) and the information of the different soil types were noted and mapped. Samples of topsoil and subsoil were taken at four localities and the soils analyzed (Fey, 2010; Le Roux, 1999). The series, horizon and type of soil was then assessed (⁴J. Dreyer pers. comm.). In association with the information obtained from the maps point data was also collected (39 samples) at 4 of our Bullfrog sites.

2.8.3. Creating geographic maps

A Desktop study (using GIS mapping) was conducted to determine possible preferences in soil utilization by Bullfrog. Maps were created from shape files in order to provide visual information of the research sites, Bullfrog distribution and to predict other possible sites. Esri's ArcGIS (Desktop) program was used to design the maps. The land type shape files of South Africa and distribution data for *Pyxicephalus adspersus*, historical data obtained from the South African Frog Atlas (Minter

⁴ Jasper Dreyer - Soil Sciences (NWU): Jasper.Dreyer@nwu.ac.za

et al., 2004), were layered onto the maps. Annual Rainfall was also compared to distribution data for *Pyxicephalus adspersus*.

2.9. Amathole survey

In collaboration with the Endangered Wildlife Trust (EWT), we also aimed to locate another elusive species with our sniffer dog (Tarrant *et al.*, 2015b). Our focus was to locate the Amathole toad (*Vandijkophrynus amatolicus*). EWT was relied upon to provide a scent samples (skin swabs) of the target species, but they were unable to locate a specimen in time. Instead, they provided a scent sample of the closely related, Karoo toad (*Vandijkophrynus gariensis*) (Frost *et al.*, 2006). This sample scent was then used to condition the dog, during a period of two weeks. During this time plank training, dilutions and outdoor training took place. An attempt was made to find the Amathole toad in the Hogsback area (Eastern Cape) during the inactive season in 2015. The same survey methods were employed as with the Bullfrog a survey, except that transects were parallel to streams at historical Amathole toad sites (APPENDIX D) and the scent of Karoo toad was used as a sample scent for our sniffer dog.

2.10. Amphibian chytrid trials

Plank experiments were conducted to determine if sniffer dogs can be used to detect the scent of the fungal pathogen, amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). Every experiment was repeated at least 20 times. The *Bd* isolate MG09 (*Bd*GPL – global panzootic lineage from South Africa) was used in these experiments, and was incubated at 21°C on agar plates. Zoospores were harvested by flooding the plates with 5 mL sterile culture media for 30 minutes, and decanting the liquid into a clean culture flask. Zoospore density was calculated by numeration on a haemocytometer.

Using the calculated density, a dilution was made of 100,000 zoospores/mL by adding sterile media. This dilution was then used to condition the sniffer dog through plank training. After this, disturbances were introduced into the experiment (e.g. empty culture flasks and sterile culture media). Swabs were also taken from the 100,000 zoospores/mL dilution to create a *Bd* swab test for the dog. The final *Bd* trails were done using different dilutions (e.g. 100,000, 10,000 and 1,000 zoospores/mL). All of these preliminary tests were performed under lab conditions and conducted to investigate if it might (in the future) be feasible for a sniffer dog to detect a disease on infected frogs.

2.11. Educating and creating awareness

A vital component of this project was informing and educating the public on the use of this novel technology and to promote amphibian conservation. Both mainstream- and social media along with scientific platforms were used to convey the applicability of this project to conservation ecology (APPENDIX H). Public interests were also tracked during this study by taking note of all media coverage this project generated, creating a blogger Facebook page and monitoring the growth in interest of this page (APPENDIX C). We also created awareness for amphibian conservation by presenting talks and demonstrations for students from other schools and universities (Figure 17).



Figure 17: Jessie the sniffer dog with some of the students after a demonstration for Jim Fouché High School, Bloemfontein.

3. Results

3.1. Olfactory tests with diluted scent

Initial conditioning of the dog was performed using meat treats and tea as target scents. After the dog achieved 100% accuracy with these training tests, olfactory tests were done to test the dog's ability to detect frog scent. The scents for this experiment were created in dilution factors of ten. The dog was able to detect the frog scent that was diluted up to 1:100,000. Remarkably, the dog achieved 100% accuracy for all of the dilutions, except for the strongest scent (1:1 dilution), for which a 93.33% accuracy was achieved (Figure 18).

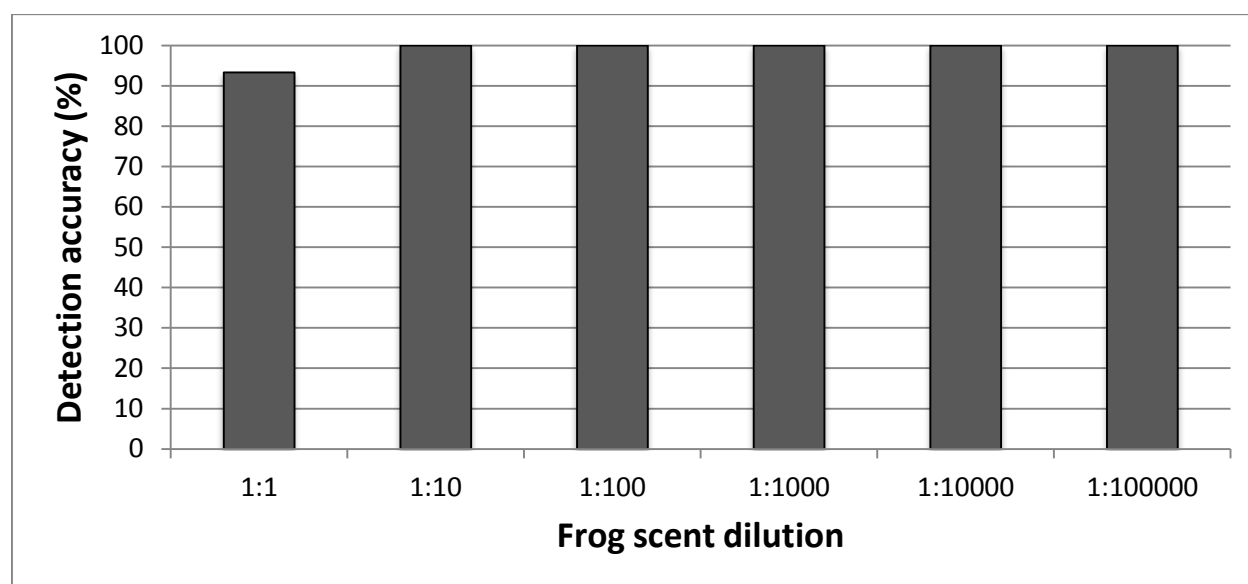


Figure 18: Sniffer dog detection accuracy for a variety of diluted frog scent.

3.2. Effect of disturbances on detection accuracy

3.2.1. Frog scent disturbance

Eight frogs from four species were used to test whether the scent of non-target species influenced the accuracy of detection by the sniffer dog. Two of the frog species were used as disturbances (*Amietia quecketti* and *Xenopus laevis*). The specimens used from these two species had approximately the same body mass as our target species specimen, *Pixycephalus adspersus*, but the third specimen *Amietophryus maculates* (toad species), had a greater body mass (Table 3). These same individuals were used in both the experiment where live frogs were used as targets, as well as the experiment for which frog scent was tested as a disturbance.

Table 3: Body measurements of *Pyxicephalus adspersus* and other frogs used as disturbances during sniffer dog disturbance testing.

Frog species	Snout-vent length (mm)	Body mass (g)
<i>Pyxicephalus adspersus</i>	26	1.6
<i>Pyxicephalus adspersus</i>	28	2.8
<i>Amietia queckettii</i>	25	1.6
<i>Amietia queckettii</i>	24	1.3
<i>Amietophrynus maculatus</i>	45	7.5
<i>Amietophrynus maculatus</i>	51	9.7
<i>Xenopus leavis</i>	26	1.8
<i>Xenopus leavis</i>	22	1.1

For the dilution tests 200 targets were used during the setup for each type of scent (species) and *P. adspersus* samples were placed in 20 of the targets. The same procedure was used for the live frog tests. This means that this experiment consisted out of 1000 possible targets for the live frog tests and a 1000 for the dilution tests.

Ten different tests with frog scent were conducted, for which the scent was used as a disturbance (five using live animals and five using a 1:500 diluted scent). The sniffer dog was able to detect the juvenile *P. adspersus* 98% of the time when other live frogs were used as disturbances. The dog only indicated falsely on one other species (*Amietia queckettii*) 1% of the time, and indicated on the target containing water 2 % of the time (APPENDIX G). Because all of the scents (except *P. adspersus*) are seen as disturbances for these experiments only 2% missed indications and 3% false indications occurred during this live amphibian tests (Table 4).

The results from the diluted scent experiment was very similar to the live amphibian experiment, with the sniffer dog only indicating 1% falsely on the scent of one other species, this time *Xenopus leavis* (Table 4). The dog also achieved 100% detection accuracy on the *P. adspersus* scent during this experiment. No other false indications were made on any other type of target and therefore no missed indications were made for any of frog dilution disturbance tests.

Table 4: The indications made on each type of target during the live amphibian and scent tests.

Live frog species	Test 1	Test 2	Test 3	Test 4	Test 5	Average%
<i>Pyxicephalus adspersus</i>	20	18	20	20	20	98
<i>Amietia quecketti</i>	0	1	0	0	0	1
<i>Amietophrynus maculatus</i>	0	0	0	0	0	0
<i>Xenopus leavis</i>	0	0	0	0	0	0
Negative (H ₂ O)	0	1	0	0	1	2
Diluted frog scent detection	Test 1	Test 2	Test 3	Test 4	Test 5	Average%
<i>Pyxicephalus adspersus</i>	20	20	20	20	20	100
<i>Amietia quecketti</i>	0	0	0	0	0	0
<i>Amietophrynus maculatus</i>	0	0	0	0	0	0
<i>Xenopus leavis</i>	0	0	0	1	0	1
Negative (H ₂ O)	0	0	0	0	0	0

3.2.2. Human scents disturbance

Various human scents were implemented as disturbances during the live *P. adspersus* and diluted scent experiments. For the dilution tests 200 targets were used during the setup for each type of human scent experiment and *P. adspersus* samples were placed in 20 of the targets. The same procedure was used for the live frog tests. This means that this experiment consisted out of 1000 possible targets from the live frog tests and a 1000 for the dilution tests.

A100% detection accuracy was achieved in the presence of all types of human scent disturbances, except for the male scent disturbance during the live amphibian test, when detection accuracy was 90% (Figure 19). The sniffer dog, however, still managed to reach 95% average detection accuracy during all of the experiments where male human scent disturbances were used. A100% detection accuracy was also achieved during the control test when gloves were worn and 70% alcohol was used as disinfectant during the setup of the experiment (no human scent present). The experiments where no human scents were present were referred to as the control tests.

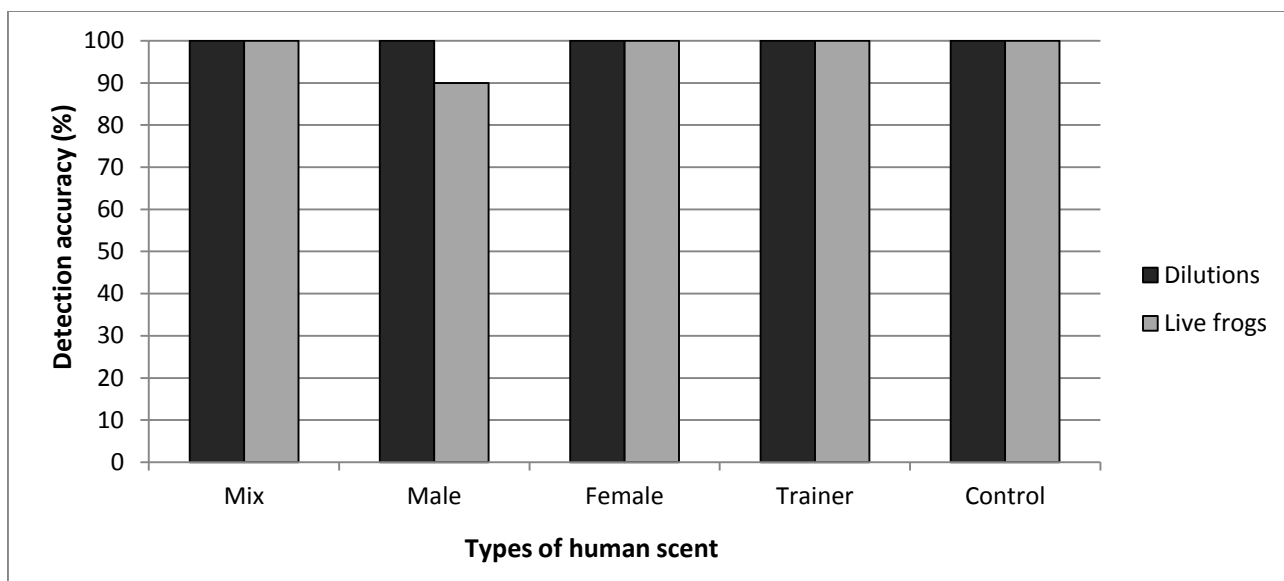


Figure 19: The average percentage accuracy achieved for each method (when all of the missed indications are incorporated).

3.3. Ability to track frog scent and detect buried frog scent

Multiple scent tracking trails were performed to enhance the dog's ability to detect scents in the field. Recording tracking performance was not easy, due to a lack of quantifiable data. However, it is possible to interpret the results in a descriptive manner. The results from six of these tracks are summarized in Table 5. Variables that were noted include the track length and shape, weather conditions, and location and age of the track. Age of the track (in minutes) refers to the elapsed time from when the track was laid and when the dog started to track the scent. The duration from the moment the scent was placed until the search commenced varied between 20 and 40 min and the track length ranged between 10 and 17 m. Three basic shapes were used for the layout of all of the tracks. During the entire above mentioned trails, the dog was always able to detect the target scent.

The dog was also able to locate a live buried *P. adspersus* both in the presence and the absence of disturbances (e.g. water filled and empty containers, other frog species, etc.). Initially the target was buried just below the surface (5 cm) and the depth adjusted in 5 cm increments to a depth of 30 cm. The detection time for the 30 cm targets varied considerably, but the targets were successfully located in every instance (Table 6). A video recording of a rapid detection in the sandbox is available as electronic Appendix.

Table 5: Detailed results of all recorded scent tracking trials. NWU campus refers to a variety of locations on the Potchefstroom Campus of the North-West University.

Location	Age of track	Distance	Basic shape	Surface	Weather
NWU campus	20 min	15m	Straight line	Grass	Cold and sunny
NWU Campus	40 min	14m	1 turn (90°)	Grass	Sunny
NWU Campus	35min	17m	1 turn (L)	Sandy soil	Sunny
Botanical garden	20 min	13m	2 turns (Z-shaped)	Short grass	Windy
Botanical garden	25 min	10m	1 turn (L)	Tall grass	Windy
NWU Campus	40 min	17m	1 turn (L)	Short grass	Cold and sunny

Table 6: The details about disturbances as well as results of all recorded sandbox trials.

Date	Disturbance	Time
5-Jul-14	H ₂ O filled container	120 seconds
14-Jul-14	empty container	34 seconds
15-Jul-14	empty container	30 seconds
15-Jul-14	empty container	60 minute
15-Jul-14	live frog in container	12 seconds

Apart from all of the recorded scent tracking and the sandbox trails, other detection tests were also conducted. The goal of these tests was to keep the dog sharp and accurate in-between fieldwork and experiments. Although these tests were not recorded, it included targets that were buried at 30, 60 and 90cm under the ground. During the entire above mentioned trails, the dog was always able to detect the target scent.

3.4. Ways to preserve frog scent

During the normal evaluation of the scent preservation experiments no significant changes were recognized (APPENDIX G). The dog was still able to indicate on more than 80% of all the targets presented after 6 months, but to gain more specific results for this experiment each run was split into two scoring opportunities (doubling our dataset). MI (missed indications) were also analysed to identify smaller changes between different tests. Results indicated that the least missed indications were made with the diluted swab method, with only 20.1% of all MI made during this type of test. The most MI were made during the swab test (29.1%), while both 1:1,000 dilutions (4°C and -20°C) had almost exactly the same percentages of mistakes, namely 24.87% and 25.93%, respectively.

Detection accuracy decreased over time for each preservation method (Figure 20). The best results were consistently attained from the diluted swab method, whereby a swab was stored for a specific time period and diluted in water before the test. The efficacy (using the split data) for the diluted swab was at 85% detection accuracy, after one month of preservation, and dropped to 55% after six months of preservation. Although 70% detection accuracy was achieved from preserving a swab at 4°C for one month, accuracy drastically dropped to 40% after five months of preservation.

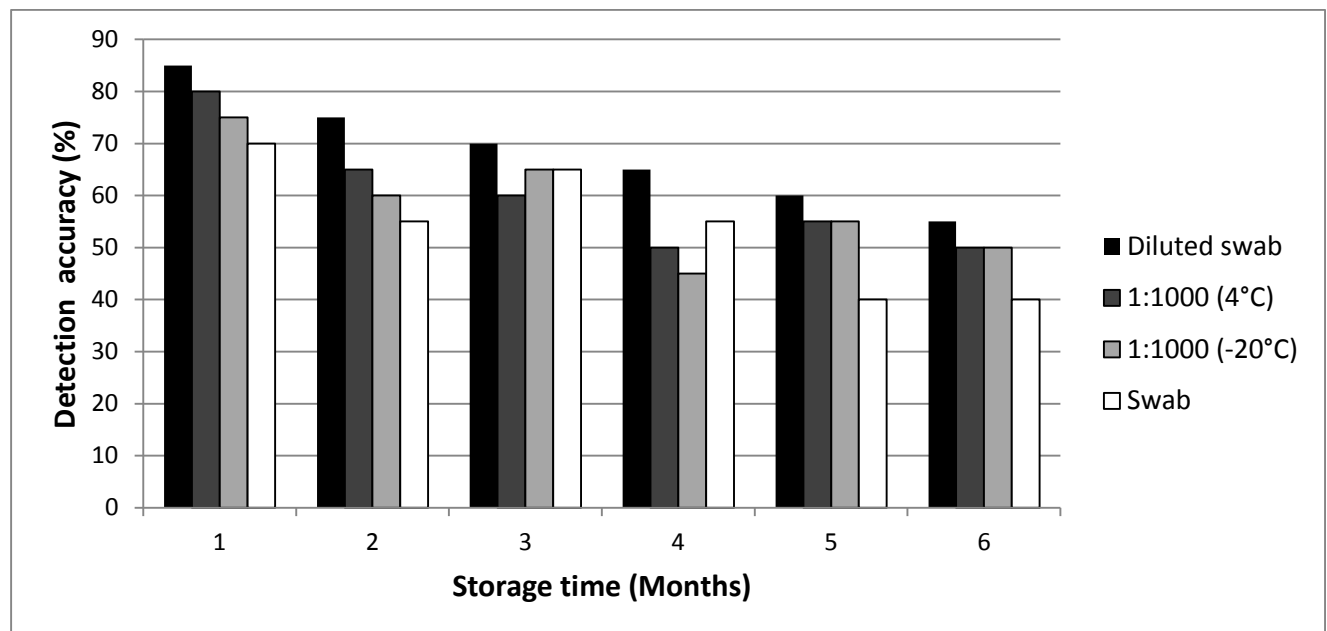


Figure 20: Sniffer dog detection accuracy of frog scent that has been preserved using different methods.

3.5. Comparison of detection accuracy for different types of tests

Figure 21 represents the total detection accuracy in relation to missed indications, for all types of plank scent experiments (where the Bullfrog scent was the target) for the duration of this study. The best results were achieved during the amphibian-scent dilution-disturbance tests, where the dog was able to indicate on the target, 100% of the time. The results also show that it was generally more difficult for the sniffer dog to positively indicate on live frogs and dried skin, achieving only 80% and 78% accuracy respectively. Except for these two experiments, all other experiments achieved accuracies higher than 92%.

