



## CONTRIBUTED PAPER

# Scent of a frog: Can conservation detection dogs be used to locate endangered amphibians in the wild?

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## Abstract

In recent years, the potential to locate endangered animals using scent trained detection dogs (conservation detection dogs) has gained attention. Among vertebrates, conservation detection dogs have demonstrated a remarkable capacity to detect the scent of endangered mammals, reptiles, and birds, but their use in detecting amphibians is only beginning to be explored. A lack of work in this area is surprising given that amphibians are declining faster than any other vertebrate taxa. Moreover, amphibians are generally small, highly cryptic and breed in complex habitats, making them difficult to locate for the purpose of monitoring or establishing conservation breeding programs. This study aimed to provide a preliminary investigation into whether conservation detection dogs can be imprinted on the scent of the critically endangered Baw Baw frog (*Philoria frosti*) under captive conditions, and then effectively trained to locate wild frogs in their complex natural habitat. Two conservation detection dogs were trained to identify and locate *P. frosti* scent under controlled conditions before assessing their ability to locate wild *P. frosti*. Both conservation detection dogs were effective at locating *P. frosti* scent under controlled conditions, and also demonstrated an ability to detect live frogs under natural conditions. From an applied perspective, our findings provide new evidence that conservation detection dogs are capable of learning to detect the scent of small, cryptic anuran species. They also indicate that detection dogs are capable of locating frogs in highly complex forest habitat, confirming their untapped potential to aid in the management of imperiled species that have previously proven difficult to detect, monitor, and protect. We discuss the limitations of our approach and provide recommendations to help direct future amphibian conservation detection dog research.

## KEYWORDS

amphibian, breeding, conservation, cryptic, detection, dog, endangered, recovery, scent, translocation

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## 1 | INTRODUCTION

Amphibians are declining faster than any other vertebrate taxa, with recent IUCN data suggesting that a staggering 53% of evaluated species may be threatened with extinction (IUCN, 2019). In response to this amphibian extinction crisis, conservation managers are heavily focused on protecting species in rapid decline (Zippel & Mendelson, 2008). One increasingly considered management option is the establishment of ex situ conservation breeding programs (CBPs; Gascon, 2007). Globally, CBPs have assisted with the recovery of a diversity of endangered amphibians, and their value is widely recognized. Nevertheless, acquiring sufficient numbers of wild individuals to establish captive populations can be extremely challenging. Traditionally, amphibians are located and collected using a combination of pit trapping, spot lighting, and acoustic tracking (Rödel & Ernst, 2004). However, when numbers of individuals become critically low, or a species is highly cryptic and/or resides in complex habitats, protracted search times can render the establishment of a breeding program very difficult. The investigation of novel techniques to assist with the detection of individuals should therefore be prioritized.

In recent years, the potential to locate endangered animals using scent trained detection dogs (Conservation Detection Dogs) has come into focus. Dogs have an area of olfactory epithelium up to 50 times greater than that of humans, and therefore have a remarkable capacity to reliably detect scents at extremely low concentrations (see Thorne, 1995). Dating back to the early 1700's, scent trained dogs were being used by monks at the St. Bernard Hospice in Switzerland to search for lost or stranded travelers (Barwig et al., 1986). While scent-trained dogs are still widely used for such search and rescue purposes, they are becoming increasingly used to locate cryptic objects (Browne et al., 2006). Some of the best known examples include the use of detection dogs to expose and locate drugs, firearms, contraband plants and foods, and even cancer (Adamkiewicz et al., 2013; Browne et al., 2006; McCulloch et al., 2006; Pickel et al., 2004; Wasser et al., 2004; Witherington et al., 2017).

In the context of wildlife management, the use of detection dogs for conservation purposes dates back to the 1890's when New Zealand managers trained dogs to assist with the location of endangered Kiwi and Kakapo (Beebe et al., 2016). There is now broad recognition that detection dogs offer a promising field-survey method, with real potential to increase detection accuracy, while reducing survey bias and time required to locate target species (Cristescu et al., 2015), as well as complimenting existing survey and detection methods to increase efficacy. In this regard, detection dogs have

proven to be significantly more successful than surveys by humans when identifying animal scats (Arandjelovic et al., 2015; Cristescu et al., 2015; Long et al., 2007), resulting in improved population estimates (Arandjelovic et al., 2015; Cablk & Heaton, 2006; Colbourne, 1992). Detection dogs have also demonstrated their superiority compared to human led detection teams (Kapfer et al., 2012; Nussear et al., 2008), live-trapping approaches (Duggan et al., 2011) and various other survey practices, including remote camera trapping and hair analyses (Long et al., 2007). For the purpose of wildlife conservation specifically, detection dogs are increasingly being used to track live animals, detect invasive species, facilitate presence/absence surveys, and even detect pathogens (Dematteo et al., 2009; Goodwin et al., 2010; Leigh & Dominick, 2015; Rutter, Howell, et al., 2021; Rutter, Mynott, et al., 2021; Witherington et al., 2017). As recognition of this broad utility increases, conservation detection dogs are expected to assist with the management of various taxa.

