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SPECIAL ISSUE: DOGS AND CONSERVATION: CURRENT AND EMERGING CONSIDERATIONS

A case study in canine detection of giant bullfrog scent

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Abstract. Accurate survey methods are required for any wildlife research to yield reliable population data. This constraint finds significance in amphibian research that involves a highly threatened group of animals with a large proportion of cryptic species not easily detected by conventional survey methods. Across a growing spectrum of zoology research, survey outcomes are benefitting from the efficacy of scent detection dogs in assisting with species detection. We investigated the ability of a scent detection dog to locate and identify traces of giant bullfrog, *Pyxicephalus adspersus* scent and investigate methods of preserving frog scent for use in subsequent conditioning training of dogs. The scent detection dog was able to detect 100,000 times diluted scent with 87% sensitivity and 84% efficacy. High specificity (98,6%) was also achieved while presented with the challenge of detecting *P. adspersus* scent amid that of other frog species. Detection sensitivity was negatively correlated with scent preservation time but yielded the highest sensitivity for samples that were preserved as skin swabs stored at 4 °C and diluted shortly before use. Conservationists, scientists, and customs officials alike can benefit from scent detection dog detection of amphibians through enhanced sample acquisition rates with reduced collection biases.

Key words: anurans, improved location, olfaction, positive reinforcement, sniffer dog

Introduction

Wildlife surveys are a fundamental component of field biology that yield data required to answer research questions in taxonomy, ecology, conservation, and epidemiology. The selection of survey methods will directly influence the accuracy and comprehensiveness of survey outcomes. For this reason, the need for standardization of field techniques has been echoed by several authors across various taxonomic disciplines (e.g. Heyer et al. 1994, Adis et al. 1998, Garden et al. 2007). Because most amphibians have terrestrial and aquatic life cycle phases, and have a tendency to hibernate, the use of a single survey method limits the ability to obtain comprehensive community data.

A few methods that are often recommended as standard field techniques for amphibians include visual encounter surveys, acoustic monitoring surveys, pit-fall traps or other traps, and dip-netting for larvae (Fellers & Freel 1995, Doan 2003, Rödel & Ernst 2004). However, there are many examples of cryptic amphibians that could easily be overlooked

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or underrepresented using these techniques such as non-vocal species, species lacking an aquatic phase, predominantly fossorial species, and extremely rare species. Two types of limitations can result from conventional surveys of species that fit this description: 1) under-representation of individuals in a population often skews population estimates; and 2) non-random distribution of observer effort leads to biased data (McKenzie et al. 2006, Crall et al. 2011). Conventional surveys are also conducted using time-constrained or area-constrained techniques amid the global amphibian decline phenomenon that necessitates accurate and timely data generation (Campbell & Christman 1982, Corn & Bury 1990). Global amphibian populations have shown a concerning decline over the past three decades, which have led to species extinctions in some instances. This has resulted in a greater percentage of amphibians being threatened than any other vertebrate group (Beebee & Griffiths 2005, Grant et al. 2016). Nevertheless, the advancement of survey methods and equipment has generated an increase in amphibian discoveries and knowledge of their ecology (Yetman & Ferguson 2011). Consequently, the current world count for amphibian species stands at 8211, with new species still being discovered (Frost 2020). However, a lack of biological information (such as anatomy, behaviour and distribution) inhibits mitigation efforts of amphibians that are going extinct. Nori et al. (2018) determined that targeted surveys from only a small fraction of the world area could yield biological information to resolve the conservation status of 80% of data deficient amphibians. Applying efficient biological data collection methods is paramount to achieving a more effective conservation strategy for amphibians worldwide.

