# Alarm calls or predator calls: which elicit stronger responses in ungulate communities living with and without lions?

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**Abstract** Alarm calls and predator vocalizations convey information on predator presence and potential risk. Generally, prey employ anti-predator behaviours more in response to alarm calls. However, occasionally prey respond more to the vocalizations of specific predators. A key question is, do prey still respond to alarm calls and predator vocalizations when a dangerous predator is absent? Additionally, would the prey species' response (e.g. vigilance) differ from prey already living with these predators? Using auditory playbacks, we tested whether four-herbivore species living with lions responded more to alarm calls than lion vocalizations compared to a black cuckoo control call. Overall, red hartebeest, wildebeest and zebra had greater vigilance in response to the lion roars compared to the alarm calls. The differences in vigilance suggest that, despite the lion roars not being related to hunting, these herbivores perceived the predator vocalizations as a more immediate indicator of risk than the alarm calls. We then tested whether herbivores living with lions increased their vigilance more in response to the calls than conspecifics in a lion-free section. Despite greater overall vigilance in the lion section, gemsbok and zebra in the lion-free section significantly increased their vigilance in response to the lion roars. This indicates that species under the greatest threat from a predator (e.g. preferred prey) may maintain innate anti-predator responses to an absent but dangerous predator longer than less preferred prey. Ultimately, our results indicate that cues from dangerous predators can have greater effects on anti-predator behaviours than alarm calls for some prey species.


**Keywords**: anti-predator behaviour, auditory cues, predator-prey interactions, vigilance

## Introduction

A key challenge faced by prey species is the need to manage predation risk (Lima and Dill 1990). Proactively, they can do this by moving in groups, increasing their vigilance, and limiting the time they spend in dangerous areas across the landscape (Caro 2005). Additionally, they can react to cues of immediate predation risk by responding to the alarm calls of conspecifics or heterospecifics, and to the vocalizations of predators themselves (Blumstein et al. 2008; Hettena et al. 2014; Magrath et al. 2015; Meise et al. 2018). However, the degree to which prey respond to these different auditory signals will vary depending on the reliability of these cues as indicators of risk (Kitchen et al. 2010; Palmer and Gross 2018; Rainey et al. 2004) and previous experience with predators (Blumstein 2006; Carthey and Blumstein 2017). Moreover, some prey species use referential alarm calls which denote predator type and evoke predator-specific anti-predator responses, while others respond to urgency-dependent alarm calls from conspecifics rather than calls for specific predators (Furrer and Manser 2009; Manser 2001).

Generally, prey tend to respond more to conspecific and heterospecific alarm calls than they do to predator vocal cues (Magrath et al. 2015; Schmidt et al. 2008). For example, Eastern chipmunks (*Tamias striatus*) decreased their feeding effort in response to heterospecific titmouse (*Baeolophus bicolor)* alarm calls but not to the direct call of their primary predator, the broad-winged hawk (*Buteo platypterus*; Schmidt et al. 2008). Similarly, coots (*Fulica atra*) spent significantly more time vigilant in response to the alarm calls of conspecifics compared to a predator call (dog bark; Randler 2006). In another example, Tammar wallabies (*Macropus eugenii*) responded to the playbacks of conspecifics' foot thumps, as an anti-predator signal, but did not respond to the vocalization of a resident predator the wedge-tailed eagle (*Aquila audax*; Blumstein et al. 2000).

A potential reason that prey may react more to alarm calls than predator vocalizations is that they tend to be indicators of greater risk (Barrera et al. 2011; Magrath et al. 2015). Many predators rely on stealth and surprise while hunting (Preisser et al. 2007). Thus, hunting predators are unlikely to give away their location, proximity, and potential identity to prey species by calling (Barrera et al. 2011). As a result, the vocalizations of predators tend to suggest that the predator is not hunting, and thus deemed to be less of a threat by prey (Barrera et al. 2011; Hettena et al. 2014). In contrast, alarm calls provide information on predator detection, state (i.e. actively hunting), and identity, thus providing public information on local risk (Kitchen et al. 2010; Palmer and Gross 2018; Schmidt et al. 2008). Therefore, prey species should perceive greater predation risk and increase their investment in antipredator behaviours (i.e vigilance; Schmidt et al. 2008) more in response to alarm calls than in response to predator calls .