Across terrestrial vertebrates, conservation detection dogs have been effectively used to assist with the management of a diversity of species (Grimm-Seyfarth et al., 2021), with outstanding examples coming from work with mammals (Cristescu et al., 2015; Duggan, 2012; Gsell et al., 2010; Reed et al., 2011; Reindl-Thompson et al., 2006; Smith et al., 2003; Wasser et al., 2004), birds (Colbourne, 1992; Robertson & Fraser, 2009; Wasser et al., 2012) and reptiles (Browne et al., 2015; Cablk & Heaton, 2006; Stevenson et al., 2010). More recently, evidence has emerged that detection dogs might also assist with amphibian management and conservation. Detection dogs have been effectively trained to identify (and indicate on) the scent of invasive cane toads (*Chaunus marinus*) in Australian and New Zealand (Powers, 2018), smooth newts (*Lissotriton vulgaris*) and great crested newts (*Triturus cristatus*) in natural terrestrial habitats in Europe (Grimm-Seyfarth et al., 2021; Stanhope & Sloan, 2019), California tiger salamander (Powers, 2018), and South African giant bullfrogs (*Pyxicephalus adspersus*), both under laboratory conditions (Matthew et al., 2021) and in the wild (Matthew, 2016, MSC thesis). Through this research, evidence has also emerged that detection dogs can learn to recognize amphibian odors at very low concentrations (Matthew et al., 2021), detect preserved scent (Matthew et al., 2021), target living individuals over residual odors (Grimm-Seyfarth et al., 2021), detect scent through soil substrate (Glover et al., 2023) and distinguish between the scent of target and non-target amphibian species (Powers, 2018; Matthew et al., 2021; Stanhope & Sloan, 2019). Despite these findings, the role of detection dogs in amphibian conservation remains relatively unexplored

compared to other vertebrate groups. This may be because amphibians with typically small and cryptic, and many species spend extended periods underground, in tree canopies, or in highly complex habitats (Arandjelovic et al., 2015; Leigh & Dominick, 2015; Reed et al., 2011), so there may be some reservation about employing the services of conservation detection dogs. However, this may be an oversight because many amphibians use chemosignals in communication and defense, and they are highly aromatic (Apponyi et al., 2004; Byrne & Keogh, 2007), which should facilitate detection by dogs (Matthew et al., 2021). Consequently, we believe there is a need for more studies aiming to assess whether conservation detection dogs have the capacity to reliably detect small cryptic amphibians in complex natural habitats.

Here, we aim to conduct a preliminary study to explore the potential for conservation detection dogs to be trained to locate the critically endangered Baw Baw frog (*Philoria frosti*), aligned with the objectives of a CBP. This species occurs within highly complex high-altitude forest habitat in the Mt Baw Baw plateau and breeds in deep terrestrial burrows located among dense vegetation during a compressed breeding season (approx. 5–6 weeks) with low temperatures (between approx. 5°C–12°C), making human surveys challenging. We hypothesize that dogs will be able to be trained to recognize the scent of *P. frosti* in a captive setting, and that this training will facilitate directed searching and successful detection of frogs in remnant wild populations.

## 2 | METHODS

### 2.1 | Study species

*P. frosti* is categorized as critically endangered (2004, IUCN red list assessment). The species is a medium sized stout terrestrial frog with large parotoid glands that produce odiferous chemicals (yet to be characterized), as is characteristic of many Australian Myobatrachid frogs (Daly et al., 1990). The species was confined to an area of 135 km<sup>2</sup> of the Mt Baw Baw plateau in Victoria, Australia (Hollis, 2004), but following recent declines (linked to anthropogenic habitat destruction, habitat modification by feral deer and cattle, increased UV-B radiation, increased fire frequency, reduced rainfall, and the spread of amphibian chytrid fungus and Chytridiomycosis disease) is now restricted to small patches of protected montane gully habitat between 1000 and 1300 m (Scheelings, 2015). The threat of extinction in the wild is now considered imminent (Scheelings, 2015). Breeding habitat relies on wet soak and seepage lines underneath

vegetation, fallen logs and rocks. Breeding occurs annually, and the breeding season extends for approximately 6 weeks during October and November. Male *P. frosti* typically call close to a burrow entrance with call sites associated with complex substrate comprising of rock, mud, and vegetation. Burrows may be shallow (<10 cm deep) or up to 1 m in depth with multiple retreat opportunities (Hollis, 2004). Males produce a distinct advertisement call, described as a short clunk, but also produce a territorial call elicited during agonistic interactions, described as a growl (Littlejohn, 1963). Calling activity can occur at any time, though is often more frequent during warmer daylight hours (Hollis, 2004). Females deposit 50–185 eggs in the burrow in a transparent foam nest produced by the female by beating air bubbles into oviducal secretions. The embryonic period last 5–8 weeks before tadpoles hatch into the foam nest. Larvae are non-feeding and metamorphose at the oviposition site after 5–10 weeks (Littlejohn, 1963; Malone, 1985a, 1985b).

### 2.2 | Captive frog population

Two captive frogs (one male and one female) reared from wild caught eggs (collected from Mt Baw Baw in 2014) were used for live animal scent training. The frogs were sexually mature, and the female was slightly larger (12 g) than the male (8 g). Both frogs were housed with conspecifics ( $n = 4$ ) in the “World of Frogs” exhibit at Melbourne Zoo, Australia, and were not part of the managed CBP.

### 2.3 | Wild frog population

One breeding site (approximately 15 m<sup>2</sup>) was utilized to conduct field scent trials. This site was chosen because it contained a remnant population of *P. frosti*, with calling males present during the study period. Also important, in 2013 and 2014 the site had been the focus of egg collections for the CBP and had previously experienced disturbance by managers searching for frogs and eggs. Due to the rapidly declining wild population, coupled with the unknown effect of dogs on *P. frosti* habitat, the decision was made to avoid disturbance to more pristine sites.