Dogs formally trained in scent detection methods and used with systematic search tactics, offer biologists an effective, alternative method for locating wildlife or other biological scents, such as scat and plant species (Hurt & Smith 2009). Canine olfactory detection of biological scents is based on the principle that organisms produce characteristic volatile organic compounds that are detected by the canine olfactory system. Depending on the breed, a dog's sense of smell is estimated to be up to 10,000 times more enhanced than a human's ability (Craven et al. 2007). Consequently, conservation dogs (dogs trained to detect wildlife scents, e.g. scat) are used for locating countless protected native species and searching for introduced pest species from major vertebrate groups including mammals (Arnett 2006, Beckmann 2006), birds (Homan et al. 2001, Browne & Stafford 2003), and reptiles (Vice & Engeman 2000, Cablk et al. 2008), as well as several introduced insect species (Lin et al. 2011, Hoyer-Tomiczek et al. 2016). Notwithstanding that area surveyed and efficiency of a survey can be greatly improved by using scent detection dogs as opposed to conventional survey methods this method remains relatively unexplored for the detection of amphibians, especially in peer-reviewed literature. Encouraging though is that several recent reports on social media have indicated that training is underway and scent detection dogs are being deployed in amphibian conservation programs (unpublished data). These programs include the detection of frogs e.g. baw baw frogs *Philoria frosti* (Spencer, 1901) in Victoria Australia, salamanders e.g. Jemez mountain salamander *Plethodon neomexicanus* (Stebbins & Riemer, 1950) in New Mexico USA, and newts e.g. crested newts *Triturus cristatus* (Laurenti, 1768) in the UK.

Training dogs for scent detection typically requires operant conditioning; a method by which consequences of initially spontaneous behaviour may reinforce or inhibit the recurrence of that behaviour (Blackman 1983). Reward-based training, is widely regarded as the best way to train dogs (Blackman 1983, Geller 2008, Hiby et al. 2004). This study evaluated the potential for using a scent detection dog to detect the presence of the giant bullfrog *Pyxicephalus adspersus* (Tschudi, 1838) in laboratory conditions. Even though, this burrowing species is listed as Least Concern by the IUCN due to it having a wide distribution, in the Gauteng Province its numbers have declined due to severe degradation of its habitat (Van Aardt & Weber 2010, Thomas et al. 2014). Given these circumstances, *P. adspersus* is 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. However, because of their fossorial behaviour, bullfrogs can easily be missed during a single-event biodiversity inventory survey (Yetman & Ferguson 2011), making this species a good test case for testing the efficacy of sniffer dog detection. We used operant conditioning to train our dog to indicate on giant bullfrog scent in scent line-ups. We quantified the sensitivity and efficacy of the dog to detect a) diluted and b) preserved bullfrog scent. We also determined the specificity for discriminating the scent of giant bullfrogs and discuss some practical considerations for dogs to detect amphibians in nature.

Material and Methods

Collection and husbandry of amphibians

Wild-caught frogs were kept in captivity to ensure that fresh scent was readily available for experimental scent detection trials. Two adults of our positive target species (*P. adspersus)* were collected on a farm near Ventersdorp, and two adults each of the semi-aquatic Delaland's river frog, *Amieta delelandii* (Duméril & Bibron, 1841) and the aquatic African clawed frog, *Xenopus laevis* (Daudin, 1802) were collected from Potchefstroom, North-West Province (Permit no. 028NW-11). Also, two adult terrestrial flat-backed toads, *Sclerophrys pusilla* (Mertens, 1937) were collected from Ndumo Game Reserve (permit no. OP526/2014). The three negative target species were selected because they represent three different habitat types and occur sympatrically with *P. adspersus*. Their selection would, therefore, be transferable should *P. adspersus* detection by dogs be tested *in situ*. Frogs were kept in pairs, by species, in glass aquaria with a coarse sand substrate and enriched with a water bath and PVC pipe for shelter. Frogs were fed live crickets three times a week and the enclosures cleaned once every week. Frogs were handled with disposable latex gloves to prevent contamination with human scent. Frog scent was collected on a sterile cotton swab by first rinsing the frog in slow running, filtered, tap water, followed by gently stroking the dorsal and ventral skin with the swab for 10 seconds.