Still, some studies have found that the vocalizations of predators elicit a strong anti-predator response (e.g. vigilance) in prey species compared to their baseline activity (e.g. Hettena et al. 2014; Karpanty and Wright 2007). For example, racoons have been found to spend less time foraging after hearing dog barks (Suraci et al. 2016). In addition, elephant (*Loxodonta africana*) herds increased their bunching behaviour and alertness in response to male lion (*Panthera leo*) roars (McComb et al. 2011). Furthermore, black-casqued hornbills (*Ceratogymna atrata*) increased their alarm call rates (an anti-predator behaviour) in response to crowned eagle (*Stephanoaetus coronatu*) shrieks (Rainey et al. 2004). A unifying feature of these different predators is that they all present a significant risk to the prey species studied (Rainey et al. 2004; Suraci et al. 2016). Thus, it seems that cues from dangerous predators can also signal risk and trigger increased anti-predator responses in prey species. A key question that arises is, when faced with dangerous predators, to what degree do prey species respond to the predator calls compared to conspecific and heterospecific alarm calls?

In several locations worldwide, predators are moving back into ecosystems, either via natural range shifts (Banks et al. 2002) or through reintroductions (Hayward and Somers 2009). As a result of these movements, prey species are now coming into contact with predators that historically have been absent on the landscape for several generations (Mech et al. 2001; Sand et al. 2006; Sih et al. 2010). In some cases, prey have lost their anti-predator responses to these 'returning' predators (Blumstein 2006; Carthey and Blumstein 2017), while in other situations, they may still react to the cues of these predators (Blumstein et al. 2009; Chamaillé-Jammes et al. 2014). Potential explanations for the retention of these anti-predator behaviours are that they are learned behaviours and thus persist within prey populations when other predators are present on the landscape (i.e. Multipredator hypothesis; Blumstein et al. 2009). Thus, the risk from extant resident predator species may be sufficient for prey species to retain appropriate anti-predator responses to the absent predator (Blumstein 2006; Carthey and Blumstein 2017). Alternatively, these prey species may have retained appropriate anti-predator behaviours over a few generations (Chamaillé-Jammes et al. 2014), possibly through genetic inheritance (Dalerum and Belton 2014).

In African savanna systems, lions (*Panthera leo*) are key apex predators (Davidson et al. 2013; Owen-Smith and Mills 2008). Due to their large size and cooperative hunting strategies, they present a significant risk to a broad range of large herbivores (Hayward and Kerley 2005; Scheel and Packer 1991). In addition, lions are stalk and ambush predators that opportunistically use ambush sites to target and kill prey (Hopcraft et al. 2005). As such, prey species need to employ and maintain anti-predator behaviours to minimize this risk (Courbin et al. 2016; Traill et al. 2016; Valeix et al. 2009). However, do prey species that no longer interact with lions on the landscape still respond to cues from this dangerous predator?

To address these questions, we first compared changes in vigilance as a primary adaptive anti-predator response (Beauchamp 2015) in four ungulate species (i.e. gemsbok –

*Oryx gazelle*; zebra – *Equus quagga*; red hartebeest - *Alcelaphus buselaphus caama,* and wildebeest - *Connochaetes taurinus*) living with lions in response to conspecific and heterospecific alarm calls and lion roars. We predicted that all four herbivore species would spend a greater proportion of time vigilant in response to the alarm calls (as indicators of greater risk) of zebra and wildebeest (preferred prey of lion) compared to lion roars. Alternatively, herbivores may show a greater increase in vigilance in response to the lion roars, as they indicate the presence (immediate risk) of this dangerous ambush predator. Next, we investigated whether individuals with no direct physical exposure to lions (for at least 100 years) responded to lion roars, and conspecific and heterospecific alarm calls, by recording changes in vigilance levels. We predicted that if these herbivores retained their anti-predator behaviours for lions (an absent but dangerous predator, as has been shown elsewhere; Dalerum and Belton 2014), then, similar to those individuals living with lions, they would react strongly towards the lion roars. Alternatively, if they had lost their predator recognition of lion calls due to a lack of associated predation events, then they should not react to lion roars, but still react to the alarm calls as they would still function as indicators of general risk (presence of cheetah and wild dogs).