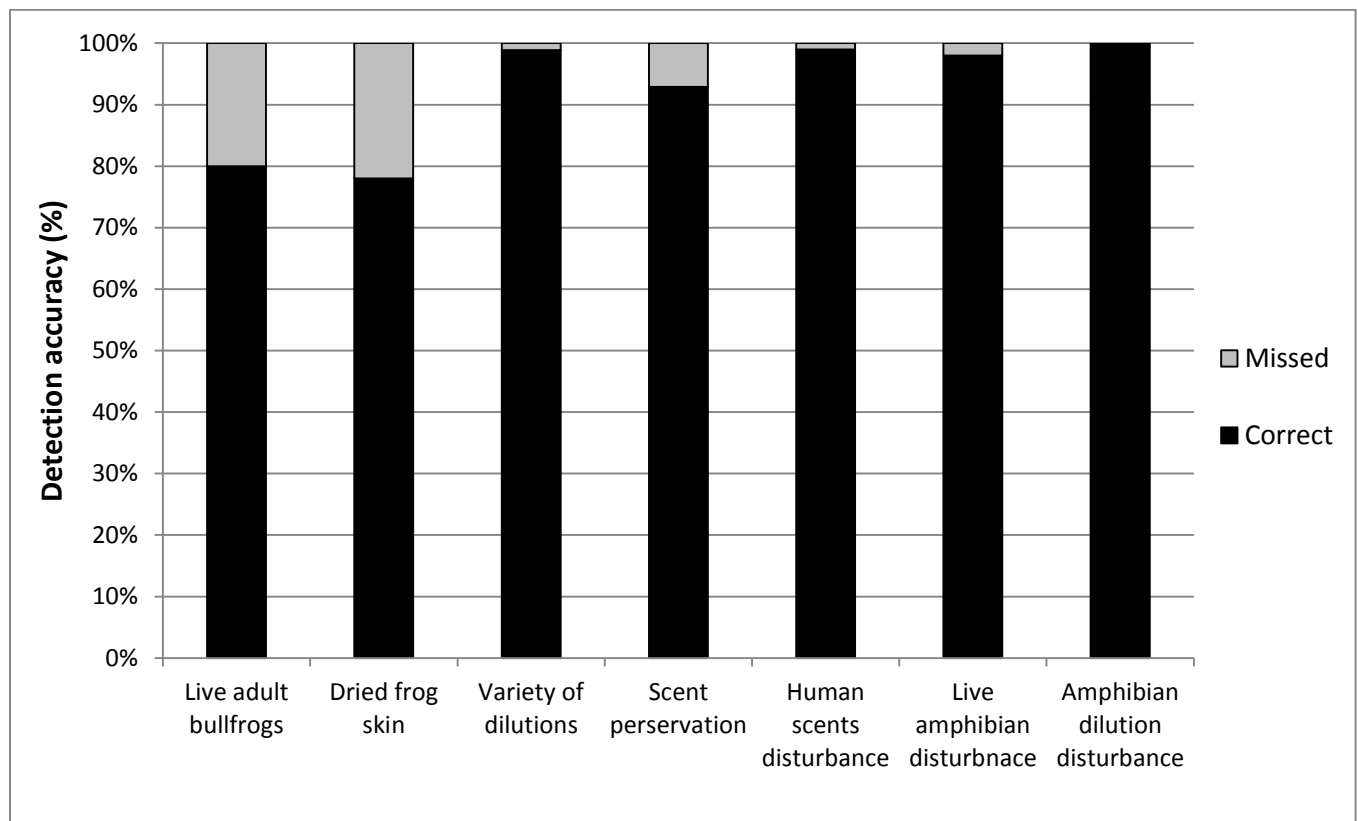


Figure 21: The total detection accuracy made for each type of bullfrog test for the entire course of the project.

3.6. Role of training plank on outcome of detection accuracy

In order to establish the effectiveness of plank training as experimental tool, it was necessary to determine whether the relative position of the target and the search direction across the plank influenced the outcome of the search pattern of the sniffer dog. For this reason, all of the single target plank experiments were analysed across the duration of the project. Emphasis was placed

on all of the missed indications (the positive target missed) and false indications (when the dog incorrectly indicated on something other than the positive target) to determine to which degree the plank had an effect on the results during target runs.

3.6.1. Outcome of false indications

Analysis was done on the false indications (FI), relative to the position of the positive target. Although the contribution of FI was examined separately for each experiment, the goal was to test any general patterns in FI that may be attributed to plank training methodology. The results indicated that all of the FI were on targets that fall within one of three categories: 1) adjacent to the positive target; 2) the position of previous positive target (preceding run); and 3) disturbances intentionally placed to serve as a distraction. The combined results of FI for all experiments were grouped for this analysis as shown in Table 7. The total number of FI for all the single-target plank experiments was 59 from a possible 10,260 chances to indicate on a negative target. This accounts for a mere 0.595% of all possible FI. Of these FI, almost two-thirds (57.62%) belonged to the *adjacent to positive target* category. Every type of experiment had at least one FI, although the majority of FI were made during the Dilution Range test (1.2%). The FI for all other tests were extremely low ranging between 0.11 and 0.33%.

Table 7: Probable causes of false indications from all of the experiments combined.

False indications (FI)	Experiments					
	Bd tests	Amphibian scent dilutions	Live amphibians	Scent preservation	Dilution range tests	Total
Max possible no. FI	900	900	900	4320	3240	10260
Adjacent to positive target (%)	0 (0)	0 (0)	1 (0.1)	7 (0.16)	26 (0.8)	34
On position of previous target (%)	0 (0)	0 (0)	2 (0.2)	4 (0.09)	13 (0.4)	19
Target with disturbance (%)	1 (0.11)	1 (0.11)	1 (0.1)	0 (0)	0 (0)	3
Unknown reason (%)	0 (0)	0 (0)	0 (0)	3 (0.07)	2 (0.06)	5
Total no. FI (%)	1 (0.11)	1 (0.11)	4 (0.44)	14 (0.32)	41 (1.2)	61(0.595)

3.6.2. Outcome of missed indications

Missed indications (MI) occurred during both the forward and return directional runs on the plank. A sample experiment was used to evaluate the MI (APPENDIX G). The preservation experiment (mentioned under 3.4) was used as the sample experiment. It was the largest experiment conducted during this project and took place over a period of 6 months with 4320 targets (480 positive targets). It is important to note that for the return runs, the actual position of the positive target on the plank did not change, but the position relative to the start of the plank (dog's perspective) did, in fact, change. It was actually a mirror image to the forward-run, and can therefore be considered to be in a different position, especially since a short recovery time (five minutes) was allowed between the two directional within a run.

The sniffer dog was evaluated twice within a run (but only scored once per run) which provides the dog with two opportunities to indicate, but also gives data and reasons for each miss. The majority (63.8%) of these MI occurred during the first directional movement on the plank, but the sniffer dog continued to indicate on the positive target 38.5% of the time on the return movement following a MI forward run. Evidently, the dog was able to indicate 95.3% of the time correctly within a run (either during forward or return movement).

Results (in Table 8.1) indicated that the most MI were made during the last month (21%) compared to the smallest amount of MI (9.5%) that were made by the dog in the first month of the experiment.

The direction of the movement of the different runs were combined with the position of the targets (1-10) to determine if the position of the target on the plank, from the dog's perspective, plays a role in the prevalence of missed indications. Table 8.2 shows results from both directions, with consideration of the dog's position. According to Table 8.2, 65% of the total MI were made by the dog on the positive targets that were placed in the first four containers from the starting position.

Table 8.1: The percentage missed indications (MI) within our sample experiment

Category	Frequency table: MI per month			
	Count	Cumulative Count	Percent %	Cumulative percent %
1	18	18	9.52	9.52
2	29	47	15.34	24.87
3	28	75	14.81	39.68
4	37	112	19.58	59.26
5	37	149	19.58	78.84
6	40	189	21.16	100
Missing	0	189	0.0	100.0

Table 8.2: The percentage missed indications (MI) in relation to the dog's perspective

Category	Frequency table: MI per hole			
	Count	Cumulative Count	Percent %	Cumulative percent %
1	3	3	1.59	1.59
2	54	57	28.57	30.16
3	30	87	15.87	46.03
4	36	123	19.01	65.08
5	22	145	11.64	76.72
6	18	163	9.52	86.24
7	14	177	7.41	93.65
8	4	181	2.12	95.77
9	7	188	3.70	99.47
10	1	189	0.53	100
Missing	0	189	0.00	100

3.7. *Pyxicephalus adspersus* habitat utilisation

The substrate in which *P.adspersus* (and other species) burrow may be a determinant in their selection of habitat, localities and distribution. The ability of the sniffer dog to locate Bullfrogs in their natural environment was tested and the dog was also used to confirm if the frogs occur in expected soil patterns as predicted with the use of spatial data.

3.7.1. Locating Bullfrogs with a sniffer dog

During the Bullfrog's active season, four temporary pans were surveyed on a farm near Ventersdorp. While searching the pans with the sniffer dog (on a 10 m leash), three male Bullfrogs were found, one individual at three of the sites, each. All of the male Bullfrogs were in the water, guarding several schools of tadpoles. The sniffer dog was able to track one of the male's movements across shallow water. The other Bullfrogs were hiding among vegetation at two of these sites. The searches for this expedition took over an hour per site and the dog rested during transport between sites.

During the inactive season (roughly six months post breeding season), when Bullfrogs hibernate underground, the field sites were surveyed at Bloemfontein, Viljoenskroon, Diepsloot and Heidelberg. A 100m transect was surveyed on four sides of each temporary pan, starting at a distance of 400m away from the pan. The ground was excavated at a number of locations where the sniffer dog indicated. The shape of a typical hole resembled a cylinder, 1 m deep with a radius of 0.5 m. Despite the efforts, no animals were found at either the Bloemfontein, Viljoenskroon or Heidelberg sites. Difficulty in digging up Bullfrogs was exacerbated by the fact that these frogs are known to dig down, up to two meters perpendicular to the surface, depending on soil conditions. They can also move horizontally or diagonally underground in an effort to find a suitable hibernacula.

The fourth site near Diepsloot, Gauteng was the location of a former 5-year Bullfrog monitoring study (Yetman and Ferguson, 2011a; Yetman and Ferguson, 2011b). An advantage of this site was that the exact localities of individual Bullfrog hibernacula had been previously recorded. According to Yetman and Ferguson (2011a), most of the Bullfrogs were very loyal to their burrow, which means that the geo-reference data from the previous study can be used to direct the searches in the area. The sniffer dog indicated at three of a possible five locations corresponding with the GPS-coordinates from the former study. It was confirmed that two of the locations where the sniffer dog indicated, were in the vicinity of burrows that were used for five years by the most loyal frogs, one

female and one male (⁵C. Yetman pers. comm.). Unfortunately our excavation method failed to locate individuals at the Diepsloot site.

The last site that was surveyed, was close to the Diepsloot sites, about 1 km from the former monitoring site and consisted of large sandy enclosures where Bullfrogs could burrow. The sniffer dog indicated in a Bullfrog holding area where, when dug out by hand, a tunnel with loose soil could be felt. Pursuing the “tunnel” with loose soil, a hibernating, male Bullfrog was discovered (Figure 22)



Figure 22: The first glimpse of the male Bullfrog in excavated hibernacula.

The hibernaculum was carefully excavated until it was possible remove the frog from its burrow. The Bullfrog was an adult, male that weighted 680 grams (Figure 23a). The Bullfrog was approximately 15.5 cm in length (snout-to-vent), buried about 15 cm underground and had a thin layer of cocoon (skin layer) on his ventral surface (abdomen area).

⁵ Caroline Yetman - Natural Scientific Services: caroline@nss-sa.co.za



Figure 23: Photos of the male Giant Bullfrog (a) removed from the ground, at location where the sniffer dog indicated (b) placed back into the borrow after measurements were taken.

After all the necessary measurements were taken and all additional information was recorded, the Bullfrog was carefully returned to his burrow (Figure 23b) and covered with soil and other organic materials.

3.7.2. Terminology associated with Land Type data

Soil classification and Land Type data analysis uses certain terms that have a specific meaning in the context geology and soil science. Realizing that this study contains components that differ slightly from the more common environmental research projects, an outline of the common terms used for identification and classification of soils are given Table 9.

Table 9: Geological terminology used during soil classification(Van der Watt and Van Rooyen, 1990).

Land Type	A class of land with specified characteristics. In South Africa it has been used as a map unit denoting land, 1:250 000 scale, over which there is a marked uniformity of climate, terrain form and soil pattern.
Broad soil patterns	Detailed Land Types, grouped according to similar morphological attributes.
Condensed soil patterns	Less detailed Land Types, grouped together by means of overlying primary similar morphological attributes for the purpose of this study.
Soil Form	Specific name provided to a soil type by South-African soil classification
Horizon	A layer of soil or soil material approximately parallel to the land surface and differing from adjacent genetically related layers in physical, chemical and biological properties or characteristics.
Terrain morphology	Position within a landscape with regard to the surrounding physical features

	(Figure 27).Area of the land surface with homogenous form and slope.
Mottles	Soil with variegated colour patterns. "Background colour" is referred to as the matrix, while the spots and blotches of colour are mottles.
Reduction	The reaction of hydrogen with another substance in the soil.
Apedal	Soil particles are without structure. Well-formed structure cannot be detected.
Plintic	In a soil, a material consisting of a mixture of clay and quartz with other diluents. Repeated wetting and drying changes plinthite to ironstone hardpan or irregular aggregates.
Catena	These soils occur under similar macroclimatic conditions, but have different characteristics due only to variation in topography and drainage.

3.7.3. Relationship of historic Bullfrog spatial data with rainfall and land type

The distributional range of Bullfrogs is mostly confined to central South Africa and the western interior (Figure 24). Most of the Bullfrog distribution overlaps firstly with the medium annual rainfall belt (approximately 840 mm), and secondly with the low annual rainfall belt (approximately 100 mm).

The distribution map for Bullfrogs indicated that the species' historic locations are associated with 25 out of a possible 28 Land Types (Figure 25). Only 2.28% of the area provided us with insufficient data. This means that Land Type data was unavailable for specific locations as indicated by the grey areas on the maps. However, Bullfrogs are not distributed evenly among land types. The highest percentage of Bullfrog locations were associated with the Ae type of soil (13.51 %), while less than 1% of Bullfrog locations was found on Ac, Ad, Ha, Hb and Ic types of soil (Table 10).

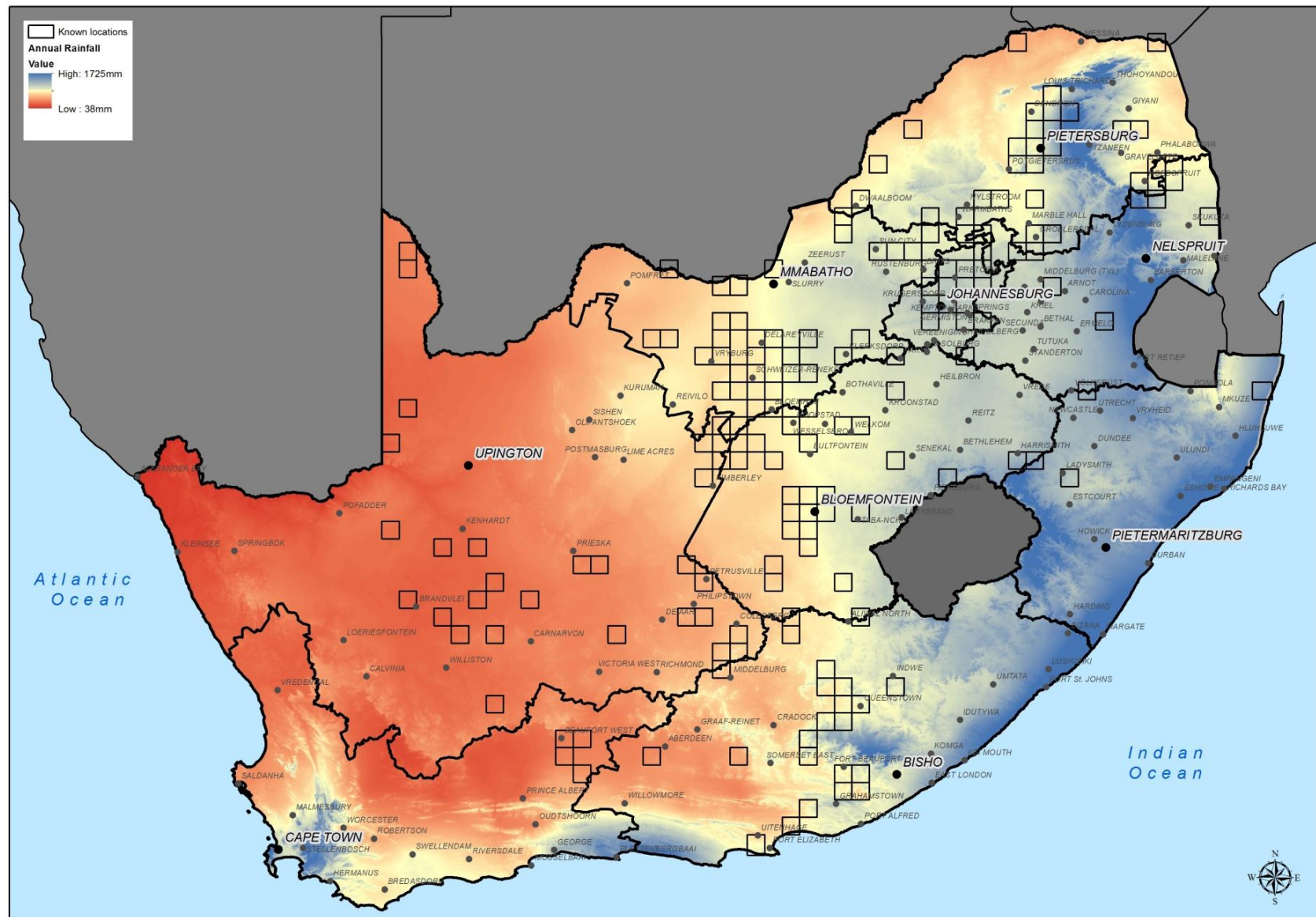


Figure 24: Rainfall map of South Africa, indicating the distribution of Bullfrogs. Rainfall data obtained from the South-African weather station and Bullfrog distribution data obtained from the South African frog atlas (Minter *et al.*, 2004).

Table 10: Broad soil pattern utilization of Bullfrogs according to their distribution in South Africa.
Land type information was obtained from Land Type Survey Staff (1972-2002).

Summary broad soil patterns according to land type data	Area of occupancy (m ²)	Percentage utilized by Bullfrogs
Ab - Red, dystrophic and/or mesotrophic	167077.57	1.46
Ac - Red and yellow dystrophic and/or mesotrophic	67273.02	0.59
Ad - Yellow, dystrophic and/or mesotrophic	5624.15	0.05
Ae - Red, high base status > 300 mm deep (no dunes)	1580033.76	13.83
Af - Red, high base status > 300 mm deep (with dunes)	214611.54	1.88
Ag - Red, high base status < 300 mm deep	474321.05	4.15
Ah - Red and yellow, high base status, usually < 15% clay	845794.33	7.40
Ai - Yellow, high base status, usually < 15% clay	322081.02	2.82
Ba - Dystrophic and/or mesotrophic; red soils widespread	499098.52	4.37
Bb - Dystrophic and/or mesotrophic; red soils not widespread	522676.60	4.57
Bc - Eutrophic; red soils widespread	447674.99	3.92
Bd - Eutrophic; red soils not widespread	732235.82	6.41
Ca - Undifferentiated	368399.13	3.22
Da - Red B horizons	689486.42	6.03
Db - B horizons not red	426327.39	3.73
Dc - In addition, one or more of: vertic, melanic, red structured diagnostic horizon	290481.86	2.54
Ea - Undifferentiated	472440.65	4.13
Fa - Lime rare or absent in the entire landscape	438539.14	3.84
Fb - Lime rare or absent in upland soils but generally Present in low-lying soil	1018804.60	8.92
Fc - Lime generally present in the entire landscape	990322.67	8.67
Ha - Regic sands dominant	56261.97	0.49
Hb - Regic sands and other soils	60689.71	0.53

la - Undifferentiated deep deposits	165031.28	1.44
lb - Rock areas with miscellaneous soils	556276.37	4.87
lc - Rock with little or no soil	16007.24	0.14
Total	11427570.78	100.00
Insufficient data	266271.53	2.33

The first letter of the soil pattern codes are representative of a specific group of patterns with the same attributes. Therefore, the broad soil patterns were combined into more condensed soil patterns and which made it clear that Bullfrogs are mostly associated (over 80%) with four of the nine land types, namely A, B, D and F (Table 11).