In accordance with South African legislation on the use of animals in research, no part of this study included any harm to any animal. This study was approved by the Biodiversity and Conservation Ecology Ethics Advisory Committee in 2014. Where possible, dilutions and other scent samples (such as swabs) were used to minimize the need for handling frogs, and to reduce the number of individuals required. The detection dog also never came into direct contact with the frogs due to the non-visual training techniques. All animals were kept in captivity following this study and were used for other research, as well as educational purposes, by the African Amphibian Conservation Research Group, at the North-West University.

Detection dog

Our dog was selected from a litter of sheep herding Border collies based on her temperament, strong motivation to learn, boldness and high drive; all qualities that indicate trainability for scent detection work. A "drive" (prey drive) can be described as an inborn predator behaviour (Marschark & Baenninger 2002), which is a beneficial characteristic for training of working dogs. Trainers should take precaution to avoid the dog from touching the target species (encourage passive indication) in order to prevent harm to the target during field surveys as a result of the prey drive. The boldness test by Svartberg (2002), indicates that dogs that are bolder can achieve higher performance as working dogs. Our Border collie was one year old when we conducted the research and was the only dog used as an experimental tool in this study. We only used one dog because we treated the research as a case study to test our methodology, but all tests were designed to be replicable. Our detection dog worked no more than three hours a day. This was divided into a 90 min morning session and 90 min afternoon session, with at least one hour break in-between. During experiments, the dog always had access to clean water, and received a reward (such as treat or toy) for indicating on the positive target scent, hidden in containers to simulate a buried frog. Our detection dog also received high-quality food daily and lived with the handler (Clark & Boyer 1993).

Scent detection training

We used the principles of operant conditioning as a mechanism for the scent detection dog training (Blackman 1983). The dog was taught to search for and indicate on target scents that started with

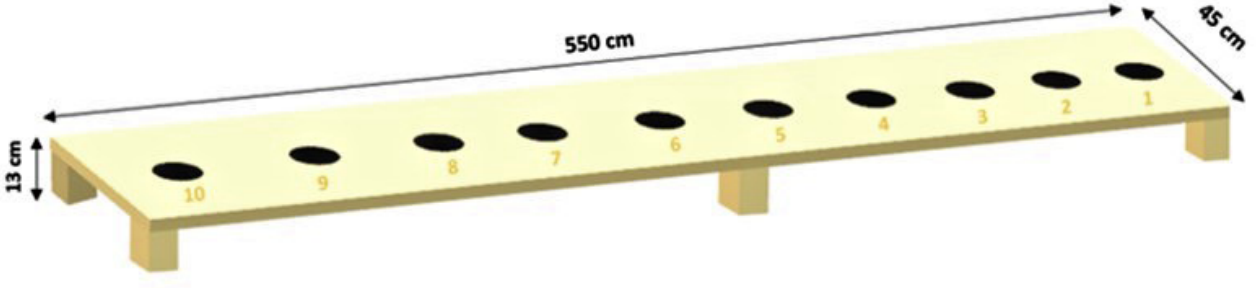

Fig. 1. Platform plank structure measurement and design. The holes for containers were evenly spaced (53 cm apart).

Table 1. Terminology related to training of a scent detection dog; as used in this study.

Term	Description
Indication	An operant conditioned response (behaviour), such as sitting, pointing, or lying down presented by the detection dog, directed at the location of a target.
Targets	Defined as all the possible locations where samples could be hidden. This includes negative- and positive targets.
Positive target	A target location containing the scent sample the dog is being trained on (example: bullfrog scent). The dog is trained to show an indication for this target.
Negative target	A target location containing scent that does not match the positive target nor that of the controls. These can also be seen as a type of disturbance.
Control	Controls refers either to clean (empty) target containers or containers with distilled water in. Water controls were used in live-frog and dilution experiments, as the positive targets also have a water component.
Disturbance	Can be anything that is seen as a distraction or obstacle for the dog during training or testing. These include other scent samples (such as negative targets), weather conditions and the presences of other humans or animals.
Miss	A lack of indication on a positive target for whichever reason.
False indication	Refers to an indication made on a negative target.
Test run	One test run is a session where the dog examines 10 targets on the platform, in one direction. This provides the dog with one opportunity to indicate on the positive target. The dog is also scored once. After each run, all the targets are rearranged.

treats, after which the dog was introduced to the target species odour (*P. adspersus* scent). Definitions of common terminology used in in the training methodology of this study are summarised in Table 1.