## Materials and Methods

We conducted this study in Tswalu Kalahari Reserve, Northern Cape, South Africa (S 27°13'30" and E 022°28'40"). The reserve is divided into two separate, but adjacent sections that contain the same herbivore species but different predators. These sections are separated by fences, a road, and a 60 m buffer zone (Fig. 1). The only predators in the eastern section (20 000 hectares) of the reserve are 24 lions, while the western section (80 000 hectares) supports a minimum of 10 cheetahs (total population size is unknown, but these individuals seem to be resident in the area around the waterholes) and a pack of 14 wild dogs (i.e. lion-free; Makin et

al. 2017a; Makin et al. 2017b). Leopards (*Panthera pardus*) were infrequently observed within the reserve, and thus unlikely to be resident. Both sections contain populations of brown hyena (*Hyena brunnea*). However, they are largely scavengers and don't pose a threat to large herbivores (Skinner and Chimimba 2005). Herbivores living in the cheetah and wild dog section have not come into direct contact with lions for over 100 years (Roxburgh 2008). However, it is likely they periodically hear lion roars from the eastern section of the reserve where they were reintroduced in 2001 (Roxburgh 2008). Within Tswalu, waterholes are widely distributed across the landscape and offer the only source of permanent ground water. Thus, they are heavily utilised by herbivore species. We selected waterholes where the surrounding vegetation density was alike (van Rooyen and van Rooyen 2017), thus providing herbivores with similar sightlines and escape opportunities.

For this study, we limited our data collection to four herbivore species that were found in both predator sections. These included gemsbok, red hartebeest, zebra and wildebeest. Calculated herbivore species densities were based on aerial census data were 1.3 and 2.4 gemsbok/km$^2$, 0.63 and 1.2 red hartebeest/km$^2$, 1 and 0.8 wildebeest/km$^2$ and 0.2 and 1 zebra/km$^2$ within the lion-free and lion sections, respectively. To quantify how these herbivores adjusted their vigilance in response to differences in perceived predation risk, we used a playback experiment to manipulate the auditory landscape of fear. Herbivore alarm calls (zebra and wildebeest) and predator calls (lion roars) were played at eight different waterholes (five within the lion-free section and three within the lion section; Fig. 1) from January 2015 to April 2015 (see below).

We used zebra ('Kwa-ha' sounds and loud snorts) and wildebeest (grunts and snorts) alarm calls (Estes 1991) because they were two commonly occurring herbivore species found at waterholes across both predator sections of the reserve. In addition, both zebra and wildebeest have distinctive alarm calls/snorts and frequently call when they have detected a

potential threat (Stensland et al. 2003). Moreover, other large herbivore species have been found to eavesdrop on these alarm calls (Meise et al. 2018; Palmer and Gross 2018). As a predator cue, we used lion roars. Lions are ambush predators, thus they rely on silence and stealth to hunt prey (Schaller 2009). Moreover, lions tend to roar to advertise territorial ownership, locate pride members, strengthen bonds, and intimidate rivals (Pfefferle et al. 2007). As a control, we used black cuckoo (*Cuculus clamous clamous*) territorial calls, which do not have an alarm function, as they are a resident bird species that called frequently around the waterholes in both predator sections. Whereas some prey species have predator-specific alarm calls (i.e. different calls for terrestrial vs aerial predators; Enstam and Isbell 2004), zebra and wildebeest seemingly have general alarm calls for all large predators to warn of predation risk (Estes 1991; Leuthold 2012; Palmer and Gross 2018). To date, no studies have found that zebra and wildebeest employ referential alarm calling  in response to different predators (Palmer and Gross 2018). This, combined with our own experiences with these herbivores, makes us confident that zebra and wildebeest use general alarm calls. There may, however, be some degree of urgency relayed in these calls based on frequency of calling, but no work to our knowledge has been done on this.

We used three exemplars of each call type to prevent any impact of pseudoreplication on our study design (Kroodsma et al. 2001). In addition, we randomized the order in which calls were played at waterholes, such that the same playback treatments were not played consecutively (Hettena et al. 2014). We played the different calls through two Boashan horn speakers (Model: SK-610; Frequency response 315 – 12500Hz) attached to short stakes (~ 1 metre) set out near bushes (100 metres) away from the waterhole (Fig. 2). Playback calls were obtained through the Macaulay Library (Catalogue Numbers: Lion Roars: 221444-221446, Zebra: 126396-126398, Wildebeest: 13894-13896, black cuckoo calls: 89721351-3; Cornell Lab of Ornithology, Ithaca, USA; Fig 2). The frequency response of the speakers covered the

spectral envelope of the lion roars (400 - 8000Hz), which can occur at very low frequencies (Ananthakrishnan et al. 2011).