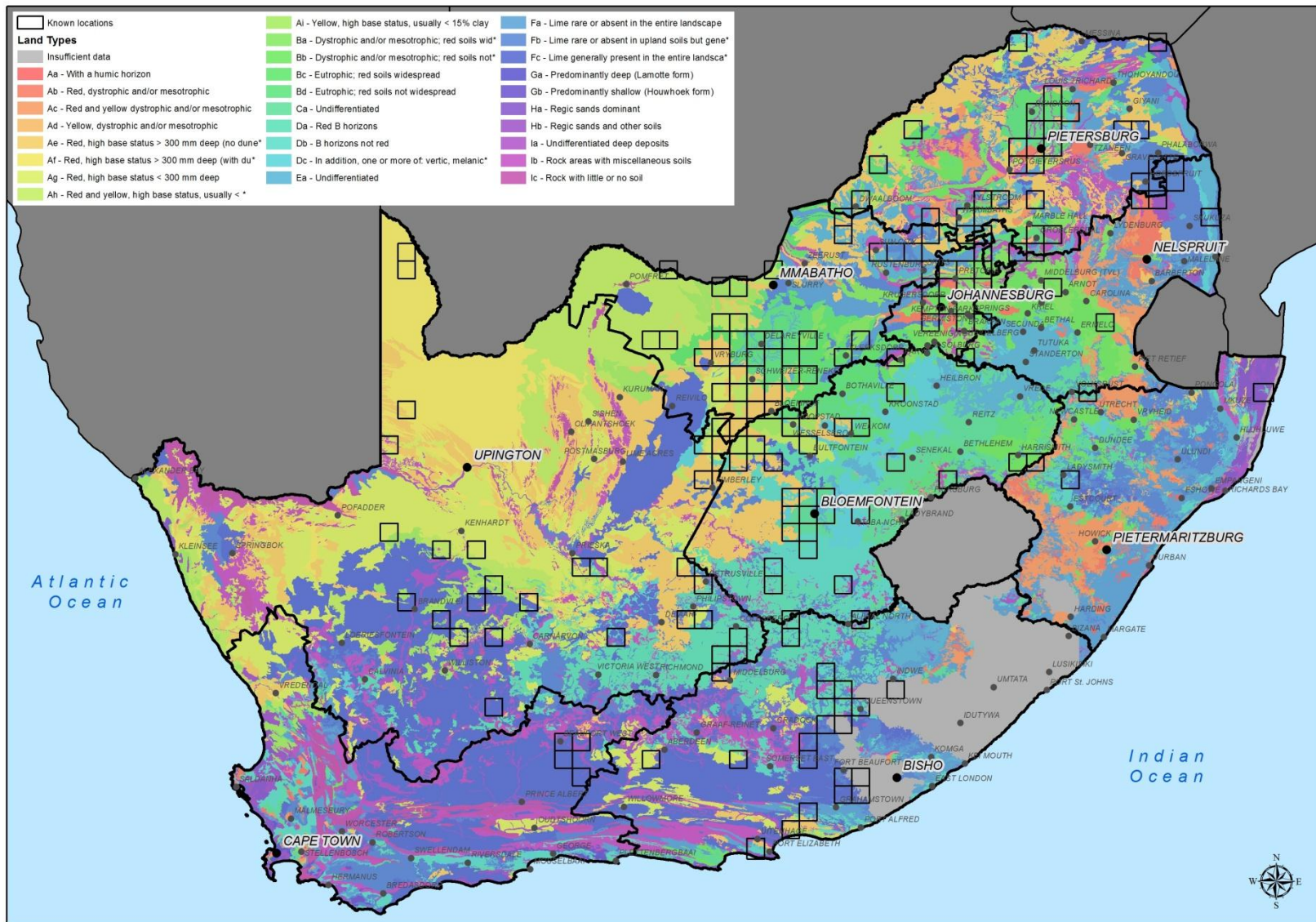


Figure 25: Detailed land type map of South Africa indicating the distribution of Bullfrogs. Land type data obtained from Land Type Survey Staff (1972-2002) and Bullfrog distribution data obtained from the South African frog atlas (Minter *et al.*, 2004).

Soil pattern G (soils with diagnostic ferrihumunic horizon) was the only pattern on which Bullfrogs did not occur, while the remaining patterns had only some association with Bullfrogs.

Soil pattern A refers to red-yellow soil that has no defined structure and is seen as dry soils. B and C both refer to Plintic Catena which refers to a soil type that is periodically wet and dry (Fey, 2010). Soil pattern D is associated with strong structured B Horizon soils, while soil patterns E can be described as a strong structured A (and B) soil with high percentage of clay. F is shallow soils with hard rocks and G is an A-Horizon soil with a lot of organic material (usually found under colder conditions).

Soil pattern H is associated with young sands that are transported by wind (e.g. dunes, desserts and coasts). Miscellaneous (I) soil patterns are defined as all other types of soils that do not qualify for any other of the previously mentioned soil patterns. Condensed soil patterns B and C can also be combined as both refer to Plintic Catena soil morphology with minor differences. This would make Plintic Catena (C+B) the second most utilized type of soil (Table11).

Table 11: The distribution of Bullfrogs by land types condensed to main soil patterns.

Condensed soil patterns	Short description	Percentage %
A	Red-yellow Apedal, Freely drained soil	31.44
B	Plintic Catena: Upland duplex and Malagaltic soil rare	18.83
C	Plintic Catena: Upland duplex and common	3.15
D	Prismacutanic and/or Pedocutanic diagnostic horizon dominant	12.03
E	One or more of: Vertic, Melanic, Red structured diagnostic horizon	4.04
F	Glenrose and/or Mispan forms (other soils may occur)	20.93
G	Soils with diagnostic Ferrihumunic horizon	0.0
H	Grey Regic sands	1.0
I	Missellaneous land classes	6.3
	Total	100

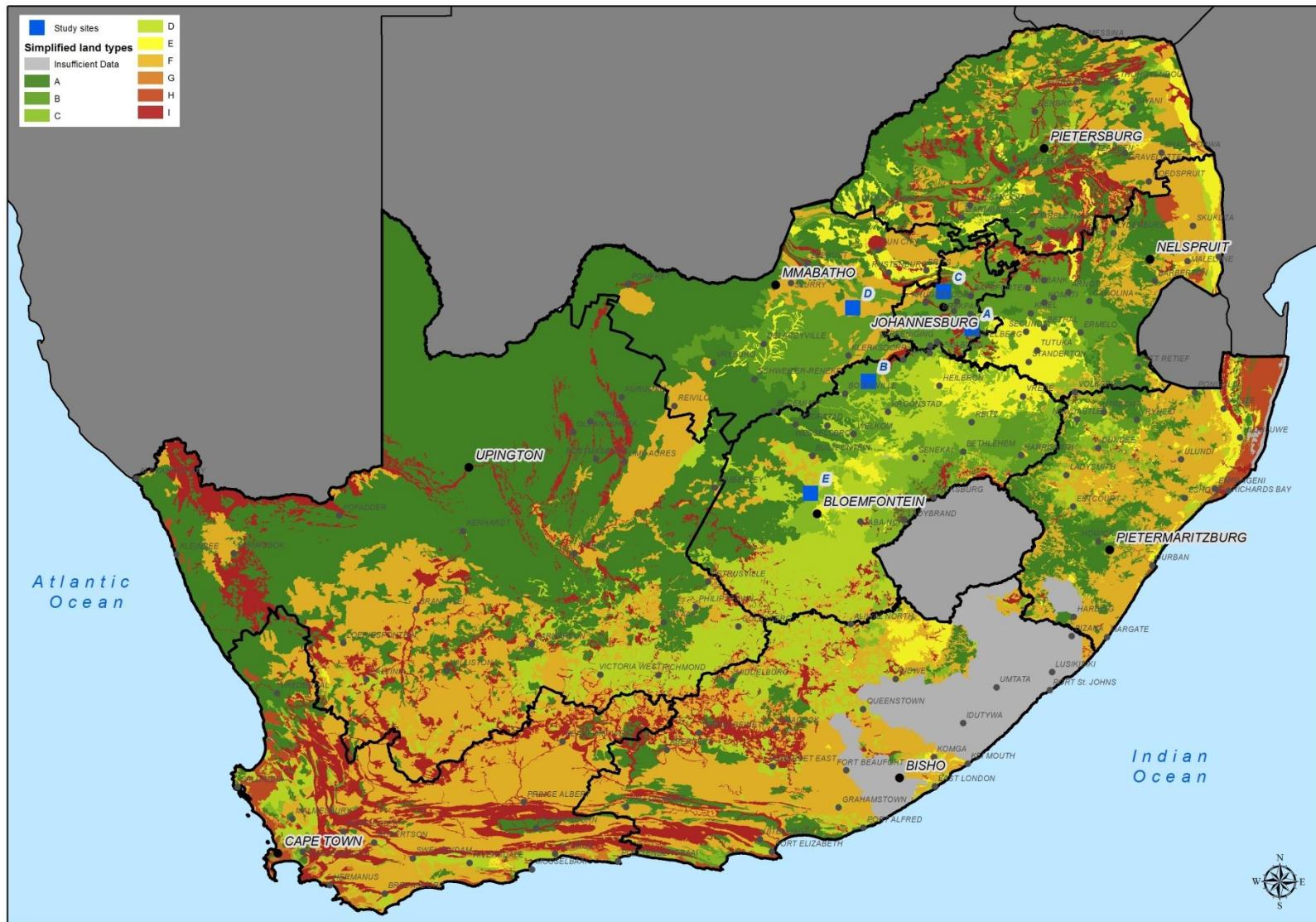


Figure 26: Map of condensed soil patterns of South Africa, indicating the location of Bullfrog study sites. Sties A = Heidelberg, B = Viljoenskroon, C = Diepsloot, D = Ventersdorp, E = Bloemfontein. Land type data obtained from Land Type Survey Staff (1972-2002) and condensed according to the broad soil patterns.

3.7.4. Land type classification with collected samples

Table 12 provides the name, coordinates and information of the locations at four of our Bullfrog sites where soil samples were taken from. These samples were used to determine the Land Type utilization of Bullfrogs. Terrain morphology units refer to the profile and slope of the land (Figure 27).

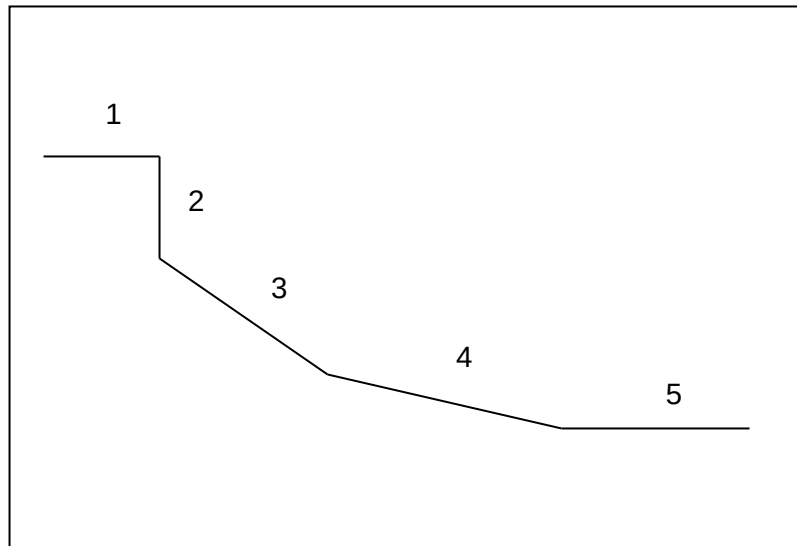


Figure 27: Terrain morphology units: 1) Top (crest), 2) Free hanging (scarp), 3) Middle slope, 4) Foot slope, 5) Valley. Figure adapted from Macvicar (1991).

The analysis of the soil sample taken from four of the Bullfrog field sites indicated that five relevant diagnostic horizons were present (Table 12). In the Diepsloot area E-Horizon was found, while a soft plinthic B-Horizon was found at the Heidelberg and Viljoenskroon sites. At the Ventersdorp sites Orthic A-Horizon was found. G-Horizon was found in terrain morphotype 5 at Ventersdorp, Viljoenskroon and Heidelberg.

E-Horizon and soft plinthic B-Horizon have similar attributes. E-horizon is recognized by its distinct grey colour. It can develop in two possible ways, but most commonly by means of reduction (Le Roux, 1999). The E-Horizon is a very soft soil, when wet, and can become quite hard when dry. The soft plinthic B-Horizon soil can be identified by grey, red, black and yellow mottles. Unlike E-Horizon this type of soil develops from shifting water tables. Both of these types of soils are periodically saturated with water. The G-Horizon is a poorly drained soil and almost always permanently saturated. It is mostly found in terrain morphotype 5 and is

associated with areas where pans, wetlands and other water bodies occur. When dry, the G-Horizon will be extremely hard and digging in these soils will be almost impossible (Le Roux, 1999).

Table 12: Characteristics of soil samples from four of the Bullfrog study sites.

Location	Observ. no.	Soil form	Relevant diagnostic horizon	Depth (mm)	Terrain unit	Coordinates	
						Latitude	Longitude
Diepsloot	D1	Longlands	E horizon	300-1000	4	S25.93961	E28.02344
Diepsloot	D2	Wasbank	E horizon	250-900	4	S25.93838	E28.02423
Diepsloot	D3	Wasbank	E horizon	200-550	3	S25.93888	E28.02420
Diepsloot2	BPO1	Cartref	E horizon	150-300	3	S25.94788	E28.02268
Heidelberg	H1	Avalon	soft plinthic B-Horizon	400-800	3	S26.45773	E28.43408
Heidelberg	H2	Avalon	soft plinthic B-Horizon	400-600	4	S26.45831	E28.43335
Heidelberg	H3	Sepane	G horizon	350-400	5	S26.45948	E28.43262
Viljoenskroon	Vil1	Avalon	soft plinthic B-Horizon	600-1100	4	S27.22497	E26.95050
Viljoenskroon	Vil2	Tukulu	soft plinthic B-Horizon	800-1200	4	S27.22414	E26.95056
Viljoenskroon	Vil3	Westleigh	soft plinthic B-Horizon	600-800	5	S27.22267	E26.93353
Viljoenskroon	Vil4	Katspruit	G horizon	300-600	5	S27.21478	E26.97017
Ventersdorp	Polka4	Hutton	Rocky/stony red Apedal B-Horizon	0-200	1/4	S26.14642	E26.71947
Ventersdorp	Polka3	Glenrose	Lithocutanic B-Horizon	0-200	1/4	S26.15861	E26.69619
Ventersdorp	Polka3.1	Hutton	Rocky/stony red Apedal B-Horizon	0-200	1/4	S26.15953	E26.69756
Ventersdorp	Polka1	Glenrose	Lithocutanic B-Horizon	0-150	1/4	S26.17231	E26.72569
Ventersdorp	Polka2	Katspruit	G horizon	100-350	5	S26.16872	E26.73036

Rocky/stony red Apedal B-Horizon (found at the Ventersdorp sites) has a simple red colouration and Apedal structure. This Horizon has a very fast water absorption rate (typically less than 3 seconds). Soil from the Horizon is never (or very seldom) saturated with water. Lithocutanic B-Horizon was also found at Ventersdorp. This type of Horizon contains hard rocks and is usually associated with young landscapes. Lithocutanic B-Horizon is normally less permeable for air, water and plant roots.

Four of the five Bullfrog field sites (Heidelberg, Viljoenskroon, Diepsloot and Bloemfontein) are associated with condensed soil pattern B (plintic catena: upland duplex and malagaltic soil rare) (Figure 26). According to the detailed land type maps of South Africa (Land Type Survey Staff, 1972-2002) these study sites are found within the following specific land types: Bloemfontein (bd14), Diepsloot (bb2), Heidelberg (bb3), Ventersdorp (fa15) and Viljoenskroon (bd14).

The soil classifications made confirm that 38, 1% of the soil description correlates with land type data bb2 and bb3 for the Bullfrogs sites close to Heidelberg and Diepsloot. Even though Bloemfontein was not sampled, Viljoenskroon soil classification confirmed that 66, 8% of the bd14 land correlates with the soils found at this site. At Ventersdorp, 87.8% correlates with the fa15 land type(Land Type Survey Staff, 1972-2002).

3.8. Detection of other frog species - Amathole toad survey

Unfortunately, the scent of an Amathole toad was not available prior to the survey to condition the sniffer dog. Instead, the scent of a closely related frog, the Karoo toad (*Vandijkophrynus gariensis*), from the Hogsback area was used for conditioning the dog. No Amathole toads were found during four days of searching, not by the sniffer dog or through conventional survey methods. However, the sniffer dog was able to detect other frog species that were present at the Hogsback field sites, including the common river frog (*Amietia queketti*) and the clicking stream frogs (*Strongylopus grayii*) that were concealed in grass tufts and fallen logs. In the Katberg area, the list of detected species included the Karoo toad (*V. gariensis*), *A. queketti* and *Cacosternum thorini*. When the Amathole toad scent is obtained in future, more surveys can be done during the breeding season, when the likelihood of finding these toads is higher (APPENDIX F).

3.9. Detection of *Batrachochytrium dendrobatidis*

Experiments with *B. dendrobatidis* (*Bd*) were conducted in three phases, i) two conditioning tests (100,000 zoospores/ml); ii) two tests with swabs dipped in *Bd* culture and; iii) a combination of dilution tests (two of each dilution and six in total). Overall, great success was achieved in detecting the scent of *Bd*. The sniffer dog was, after introduction to the new scent (the conditioning phase), able to achieve a 100% detection accuracy for all types of *Bd* tests (Figure 28). Overall, ten tests were performed for this component of the research. During all of the preliminary *Bd* experiments, the dog only made 0.55% false indication, of which all occurred during the dilution tests (Figure 29).

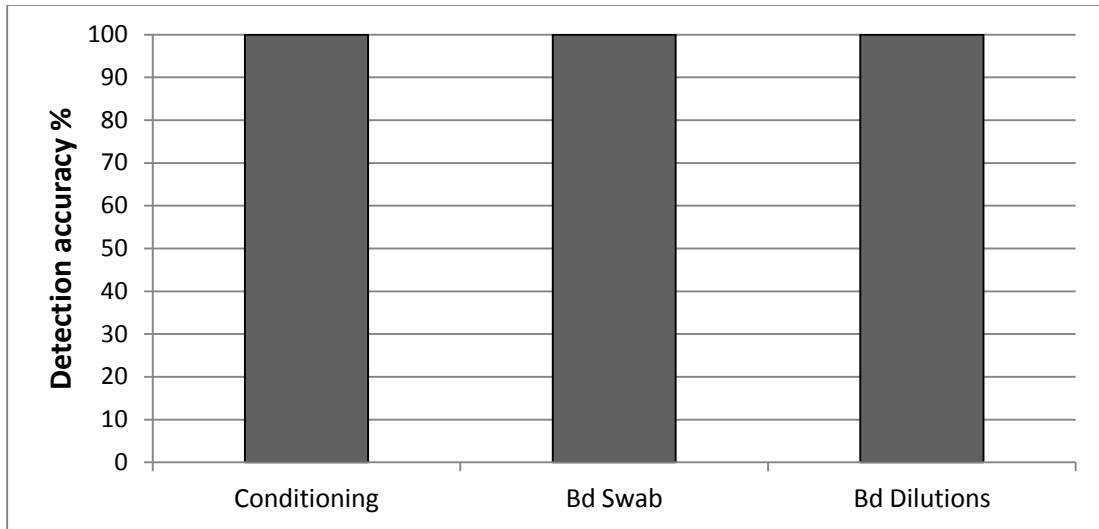


Figure 28: Average detection accuracy from all different types of *Bd* tests performed.

The sniffer dog was able to detect 100% of all positive dilution targets, ranging from a 1,000 zoospore/mL dilution to a 100,000 zoospore/mL dilution. During the 10,000 zoospores/ml experiment, one false indication was made, which accounted for mere 1, 6% of indications for all dilution tests combined. The false indication was made on the *Bd* negative disturbance (control). The control consisted of the same components as the *Bd* positive target (including the same containers and liquid media) apart from the actual culture.