A raised platform (used as a training aid) with evenly spaced, 6 cm diameter holes was used to create a "false bottom". The platform allowed 10 spaces for positive- and negative targets to be concealed in containers below the work surface

Fig. 2. Platform indicating how containers with breathable lids were placed below the surface and how the platform was used by the detection dog for operant conditioning and during tests.

(Fig. 1). The equipment was specifically designed keeping in mind that *P. adspersus* was a burrowing species, and thus the platform did not provide the dog with any visual confirmation. Using 10 target spaces made quantification of data easier and was consistent with other studies that made use of similar training aids (Fischer-Tenhagen et al. 2011, Johnen et al. 2013). For experimental purposes, the holes were numbered 1 to 10. Thus, one run was performed by navigating the scent detection dog across numbers 1 to 10.

The positive target odour was placed in one of the containers while the remainder of containers were left empty. The dog was guided to investigate each opening of the raised plank and encouraged to smell (Fig. 2). The dog was trained to indicate by touching the lid of the target container with her paw and assuming a sitting position. Concurrently, negative punishment (removal of the reward) was employed when the dog indicated on a negative target, resulting in a decrease in that behaviour. Clicker training was used in conjunction with the reward to increase the dog's precision when indicating the positive target during platform training (Cornu et al. 2011). Detection of a target sample was positively reinforced through clicker training, where the "click" sound was followed by a reward (small pieces of food or a play object) used as conditioned secondary reinforcer to increase the dog's behaviour, as part of operant conditioning (Fjellanger et al. 2002, Cornu et al. 2011). The dog quickly learned to associate target species' sample detection with the reward, which sustained a strong motivation level for the dog to locate the scent again (Wasser et al. 2004).

Frog scent detection tests

Each test consisted of two or more variations (e.g. scent dilution sensitivity, consisted of six different dilutions) and each variation was replicated at least 20 times. Scent from frogs was collected by swabbing their ventral and dorsal surfaces for 10 seconds. There was always only one positive target (*P. adspersus* scent) during each test run. The other nine targets consisted of negative targets and controls. For all types of test, all of the targets were switched by volunteers to a random, non-patterned position, between every test run. Care was taken to touch all of the 10 containers during switches while wearing disposable gloves in order to eliminate human odour as a possible confounder. The platform and containers were also cleaned with 70% ethanol wipes between each run. The detection dog and handler left the training room during switches to ensure a double-blind setup.

Scent dilution sensitivity test

A fresh *P. adspersus* skin swab was allowed to soak in 1 ml of distilled water for 1 min, which served as the positive target concentrate (1:1). Five consecutive exponential dilutions were then prepared with distilled water (1:10-1:100000). Each of the six concentrations served as a different treatment within the dilution test and consisted of 30 test runs, where each test run had two positive targets in the line-up. The eight negative target containers contained distilled water only and served as the controls. The dog was scored for each positive target independently, thus receiving a score of either 0, 1 or 2 per test run. This accounted for 60 positive targets and 240 negative targets for each dilution treatment, and a total of 1800 possible targets (with 360 positive targets in total) for all six dilution treatments.