We played the calls at amplitudes deemed to simulate the natural call of the animal and this was maintained throughout the duration of the study (100 decibels – lion roar; Webster et al. 2012), 75 decibels – zebra and wildebeest, 65 decibels – black cuckoo control). The peak intensity of calls (dB) were checked at 1 m away from the speakers using a handheld Lutron Digital Sound Level Meter (Model: SL-4001). The calls were played from a 5-core Sound of India amplifier (Frequency: 25Hz – 20KHz; Model number: 5CA-4040) powered by a 12 V car battery (Leisure Pak, Model: FNL 464). During observations we positioned ourselves within a portable bush-hide located 100 m away from the waterhole which was sufficiently far enough away to minimize the effects of potential observer disturbance (Fig.2; Khoury 2013).

We played the different randomly selected playback calls once the focal herbivores had moved towards the waterhole and began drinking. Playback calls (20 seconds in length) were played every ten minutes at the waterhole for 2 hrs for a single exemplar of each call type (Khoury 2013). On average, the different herbivores spent less than 20 minutes at the waterholes (Table 1). Thus, we limited our data collection on vigilance behaviour to the first two playbacks of each call type that the different herds were presented with (i.e. a twenty-minute period, see details below). Moreover, we only recorded and analysed data from single groups of herbivores utilising the waterhole during the 20-minute playback period. While multiple groups of mixed species were recorded utilising waterholes together, these data were not included in the analyses.

To prevent waterholes from being considered as consistently dangerous, thereby reducing the chances of herbivores using them, we did not play calls at each waterhole consecutively, but rather randomized the days when playback calls would be played. This meant that each waterhole had a minimum of two rest days between playbacks when no calls

were played. Thus, for each waterhole, 1 of 3 exemplars for a call type (conspecific and heterospecific alarm calls, lion calls, and black cuckoo calls) was randomly selected and played once every ten minutes over a 2-hour period for a given day at a particular waterhole. Thus, a total of 8 waterholes, 4 different call types and 3 repeats of each call type were used, totalling 96 random playback combinations. The total number of times the species were presented with a playback of each call type for the different predator sections varied depending on the presence/absence of the species at a waterhole on a given day for the 2-hour observational period (Table 1). As a result, not all the species were exposed to the same number of playbacks for each treatment.

Vigilance behaviour is defined as a primary adaptive response to reduce perceived predation risk through actively scanning the environment for potential threats (Delm 1990). Thus, while vigilance behaviour can have other functions such as searching for mates and resources (Beauchamp 2015), within the context of this study, vigilance behaviour to reduce risk is key, as waterholes are dangerous areas where predators target prey species. As such, in our study, vigilance was defined as the focal animal standing, head up, and actively scanning their environment for potential threats (Beauchamp 2015). To determine how herbivores responded to the different playback calls, we recorded the vigilance of individuals compared to the control call (black cuckoo) to assess the magnitude of change in perceived predation risk at waterholes (Beauchamp 2015; Delm 1990).

We used the focal sampling technique to monitor the vigilance behaviour of one focal individual per group (Altmann 1974). For each individual, we recorded the proportion of time spent vigilant at waterholes for a maximum of 20 minutes, or over the entire duration if they spent less than 20 minutes at the waterholes (Périquet et al. 2010). The vigilance behaviour of one individual was recorded for 20 minutes during which each playback call was played twice, once as the group reached the waterhole, and again after 10 minutes. Within each 20-minute

observational period, the total amount of time the focal individual within the group was vigilant was recorded using a stopwatch.