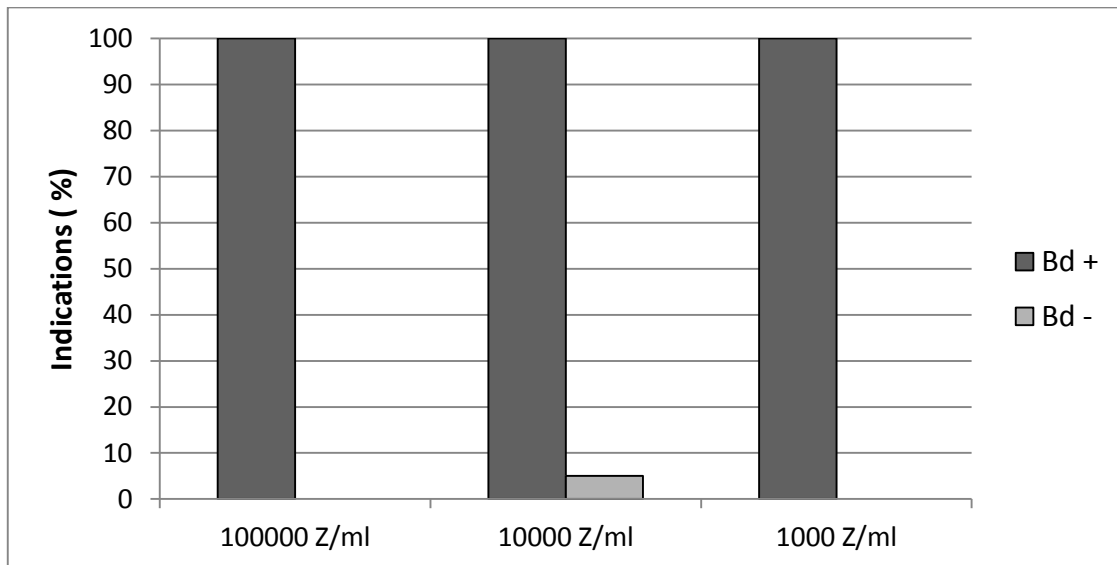


Figure 29: Presentation indications made by the sniffer dog on a variety of *Bd*+ dilutions as well as *Bd*- targets within each experiment.

3.10. Engaging with the public

3.10.1. Facebook pages and posts

For this study two Facebook pages were created and monitored to serve as an interactive and continuous platform for engaging with the public about the project (Table 13). The first Facebook-group was created in 2014 to provide information on amphibian conservation and research. Its focus was on projects and other activities associated with our research group, the African Amphibian Conservation Research Group (AACRG), which included the sniffer dog project. Since the page was created it has obtained 449 likes, with the most popular post achieving a total of 39 likes, comments and shares on a post (combined).

The second page named, "Jessie the Border collie", was created a year later, in 2015, and was dedicated to activities relating exclusively to the sniffer dog project. This page focused on aspects associated with conservation research and awareness. It was used as a tool to give the sniffer dog a personality in the eyes of the public. These posts were also made from the dog's perspective, while sharing day-to-day experiences. The page has achieved 310 page likes, while the most popular post received a staggering 3388 overall likes, comments and shares (Table 13).

Table 13: General information on the two compared Facebook-pages.

Name of page	Logo	Date Created	Page likes*	Most popular post (combined likes, comments, shares)
African Amphibian Conservation Research Group		2014/04/10	449	39
Jessie the Border collie		2015/01/28	310	3388

**Results at time of submission*

The top five posts from the “Jessie the Border collie” page, with regards to the total people reached and reflected the type of information that is popular with the public, as follows:

- ❖ A post on the sniffer dog activities on the NWU Potchefstroom campus received the 5th largest reaction (Figure 30). A total of 613 people were reached by this post. The post emphasized the fact that Jessie was working on the university campus. A picture of the dog in working attire in front of the main campus building was included in the post.

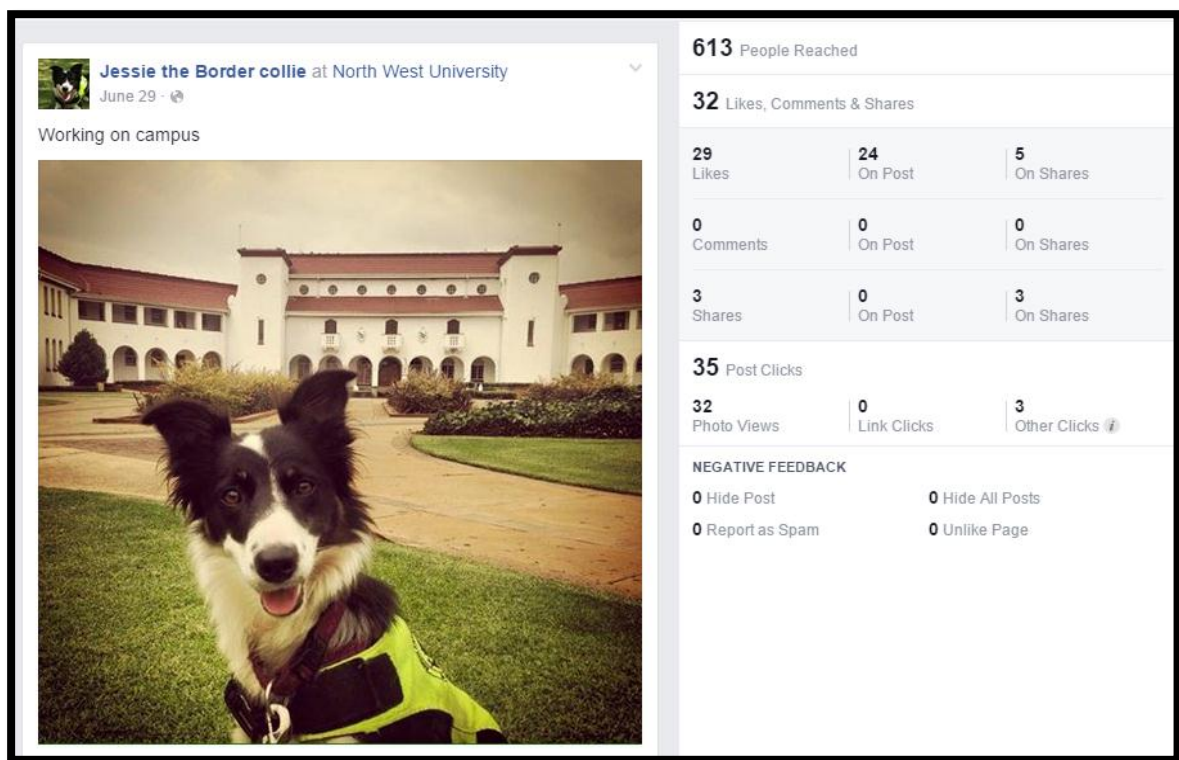


Figure 30: Web-grab image of information on one of the top five posts that reached the most people (5th popular post).

- ❖ A video post on the sniffer dog actively searching for frogs during a fieldwork session received the 4th largest reaction (Figure 31). A total of 988 people were reached by this

post. The video was made using an action camera attached to a harness on the back of our sniffer dog.

- ❖ A post about the sniffer dog's demonstration for a group of school students received the 3rd largest reaction (Figure 32). A total of 1018 people were reached by this post. The post provided a description of the event and a picture of our sniffer dog (and handler) with the students.

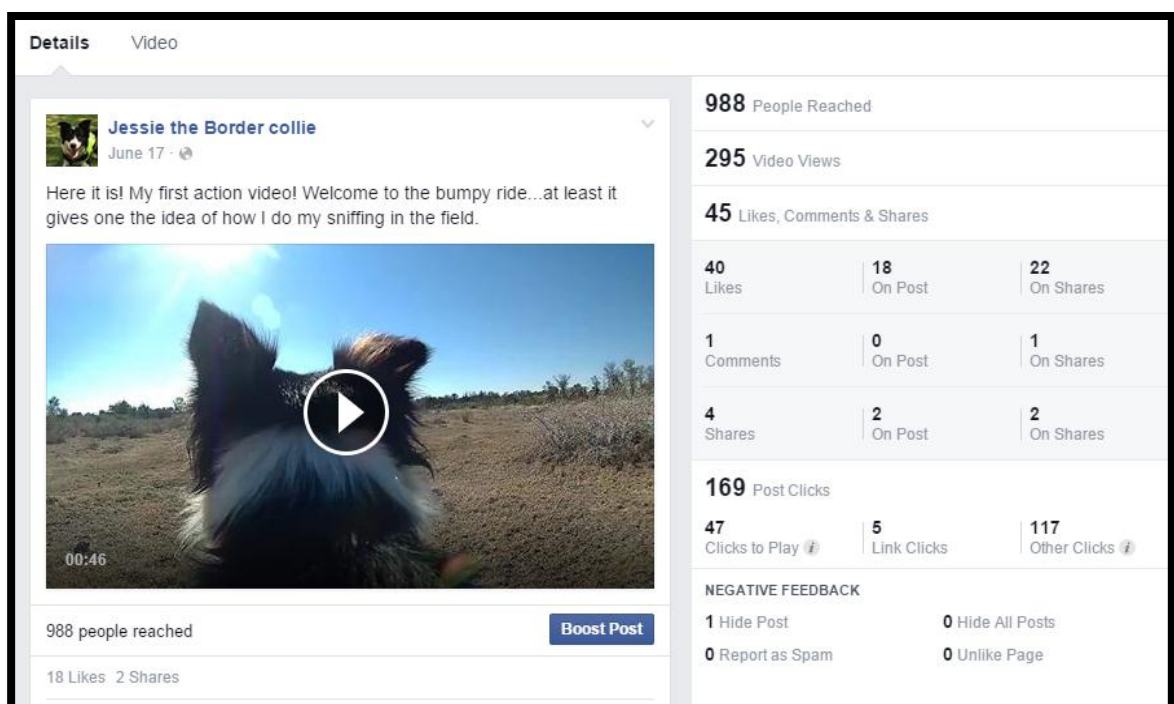


Figure 31: Web-grab image of information on one of the top five posts that reached the most people (4th popular post).

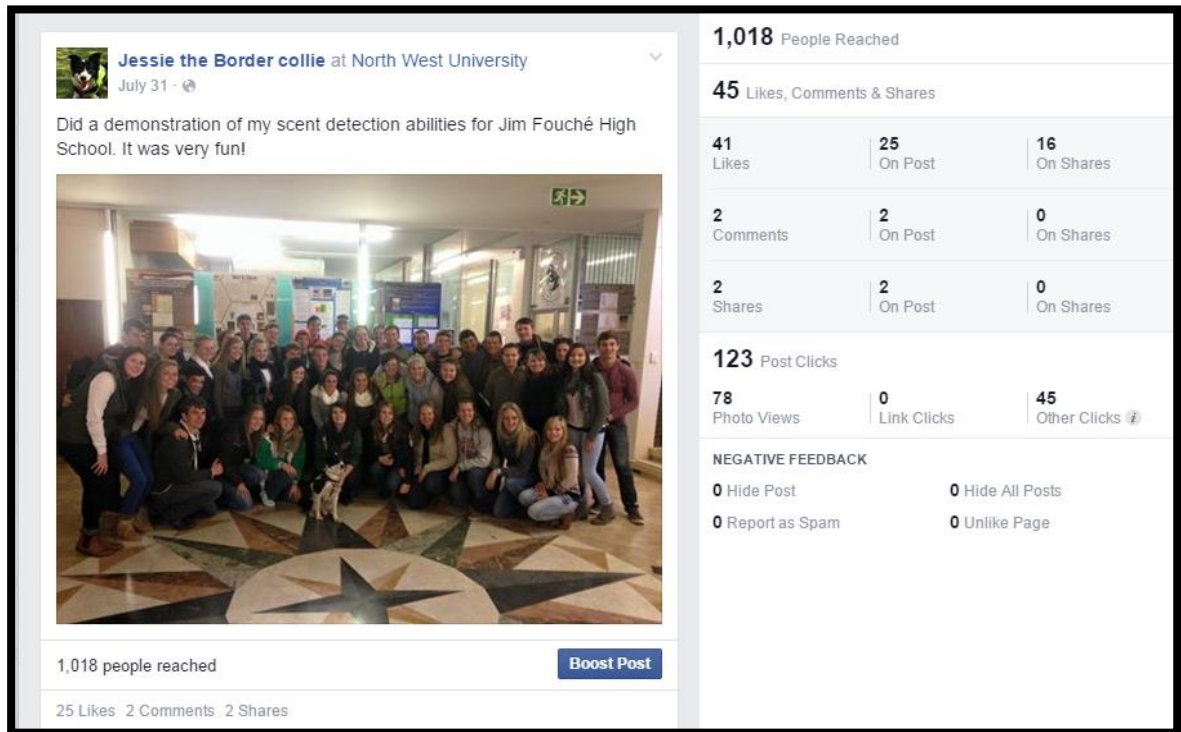


Figure 32: Web-grab image of information on one of the top five posts that reached the most people (3rd popular post).

- ❖ A post about the sniffer dog finding her first wild buried bullfrog during a field survey received the second largest reaction (Figure 33). A total of 11,992 people were reached by this post. The post was accompanied by five photos and an explanation of how deep the frog was buried. Only limited information about the circumstances surrounding this find was revealed by the post.
- ❖ A post of the sniffer dog's nose received the largest reaction (Figure 34). A total of 54,510 people were reached by this post. The post was accompanied by one photo and the picture's caption referred to her nose as her "secret weapon". This specific post was shared 172 over a three month period.

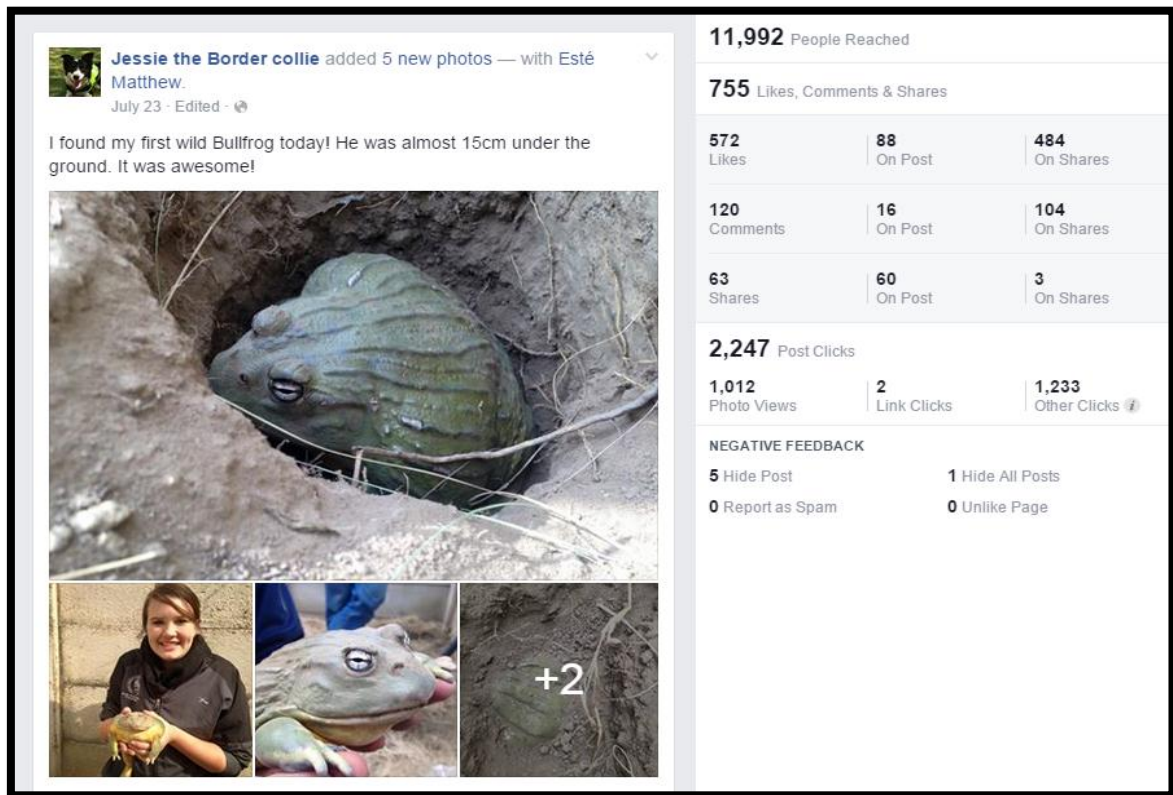


Figure 33: Web-grab image of information on one of the top five posts that reached the most people (second most popular post).

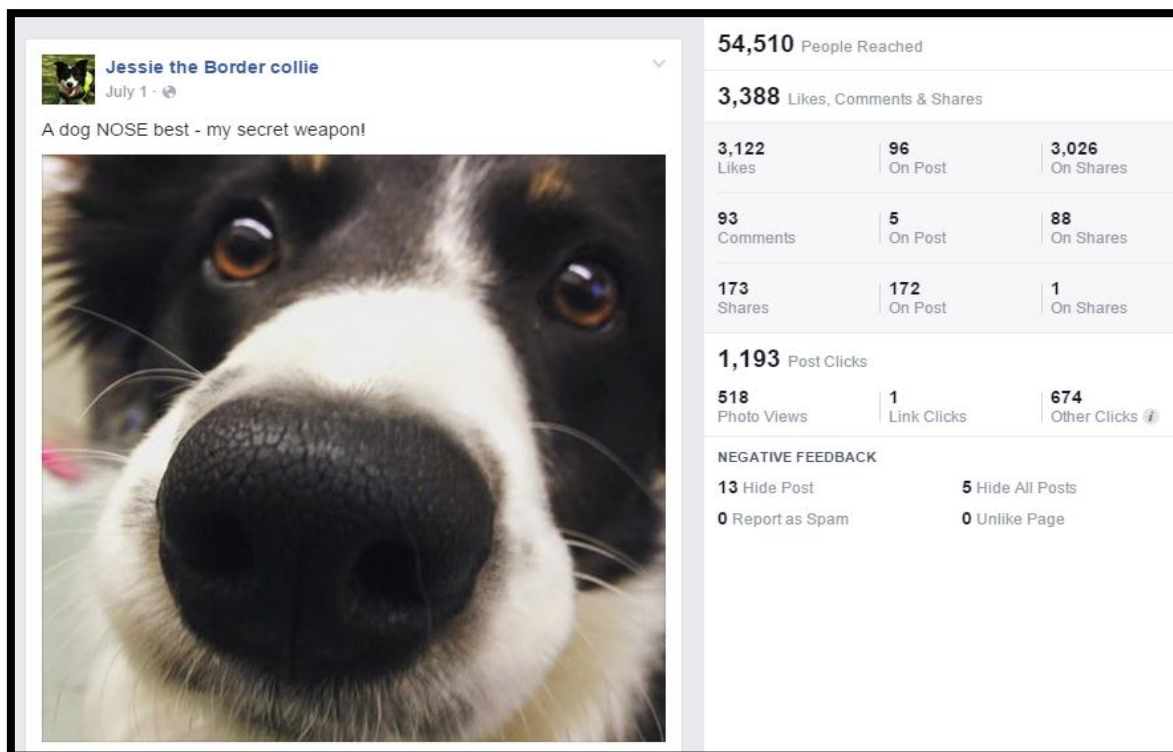


Figure 34: Web-grab image of information on one of the top five posts that reached the most people (most popular post).

The Top 10 posts from the “Jessie the Border collie’s” page, with regards to the likes, comments and share (LCS on post) are compared in Table 14. Each post is also placed in a category according to an emotional trigger, work related, miscellaneous or educational/awareness. Based on the LCS (on post), each post was awarded a rank within the top 10. The first five posts in these results are not the same as the top five for reaching the most people overall. None of the miscellaneous posts reached the top ten posts on the page. Detailed results can be found in APPENDIX G.

**Table 14: On post information for the top 10 posts form
the *Jessie the Border collie* Facebook page.**

Ranking	Date	Description	Type	On Post				
				Pictures	Likes	Comments	Shares	Total (LCS)
1	01-Jul-2015	Dog nose photo (secret weapon)	Emotional trigger	1	96	5	172	273
2	23-Jul-2015	Found first wild bullfrog	Work related	5	89	15	60	164
3	07-Apr-2015	Trainer in leaving	Emotional trigger	1	47	4	0	51
4	01-Oct-2015	Throwback photo (3months)	Emotional trigger	1	37	1	0	38
5	03-Sep-2015	Going to vet	Emotional trigger	1	34	3	0	37
6	03-Sep-2015	Update on experiments	Work related	2	31	2	0	33
7	14-Feb-2015	Live frog experiments	Work related	5	27	3	0	30
8	31-Jul-2015	Demonstration for school	Educational/Awareness	1	25	2	2	29
9	07-Mar-2015	Proof of posting	Emotional trigger	1	29	0	0	29
10	11-Sep-2015	Article on fieldwork	Work related	1	27	1	0	28

The post about the detection of the first wild bullfrog was monitored 4 times over a period of 32 days. The largest number of people was reached during the first week that the article was posted (Figure 35). During the next two weeks this number only gradually increased, and during the fourth week the number of people reached started to plateau.