### 2.4 | Dog breed and training history

Two border collies were used for scent detection training. Both dogs were desexed males, aged 5 years old (Dog 1, “Rubble”) and 2 years old (Dog 2, “Uda”), and had previously been used for a wide range of scent detection

activities, including koala and quoll scat detection and animal mortality scent detection on windfarms (<https://www.canidaedevelopment.com.au/services>).

At the time of the study, dog 1 had been operational for 4 years and dog 2 for 12 months. The dogs were first assessed by a professional dog trainer at approximately 12 months of age at Canidae Development Australia. Food rewards were used during early training. Neither dog had previously worked with amphibians nor been exposed to amphibian odors. Both dogs had been trained through positive reinforcement.

## 2.5 | Trainer history

The study involved two experienced trainers/dog handlers. Trainer 1 had a history of over 10 years training conservation detections dogs. Trainer 2 had a history of approximately 6 years training dogs. Dog 1 had 4 years' experience in the field largely on wind farms searching for deceased wildlife, while dog 2 had 1.5 years' experience in the same field. For the duration of this study, training on Baw Baw frog scent was the only target odor for both dogs.

## 2.6 | Experimental design

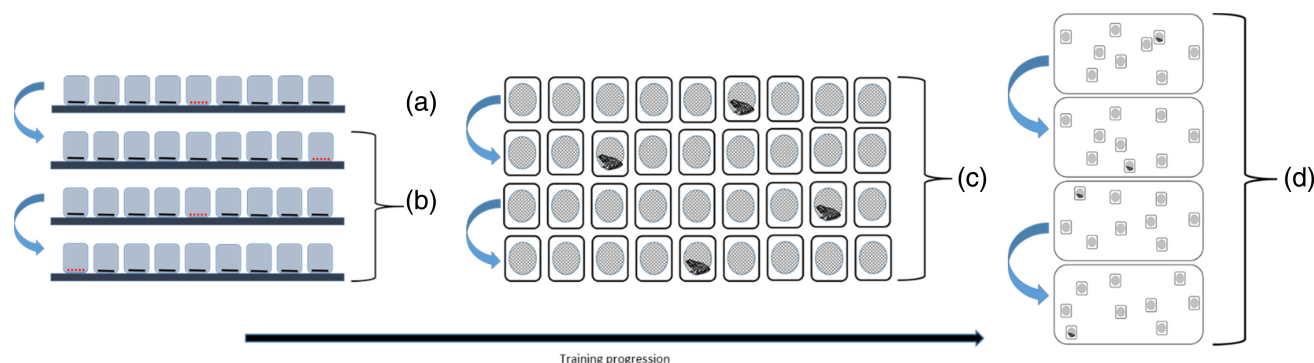
### 2.6.1 | Scent training in the lab

Scent training at Melbourne Zoo was first conducted by training the dogs on *P. frosti* scent (collected using skin swabs) before subsequent training using live animals. This approach was based on an ethical decision to minimize the number of frogs used in the study.

Scent training using the skin swabs from the Melbourne Zoo captive animals started approximately 2 months prior to the first field trials. Training was only conducted when ambient Melbourne temperature was under 15°C. The ad lib scent containers (swabs and live frogs) were placed in shade to ensure animals did not exceed maximal thermal tolerance. During training wind speed was light, ranging between 7 and 12 km/h from an east-south east direction. The successful use of skin swabs to induce scent recognition has been previously reported for reptiles (Browne et al., 2015; Cablk & Heaton, 2006). The training protocol involved using a cotton tip applicator to swab each frog five times on the dorsal surface, and five times on the ventral surface (total of 10 sweeps per frog). Swabbing on the dorsal surface involved sweeping snout to vent twice along each dorsal side (ensuring to sweep the full length covering the parotid gland) and once along the spine. Swabbing on

the ventral surface involved sweeping twice on each side, and once centrally. Swabs were sealed in a ziplock plastic bag and stored in a refrigerator prior to commencing training. The skin swab (scent) was then sealed in a custom-designed PVC container with a small (5 mm) hole drilled into the top to allow scent permeation. The scent-positive container was then placed among other-wise identical containers containing non-scent swabs to create a scent board (Figure 1a). Of note, non-scent swabs were treated in the same fashion as scent-positive swabs (i.e., stored in ziplock plastic bags and refrigerated prior to training) to avoid odor confounds. This method prevented the dog targeting the scent of the swab rather than the frog (i.e., provided a control for the swab odor). To prevent human scent contaminating the sample container (and biasing decisions made by the dogs), each non-sample container was also handled by the trainer. At no point did the experimenter touch the sample containers or the scent swab during testing. The dogs were systematically tested, and swabs were changed between dogs, though the containers remained the same throughout testing. All containers were cleaned between dogs using a dilute spray of F10 and water (1:500) to ensure that any odor cues left by the previous dog did not influence search patterns. Each dog was allowed to sniff the scent board (row of containers) and was given the command to identify the scent of the frog. The dogs were rewarded (with a piece of dry cooked beef) each time they performed a positive "alert" on the target scent. Positive alerts involved the dogs repeatedly touching their nose to the scent container (Figure 2). Each time the dog positively identified the correct container it was moved to an alternative position on the scent board as was a subset of the non-target containers and the dog was again given the command to identify the scent (Figure 1b). This process ensured that each training exercise returned 100% accuracy before moving the scent-positive container to a different position on the scent board. Each training exercise lasted a maximum of 10 min, with three 10-min sessions completed before moving to live frog scent training. To prevent fatigue, each dog was rested for approximately 15 min between training sessions. Swab scent training was conducted twice at Melbourne Zoo, and the entire training session was completed in 2.5 h. To ensure double blinding, throughout scent swab training the dog trainer and all assistants were unaware of the location of the scent positive swab. Only the frog biologists overseeing the project had knowledge of where each sample was located.