Species identification test

The test for species-specific detection had two treatments, one performed with diluted frog scent (1:500) and the other using live frogs as targets. For the tests, each test run of the variation with diluted scent consisted of one container with diluted *P. adspersus* scent. The remaining nine target containers consisted of three negative targets – one diluted scent (1:500) each of *A. delalandii*, *S. pusilla*, and *X. laevis*, and six containers that served as controls, filled with distilled water only. For the live frog tests, each test run consisted of one container with a live *P. adspersus* as a positive target, in 20 ml of distilled water. Similarly, the three negative targets contained one frog each of *A. delalandii*, *S. pusilla*, and *X. laevis* in 20 ml of distilled water. The remaining six controls were filled with 20 ml of distilled water only. Each run, therefore, included all four frog species, and the dog was expected to discriminate between species and only indicate on the positive target. The other three species were regarded as disturbances. Each test run for treatments was replicated 100 times accounting for a total of 100 positive targets (out of a possible 1,000 targets) per variation. Specificity towards target species was calculated for this test only in addition to other calculations (see section on data analysis).

Scent preservation test

For this test we made use of four preservation treatments prepared in the following way. Twelve

replicate 1:1000 dilutions of *P. adspersus* scent samples were prepared in 50 ml plastic centrifuge tubes; six replicates were stored at 4 °C (diluted treatment) and six were stored at –20 °C (frozen treatment). Twelve skin swabs from the same individual were also stored dry at 4 °C; six of these swabs were used as a direct target (dry swab treatment), while six were diluted 1:10 (diluted swab treatment) 30 minutes before use. This accounted for six samples per treatment, and a total of 24 samples. Once a month, for six months, one sample from each of the four preservation treatments was subjected to scent detection testing and represented the positive target. Before the start of each test, the samples were acclimated at room temperature (20 °C) for 30 minutes. Each treatment of the preservation method test was replicated 20 times (20 runs), accounting for 20 positive targets out of 200 possible targets for each of the six months. This accounted for a total of 480 positive targets (20 positive targets × 4 preservation treatments × 6 months), out of a possible 4800 targets. The positive target was thus the scent sample (*P. adspersus* scent), while the nine remaining target containers were the controls, filled with distilled water when positive targets were derived from diluted swabs, and contained only sterile swabs when positive targets were derived from dry swabs.

Data analysis

Sensitivity and efficacy were calculated for all three tests as a proxy for the success of the dog's effort (Marschark & Baenninger 2002). A *Correct Indication* was recorded if the dog alerted the handler and indicated at the positive target location. A *Miss* was recorded if the positive target was not detected (no indication), while an *Incorrect Indication* referred to any indication made at a negative target location.

We defined sensitivity as the accomplishment of the purpose, namely to find the positive target. Sensitivity was determined by the number of correct indications compared to the number of positive targets, calculated as *Correct Indications/Positive Targets*. Misses were accounted for by the number of ignored positive targets compared to the total number of positive targets. This was also seen as an indication of the reliability of the dog to detect the target scent.

We considered effectiveness as the degree to which the dog successfully achieved the correct indications, while accounting for the number of incorrect indications in relation to the number of positive targets. Effectiveness was thus calculated as *Correct Indications/(Positive Targets + Incorrect Indications)*.

Lastly, we determined specificity towards the target scent when other frog scents (*Disturbances*) were present by determining the number of positive targets correctly identified by the dog, and the number of indications on negative targets in relation to the total number of positive targets, thus accounting for *Disturbances* ignored (true negatives). Specificity was thus calculated as *Positive targets/(Correct indications + Incorrect Indications)*.

GraphPad Prism™ was used to analyse the data. Correlation analysis was used to evaluate the relationship in sensitivity between the variations within tests. One-way ANOVA calculations were used to compare the different tests and determine the statistical differences between the tests in terms of sensitivity and efficacy. One-way ANOVA comparisons were also used to determine the significant difference in sensitivity over time, regardless of the preservation method.