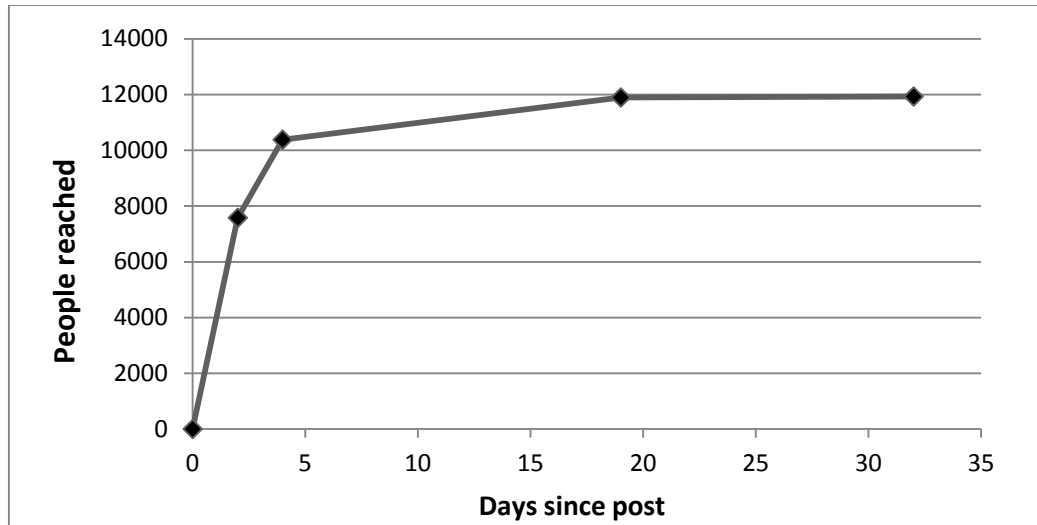


Figure 35: Information on first bullfrog post - people reached over 32 days.

3.10.2. Types of media coverage

This study also gained a lot of public interest in other forms of public media such as the printed and audiovisual sectors (APPENDIX E and H), with websites and newspapers having the highest percentages (Figure 36). Twitter was the social media component that was used least frequently by the public to cover this project.

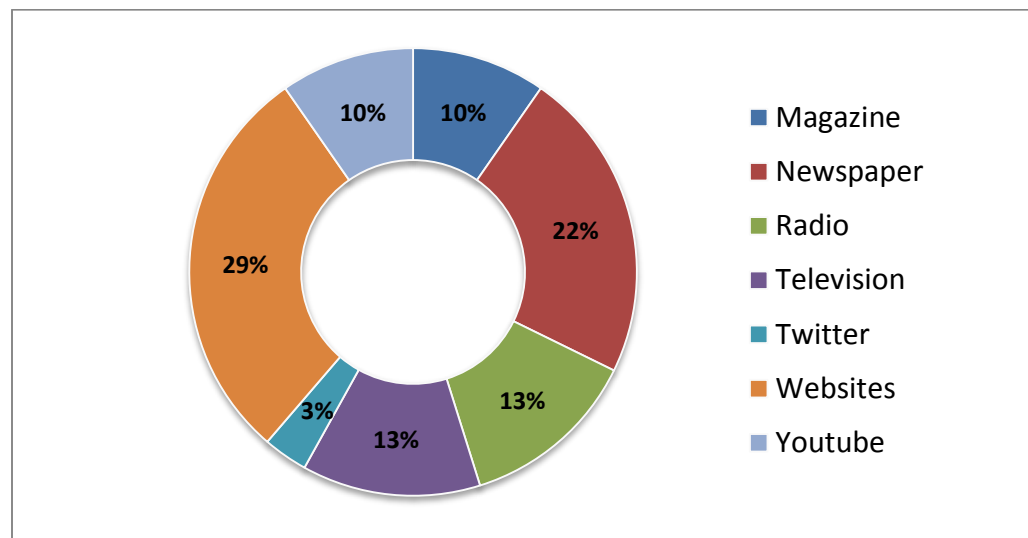


Figure 36: Ratios of the sniffer dog coverage within the various media sectors. Facebook posts are excluded from these data.

Table 15: Timeline of the types of media coverage on the sniffer dog project.

Publication date	Media type	Publisher
13-Mar-14	Newspaper	Sunday Times
23-Mar-14	Newspaper	The Citizen
23-Mar-14	Website	Maroela Media
23-Mar-14	Radio	JacarandaFm
23-Mar-14	Website	News 24
24-Mar-14	Radio	OFM
24-Mar-14	Website	OFM
25-Mar-14	Website	The Dodo
25-Mar-14	Website	iOL
10-Apr-14	Newspaper	Die Burger
10-Apr-14	Newspaper	Volksblad
10-Apr-14	Website	Newsroom NWU
15-Apr-14	Facebook	African Amphibian Conservation Research Group
15-Apr-14	Television (+ Youtube)	eNews (Afrikaans)
15-Apr-14	Television (+ Youtube)	eNews (English)
17-Apr-14	Newspaper	Potch Herald
22-Apr-14	Newspaper	Die Vryburger
28-Apr-14	Radio	Puk Fm
29-Apr-14	Website	TV Stream Times
29-Apr-14	Television (+ Youtube)	Dagbreek
30-Apr-14	Radio	Pretoria FM
9-May-14	Youtube	Pukke TV
29-Jul-14	Newspaper	Rapport
9-Feb-15	Magazine	Froglog 113
12-Apr-15	Twitter	Chytrid Crisis
25-Apr-15	Website	Chytridcrisis
8-Jun-15	Website	Amphibians.org
25-Jul-15	Magazine	Froglog 115

13-Aug-15	Television	Supersport (Channel 210)
1-Dec-15	Magazine	Canine Zone

The first newspaper article on the study was released on the 13th of March 2014. This was followed by a rapid wave of media coverage in published and aired media (Table 15 and APPENDIX B). More details, links and comments are available in (APPENDIX G). A popular article on the profile of our Border Collie has been accepted for publication in the December edition of *Canine Zone Magazine*.

Therefore, by the end of 2015 the study will have received 30 different instances of media coverage (Figure 37). In 2014 more media coverage had been received than in 2015. Generally, the most events concerning the study were spread via websites. The same number of media events was covered by newspapers as for websites in 2014, while the most media coverage in 2015 was achieved through magazine articles.

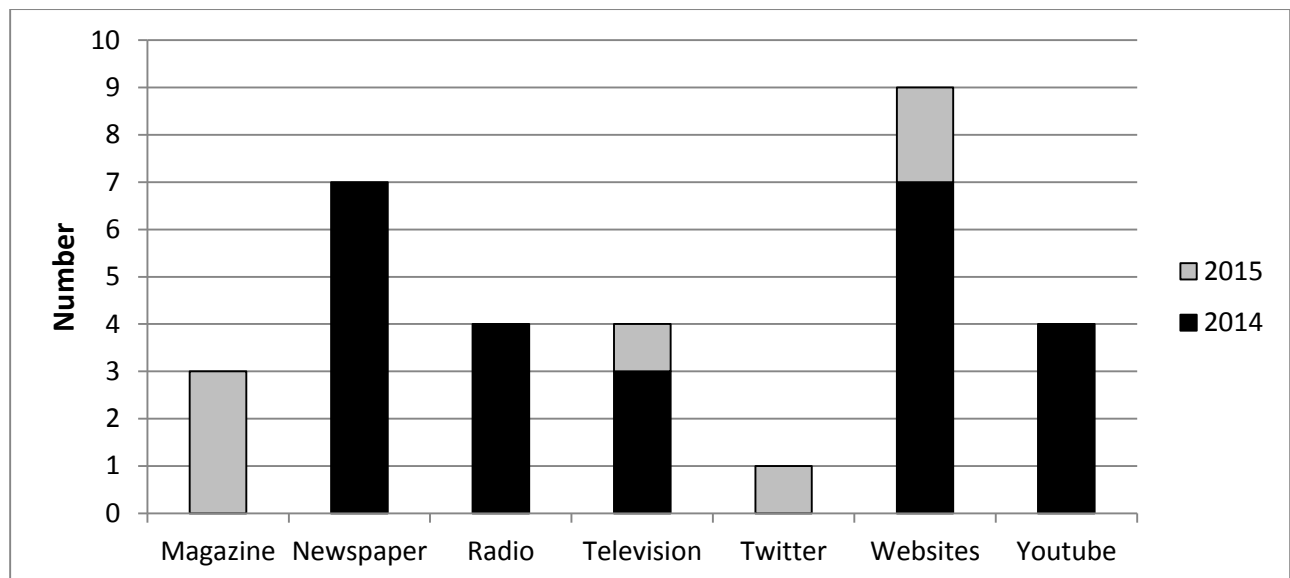


Figure 37: Number of events that received attention in the media (according to types) over the two years that the project was running.

3.10.3. Presentations and demonstrations

A total of nine presentations about the study, including demonstrations with the sniffer dog, were conducted during 2014 and 2015, in order to create additional awareness of amphibian conservation (Table 16). Particular attention was paid to local communities and schools, but a few international events also took place. Demonstrations did not always form part of these events (in 44% of events), while presentations were delivered at 88% of the events. Many people were reached through these presentations and demonstrations and attendants varied between 40-80 people.

Table 16: Information on the events where presentations and/or demonstrations were performed.

Date	Event	Presentation	Demonstration
20-Jun-14	Masters colloquium, Potchefstroom	Yes	No
16-Oct-14	Vaalpark Primary School, Sasolburg	No	Yes
5- Nov-15	Symposium - Howick, KZN	Yes	No
10-Feb-15	Students from America (California)	Yes	Yes
27-Feb-15	Kroonstad High School	Yes	Yes
12-Apr-15	ACRS symposium, Cambridge UK	Yes	No
16-Apr-15	Institute of Zoology, London UK	Yes	No
31-Jul-15	Jim Fouché High School, Bloemfontein	Yes	Yes
18-Sep-15	Cocekani High School, Jouberton	Yes	No

4. Discussion and conclusion

4.1. Efficacy of sniffer dog to detect frog scent

According to Johnen *et al.* (2013) dogs have been used for a variety of scent related jobs. These jobs include drug detection, explosives, forensics and hunting (Adamkiewicz *et al.*, 2013; Browne *et al.*, 2006; Hurt and Smith, 2009). Dogs are also use to screen for cancer (Walczak *et al.*, 2012) and to locate bigger wildlife by means of detecting scat (Wasser *et al.*, 2004). The training method, called Operant conditioning, is an affective and reliable way to train scent detection dogs (Blackman, 1974; Jezierski *et al.*, 2010; Johnen *et al.*, 2013). Even though scent detection dogs have been applied to detect a variety of scents, the application of amphibian scent detection has not been fully explored (no publications available).

It is important to keep in mind that all of the training trails in this study were not time-dependant, with regards to the time it took the dog to learn a new component. Even though our sniffer dog was a fast learner, we do not recommend giving a set time for training and experiments, because every dog's abilities are different. Instead, our dog was trained until a satisfying result (more than 90% correct identification) was obtained. Some training and experiments (of different kinds) were often completed on the same day.

4.1.1. Scent dilutions

Previous studies have confirmed the legitimacy of canine scent detection (Jezierski *et al.*, 2010; Johnen *et al.*, 2013). The dilution experiments were introduced to provide a better understanding of the sniffing abilities of a sniffer dog and to increase sensitivity for Bullfrog scent. It was agreed upon that the 1:100,000 dilution would be the cut-off for the dilution test, due to the assumptions that this simulated the most diluted frog scent that the dog would encounter in the field. This assumption was made due to the fact that a live bullfrog's scent (above ground) would be stronger than our 1:1 scent on a swab and no published work about the movement of scent through soil was available. Although the dog achieved 100% accuracy with the 1:100 000 dilution, the experiment was stopped and the dog was ready to progress to the next step in her training. Even though the dog only achieved 93.33% accuracy with the 1:1 dilution, it was found that the plank equipment could partly explain the results. Because the targets on the plank are situated close together (53 cm apart), the dog could have picked up the

scent too early, and therefore causing the dog to indicate on the false targets before the positive target. No previously published work was found where dilutions of the target scent were presented to sniffer dogs. The only published work available is about the use of diluted urine samples used during scent discrimination trails with mice (Osada *et al.*, 2003). The use of diluted amphibian scents was vital to the success of this project. Working with dilutions increased our sniffer dog's sensitivity for Bullfrog scent. By using dilutions, it was possible to train without any visual cues, which in return made it possible for our dog to find wild Bullfrogs.

4.1.2. Influence of disturbances

Amphibian scent disturbances

Scent detection dogs can also be influenced or distracted by environmental factors and other disturbances (Jeziński *et al.*, 2010; Reed *et al.*, 2011; Thesen *et al.*, 1993). For this reason, disturbances and distractions were also added to our experiments to help the dog focus on the target scent in any situation (Batt *et al.*, 2008). During the experiments where live frogs were used as a disturbance, the detection accuracy was slightly lower than with the dilution test. The dog detected 98% of the Bullfrog scents, but also indicated on *Amietia quecketti* (1%, or one indication) and on containers with water in (2%, or 2 indications). In the case of the false indication on *Amietia quecketti*, the indication was also made on the previous position of the positive target. This test might also have been more difficult for the sniffer dog as it was the first experiment where multiple frog species were included in the same line-up (Schoon, 1996). The dog only made one false indication on the scent of another species that was placed in the test as a distraction/disturbance (*Xenopus leavis*). Also, as indicated on the marking sheet, the dog was tired during this specific test.

As a variety of species can be found in the same habitat, it was important to train our sniffer dog to focus on the scent of one species while confronted with other species in the area. This test proved that even with a variety of species in the line-up, the dog could locate the scent of the Giant Bullfrog, which also means that the dog will be able to find Bullfrogs in between other frogs, without indicating on the other species (increased the dogs specificity).

Human scent disturbances

It is known that dogs can discriminate between scents from identical twins (Berns *et al.*, 2015; Pinc *et al.*, 2011). Therefore it was important to determine if the presence of different human scents affected the dogs performance (as well as the absences of human scent) .The results for this experiment indicated that human scent disturbance is not a major distraction for our sniffer dog. The dog was able to indicate on 99% of the bullfrog scents during all the tests in this experiment. It is likely that the lower detection accuracy observed for the male scent test (95%) was caused by the dog's emotional attachment towards the male volunteer (Bennett and Rohlf, 2007; Clark and Boyer, 1993), because the dog was familiar with this person on a non-professional level. In this specific case, the position of the false indications was one of the positions on the plank that have previously been associated with as false indications (see section 4.2) and is one of the positions that caused technical difficulties in the past.

4.1.3. Tracking ability above and below ground

Previous research has shown that dogs are able to pick up on scents underground (Browne *et al.*, 2006; Cablk and Heaton, 2006) and they have the ability to track scents (Thesen *et al.*, 1993). Although scent tracking and depth detection are very important aspects of the training, it is difficult to record results to validate the success of these exercises. The results only show that a range of trails were completed and that the dog was trained under a variety of conditions, including: different weather conditions, surfaces and times of the day. The setups of the tests were also dissimilar in most cases. The dog was always allowed enough time to indicate on the correct target (theoretically achieving 100% for detection accuracy). If a false indication did occur during the depth training the sniffer dog was simply not rewarded and the search was continued. No false indications occurred during the scent tracking trails, regardless of how long it took for the dog to locate the target. Without these vital components as part of the training routine, the sniffer dog would not be able to locate buried frogs and would also not be able to track scents in a field to locate the strongest point (Thesen *et al.*, 1993) as most frogs are active at night time and hide during the day (Buchanan, 1993). Because frogs are burrowed in the ground or hiding underneath rock and logs during our daytime fieldwork, their tracks may be weak, but a lot of scent can be found at the location of their hideout. Training the dog to track also allowed us to find active frogs.

4.1.4. Preserved scent

A limited amount literature exists on studies that involve the preservation of animal scent. One known method includes freeze-drying liquid scents, for example buck urine in order to preserve the scent (Kennedy, 2013). Because equipment used for freeze-drying is not readily available, this study focused on four simplified ways to preserve frog scent. For these experiments the sniffer dog achieved more than 80% detection accuracy for each method tested, after the six months period. Both the diluted swab method and the 1:1,000 dilutions (at 4°C) reached 95% average detection accuracy. Because the interest of this study was more on the distinct differences between preservation methods, the data was divided into both forward and return runs, in an effort to yield more detailed results. What was clear from these results was that the scent strength decreased in relation to length of preservation, regardless of which preservation method was used. The scent was, however, best preserved by using a Fridge (4°C). The two methods included a 1:1,000 dilution and a swab that was kept under the same conditions, and then diluted before each test. Even though the scent weakened over time, the sniffer dog was still able to yield reliable results, even after six months. The scents of animals as well as the scent samples are not always readily available, especially with elusive species. For this reason, it is vital to know how to preserve a scent over time so that the sniffer dog can be trained on the scent, even if scent was taken months earlier. Sometimes, scent has to be obtained by a third party and sent to a facility where the sniffer dogs are trained. This method of preservation for scents can, therefore, play an important role for future research and method development.

4.1.5. Comparison of detection accuracy for different types of tests

The average detection accuracy for the dried skin tests and the live frog tests are notably lower than the other types of tests. The best explanations for these results are that both of tests have drier positive targets (less scent) and fewer tests were done with these types of targets. For example, only two tests were done using dried skin while 10 tests were done using amphibian scent, as disturbances during this study. The reason that fewer dried skin tests were done is, because the dried skin was not always available to work with. Only when the captive Bullfrogs, in the lab, shed their skin were they able to be collected and dried to be used in experiments. Remarkably, the dog was able to detect 93.35% of all positive targets throughout this study.

4.2. Effective use of wooden plank equipment

False indications and missed indications have often occurred in previous studies (Jeziński *et al.*, 2010; Lesniak *et al.*, 2008). The causes for these mistakes have often been cues from the handlers, if they had knowledge about the location of the target (McKinley and Sambrook, 2000; Miklösi *et al.*, 1998; Pfungst, 1911). During this study, the possible consequences of the *Clever Hans effect* were eliminated by training methods. However, FI and MI did occur and for that reason it was important for us to evaluate the equipment used (the wooden plank).

False indications (FI)

Even though in total only 0.42% false indications were made, most of the false indications could be explained by the position of the target and/or the setup of the experiment on the plank equipment. A total of 93.6% of all false indication could be ascribed to one of three predicted causes: 1) adjacent to the positive target; 2) the position of previous positive target (preceding run); and 3) disturbances intentionally placed to serve as a distraction.

Due to the design of the plank equipment, which only provides a 53 cm gap between targets, false indications were found to occur when the false target was located next to the positive target. Some of the targets were located less than 20 cm from each other (positions five and six on the plank), because this was where the two planks were fitted together. It appeared as if the scent from the positive target might have already been picked up by the dog on the target position preceding the positive target, because of the small distance between these targets. During the six month preservation of scent experiments, 64% of the false indications could be explained due to this specific cause. A similar outcome was obtained for the Bullfrog dilution tests (63% of false indications).

A second explanation for the occurrence of false indications has to do with the position of the positive target during the preceding run, for two reasons. Firstly, it is likely that some of the food rewards (e.g. meat treats) could have dropped between the container and the plank when the trainer/handler rewarded during the previous run. For this reason, the plank was always cleaned after every five runs, but in-between these runs, the plank was only cleaned on the surface, to complete a set of experiments within a reasonable time and to prevent the dog from tiring. Secondly, it might be that the dog was able to still pick up on the scent at a specific position after the scent was moved to a new position on the plank (Browne *et al.*, 2006). This assumption is purely based on the behaviour of the dog, as the real scent ability of a sniffer dog

is still unknown. For the position of the positive target in the previous run, 67% of false indications from the live amphibian test and 32% from Bullfrog dilution tests were made.

Lastly, false indications were found to be based on the position of a disturbance (excluding water) intentionally placed in a position to distract the dog (Rooney and Bradshaw, 2004). Since disturbances were intentionally used to test the dog, it is fair to assume that this could result in false indications. During the amphibian dilution experiments and the *Batrachochytrium dendrobatidis* (*Bd*) experiments, only one false indication for each was made on a disturbance. In these cases, the disturbance was the scent of another frog species and a *Bd* negative target (sterile media) respectively. One of the three false indications made during the live amphibian experiments could also be explained by this cause.