Once the initial scent training using skin swabs was completed, training progressed to using live *P. frosti* at Melbourne Zoo. Individual frogs were contained for approximately 10 min in a specially designed sealed



**FIGURE 1** (a) Scent board with ventilated PVC containers (gray boxes) used for detection dog scent training. The scent swab (red dotted line) is placed among non-scent swabs (black lines) as the target odor for the detection dog to positively identify before being moved to another position on the board and the process repeated (b). Similarly, the next training progression utilized live frogs placed in ventilated tubes among non-scent tubes and randomized following positive identification (c). Final controlled training phase (d) using live frogs in a larger more complex area.



**FIGURE 2** Image of conservation detection dog "Rubble" demonstrating a positive "alert" of *Philoria frosti* skin swab hidden in the scent board during training.

opaque plastic tube to allow scent to permeate into the environment without the dogs making contact with or seeing the live frogs. The frogs remained in the tube for the duration of the training session, and only one frog was used per 10-min session. The tube consisted of a 100 mm diameter section of PVC tube approximately 150 mm long, with each end capped with perforated drainage gutter and fly mesh with small ( $1 \text{ mm}^2$ ) aperture to block visualization of the frog (Figure 3). This approach has previously been used when training dogs to detect the scent of mammals (see Duggan et al., 2011). As described above for the scent-swab training, the container holding a frog was randomly placed in a line among nine empty but otherwise identical, containers. To prevent human scent being used by dogs to identify the sample container (or influence a dogs' behavior in any way), each non-sample container was touched by the trainer in a standardized fashion. At no point did the experimenter touch the tubes. The dogs were systematically tested, with the older dog first. Frogs were

changed between dogs, though the frog positive tube remained the same. Each dog was given the opportunity to investigate the containers and were rewarded each time they performed a positive alert on a container holding a frog. This process ensured that each training exercise returned 100% accuracy before randomly moving the tubes to another configuration and testing the dog again (Figure 1c). This process was only conducted twice with each dog before moving to more complex training. Live scent training was made more challenging by randomly placing containers within a complex habitat. Specifically, the containers were spread over a larger search area (approximately  $12 \text{ m} \times 20 \text{ m}$ ) and were hidden among garden vegetation at Melbourne Zoo. As with previous training methods described above, all containers were touched by the trainer, and each dog was given a command to identify the container holding a frog. After each positive identification, the "frog positive tube" was randomly moved within the search area (Figure 1d), as was a subset on non-scent tubes. Each training exercise lasted a maximum of 10 min, with four 10-min sessions completed for each dog. As for earlier training, to prevent fatigue, each dog was rested for approximately 30 min between training sessions. Five discrete live frog training days took place over a period of 2 months, including on the morning of departure for field trips to Mt. Baw Baw. To ensure double blinding, throughout live frog scent training the dog trainer was unaware of the location scent positive swab. Only the frog biologists overseeing the project had knowledge of where each sample was located. The live frog scent training was based on the assumption that live captive frogs would have a similar or identical scent profile to wild frogs and elicit the best performance from the dogs when applied to a field setting. While dogs are likely to be trained most effectively



**FIGURE 3** Tube used for live frog scent training preventing contact between frog and dog while allowing scent permeation. Consisting of 100 mm section of PVC tube approximately 15 cm long, with each end capped with perforated drainage gutter and fly mesh.

using live wild animals (DeMatteo et al., 2019), this was not possible in the present study due to the critically endangered status of the study species, and ethical constraints. Of note, past studies have effectively trained dogs to search for wild animals following scent training using captive animals in a controlled environment (see Cristescu et al., 2015; DeMatteo et al., 2009; Oldenburg Jr et al., 2016).

## 2.6.2 | Environmental acclimatization

Between October 30 and November 2, 2017, a field trip to Mt. Baw Baw was undertaken with two dogs, two handlers and four zoo staff for 3 days to allow the dogs to gain familiarity and exposure to field conditions. Exposure to field conditions prior to conducting trials has been noted as an important factor for training during field deployment (Arandjelovic et al., 2015; Reed et al., 2011). This consisted of short walks (20–30 min) in appropriate *P. frosti* habitat to allow the dogs to gain familiarity with habitat scent (e.g., flora and fauna odors) and complexity (e.g., depth of forest floor). The two detection dogs and investigation team visited four historic *P. frosti* sites and one extant site over 2 days. Daytime temperatures were  $-3^{\circ}\text{C}$ – $4^{\circ}\text{C}$  with medium to heavy snowfall.

## 2.6.3 | In situ scent training

Owing to limited access to wild frogs for in situ scent training, additional skin swab training was conducted in situ with captive *P. frosti* skin swab containers. Each dog was tasked with identifying the positive skin swab container hidden at ground level in vegetation within a search area of approximately  $10\text{ m}^2$ . Training protocols were the same as for the ex situ trials described above. Each dog conducted training on two consecutive days during the environmental acclimation period, then one training session per dog prior to each field detection trial day. Training lasted for 10 min per session.

## 2.6.4 | Field detection trials

Between November 14 and 17, 2017 a trip to a known *P. frosti* breeding site at Mt. Baw Baw was undertaken to assess whether the dogs could detect live animals in situ. The trip involved four Melbourne Zoo staff, two dogs, and two dog handlers. In situ trials were conducted during a period of peak *P. frosti* calling activity.