Results

The scent detection dog was able to detect *P. adspersus* scent samples that had been diluted up to 100,000 times. Detection sensitivity for *P. adspersus* scent varied between 72 and 87% for the various diluted samples, but sensitivity was not correlated with dilution in any way ($R^2 = 0.01$, Table 2). On average 21% of positive targets were missed for the combined dilutions. The efficacy of detecting diluted scent was slightly more variable and ranged between 64 and 84%. However, no statistically significant differences existed for either the sensitivity ($p = 0.467$) or efficacy ($p = 0.241$) for detecting diluted scent, and the highest detection success was achieved for the 1:100000 dilution. A difference of less than 10% between overall sensitivity and efficacy towards diluted scent suggests that incorrect indications are not a significant factor in quantifying overall performance.

When presented with the challenge of detecting *P. adspersus* scent amid other amphibian scents (species identification test), including *A. delalandii*, *S. pusilla* and *X. laevis*, specificity for *P. adspersus* was 99% regardless of whether diluted frog scent or live frogs were used as negative targets/

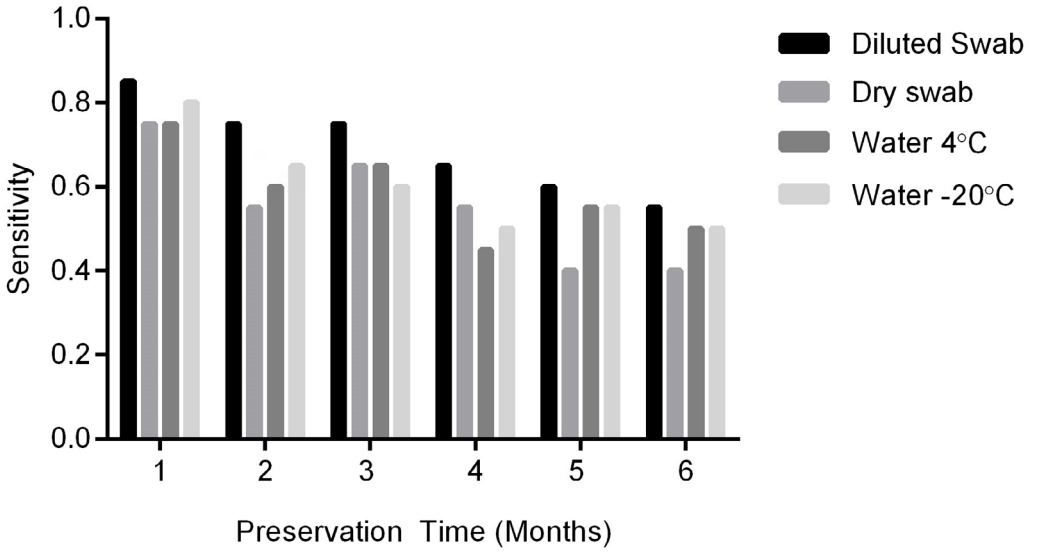

Fig. 3. Detection sensitivity of *Pyxicephalus adspersus* scent that has been preserved through various methods over six months.

disturbances. The scent detection dog indicated 148 times, of which 146 correctly, out of a possible 200 positive targets (*P. adspersus* scent) for the two treatments combined. When diluted frog scent was used the scent detection dog indicated incorrectly once on a control target and incorrectly once on *X. laevis* when live frogs were used. The average positive targets missed (27%) in the species

Table 2. Test results for the various *Pyxicephalus adspersus* scent detection tests, demonstrating the proportion of targets correctly (indicated on positive target) or incorrectly indicated (indicated on negative target or control), or missed (did not indicate on positive target), as well as the sensitivity and efficacy values for each test. N = no. of targets; N*p* = no. positive targets.