Even though false indications were made, the percentage of FI was minute. The statistical analyses showed that the percentage was lower than 0.6% (for all plank experiments), random mistakes and irrelevant to the outcome of this study.

Missed indications (MI)

Missed indications are caused by a variety of factors including disturbances, the experimental setup and even the dog's (and trainer's) emotional condition (Jezierski *et al.*, 2010). Apart from the experimental setup, most of these factors cannot be controlled. Missed indications were not a concern for this study due to the fact that if the dog would miss one Bullfrog in a site it would not affect our results. The sniffer dog did not have to find all of the Bullfrogs at a site for this study.

One of the factors that was applicable to our experiments was the way in which the plank equipment was used. The direction of movement across the plank, along with the position of the positive target (from the dog's perspective), provided some technical difficulties. Because the position of the first target on the plank is only a few centimetres (7,5 cm) from the edge of the equipment (which is raised 130 mm from the ground), the dog had to focus on climbing up onto the equipment while moving over the first targets. Statistical analyses proved that the first four targets (from the dog's perspective) was more difficult for the dog to indicate. 65% of the MI were made on the first four targets. It also shows that the scent from the preservation experiment did decrease over time due to the fact that more missed indications occurred with each passing month.

Because of this occurrence, the sniffer dog had two opportunities to indicate on the target (either/or during forward a return runs), while only scoring the dog once. This scoring practice is deployed by most sniffer dog trainers when working with plank equipment and training boards (Johnen *et al.*, 2013). It gives the sniffer dog the ability to scan (or check) all of the targets and then return to indicate on the positive target. For example, sniffer dogs used for airport luggage are often observed scanning all of the items and returning to items that contain the target scent.

The first and the last target holes on the plank were also used less to provide the dog with a fair chance to indicate. Despite the fact that the equipment could not be improved (during this study), the sniffer dog was still able to locate positive targets with only minimal MI and FI occurring within the experiments. The plank equipment was therefore still a vital and effective training tool and delivered accurate results.

4.3. Detection of *Pyxicephalus adspersus*

The training phases of this study took longer than was expected, in terms of training the dog to be sensitive enough under a variety of conditions. This resulted in each field site being visited a maximum of three times. Although the sniffer dog indicated at all of the field sites where Bullfrogs were known to occur, it was physically impossible to dig enough holes deeper and wider than 50 x 50 cm, This was because, in most cases, the soil was quite compacted at the sites. This also seems to be case in other similar studies on the ecology of Bullfrogs (Yetman and Ferguson, 2011a; Yetman and Ferguson, 2011b; Van Aardt and Weber, 2010). Contrary to this, the Bullfrog that we did manage to dig up was buried close to the surface in sandy soil, which made digging by hand a lot easier. The confirmation of the presence of Bullfrogs, after indication by the sniffer dog, is what makes this method hard to implement practically. Confirming the presence of species that are accustomed to hiding beneath rocks and logs or within leaf litter, is likely to be a lot easier and, thus, this method is more applicable to species that display this kind of behaviour.

Also, little is known about the distribution of scent through soil and how long scent can remain in an area after an animal moves away from that area. From this study, it appears that the sniffer dog targets the scent that filters through from the burrow. This assumption seems realistic, as it would be expected that the strongest scent (above ground) would be located where the frog started excavating the burrow. This could be another reason why digging at the point of indication by the sniffer dog was unsuccessful, because the frogs may have moved significantly underground from the burrow entrance.

Bullfrog association with rainfall land type

The annual rainfall map suggests that most Bullfrog habitat sites fall in the medium rainfall belt. This seems realistic as too much rain might drown the frogs, while drought might cause them to dehydrate. Even though the range of bullfrogs extends into very high and very low rainfall areas, it can be more related one significant rainfall event rather than annual rainfall. The Bullfrogs only require one heavy rainfall event for them to emerge from their hibernacula for the breeding season.

Using the information obtained from the Land Type maps, it was possible to determine that more than 70% of historic Bullfrog records are associated with three main types of soils (A, B and F). These types of soils are: 1) a red-yellow Apedal that is freely drained, 2) a Plintic catena (soft and periodically saturated), and 3) a dry Glenrose or Mispan soil form (Fey, 2010). Occurrence in soil types 1 and 2 can be explained by the fact that the frogs would require a certain amount of moisture to prevent dehydration, as well as low oxygen levels, during their long aestivations (Van Aardt and Weber, 2010), while never having the possibility of drowning (Schmidt, 2006; Thomas *et al.*, 2014). The hardness of the dry soils of type 3 could serve as a protective measure for the frogs. This type of soil is hard enough to protect them from most predators during aestivation, but becomes soft enough for them to emerge or burrow into if sufficient rainfall occurs. All of the field sites had either soil type B or F.

The soil samples taken from the field sites concluded that most locations had an E- Horizon or a soft Plintic B-Horizon. Both of these soil types are periodically saturated, providing the Bullfrogs with moisture and making it easier for them to emerge after rainfall. Three of the four sites also had a G-Horizon present, which is associated with pans and long-term water bodies. This is relevant, as the frogs would require the areas containing G-Horizons as breeding sites. The Ventersdorp site was the only one with the F-type soil. It was unexpected to find such a dry and rocky type of soil near the Bullfrog breeding sites. It is assumed that the reason why Bullfrogs are able to utilize this type of soil, includes the fact that this soil does become soft after sufficient rainfall, which would allow the frogs to burrow. Hardening of soil during dry months will provide protection, while the rocks can provides additional protection when the frogs are able to dig in-between rock particles.

4.4. Detection of *Vandijkophrynus amatholicus*

In 2015, an attempt was made to locate the Amathole (Tarrant *et al.*, 2015a; Tarrant *et al.*, 2015b). Unfortunately, the correct scent was not provided to train the dog with, as this may have been the difference between finding and not finding the toads. During this survey a closely related toad's scent was used (Frost *et al.*, 2006). Our results show that dogs can distinguish between different species from different genus, but no previous studies have tested if a dog can use their olfactory systems to identify comparisons between species within the same genus. Even though it is known that dogs can discriminate between different human individuals (Curran *et al.*, 2010; Pinc *et al.*, 2011). Therefore, it was on a trail bases that *Vandijkophrynus gariensis* scent was used to try to find *Vandijkophrynus amatholicus*.

The other species that were found during the survey was found by sight and not by scent. Although this trip was unsuccessful, the plan is to return to the site, with the sniffer dog, during the active season, when the likelihood to find toads are higher. EWT did manage to find an Amathole toad in October 2015 and were able to collect a scent sample on a swab. Training the sniffer dog on the correct scent should further increase the chances of detecting the toads during future surveys.

4.5. Potential for amphibian disease sniffer dog

The spread of diseases has also been associated with amphibian declines (Conradie *et al.*, 2011; Fisher *et al.*, 2009; Beebe and Griffiths, 2005; Stuart *et al.*, 2004). Strong evidence suggest that the extreme decline in amphibians can be explained by the infectious waterborne disease *Batrachochytrium dendrobatidis* (*Bd*), also referred to as amphibian chytrid (Berger *et al.*, 1998; Blaustein *et al.*, 2005; Ouellet *et al.*, 2012).

The sniffer dog used in this study was able to detect all of the *Bd* targets in the experiments. The dog was also capable of indicating on targets that only contained 1 000 zoospores/ml. Only one false indication was made, by the dog, on a container with sterile media. Even though the results are only for experiments where pure *Bd* cultures were used, it is likely that a sniffer dog will be able to detect the scent of *Bd* on a frog. Because the dog used in this study was primarily trained to detect different species of amphibians, it would have been risky to retrain it to detect a different type of scent on live amphibians, without focussing on the scent of the animals themselves. Further research is however required to test the limits of detecting diluted *Bd* scent, and to test for *Bd* on infected frogs.

These experiments were conducted in order to determine whether it would be possible for a sniffer dog to detect the scent of this eminent amphibian pathogen. This application holds great promise for: future development of diagnostic technologies, determining *Bd* distribution, implementation at customs check points (e.g. harbour ports and airports) and many more.

4.6. Public interest in conservation/detection dogs

The collection of ecological information is not the only important component for an effective biological conservation initiative. An often overlooked important component, is public involvement and adherence to protection programs, because awareness, knowledge and perception play a fundamental role in the community's willingness to protect species (Smith and Sutherland, 2014; Vincenot *et al.*, 2015). Thus, to ensure successful conservation intervention it is vital for scientists to engage with the general public through public and social media about contemporary conservation issues (Papworth *et al.*, 2015).

A lot of public interest and awareness was generated for the duration of this study through two Facebook pages (combined page-likes of 759). Despite the fact that the *Jessie the Border collie page* has been active since the beginning of 2014, the posts on this page has reached substantially more people than the basic amphibian research page. The best post reached over 54 000 people. Posts with emotional triggers and posts that are activity-related seems to have attracted the most attention, which can be explained by the relationship dogs have with humans (Bennett and Rohlf, 2007; Clark and Boyer, 1993). The majority of public interest for a post takes place within the first 20 days. It is therefore important to have regular updates at least once a fortnight (every 2 weeks) and more frequently when posts are not generating the anticipated interest. Public media also played a big role in the education/awareness component of the study, with newspapers and websites generating the biggest interest. At least 450 people were directly reached through practice demonstrations and presentations. Even though there is uncertainty about the proportion of public interest that will be turned into active conservation action, awareness and education is often the first step towards creating change through some level of community involvement (Warner *et al.*, 2014).

4.7. Concluding remarks

Unlike working with humans and machinery, working with a sniffer dog provides its own challenges, but also a lot of benefits. These advantages and disadvantages are briefly discussed below followed by recommendations for future projects.

4.7.1. Advantages

There are many advantages when working with a sniffer dog. First, a sniffer dog costs little to train and maintain and in most cases the dog provides a calming atmosphere when working with it. Second, medium sized dogs are also easy to travel with, to and from sites. The sniffer dog is able to cover larger areas in a short time and is the best tool for detecting and identifying scents (there is no better method). A dog is also easily rewarded with a toy or treats for doing extraordinary work. Lastly, after the dog is trained, it is reasonably easy to train on other species, as long as you have a sample scent, and also holds the possibility to train the dog on multiple scents. The potential for sniffer dog research is almost endless. Because dogs have a charismatic presence (Archer, 1997), it is also easier to generate public interest and receive media coverage.

4.7.2. Disadvantages

The disadvantages of using a sniffer dog, is that it requires patience and continued dedication from the trainer. It is always difficult when working with live animals, as they do get tired and frustrated, and it is important to be aware of these aspects in order to optimize training and better experimental results (Adams and Johnson, 1994).

Dogs also need to be taken outside for bathroom breaks when working long hours inside (this includes cleaning up after them). Their health, exercise, discipline, affection and nutrition have to be taken into consideration (Clark and Boyer, 1993). Because it is difficult to communicate with dogs, it is hard to determine when they pick up on the wrong (not the target) scents and other things that distract them. Because dogs can easily get bored, it takes continuous effort to keep training and exercise routines interesting and challenging for the dog.

4.7.3. Recommendations and future studies

Firstly, regarding the plank equipment that should be modified for future experiments. The ends of the plank equipment should be extended with at least 50 cm on each side for the dog to climb onto the plank before encountering the first target. A suggestion can also be made to have more distance between targets 5 and 6 where the two parts of the plank fit together.

Secondly, a better digging method is recommended for future field surveys on burrowing frog species, because most alternative invasive methods might cause injuries to the frogs. This component proved to be the most difficult during the entire study.

As conflicting interests occur between amphibians and amphibian diseases, a dedicated dog for amphibian disease detection should be used. Future research on amphibian disease detection should involve a dog that has not been trained on any other scents (especially not amphibian scent). Keeping this in mind and from our *Bd* trails, it would seem very plausible to train a new dog to indicate of infected frogs.

The sniffer dogs can have a tremendous positive impact on future research and conservation actions. Sniffer dogs can also be implemented for various aspects of environmental sciences, as this type technology is nowhere close to reaching its full potential. Every result helps to improve sniffer dog training methods and other applied problem areas. Using sniffer dogs to create awareness for other, often neglected conservation programs, seems to be a very effective strategy. This study will also serve as a stepping stone for other future related initiatives in wildlife conservation. It is also concluded that sniffer dogs can be a promising herpetological tool for future research, ecological studies, surveys and conservation strategies.

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6. Appendices

APPENDIX A: Marking sheet for experiments.

Plank

Key	
X	Good indication
O	Missed/inconclusive
Z	False indication
	Positive sample

Type of test: _____

Date: _____ Time: _____ Disturbances: _____

Test	Setup										Results		Comment	
											→	←	→	←
A														
B														
C														
D														
E														

Discussion:

Result:

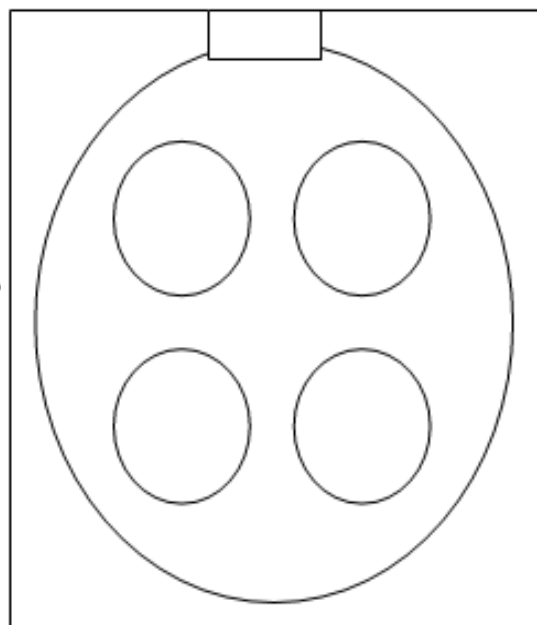
Sandbox

Key	
X	Good indication
O	Missed/inconclusive
Z	False indication
	Positive sample

Type of test: _____

Date: _____ Time: _____ Disturbances: _____

Test Setup:



Result	Comment
1.	
2.	

Discussion:

Result:

Type of test: _____

Date: _____ Start Time: _____ Disturbances: _____

Key	
X	Good indication
O	Missed/inconclusive
Z	False indication
	Positive sample

Test Setup:

Scent track information

Steps	
Shape	
End Time	
Weather	
Surface	

Discussion:

Result:

APPENDIX B: Examples of newspaper articles



SLIPPERY CUSTOMER: Esté Matthew and her border collie, Jessie, who is being trained to sniff out 'Pyxicephalus adspersus', the giant bullfrog

Picture: WALDO SWIEGERS

Dog takes leap of faith for frogs

Border collie being trained to protect threatened species, writes **Jan Bornman**

SHE is only six months old, but Jessie, a thoroughbred border collie, is already being billed as the potential saviour of the African bullfrog.

The offspring of two hard-working herding dogs, Jessie is being trained to sniff out the giant frogs, which are a protected species.

Experts believe they are gradually being wiped out by large-scale construction in Gauteng — and training Jessie could also pay off for developers.

But because a dog her age has a tendency to attack anything that moves, Jessie first needed some basic training.

We've got to make the right decision for conservation

She kicked off her year-long training stint with no more than a cup of tea last week — or, rather, several over a few days.

Her sense of smell is boosted with tea, which is presented more diluted each time as a way of managing her response to her first surprise encounter with the frog.

Using live frogs from the start might startle her and she might attack, said her Swedish trainer, Peter Bergman. "You must constantly challenge the dog, otherwise it will get bored and not cooperate," he said.

The idea to train Jessie came from her owner, Esté Matthew, a master's student in zoology at

North-West University, after she attended a lecture by Bergman last year.

Bergman, a geologist, has been using his five-year-old Alsatian, Rex, to do mineral exploration all over the world. He is helping Matthew and her supervisor, Professor Ché Weldon, to train Jessie and is also teaching them to train other dogs.

However, Bergman's first thought about the idea was: "A frog is a frog. Who gives a f**k about a frog?" But he eventually agreed.

But for Weldon, who works in the African Amphibian Conservation Research Group at the university, finding endangered frog species is not just about conservation.

He said developers were spending huge amounts of money on environmental-impact assessments, and sometimes projects folded as a result of the belated discovery of the creatures. Dogs were a much cheaper option in this regard.

"We've got to make the right decisions for both human development and the conservation of nature."

He said training dogs to help trace the frogs could also lead to breakthroughs in research into the Amatola toad, a species so rare that it was thought to be extinct between 1998 and 2011, when it was spotted for the first time in 13 years.

Comment on this: write to tellus@sundaytimes.co.za or SMS us at 33971

www.timeslive.co.za

Skaaphondjie se skerp reuksin help natuur bewaar

NUUSREDAKSIE

'n Jong skaaphondjie word tans opgelei om die grootbrulpadda uit te snuffel as deel van 'n meestersgraadstudent se navorsing aan die Noordwes-Universiteit se Potchefstroomkampus.

Die ses maande oue Jessie het pas met haar opleiding begin om dié paddas onder die grond te kan uitruik. Die Grootbrulpadda is tans 'n beskermde spesie in Gauteng en word bedreig deur verstedeliking.

Jessie se eienaar, Esté Matthew, vertel sy het met die idee vorendag gekom en ná 'n gesprek met haar studieleier, prof Ché Weldon, het hulle 'n program uitgewerk waarmee Jessie kan help met Esté se M-studie in dierkunde.

Esté sal later uitbrei na 'n PhD-studie om te help om ook ander bedreigde paddaspesies uit te snuffel. Die hondjie kan ook opgelei word om siektes op die paddas se vel te identifiseer.

Jessie woon by 'n vriendin van Matthew, aangesien sy in Wag-'n-

Bietjie-vrouekoshuis woon.

Esté het gesê hulle is baie verras oor hoe vinnig sy gevorder het. Hulle het aanvanklik met biltong in 'n houer begin en later 'n teemengsel aangemaak en dit stelselmatig verdun tot daar net een persent tee in die mengsel oor was.

Jessie kon dit steeds uitruik.



**Dit is net fenomenaal.
Ons het geen idee hoe
sensitief haar reuksin is
nie.**

Hulle het toe met paddavelreuk begin om te voorkom dat sy skrikkerig sou wees tydens eerste kennismaking met 'n lewendige padda. Esté het gesê Jessie kan nou al een tot 100 000ste van die reuk in 'n houer onder die grond uitruik.

“Dit is net fenomenaal en ons het geen idee hoe sensitief haar reuksin is nie.”

Jessie sal binnekort gebruik word om paddas in die veld uit te snuffel. “Daar is 'n nood om die brulpaddas op te spoor voor ontwikkeling plaasvind, anders kan dit gestaak word en ontwikkelers miljoene rande kos,” het Weldon gesê.

Jessie, met haar wakker ogies en waaiende stert, kan nog 'n waardevolle instrument word om meer inligting oor paddas te bekom om die spesies beter te beskerm.



Die beskermde grootbrulpadda.



Prof. Ché Weldon en Esté Matthew saam met Jessie, die ses maande oue skaaphondjie.

Slim hond ruik waar skaars paddas skuil

Claudi Mailovich

'n Handvol biltong en verdunde tee. Dít is wat jy nodig het om 'n hond te leer om brulpaddas uit te snuffel.

Jessie, 'n tien maande oue skaaphond, moet nog net twee weke lank "skoolgaan" voordat sy waarskynlik die eerste en enigste hond in Suid-Afrika sal wees wat spesifiek opgelei is om brulpaddas onder die grond op te spoor.

Dan sal Jessie haar baas, Esté Matthew (22), 'n meestersgraadstudent in omgewingswetenskap aan die Noordwes-Universiteit, kan help met haar navorsing oor die Suid-Afrikaanse brulpadda.

Esté sê die *Pyxicephalus adspersus* leef sowat 11 maande van die jaar onder die grond en ons weet bitter min van die spesie. Hoe diep onder die grond bly hulle? Watter soort grond verkies hulle? Wat is hul gedrag?

Dit is wat Esté graag wil weet. Sy sal net antwoorde kan kry met Jessie se gawe om dié paddas te vind ongeag waar hulle wegkruip.

Uiteindelik behoort Jessie ook 'n rol te speel in die bewaring van dié bedreigde spesie omdat sy nog voordat bouwerk begin 'n ontwikkelingsperseel sal kan deursoek om vas te stel of daar enige paddas skuil.