Both dogs traveled from Mt Baw Baw alpine village by car for 20 min before walking for 45 min to reach the breeding site. The walk consisted of overgrown forestry vehicle tracks with moderate to thick under story with shallow leaf litter substrate (10–20 cm deep) before entering high elevation (approximately 1050 m) montane eucalypt forest comprising of dense mountain ash canopy cover and deep substrate (<65 cm deep) with fallen trees, branches, logs, and large boulders. Before entering the site, the dogs were rested for 15 min. Prior to entering suitable *P. frosti* habitat, both dogs were cleaned, as described below in the section on biosecurity, before walking another 20 min to the breeding site (Figure 4).

Active searching for frogs at the breeding site started at approximately 10:00 a.m., with each dog given a maximum of 20 min to locate *P. frosti* scent. Ambient temperature ranged from  $11^{\circ}\text{C}$  to  $21^{\circ}\text{C}$ , with ground temperature ranging from  $9^{\circ}\text{C}$  to  $12^{\circ}\text{C}$ . Wind was light ranging between 9 and 17 km/h from a north to east-south-east direction. The ground surface at the site was highly complex muddy substrate comprised largely of decomposing granite and organic material, and small to large granite boulders with fallen debris including logs and branches. Each dog searched consecutively to ensure a rest period between searches, minimize disturbance within the breeding site, and the opportunity to assess the dogs independently. During rest periods, dogs were not able to see the other dog searching. Each dog participated in four active search attempts on each of two non-consecutive days.

The search area concentrated on breeding habitat where male call sites had previously been identified so that positive indication of frogs could be verified by calling heard at the alerted location. Each dog was allowed

**FIGURE 4** Typical montane forest breeding habitat of *Phyllorhina frosti*. Note the complexity of understory and fallen logs, rocks and organic matter.



unrestricted access to the search area (approximately 15 m<sup>2</sup>), unless it strayed outside of suitable habitat and was called back. The area of the search area was chosen as this is indicative of a typical patch of breeding habitat. Active searching was conducted with the dogs off-leash to prevent any impediment from the lead, handler or environment, and enable a non-linear search pattern ensuring the dogs could search freely (Cristescu et al., 2015; Reed et al., 2011). Detection dogs were trained to indicate the presence of a frog (likely in an underground burrow) by touching their nose to the most concentrated source of the scent (Figure 2). While this is not a passive indication recommended for work with live animals, it was deemed that it would have little impact on frogs, which would likely already be in their burrows and poised to retreat further if threatened. The handler was also attuned to cues exhibited by the dog, including changes in behavior (such as vigorous tail wagging), changed breathing rates, or visual contact from dog to handler, indicating the likely presence of a live animal or concentrated target odor. Each dog was allowed to search until it displayed signs of fatigue (excessive panting or reduced activity) as assessed by the handler, but no trial lasted longer than 20 min. Between search trials, dogs were rested for a minimum of 20 min with adequate hydration and food provided until signs of fatigue abated. Dogs were then tasked with another search. Neither of the dogs' performed more than four searches in 3 h.

The trial search was considered a success after a minimum of three individual frogs had been detected. This number was deemed successful based on; (1) the high accuracy of detections demonstrated by the dogs under controlled settings, and (2) the low number of frogs

present at the trial site, confirmed previously during field surveys. During all field activities, only the frog biologists overseeing the project had prior knowledge of where frogs were located. The dog trainers and all other assistants/observers had no prior knowledge of frog locations, to ensure that all work was double blinded.

## 2.7 | Biosecurity

In order to meet hygiene protocols established to mitigate the potential spread of chytrid fungus, both dogs were cleaned prior to entering the field site. All organic matter was removed from the dogs, and each animal was washed with an antifungal shampoo (Malaseb™ medicated shampoo). Dogs were washed approximately 320 m from the research location. The dog's nose was not cleaned with shampoo during this process in case it caused irritation; however, it was wiped down and free of organic matter which would greatly mitigate the risk of carrying chytrid as nose touching is a positive scent "alert." The cleaning site was chosen by amphibian researchers due to (1) proximity to habitat where *P. frosti* persist and (2) separation by large natural barrier (river) which would likely prevent movement of other large wildlife that may also transfer chytrid spores. Researchers and dog trainers cleaned boots and lower portion of legs (from knee down) by using scrubbing brushes to removing organic matter then spraying with disinfectant spray (Glen 20™). With both practices there is the potential for some local site contamination. However, the use of hygiene management to prevent or limit the spread of chytrid fungus is deemed to outweigh any negative

effects associated with contamination. Water from the river was used to dilute and rinse any areas contaminated during the cleaning process. Hygiene protocols have been developed at a National level (<https://www.dcceew.gov.au/environment/invasive-species/publications/hygiene-protocols-control-diseases-australian-frogs>) and highlight the need to mitigate where possible the spread of disease.

## 2.8 | Data analysis

To compare whether there was an effect of sample type (swab versus live frog) and test day (day 1 versus day 2) on the time taken by dogs to locate samples during ex situ trials scent training in the lab, we used a linear mixed effects model (LME). For the model, the fixed effects were sample type and test day. Dog ID was included as a random effect to control for repeated sampling by the same dogs. Model estimation was made using Restricted Maximum Likelihood methods, and the significance of the tested fixed effects was assessed using F values. Data analysis was performed using JMP Pro version 16. The outcomes of trials were described using descriptive statistics (averages, ranges, proportions and percentages).