Test type	N*p*/N	Correct	Incorrect	Miss	Sensitivity	Efficacy
Scent dilution test						
1:1	0.2	43	7	17	0.72	0.64
1:10	0.2	48	8	12	0.80	0.71
1:100	0.2	49	11	11	0.82	0.69
1:1000	0.2	45	9	15	0.75	0.65
1:10000	0.2	46	6	14	0.77	0.70
1:100000	0.2	52	2	8	0.87	0.84
Species identification test						
diluted frog scent	0.1	74	1	26	0.74	0.73
live frog	0.1	72	1	28	0.72	0.71
Scent preservation test (time)						
1 month	0.1	63	7	17	0.79	0.72
2 month	0.1	51	1	29	0.64	0.63
3 month	0.1	53	4	27	0.66	0.63
4 month	0.1	43	1	37	0.54	0.53
5 month	0.1	43	0	37	0.53	0.53
6 month	0.1	39	0	41	0.49	0.49
Scent preservation test (method)						
diluted swab	0.1	79	4	41	0.69	0.66
dry swab	0.1	70	5	50	0.55	0.53
diluted (stored at 4 °C)	0.1	72	3	48	0.59	0.59
diluted (frozen at –20 °C)	0.1	71	1	49	0.58	0.58

identification test was slightly higher than for the dilution test (21%). Sensitivity was within the range of the dilution test results, and efficacy was generally high at 71 and 73% for the live frog and diluted scent, respectively.

All of the samples were still detectable after six months of preservation, although detection sensitivity was negatively correlated with time for the pooled preservation methods results ($R^2 = 0.88$, Fig. 3). Detection, independent of preservation method, was significantly more sensitive after one month of sample preservation (79%, $p < 0.01$) than after six months of preservation (49% sensitivity, Table 2). The number of targets missed increased from 21% of targets in month one to 51% of targets in month six. The highest detection sensitivity for any preservation method independent of time was obtained for the diluted swab method (66%), while very similar results were obtained for the dry swab, refrigerated scent water, and frozen scent water (58, 59 and 60% respectively). However, the four methods did not differ significantly in terms of their detection sensitivity ($p = 0.088$). The efficacy of detection across preservation time and method matched the values for sensitivity very closely. Interestingly there were very few incorrect indications (13 in total) for this test resulting in efficacy values being very similar to sensitivity values. By comparison, misses during the first three months were comparable to those of other tests but became more frequent as preservation time increased.

Discussion

Our results provide empirical evidence and agrees with other ongoing conservation programs that amphibian scent should be added to the long list of animate scent that scent detection dogs can distinguish and detect following conditioning training. We assume that the anatomical properties of amphibian skin enhances the basic physics principle of target detection by olfaction and in doing so contributes to its effectiveness. Firstly, for the dog to recognize a particular target the odour molecules must first evaporate from the integument surface of the target organism (Write & Thompson 2005). Secondly, the skin has been identified as the most likely source of odour even in tortoises that have relatively dry skin (Cablk et al. 2008). If we assume that amphibian skin functions in the same way, then its moist and permeable surface should facilitate the evaporation of odour molecules.

Having the potential to employ this search strategy, expands the arsenal of tools that customs officials or conservation scientists have to perform their respective duties. When performing critical functions with scent detection dogs such as identifying illegal consignments of amphibians in the pet trade or searching for a rare species in nature, handlers should consider the effectiveness of the dog to detect the target scent (Greatbatch et al. 2015). In our tests, the scent detection dog was able to detect frog scent samples that were diluted up to 10^5 times with relatively high sensitivity, and dilution factor was not proven to be a determinant of detection success. This outcome is encouraging for use of scent detection dogs to detect amphibians in the field since the odour of other animals is known to disperse in a gradient of concentration depending on the distance from the source and the prevailing environmental conditions (Jones 1983, Tomba et al. 2001). We also equate our dilution test to the dispersal of a scent gradient through soil in the case of fossorial amphibian species that are buried at different depths. Even though, we did not test frog detection in the field, as part of this study, conservation dogs have had success with detecting various amphibians in the UK, USA and Australia (unpublished data).