Die idee om 'n hond as deel



'n Vet brulpadda, die "prys" wat Jessie geleer is om uit te ruik.

van haar verhandeling te gebruik het by haar ontstaan nadat sy gehoor het hoe Peter Bergman, 'n Sweedse geoloog, vertel waarvoor hulle alles honde gebruik. Een daarvan was om longkanker te identifiseer.

"Ek het gedink as dit is waar toe hulle in staat is, dan sal 'n hond mos 'n ondergrondse brulpadda kan opspoor," sê Esté.

Haar studieleier, prof. Ché Weldon, moes die groen lig gee vir dié eksperimentele idee.

"Dit was baie riskant omdat ons nie geweet het of Jessie dit sou regkry nie. Maar sy het al ons verwagtings oortref."

Sy sê Jessie is aanvanklik met biltong en verdunde tee geleer om reuke op te spoor voordat sy geleer is om ook die reuk van 'n brulpadda se vel te identifiseer. Die brulpadda-reuk is mettertyd al hoe meer verdun totdat Jessie met sukses 'n reuk wat tot 'n honderdduisendste verdun is, kon identifiseer. Deesdae kan sy sonder aarseling twee brulpaddas opspoor wat in 'n toetsterrein van 25 m by 25 m versteek is deur op die gat te krap waar die padda skuil.

"Sy is nou deel van my *materials* en *methods*," sê Esté oor die papierwerk wat sy vir haar studies moet byhou en waar haar "baba" in dié skema inpas. Sy sê Jessie se werk is darem nie net tot paddas soek beperk nie. "Ek leer haar elke maand iets anders net sodat sy nie verveeld raak nie."

"Gewoonlik is skaaphonde meer 'n drukkie-soort-hond, maar sy's 'n 'ek wil iets gaan doen'-hond."



A young sheep dog is being trained to sniff out the Giant Bullfrog as part of the research of a master's student at the Northwest University's Potchefstroom Campus .

The six-month-old Jessie has just started her training to sniff out these frogs under the ground. The Giant Bullfrog is currently a protected species in Gauteng because its habitat is gradually shrinking due to urbanisation.

Jessie's owner, Esté Matthew, says she came up with the idea and after a conversation with her supervisor, Prof. Ché Weldon, they developed a programme in which Jessie could help with Esté's study in zoology.

Jessie comes from two working parents on a sheep farm near Bloemfontein. Because Esté lives in Wag-'n-Bietjie residence, Jessie is staying with a friend of Esté's. A research colleague of Weldon and geologist from Sweden, Peter Bergman, recently visited them to help with Jessie's finer training. Bergman's own dog, a German shepherd, was trained to help with mineral exploration.

Esté said they started her training two weeks ago and are amazed at how quickly she has progressed. They initially started with biltong in a container and later made a tea mixture, which they gradually diluted until there was only one percent tea in the mixture. Jessie could still sniff it out. They then started with frog-skin smell, so that she would not be anxious when she encountered a live frog for the first time.

Esté said Jessie can already sniff out one to 100 000th of the smell in a box under the ground. "It is simply phenomenal and we have no idea how sensitive her sense of smell is."

Jessie was initially trained in laboratory conditions and later in a controlled intermediate stage (mesocosm) in the outdoors. The next step will be to take her to the field in areas where they know bullfrogs occur. Weldon says the training is done in a scientific, reliable way and the same test is repeated up to forty times to ensure a reliable result.

Weldon says the African Bullfrog hibernates underground for up to eleven months of the year and is above ground for only about a month (usually November/December) to breed and to lay its eggs, if it rained sufficiently. "This makes it extremely difficult to find the frogs during the rest of the year. The frogs must be detected before development starts, otherwise the development can be stopped and this will cost developers millions of rands."

Weldon and his colleague, Prof. Louis du Preez, performed such an investigation for Transnet near Heidelberg in Gauteng two years ago, where they spotted the bullfrogs and could make the

right recommendations for the development in the area. "If we did not do the investigation in the breeding season, we would not have found the frogs."

The purpose of Jessie's training is to sniff out the bullfrogs in time, to help conserve the species and to explore the ecology around their habitat. Weldon says it will also help to determine a buffer area around the habitat, because there is no knowledge about the behaviour of the young metamorphs.

Weldon says Jessie will later also be trained to sniff out frog diseases, to help them in the diagnostic phase. Currently, frogs are caught and expensive and time-consuming molecular tests have to be done in laboratories to determine whether the frogs have certain skin infections. The fungi on the skin give off a distinctive smell, which Jessie's sensitive nose can possibly pick up.

He says Esté is focusing on the bullfrog for her MSc, but in the next phase, in a PhD study, her focus will extend to help sniff out other endangered species, like the Amatola Toad in the Hogsback area. The frog "disappeared" for twelve years, before a frog was seen again approximately three years ago."The species is not extinct, but is found in an extremely small area, and we may be able to use Jessie to search for other individuals or populations. She will help us to research the distribution and conservation status of the Amatola Toad."

Jessie, with her sparkling eyes and wagging tail, can still become a valuable tool for learning more about frogs, to protect the species better.

2014/04/10

#NWUPuk



Prof. Ché Weldon and Esté and Matthew with Jessie, the six-month-old sheep dog

APPENDIX C: Facebook Page - Jessie the Border collie

https://www.facebook.com/Jessie-the-Border-collie-710550039064555/?ref=hl



Jessie the Border collie



Jessie the Border collie

Home



Page

Messages

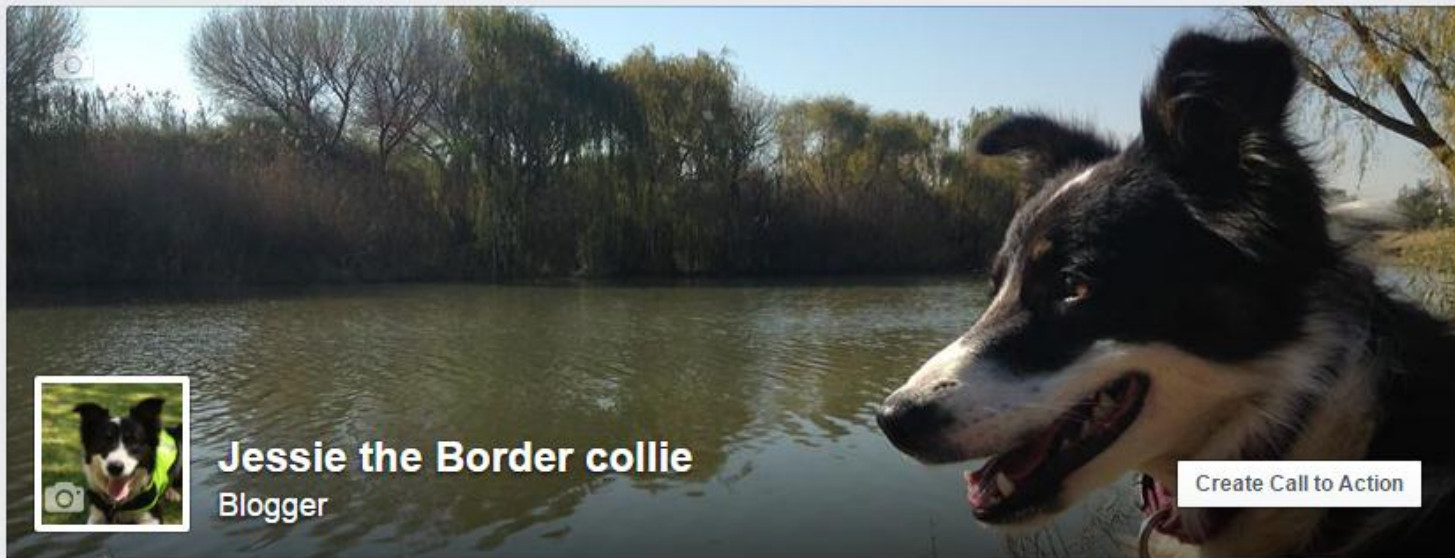
Notifications **3**

Insights

Publishing Tools

Settings

Help ▾



Jessie the Border collie
Blogger

Create Call to Action

Promote ▾

THIS WEEK

322
Post Reach

39
Post Engagement

Recent
2015

Timeline

About

Photos

Likes

More ▾

Share



306 likes 0 this week



322 post reach this week



View Pages Feed

See posts from other Pages



Reach a new milestone

500 Likes

Status

Photo / Video

Offer, Event +



Write something...



Jessie the Border collie added 3 new photos.

11 hrs · 🌐

I received some requests for more photos. Then I decided to post some pictures of me (and my furry family) from my holiday. As I am currently not

APPENDIX D: Of Dogs and Frogs: Sniffing out one of South Africa's most threatened amphibians



Jessie, the project dog, with her trainer Este Matthews searching for Giant bullfrogs. Photo: Jeanne Tarrant.

Of Dogs and Frogs: Sniffing out one of South Africa's Most Threatened Amphibians

By ¹Jeanne Tarrant, ²Ché Weldon, ³Este Matthews and ⁴Christine Coppinger

The Critically Endangered Amathole toad, *Vandijkophrynus amatolicus*, is one of South Africa's rarest frogs. Having disappeared for 13 years between 1998 and 2011, the species made it onto the IUCN's Lost Frog search campaign list in 2010. Despite a concerted search effort in August of that year (and in years prior), the toad remained AWOL and rumors of its extinction emerged. Then, in 2011 Jeanne Tarrant and Michael Cunningham turned up a single female as well as a few egg-strings and tadpoles in a near-by road puddle, sparking new hope for the existence of the species.

In 2012 a single male was located at a new location, and in 2013 a total of three Amathole Toads were found during the breeding season. So we know it's still out there, but compared to historical reports of hundreds of individuals per sighting, the Amathole Toad remains elusive. We've since developed an ecological niche model based on known records and ecological requirements and we are

using this to guide ongoing surveys.

Independently, the North-West University's African Amphibian Conservation Research Group (Potchefstroom, South Africa) has



Amathole toad (*Vandijkophrynus amatolicus*). Photo: Jeanne Tarrant.

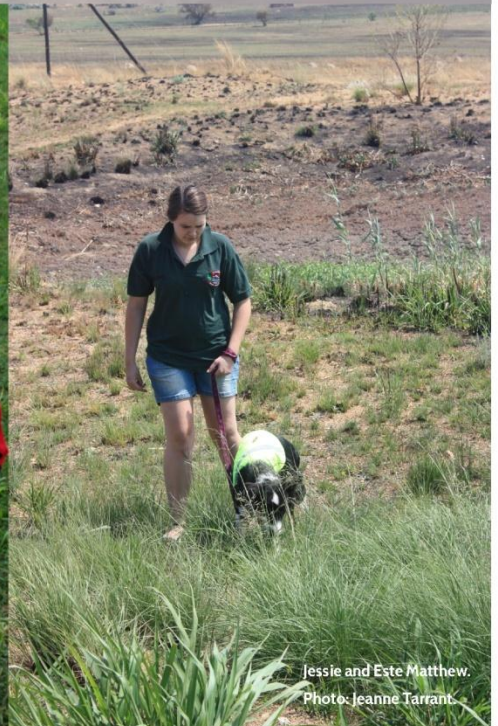
¹Threatened Amphibian Program, Endangered Wildlife Trust, Building K2, Pinelands Office Park, Ardeer Road, Modderfontein, 1609, South Africa. ²Unit for Environmental Sciences and Management, North-West University, Potchefstroom, 2520, South Africa.



Jessie. Photo: Jeanne Tarrant.



Jessie, Ché Weldon and Este Matthew.
Photo: Jeanne Tarrant.



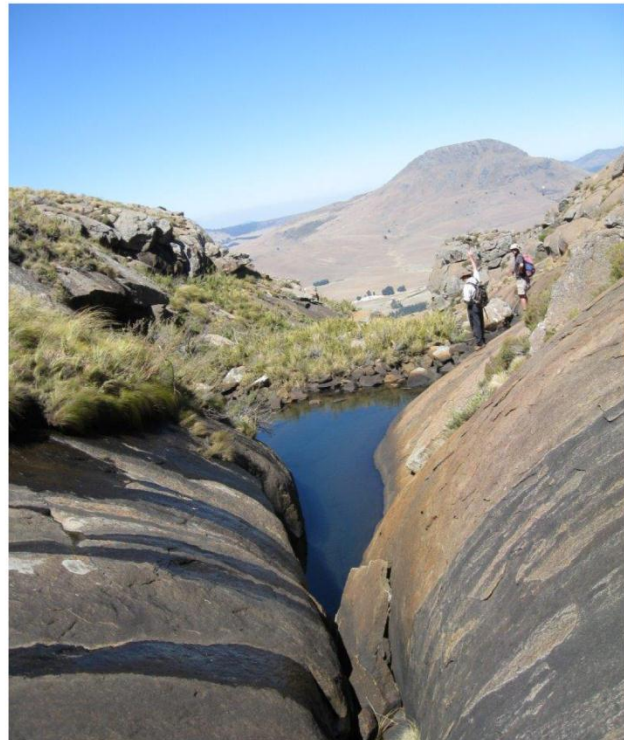
Jessie and Este Matthew.
Photo: Jeanne Tarrant.



Filming the Amathole toad. Photo: Jeanne Tarrant.

started investigating the use of sniffer dogs to search for fossorial frog species. They have successfully trialed this novel approach on the Giant bullfrog, *Ptychocheilus adspersus*, a species locally threatened in Gauteng Province. The aim of this project is to make use of this 'frog-sniffing' method to help in the search for the Amathole toad. This will be the first project of its kind in South Africa focused on a threatened frog species.

The project will take place within the toad's very limited range, restricted to the Hogsback Region of the Eastern Cape in South Africa. The sniffer dog, a frisky border collie called Jessie, will be trained prior to the trip based on scent samples obtained from an Amathole toad (or the closely related Karoo toad). Using our predictive model as a guide, we will focus our searches within areas of probable toad habitat, during the breeding season for this species with an initial field trip planned for February/March 2015). This project will provide the necessary information to more fully understand the status of the Amathole toad and to help prioritize sites for long-term protection, management and monitoring strategies. Conservation plans are currently being developed and will be made available to the relevant land-owners on whose property the species is known to occur.



Amathole toad habitat. Photo: Jeanne Tarrant.

APPENDIX E: ACRS 2015: Empowering the Future of Amphibian Conservation



More than 100 people attended the fourth ACRS, Cambridge, 2015. Photo: ???

ACRS 2015: Empowering the Future of Amphibian Conservation

By ¹Candace M. Hansen-Hendriks, ²James P. Lewis, ³Helen Meredith, ⁴Christopher Michaels, ⁴Rachael Antwis, ⁵Collin Van Buren & ⁴Chloe Weldon

The Amphibian Conservation Research Symposium (ACRS) is the only international series of conferences devoted to sharing research and strategies to empower the future of amphibian conservation. In 2015 ACRS was adopted, through a joint initiative, by the Amphibian Survival Alliance (ASA), the world's largest partnership for amphibian conservation and the IUCN SSC Amphibian Specialist Group (ASG), a global network of experts building a foundation for conservation. ACRS has remained true to its roots dating back to 2012 by bringing together amphibian conservationists and researchers from around the world to gain experience, learn new ideas and make contacts.

Each year individuals present talks and posters detailing evidence-based approaches and management strategies that promote amphibian conservation and this year was no different. Held between April 11th-12th at the University of Cambridge, ACRS 2015 was the biggest yet, with more than one hundred attendees from 55 institutions in 18 countries. The atmosphere over

the two days was buzzing with enthusiasm and energy as amphibian conservation practitioners and scientists discussed projects and planned for the future. With 17 presentations over the course of the symposium, there were a diverse array of topics covered including amphibian ecology, taxonomy, disease, captive husbandry and *in situ* management. These included talks presented by researchers like Gemma Harding (University of Kent and DICE, UK) who asked the question if amphibian reintroductions conformed to IUCN protocols and Edward Netherlands (North West University, South Africa) who assessed the effectiveness of Passive Acoustic Monitoring for determining species diversity at a locality when compared to traditional methods. The abstract program book can be downloaded [here](#).

Three key-note presentations were delivered covering three different, but equally important, areas of amphibian conservation. Sarah Thomas from the Zoological Society of London spoke on the importance of considering social aspects of conservation issues when establishing or assessing success of conservation interventions, such as what animals people are willing to help save or what social programs actually succeed in teaching people about

¹Amphibian Survival Alliance. ²IUCN SSC Amphibian Specialist Group.

³Zoological Society London. ⁴North-West University. ⁵University of Cambridge.

conservation issues. Rebecca Smith from the University of Cambridge highlighted the need for the right kind of evidence (*i.e.*, data collected from controlled studies to show what the effect of an intervention is when compared to a scenario where no intervention was undertaken) required to make decisions about the best management strategies and showed where to find it on the Conservation Evidence [website](#). Finally, Jennifer Luedtke, the Amphibian Red List Authority Deputy Coordinator, explained The IUCN Red List of Threatened Species in detail by showing what the different classifications mean and how easy it is for researchers to make much-needed assessments on their focal species by presenting a case study on the Red List status of Helen's Tree Frog (*Rhacophorus helenae*).

Talks were also given by three Future Leaders of Amphibian Conservation, the first cohort of a new program started at this year's ACRS, which aims to bring in up-and-coming leaders into the field of amphibian conservation who may not have otherwise been able to attend the event, enabling them to share their innovative work in the field. This year's Future Leaders were Arun Kanagavel from India, who presented his research as an EDGE fellow working on people's perceptions of amphibians in the Western Ghats; Esther Matthew from South Africa, who presented her research on the use of sniffer dogs as a new method for finding elusive species for monitoring; and Karla Pelz Serrano from Mexico, who presented both the social and biological work she is doing in Mexico on the Critically Endangered Lake Lerma salamander (*Ambystoma lermaense*). The attendance of these individuals at ACRS 2015 was made possible through funding from Women in Wildlife, Nordens Ark and Prof. Richard Preziosi from the University of Manchester.

In keeping with the new international theme of the symposium, ACRS 2016 is likely to be held in Potchefstroom, South Africa, in late January (details to be confirmed). This university town is home to North-West University, where the meeting will be hosted, and is located about 1.5 hours drive from Johannesburg, making it relatively accessible for Europeans, Africans, and other attendees from around the world.

ACRS 2015 would not have been the success it was without the generous support of Chester Zoo, the British Herpetological Society, VetArk Professional, Nordens Ark, Peregrine Livefoods, Arcadia, and Shepreth Wildlife Park.

As we look to the future, the ACRS Steering Committee would like to continue providing an annual award to early career conservationists as Future Leaders of Amphibian Conservation from around the world. This award not only provides the winners with an ability to present at the symposium, but, more importantly,

it provides them with an opportunity to network with other attendees and become Fellows of this new initiative. In addition to providing the support necessary for the award recipients to attend the symposium, it is our intention to also work with them to build a support network of experts, organizations and funders that will help them achieve their conservation goals and, most importantly, remain in the field of amphibian conservation for the long-term. We encourage you to be part of our long-term vision for ACRS and welcome your support for this initiative and these Fellows in future years. We are happy to [discuss these sponsorship opportunities](#) with you further.

We would also welcome general funding support for the symposium itself, which helps to keep the registration cost low, which is particularly important for students. Please contact the [ACRS Steering Committee](#) to start the conversation.

The future of amphibian conservation, research and education depends on what we do now as a community. ACRS provides all of us with a chance to work together and support the amphibian conservation leaders of tomorrow, helping to develop them into the confident, experienced and well-connected leaders we need to make a real difference for amphibians.

We would like to thank everyone who participated in this year's symposium and we hope you will join us in the years to come.



ACRS Future Leaders of Amphibian Conservation 2015. From left to right, Dr. Karla Pelz Serrano (Mexico), Arun Kanagavel (India) and Esther Matthews (South Africa). Photo: ???