## 3 | RESULTS

### 3.1 | Ex situ scent trials

During scent swab and live-scent training under controlled settings, both dogs behaviorally identified frog odor cues. In total, during the two trial days conducted at Melbourne Zoo (separated by 17 days) each dog completed 1.5 h of scent swab training and 1 h of live scent training broken into 10-min training sessions. Both dogs positively identified scent during each session (100% success rate), including in the trials where scent was placed among natural vegetation. Time taken to positively identify scent swabs ranged from approximately 5–20 s (average 15 s from eight sessions), while time taken to positively identify live frog scent ranged from 35 to 90 s (average 74 s from six sessions). Time taken to positively identify scent samples was significantly faster for swab samples compared with live frog samples (LME:  $F_{1,24.33} = 104.03$ ,  $p < .0001$ ), with no significant difference between test days (LME:  $F_{1,24.21}$ ,  $p = .80$ ).

### 3.2 | In situ scent trials

In total, active field trials within *P. frosti* breeding habitat were conducted over two non-consecutive days

(separated by 1 day due to adverse weather conditions), and each dog participated in trials on both days. During trial day one, both dog showed signs of scent indication but *P. frosti* scent presence could not be confirmed by human observers; however, positive identification of frogs (either visually or by call) was not possible. Dog 1 indicated the presence of frog scent and made positive, but not verified, indications on each of its four search attempts (100% scent indication rate). By comparison, Dog 2 only indicated on one of its four search attempts (25% scent indication rate) (Table 1).

During trial day two, both Dog 1 and Dog 2 successfully and independently located the same three frogs, and each frog was audibly verified by calling from the burrow entrance (Table 1). Dog 1 indicated the presence of frog scent and made positive and verified indications on three of its four search sessions (75% scent indication rate). By comparison, Dog 2 only indicated on one of its four search sessions (25% scent indication rate).

In total, over two trial days and eight search sessions, Dog 1 positively indicated on live frog scent in 87.5% of its search sessions (7/8 search sessions). Of the positive indications, 42.8% (3/7 indications) were positively verified by observing males calling at the burrow entrance. Conversely, over two trial days and eight search sessions, Dog 2 positively indicated on live frog scent in 50% of search sessions (4/8 search sessions) of which 75% (3/4 indications) were positively verified by observing males calling at the burrow entrance.

## 4 | DISCUSSION

Conservation detection dogs are increasingly being used to detect endangered species. Surprisingly, however, their potential to assist with amphibian conservation has been largely overlooked. The aim of the present study was to investigate whether conservation detection dogs could be imprinted on the scent of the critically endangered Baw Baw frog (*P. frosti*) under captive conditions, then effectively trained to locate wild frogs located in complex natural habitat in remnant populations. Despite the low sample sizes of the present study, scent detection trials were considered successful as each dog was able to locate frog scent under both controlled and natural conditions. Interestingly, during ex situ training, more time was taken to detect live frog scent compared to scent on skin swabs, which may be due to a difference in profile between scent on live frogs versus skin swabs. Skin swabs may have presented a more concentrated odor cue because frogs use chemicals in defense and release stronger chemosignals in response to handling, as has been reported for other toxic frogs (Myers et al., 1991). Additionally, live frog odor may take time to permeate the

**TABLE 1** Detection trials, search sessions, and positive frog indications during active field searches at Baw Baw frog breeding site.

Trial	Dog ID	Session	Search outcome	Positive alert
Day 1	Dog 1	1	After a 10-min search positively indicated on call site however no positive visualization or calling was heard.	Yes
		2	After a 12-min search positively indicated under large boulder, could not access site effectively to search for frog.	Yes
		3	After a 15-min search positively indicated under large boulder, could not access site effectively to search for frog.	Yes
		4	After a 2-min search positively indicated on call site however no positive visualization or calling was heard.	Yes
	Dog 2	1	Actively searched for 20 min over a range of approximately 15 m <sup>2</sup> no positive indications made.	No
		2	Actively searched for 20 min over a range of approximately 15 m <sup>2</sup> no positive indications made.	No
		3	Actively searched for 20 min over a range of approximately 15 m <sup>2</sup> no positive indications made.	No
		4	After a 10-min search positively indicated on a call site however no positive visualization or calling was heard.	Yes
Day 2	Dog 1	5	Actively searched for 20 min over a range of approximately 15 m <sup>2</sup> no positive indications made.	No
		6	After a 6-min search positively indicated on a call site, frog audibly verified from burrow entrance.	Yes
		7	After a 3-min search positively indicated on a call site, frog audibly verified from burrow entrance.	Yes
		8	After a 9-min search positively indicated on a call site, frog audibly verified from burrow entrance.	Yes
	Dog 2	5	Actively searched for 20 min over a range of approximately 15 m <sup>2</sup> no positive indications made.	No
		6	After an 11-min search positively indicated on a call site, frog audibly verified from burrow entrance.	Yes
		7	After a 5-min search positively indicated on a call site, frog audibly verified from burrow entrance.	Yes
		8	After a 4-min search positively indicated on a call site, frog audibly verified from burrow entrance.	Yes

skin and be released into the air. Therefore, the frog may need to be held in the container for a period of time prior to the scent trial commencing to allow the scent to concentrate. Regardless of the cause, the difference in detection success between the skin swabs and live frogs highlights the need to ensure the training scent profile is as close to the target scent as possible, and this should be an important consideration when establishing a training methodology. Additionally, due to ethical restrictions pertaining to the use of live frogs, the amount of training time, and therefore positive reinforcements on live frog scent, was limited. This too may have influenced the dog's ability to detect live frog scent. Nevertheless, the speed that both dogs could be trained to detect

*P. frosti* scent is noteworthy. Following just a few hours of training it was possible to operantly condition both dogs to detect the scent of frogs in captivity, and in the wild under challenging environmental and climatic conditions.