The sensitivity and efficacy values obtained from our study suggest that employing scent detection dogs in search and monitoring programs involving *P. adspersus* holds great potential, but this ultimately requires testing and validation in field settings. An important consideration that will likely influence the probability of detecting a target in the field is the variation in habitat types that frog species with different life strategies utilize. The majority of frog species can be detected in terrestrial habitats, but a large portion of species are semi-aquatic, fossorial, or arboreal, and only a small percentage of species have adapted to a strict aquatic mode of existence. According to Wasser et al. (2004), a desired probability of detection can still be achieved under variable circumstances by considering species and habitat when tailoring a search strategy. Indeed, dogs have proven their ability to search and locate scat of the aquatic North Atlantic right whale, *Eubalaena glacialis* (Müller, 1776) and have shown great success in locating three-toed box turtles, *Terrapene carolina triunguis* (Agassiz, 1857) that are associated with wetlands (Schwartz & Schwartz 1974, Rolland et al. 2006); thus demonstrating that accessible aquatic habitat does not necessarily exclude detection. Dogs are perhaps best known

for their ability to detect buried human remains (Alexander et al. 2016) in a habitat that can be deemed analogous to that utilized by fossorial amphibians, although obvious differences in size and composition of the two targets exist. But this versatility for habitat type demonstrated by conservation and forensic dogs implies that, with comprehensive training, the vast array of habitat utilized by various amphibian species should not be a limiting factor for detection success.

Amphibian communities typically consist of multiple species that share parts of the same habitat, depending on the geographic location and habitat type. When searching for a specific amphibian species, its scent will likely have to be distinguished from that of sympatric species that may or may not share common odour signature elements. It is known that dogs are not only good at scent detection but also at scent discrimination or the ability to distinguish one odour from another (Lit 2009). Indications from our study are that scent detection dogs have the potential to discriminate between frog species since near-perfect specificity for *P. adspersus* was displayed despite the presence of three other species from different anuran families, to which *P. adspersus* belongs. It is hard to predict how specificity values will be affected when a dog is simultaneously presented with the scent of more closely related amphibian species, even if we assume that related species (e.g. same genus) share a greater percentage of odour signature elements than unrelated species (e.g. separate families). Be that as it may, numerous examples of conservation dogs exist where discrimination between related species resulted in 85-100% accuracy, including controlled line-ups of two species of foxes, two species of bears, and even individual Amur tiger scats (Smith et al. 2003, Kerley & Salkina 2007, Hurt & Smith 2009). However, Lit & Crawford (2006) caution that if scents cause a conflict of interest or are too contradicting it can lead the dog to indicate when no target scent is present (false indications). How this will translate to scent detection in amphibian communities, remains to be investigated.

Being able to preserve frog scent for a few months at least provides handlers with leverage to employ matching scent detection. Preserved scent of rare or threatened species would, for instance, be extremely valuable for training a dog, or for use in research and monitoring of the same species. Alternatively, there might be a need for detecting individuals of hibernating species either during or immediately following hibernation, months after the last specimen was observed. This study has shown that it is possible to detect *P. adspersus* scent that had been preserved for at least six months. Detection sensitivity towards the preserved scent samples decreased over time, presumably since concentration as a function of time has a negative relationship. The selection of the right preservation method is therefore necessary to ensure that optimal detection sensitivity towards the sample is achieved. We obtained the best results from an aqueous dilution prepared from a skin swab that had been stored dry at 4 °C, which accounts for an easily deployed method with equipment that is readily available.

The use of dogs presents a unique opportunity for conservationists and scientists to study and conserve wild amphibians and can help customs officials to curtail the illegal amphibian trade. Their incredible olfactory abilities have aided wildlife biologists with locating protected native species, searching for introduced pest species, finding nests, and searching for dying animals from natural causes or insecticides amongst others (Zwickel 1969, Browne 2005). Having successfully displayed olfactory sensitivity towards *P. adspersus* in the laboratory demonstrates promise for advancing amphibian research and conservation through the myriad applications of conservation dogs. Significantly, some of the limitations experienced with amphibian surveys have been addressed with canine detection of other biological scents through enhanced location, identification, and sample acquisition rates with reduced collection biases (Hurt & Smith 2009).

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