ACRS

Amphibian Conservation Research Symposium

APPENDIX F: Of Dogs and Frogs: Sniffing out one of South Africa's rarest toads

Of Dogs and Frogs: Sniffing out one of South Africa's Rarest Toads

By Jeanne Tarrant, Ché Weldon, Esté Matthew & Christine Coppinger

The elusive Amathole Toad, *Vandijkophrynus amatolicus*, is very possibly one of South Africa's rarest seen frogs, despite in recent years, multiple and concerted efforts to find it (1). After a thirteen year absence of it being reported, the species was 'rediscovered' near Hogsback, Eastern Cape, South Africa in September 2011 (2). This discovery sparked renewed interest in the Amatole Toad and it has since become a focal species for the Endangered Wildlife Trust's (EWT), who are working with farmers and forestry companies in the area to conserve and manage known sites for the species. Active searching during the past five years has turned up just five individual adults of the Amatole Toad, which not surprisingly, is listed by the IUCN as Critically Endangered due to its limited distribution and loss of much of its original grassland habitat.

Pairing up with North-West University's African Amphibian Conservation Research Group and Jessie the frog-finding Border collie, we set off in early March 2015 to test out a novel method for tracking down the toad. The use of sniffer dogs for conservation is on the increase, including for reptiles and amphibians. Since no Amatole toads were available prior to the survey, the scent of the closely related Karoo Toad, *Vandijkophrynus gariepensis*, was used to condition Jessie for two weeks prior to the trip. Based on a probability model developed in 2013, suitable habitat for Amatole Toad was systematically surveyed using scent searches by Jessie and her handler Esté Matthew, while the rest of the team actively searched underneath rocks and logs and around seepage areas for the toads. The signs were promising, with the grasslands lush and green and many seepages (the toads' ideal habitat) spongy with recent rains. We encountered several species of other frogs and tadpoles and after three days finally turned up a small, lumpy toad crossing a path on the Katberg Pass, another historical site of the Amatole Toad. Much excitement from the team, but unfortunately the find was that of a Karoo Toad which shares many features with the Amatole Toad but differs slightly in its ventral and dorsal markings. Unfortunately, this was the sole toad found during the four day search, which can be largely attributed to the time of year. The Amatole Toad is active primarily from September to January and our continued efforts will be focused on that time of year and employ additional methods such as pit-fall traps and automated audio recorders to sample more thoroughly. Greater success can also be expected with the use of the sniffer dog when the dog is conditioned on the Amatole Toad's scent as well as a longer period of conditioning prior to the field trip. This project was made possible thanks to an Amphibian Survival Alliance seed grant, which allowed us to survey additional areas of our probability map and identify suitable sites for future searches.

References:

1. W. Conradie, J. Tarrant, J., *FrogLog* 97, 24 (2011).
2. J. Tarrant, M. J. Cunningham, *African Herp News* 55, 1-6 (2011).



Landscape of the Hogsback Mountains. Photo: Christine Coppinger.



Jesse and Esté in the field. Photo: Jeanne Tarrant.



Searching for toads. Photo: Jeanne Tarrant.

APPENDIX G: Supplementary information to results

Missed indications (MI)- complete results

Hieronder is frekwensies van jou data - hier lyk dit of 1 en 10 die minste foute het - omdat meer gebruik het nie

Category	Frequency table: Gat (MI Dilutions)			
	Count	Cumulative Count	Percent	Cumulative Percent
1	1	1	0.52910	0.5291
2	27	28	14.28571	14.8148
3	21	49	11.11111	25.9259
4	27	76	14.28571	40.2116
5	19	95	10.05291	50.2646
6	21	116	11.11111	61.3757
7	23	139	12.16931	73.5450
8	13	152	6.87831	80.4233
9	34	186	17.98942	98.4127
10	3	189	1.58730	100.0000
Missing	0	189	0.00000	100.0000

Category	Frequency table: rigting (MI Dilutions)			
	Count	Cumulative Count	Percent	Cumulative Percent
1	105	105	55.55556	55.5556
2	84	189	44.44444	100.0000
Missing	0	189	0.00000	100.0000

Hierbo nie vreeslik verskillend nie

Category	Frequency table: maand (MI Dilutions)			
	Count	Cumulative Count	Percent	Cumulative Percent
DT1	18	18	9.52381	9.5238
DT2	29	47	15.34392	24.8677
DT3	28	75	14.81481	39.6825
DT4	37	112	19.57672	59.2593
DT5	37	149	19.57672	78.8360
DT6	40	189	21.16402	100.0000
Missing	0	189	0.00000	100.0000

Hierbo sien ek trend dat dit lyk of foute meer word , hoe later dit word - geur verdwyn

Category	Frequency table: toets (MI Dilutions)			
	Count	Cumulative Count	Percent	Cumulative Percent
1	38	38	20.10582	20.1058
2	48	86	25.39683	45.5026
3	49	135	25.92593	71.4286
4	54	189	28.57143	100.0000
Missing	0	189	0.00000	100.0000

hierbo geen verskil nie

Hieronder raak ek ontslae van rigting - rigting2 se gat 1 word dan 10 ens

Category	Frequency table: Gat_rigting: =gat (MI Dilutions)			
	Count	Cumulative Count	Percent	Cumulative Percent
1	3	3	1.58730	1.5873
2	54	57	28.57143	30.1587
3	30	87	15.87302	46.0317
4	36	123	19.04762	65.0794
5	22	145	11.64021	76.7196
6	18	163	9.52381	86.2434
7	14	177	7.40741	93.6508
8	4	181	2.11640	95.7672
9	7	188	3.70370	99.4709
10	1	189	0.52910	100.0000
Missing	0	189	0.00000	100.0000

Hieruit lyk dit asof eerste 4 gate meer foute het as latere 6 gate - sien hieronder

Category	Frequency table: Eerste4 (MI Dilutions)			
	Count	Cumulative Count	Percent	Cumulative Percent
0	66	66	34.92063	34.9206
1	123	189	65.07937	100.0000
Missing	0	189	0.00000	100.0000

'n t-toets om te bepaal of bg % verskil van 50% kry ek 'n p-waarde van 0.00001 wat daarop dui dat daar definitief meer as 50% foute in eerste 4 gate as in laaste 6 is, bewys dan dat die foute nie 'completely at random' is nie, maar meer in eerste gate voorkom.

Overall results from preservation experiments. Before data was split to pick up on significant changes.



Media coverage- details

Date	Type	Publisher	Title	Link
13-Mar-14	Newspaper	Sunday Times	Dog takes leap of faith for frogs	http://www.timeslive.co.za/sundaytimes/2014/03/23/dog-takes-leap-of-faith-for-frogs
23-Mar-14	Newspaper	The Citizen	Dog sniffs out threatened frogs	http://citizen.co.za/148414/dog-sniff-threatened-frogs/
23-Mar-14	Website	Maroela Media	Hond opgelei om bedreigde paddas uit te snuffel	http://maroelamedia.co.za/blog/nuus/suid-afrika/hond-opgelei-om-bedeigde-paddas-uit-te-snuffel/
23-Mar-14	Radio	JacarandaFm	Time: 16:00	
23-Mar-14	Website	News 24	Dog sniffs out threatened frogs	http://www.news24.com/Green/News/Dog-to-sniff-out-threatened-frogs-20140323
24-Mar-14	Radio	OFM	Time: 8:00	
24-Mar-14	Website	OFM	Border collie trained to sniff out endangered Bullfrog	http://www.ofm.co.za/article/Local-News/143807/Border-collie-trained-to-sniff-out-endangered-bullfrogs
25-Mar-14	Website	The Dodo	<u>How One Border Collie Might Save The African Bullfrog</u>	https://www.thedodo.com/how-one-border-collie-might-sa-482341569.html
25-Mar-14	Website	iOL	Jessie to sniff out threatened frogs	http://www.iol.co.za/scitech/science/environment/jessie-to-sniff-out-threatened-frogs-1.1665788
10-Apr-14	Newspaper	Burger	Hond opgelei om paddas uit te ruik	http://www.netwerk24.com/nuus/2014-04-09-hond-opgelei-om-padda-uit-te-ruik?redirect_from=dieburger
10-Apr-14	Newspaper	Volksblad	Hond leer om brulpaddas uit te ruik	http://www.netwerk24.com/nuus/2014-04-10-hond-leer-om-padda-uit-te-ruik?redirect_from=volksblad
10-Apr-14	Website	Newsroom NWU	Sheepdog trained to help protect bullfrogs	http://potchnews.nwu.ac.za/n/en/269
15-Apr-14	Facebook	African Amphibian Conservation Research Group	Bullfrogs get helping paw (eNCA)	https://www.facebook.com/permalink.php?story_fbid=246627228855626&id=245279618990387
15-Apr-14	Television (+ Youtube)	eNews (Afrikaans)	Skaaphond snuffel paddas uit	https://www.youtube.com/watch?v=h1MvoqY1aUI
15-Apr-14	Television (+ Youtube)	eNews (English)	Bullfrogs get helping paw	http://www.enca.com/media/video/bullfrogs-get-helping-paw
17-Apr-14	Newspaper	Potch Herald	Skaaphond opgelei om brulpaddas te help bewaar	https://www.youtube.com/watch?v=h1MvoqY1aUI
22-Apr-14	Newspaper	Die Vryburger	Hond beskerm Brulpaddas	http://www.dievryburger.co.za/2014/04/hond-beskerm-brulpaddas/
28-Apr-14	Radio	PukFm	Time: 13:00	
29-Apr-14	Website	TV stream times	Search: Paddakoor	http://tvstreamtimes.co/stream/paddakoor
29-Apr-14	Television (+ Youtube)	Dagbreek	Dagbreek: Dagbreker Esté Matthew en Jessie	https://www.youtube.com/watch?v=VVljxAiRMTk
30-Apr-14	Radio	PretoriaFM	Time: 6:00	
9-May-14	Youtube	Pukke TV	Skaaphond brulpaddas bewaar	https://www.youtube.com/watch?v=zp5RXXV0Cww

29-Jul-14	Newspaper	Rapport	Slim hond ruik waar skaars paddas skuil	https://af-za.facebook.com/RapportWeb/posts/10152539433823895
9-Feb-15	Magazine	Froglog	Of Dogs and Frogs: Sniffing out one of South Africa's Most Threatened Amphibians	http://www.amphibians.org/wp-content/uploads/2015/02/FrogLog113_low.pdf
12-Apr-15	Twitter	Chytrid Crisis	Sniffing out endangered #frogs with Jessie the border collie in South Africa - awesome idea! #ACRS2015	https://twitter.com/chytridcrisis/status/587209370158641154
25-Apr-15	Website	Chytridcrisis	Using Sniffer Dogs to Track Down Endangered Amphibians (and Chytrid?)	https://chytridcrisis.wordpress.com/
8-Jun-15	Website	www.amphibians.org	Amphibian Champion: Esté Matthew	http://www.amphibians.org/news/amphibian-champion-este-matthew/
13-Aug-15	Television	Supersport	Varsity football (halftime 19:15) NWU vs NMMU	

On post results for *Jessie the Border collie* page (from created date to 1October 2015)

Ranking	Date	Description	Type	Pictures	Likes	Comments	Shares	Total (LCS)
1	01-Jul-2015	Dog nose photo (secret weapon)	Emotional trigger	1	96	5	172	273
2	23-Jul-2015	Found first wild bullfrog	Work related	5	89	15	60	164
3	07-Apr-2015	Trainer in leaving	Emotional trigger	1	47	4	0	51
4	01-Oct-2015	Throwback photo (3months)	Emotional trigger	1	37	1	0	38
5	03-Sep-2015	Going to vet	Emotional trigger	1	34	3	0	37
6	03-Sep-2015	Update on experiments	Work related	2	31	2	0	33
7	14-Feb-2015	Live frog experiments	Work related	5	27	3	0	30
8	31-Jul-2015	Demonstration for school	Educational/Awareness	1	25	2	2	29

9	07-Mar-2015	Proof of posting	Emotional trigger	1	29	0	0	29
10	11-Sep-2015	Article on fieldwork	Work related	1	27	1	0	28
11	04-Sep-2015	Recovering from surgery	Emotional trigger	1	28	0	0	28
12	24-Apr-2015	Throwback photo (3months)	Emotional trigger	1	25	2	0	27
13	07-Mar-2015	Information: Amathole	Work related	2	25	2	0	27
14	09-Sep-2015	Recovering from surgery	Emotional trigger	1	25	1	0	26
15	24-Aug-2015	Update on experiments	Work related	1	24	0	0	24
16	29-Jun-2015	Working on Campus	Emotional trigger	1	24	0	0	24
17	01-Apr-2015	Not impressed face: photo	Emotional trigger	1	20	4	0	24
18	10-Feb-2015	Student demonstration	Educational/Awareness	0	22	2	0	24
19	04-Jun-2015	Throwback photo (3months)	Emotional trigger	1	23	0	0	23
20	18-Sep-2015	Social interaction with other dogs	Miscellaneous	1	22	0	0	22
21	30-Jul-2015	Working and playing	Emotional trigger	1	21	1	0	22
22	14-Mar-2015	Fieldwork : Eastern Cape	Work related	10	20	0	2	22
23	12-Mar-2015	Enjoying view from mountain	Emotional trigger	1	21	1	0	22
24	17-Feb-2015	Funny photo	Emotional trigger	1	22	0	0	22
25	28-Jan-2015	Introduction	Emotional trigger	1	22	0	0	22
26	15-Sep-2015	2 year Birthday	Emotional trigger	2	21	0	0	21

27	02-May-2015	Focus photo	Miscellaneous	1	20	1	0	21
28	01-Sep-2015	Video of lab experiment	Work related	1	19	1	0	20
29	17-Jun-2015	Action camera video	Work related	1	18	0	2	20
30	11-Jun-2015	Fieldwork: Ventersdorp	Work related	1	20	0	0	20
31	15-May-2015	Obedience training	Work related	1	19	1	0	20
32	10-May-2015	Mother's Day	Emotional trigger	1	19	1	0	20
33	24-Jun-2015	Social interaction with other dogs	Emotional trigger	1	17	1	0	18
34	15-Jun-2015	Sponsor: harness	Miscellaneous	2	18	0	0	18
35	22-May-2015	Video eNews (April 2014)	Work related	1	17	1	0	18
36	21-Aug-2015	Funny picture about Border collies	Miscellaneous	1	16	1	0	17
37	07-Feb-2015	Update on work	Work related	3	17	0	0	17
38	15-Aug-2015	Keeping people out of work	Emotional trigger	2	15	0	1	16
39	22-May-2015	Social interaction with other dogs	Emotional trigger	1	16	0	0	16
40	20-Jul-2015	Post of food	Miscellaneous	1	14	1	0	15
41	08-May-2015	Matching scent detection	Work related	4	15	0	0	15
42	04-May-2015	Trainer returns	Emotional trigger	1	15	0	0	15
43	28-Feb-2015	School demonstration	Educational/Awareness	1	15	0	0	15
44	19-Feb-2015	Swimming	Emotional trigger	1	15	0	0	15
45	29-Mar-2015	Selfie	Miscellaneous	1	14	0	0	14

46	17-Mar-2015	On Holiday	Emotional trigger	1	14	0	0	14
47	23-Feb-2015	Social interaction with other dogs	Emotional trigger	4	14	0	0	14
48	09-Jul-2015	Fieldwork: Diepsloot	Work related	2	13	0	0	13
49	12-May-2015	Update: Overall	Miscellaneous	1	13	0	0	13
50	31-Jan-2015	Training	Work related	1	13	0	0	13
51	29-Jan-2015	Playing frisbee	Miscellaneous	2	13	0	0	13
52	08-Jun-2015	Scent detection: Bullfrog	Work related	2	12	0	0	12
53	22-May-2015	Video Dagbreek (April 2014)	Work related	1	11	0	0	11
54	26-May-2015	Update on experiments	Work related	1	10	0	0	10
55	04-May-2015	Finding bullfrog scent in garden	Work related	4	10	0	0	10
56	02-Feb-2015	Vistor form UK	Emotional trigger	5	10	0	0	10
57	24-May-2015	Playing frisbee	Emotional trigger	1	9	0	0	9
58	18-Feb-2015	Update on experiments	Work related	1	8	0	1	9
59	20-Mar-2015	Thanking sponsors	Miscellaneous	4	7	0	0	7
60	26-Feb-2015	School demonstration	Educational/Awareness	1	5	2	0	7
61	12-Apr-2015	Random fieldwork video	Miscellaneous	1	6	0	0	6
62	11-Feb-2015	Froglog magazine	Work related	1	4	1	0	5
63	06-Aug-2015	Social interaction with other dogs	Emotional trigger	2	3	0	0	3
64	02-Mar-2015	Agility	Work related	3	3	0	0	3

APPENDIX H: Videos and photos (Digital media)

Dagbreker: Esté Matthew en Jessie

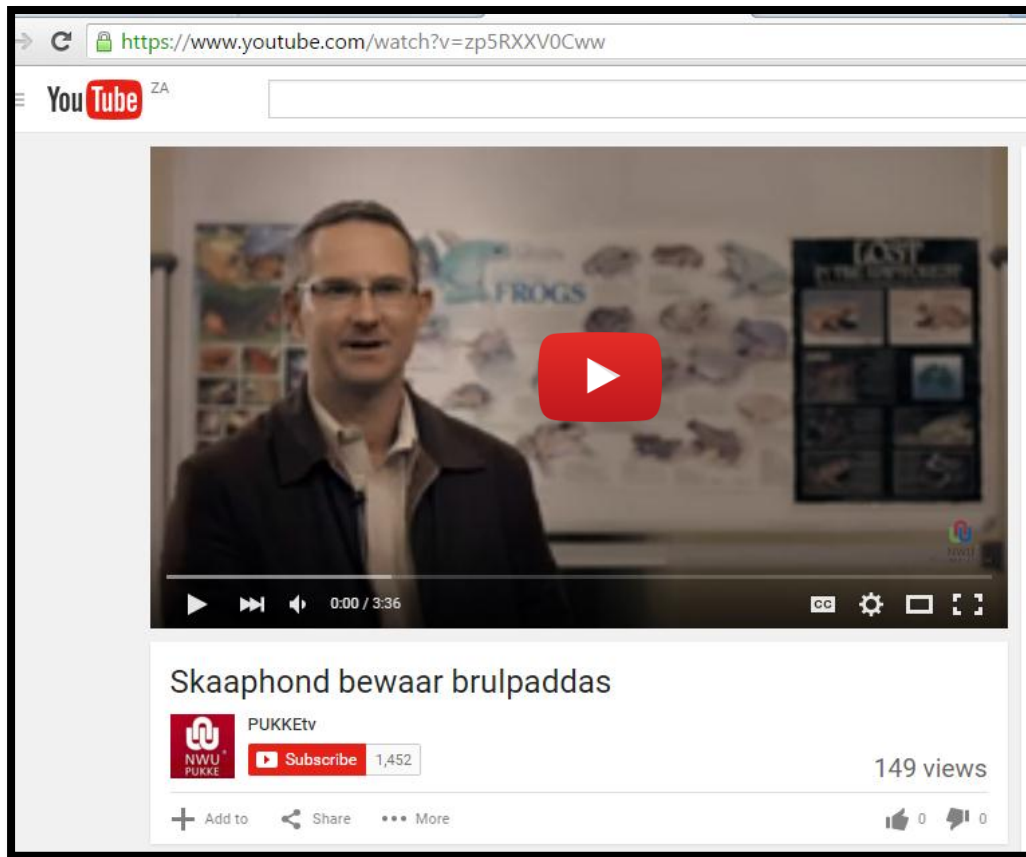


The video is a live interview in Afrikaans. It is almost 7minutes long and contains footage of a restless Jessie, her trainer and a news reporter, Riaan Cruywagen. The interview was about how we started with the project, why bullfrogs are important and more information about Jessie was revealed.

The video is available at the follow link:

<https://www.youtube.com/watch?v=VVljxAiRMTk>

Skaaphond bewaar brulpaddas



The video is a interview in Afrikaans. It is almost 4minutes long and contains footage of Jessie, and interviews with her trainer and the project supervisor (Prof. C. Weldon). The interview was recorded by the media department of the North-West University. This video also contains footage of Jessie training on the wooden plank equipment as well as in the sandbox.

The video is available at the follow link:

<https://www.youtube.com/watch?v=zp5RXXV0Cww>

Bullfrogs get a helping paw



The video is a news interview in English. It is almost 2minutes long and contains footage of Jessie, and interviews with her trainer and the project supervisor (Prof. C. Weldon). The interview was recorded by the eNews. This video also contains footage of Jessie training on the wooden plank equipment as well as in the sandbox.

The video is available at the follow link:

<http://www.enca.com/media/video/bullfrogs-get-helping-paw>

Many more photos and some additional videos are available on the *Jessie the Border collie* Facebook page:

<https://www.facebook.com/Jessie-the-Border-collie-710550039064555/>