During field trials on day one of the study, both dogs indicated live frog scent, but the presence of a live frog could not be confirmed, and the possibility of a false positive could not be excluded. By contrast, during field trials on day two the dog's positive indications were confirmed by the presence of a calling male in a burrow. One explanation for the difference in results between days is that the dogs were unsettled (or still learning what they were indicating on) during their first day searching in the field

and incorrectly identified the presence of scent (and frogs). In unfamiliar settings, it is not uncommon for detection dogs to perform sub-optimally, resulting from contextual changes and/or sensory distraction (Rutter, Howell, et al., 2021; Rutter, Mynott, et al., 2021). Saying this, we did not observe the dogs displaying any behavior to suggest they were unsettled or overly distracted. Therefore, a more likely scenario is that the dogs were effectively detecting *P. frosti* scent, but were unable to pinpoint the source. Several variables may have made scent localization difficult. One possibility is that a live frog recently moved across the indicated area and left residual scent. Another possibility is that the resident frog was residing deep in a burrow system, preventing confirmation of their presence. We do not have an understanding of how *P. frosti* respond to domestic dogs, but the flight response is a common antipredator behavior in amphibians (see Rajchard, 2006). Moreover, *P. frosti* often display retreat behavior in response to physical disturbance during collection attempts (D.G, personal observations). If indeed the dogs are eliciting a fright response this might make the use of detection dogs to facilitate capture or identification of frogs more challenging. Moreover, there might be ethical concerns if frogs are being acutely stressed by searching dogs. Assuming this is the case, there may be value in modifying how dogs are trained. One option might be to train dogs to approach the scent more slowly and indicate at a greater distance. Indeed, a recent study with great crested newts demonstrated that detection dogs could be trained to indicate at a specific distance from the odor source, potentially minimizing disturbance to the target animal (Glover et al., 2023). Disturbance might be further minimized by training handlers to recognize subtle behavioral changes in dogs that indicate changes in proximity to the odor source (Glover et al., 2023). Moving forward, we recommend additional studies aimed at empirically assessing how distance affects a dog's odor perception, and the extent to which dogs might be able to assist surveyors to locate *P. frosti* while minimizing disturbance. Such work could also consider the influence of frog sex and life stage on detectability. In the present study, all frogs detected were reproductively mature calling males. However, based on work with crested newts there is reason to suspect that mature males might be more odiferous and easier to detect than females (or smaller individuals), necessitating a more nuanced training program (see Grimm-Seyfarth et al., 2021). Underpinning such work, there would also be merit in considering the influence of search mode (i.e., on leash or off leash) and environmental variables (such as soil type and vegetation type) on individual detectability, as these factors have been shown to influence detection probabilities in newts (Glover et al., 2023; Grimm-Seyfarth et al., 2021).

Both dogs displayed an ability to operate under field conditions despite the complex and challenging habitat, as well as the long walk (totaling approximately 65 min) from the research vehicle to the study site. In part, we attribute this success to the fact that both dogs underwent an environmental acclimation period for 3 days prior to commencing field trials. Working under novel environmental conditions and physical exhaustion have both been suggested as potential factors affecting the ability of dogs to detect scent (Arandjelovic et al., 2015; Leigh & Dominick, 2015). Although only using one detection dog, Leigh and Dominick (2015) found that vegetation type and density did not affect detection rates of spotted quoll scat, however, search time and exposure training in dense habitat needed to be accounted for. Here, during environmental acclimation training, conditions experienced were harsh, including below zero temperatures and snowfall. During future conservation detection dog deployments, extended field exposure training may provide dogs with an even greater ability to operate effectively in challenging habitat under severe conditions. This training could also be modified to help dogs locate odor cues within concealed locations (simulating burrows) as the characteristics of odor cones coming from burrows is likely to be unique. Another consideration when conducting field trials is how the odor cues left by dogs might influence the behavior of dogs in subsequent searches. While our dogs were not allowed to observe each other, it is possible that their scent trails influenced search patterns and resulted in dogs locating the same frog burrows. To avoid this issue, future studies may benefit from adopting a "one dog per search zone" approach.

Although the present study suggests there is clear potential for detection dogs to assist with locating *P. frosti*, under harsh climatic and environmental conditions, to ascertain if detection dogs are cost effective, the value of this approach will need to be compared against other survey approaches. Specifically, it would be informative to compare the efficacy of detection dogs against traditional methods (particularly human surveyors), factoring in financial costs associated with dog training, housing and welfare requirements (Rutter et al., 2022). While past studies have suggested that dogs are significantly more effective than all other methods at locating cryptic wildlife (Grimm-Seyfarth et al., 2021), the extent of any such benefits would need to be formally quantified. Furthermore, any benefits would need to be weighed against the potential for negative environmental impacts associated with the use of detection dogs. In addition to the potential for dogs to cause stress to target and non-target animals, there is also a risk that dogs might predate upon wildlife, introduce disease, or cause fear-mediated behavioral changes that reduce wildlife fitness (Doherty et al., 2017). Dogs might also damage

remnant habitat critical for breeding, either directly through damage to vegetation and/or substrate, through biosecurity cleaning methods, or indirectly in various ways. For instance, dog feces and urine (which is known to contain high levels of nitrogen and phosphorus as well as a diversity of endocrine disrupting chemicals) may contaminate soil and water with lethal or sublethal consequences (De et al., 2022; Pocar et al., 2023). Undertaking studies aiming to identify such risks, followed by careful consideration of mitigation strategies, will certainly need to be a priority when evaluating whether detection dogs have a role to play in *P. frosti* conservation (both in terms of monitoring remnant populations and capturing frogs to supply genetic variation to captive breeding programs).

Importantly, we tested the potential value of using conservation detection dogs with simple training methods, and this was enough to allow the dogs to locate an extremely cryptic species in a highly complex habitat. This finding suggests that using conservation detection dogs to locate less cryptic species in less challenging habitat should also yield positive outcomes. However, in cases where the density of frogs is expected to be very low, there may be value in investigating more intensive training protocols that improve detection rates. It is likely that training will also need to be tailored to a species' natural history. For example, different protocols may be needed to effectively detect species characterized by arboreal, terrestrial and aquatic life histories (see Richards, 2018). Another consideration is whether training needs to allow dogs to discriminate between a target species and other amphibians occupying the same habitat (Stanhope and Sloan, 2019). For instance, detection dogs trained to identify a certain species might make false positive indications on sympatric congeneric species. This is not an issue for *P. frosti* because the montane forest on Mount Baw Baw is free of co-occurring amphibian species. However, for many amphibians habitats are shared by multiple species with extensive temporal and spatial overlap. In these instances, more specialized training may be needed to ensure that detection dogs can discriminate between heterospecifics (see Stanhope and Sloan, 2019). This approach seems highly feasible given that past research in vertebrates has shown that detection dogs can reliably discriminate between congeners (Rosell et al., 2019), or even reliably identify individuals within a species (Wasser et al., 2009). Of equal importance, widespread characterization of amphibian chemosignals has shown considerable interspecific variation within this vertebrate class (Iglesias-Carrasco & Wong, 2023), which will facilitate rapid species-identification training.

Amphibian CBPs are typically established with reintroduction as a long-term objective. However, the

reintroduction success of individuals in early life stages can be challenging to monitor. For example, for species that are slow to reach sexual maturity, it may be several years before individuals can be detected within natural habitat. Often, frogs can only be located when adults return to breeding sites. Detection dogs have demonstrated their value in detecting the presence and/or absence of other taxa by indicating on either live scent or scat (Arandjelovic et al., 2015; Browne et al., 2006; Colbourne, 1992). We suggest that conservation detection dogs could play a valuable role identifying the presence and/or absence of amphibians during early life stages post reintroduction and recommend further investigation into the feasibility of incorporating detection dogs into recovery plans.

One factor that may limit the training of conservation detection dogs for endangered or cryptic amphibians species may be the scarcity of individuals available for initial scent training. For many species, access to training scent may only be possible if animals are being kept in captivity. Even under this scenario, however, it is possible that scent profiles between captive and wild animals may differ. Although the present study did not compare the ability of detection dogs to discriminate between the scent of different anuran species, training with the scent of common amphibians may be a way to overcome this limitation and enable effective training when access to rare species is not possible. This approach may be most effective where there are few non-target amphibians within a search area, as is the case with *P. frosti*. Investigating the potential use of generic amphibian scents would be a valuable future-research direction.

Beyond aiding in endangered amphibian monitoring and collection, conservation detection dogs may also have a role to play in guiding the selection of management actions in the face of ongoing threatening processes. For instance, globally, amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) remains one of the key factors driving amphibian population decline, with management options to control chytrid fungus in the environment extremely limited (Mendelson et al., 2006). Detection of chytrid fungus in natural habitat relies on environmental DNA detection of chytrid zoospore, positive skin swab (from infected or non-susceptible species), or presence of disease reservoir amphibian host species (Scheele et al., 2014). Identification of environmental refugia from disease is therefore a complex task. Detection dogs have proven effective at identifying several fungal species (Kauhanen et al., 2002; Matthew, 2016) and may have the potential to play a valuable role in identifying disease free reintroduction sites.

The present study adds to a small number of studies investigating the potential use of conservation detection

dogs as a tool to assist the development of an amphibian CBP (Glover et al., 2023; Grimm-Seyfarth et al., 2021; Matthew et al., 2021; Murrell, 2022; Peacock, 2007; Powers, 2018). Our findings that dogs could be trained to locate the scent of live animals under challenging natural conditions suggests that conservation detection dogs may have the potential to serve a wide range of amphibian conservation applications, including assisting with threatened species in situ presence/absence surveys, and gathering data on species distribution, population density, and reproductive output. Conservation detection dogs may also help to locate individuals for collection for CBPs, or for wild-to-wild translocation. Moreover, simple establishment of species occupancy in the landscape can help to initiate management practices aimed at in situ conservation, such as determination of reintroduction sites, postrelease monitoring and/or detection of species that form a reservoir for disease, such as amphibian chytrid fungus.

## 5 | CONCLUSION

We have provided one of the first demonstrations that conservation detection dogs are capable of being rapidly trained using the scent of live captive frogs to detect the scent of live wild frogs. These findings have important implications for amphibian conservation programs because we have demonstrated that conservation detection dogs are able to detect cryptic amphibian species in highly complex habitat using simple training methodology. We encourage ongoing research across a diversity of amphibian species to broaden our understanding of how conservation detection dogs can assist with amphibian conservation globally.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, Deon J. Gilbert, upon reasonable request.

## ETHICS STATEMENT

Work followed protocols approved by the Zoos Victoria Animal Ethics Committee (project number ZV16011) in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes 2013, and was authorized by the Victorian Department of Environment, Land, Water and Planning, Research Permit 10,008,391.

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