Following the approach of (Périquet et al. 2010), we focused on individuals centrally located within each group. These individuals are unlikely to be attacked and/or killed before individuals on the group periphery. Thus, any increase in vigilance for central individuals likely reflects an increase in vigilance for the entire group. All individuals recorded were adults, and as females with juveniles will maintain greater levels of vigilance to protect their young, we only monitored adult females with no offspring (Makin et al. 2017b; Périquet et al. 2010). To avoid potential confounding group-size effects through recording the vigilance of individuals across different sized breeding groups, we recorded the vigilance of individuals from similar sized breeding groups (mean herd size = 8 ± 2 SE individuals) for each herbivore species. The mean proportion time spent vigilant by the herbivore species in response to each of the playback calls (i.e. wildebeest, zebra, control and lion) was then compared across the two predator sections (lion vs cheetah and wild dogs).

To test whether herbivore species living with lions responded more strongly to alarm calls, or the lion roars compared to the control call, we ran Kruskal-Wallis tests comparing the proportion time spent vigilant at waterholes for each herbivore species (gemsbok, zebra, red hartebeest and wildebeest) separately in response to the different playback calls (zebra, wildebeest, lion, control). To determine where significant differences in proportion time spent vigilant existed for each herbivore species in response to each of the different playback calls, we then ran Dunn's tests for multiple pairwise comparisons (Dinno and Dinno 2017).

Next, we compared herbivore species vigilant responses to the lion roars and zebra and wildebeest alarm calls across the two predator treatments (lion vs lion-free). To compare how the same herbivore species living across both lion and lion-free sections responded to the lion roars and alarm calls, we ran Wilcoxon Rank Sum tests on each species separately.

To avoid pseudo-replication when several groups of the same species were seen during the same 20-minute sampling period, and because group numbers were generally too low to use a random effect for group observations, we averaged the proportion time (mean ± standard error) spent vigilant by each species at each waterhole over each 20-minute sampling period. While it is possible that the same herbivores were monitored at multiple waterholes, this occurrence was likely rare, as most herbivore groups observed displayed high constancy for certain waterholes during this study (Pers obs. Makin 2015). However, the exact number of multiple individual observations is unknown as we could not identify all individual herbivores utilising waterholes over the duration of the study. All analyses were performed using R 3.25 (R Core Team 2014) using the PMCMR package (Pohlert and Pohlert 2018), MASS package (Venables and Ripley 2002), and the multcomp package (Hothorn et al. 2008).

## Results

We found that within the lion section the mean vigilant responses to the herbivore alarm calls and lion roars differed significantly compared to the control call for all four herbivore species (zebra, $X^2_{3,58} = 29.48$, P < 0.001; wildebeest, $X^2_{3,61} = 20.59$, P < 0.001; red hartebeest, $X^2_{3,31} = 17.98$, P < 0.001; gemsbok, $X^2_{3,61} = 23.87$, P < 0.001; Fig. 4). Specifically we found that compared to the control call, red hartebeest spent significantly more time vigilant in response to the lion roars (22 ± 6% up to 94 ± 6%) than either the zebra (22 ± 6% up to 43 ± 4%; $Z_{3,29} = 2.28$, P = 0.011) or wildebeest (22 ± 6% up to 33 ± 11%; $Z_{3,29} = 3.38$, P < 0.001) alarm calls (Fig. 4). In response to the zebra and wildebeest alarm calls, red hartebeest maintained similar levels of vigilance compared to the control call ($Z_{3,29} = -1.09$, P = 0.136). Wildebeest also spent more time vigilant in response to the lion roars (26 ± 3% up to 68 ± 3%) than the zebra alarm calls (26 ± 3% up to 27 ± 3%; $Z_{3,61} = 4.33$, P < 0.001). However, there was no significant difference in the vigilance response between the lion roars and wildebeest alarm calls (26 ± 3%

up to $60 \pm 5\%$; $Z_{3,61} = 1.59$, $P = 0.055$; Fig 4). Moreover, wildebeest responded more strongly to alarm calls from conspecifics than zebra alarm calls ($Z_{3,61} = 2.63$, $P = 0.005$; Fig 4).

Zebra maintained greater levels of vigilance in response to the lion roars ($18 \pm 6\%$ up to $70 \pm 9\%$) than the wildebeest alarm calls ($18 \pm 6\%$ up to $52 \pm 5\%$; $Z_{3,42} = 2.43$, $P = 0.007$) and the alarm calls of conspecifics ($18 \pm 6\%$ up to $58 \pm 4\%$; $Z_{3,33} = -2.81$, $P = 0.002$; Fig 4). There was no significant difference in the vigilance responses for gemsbok within the lion section comparing the lion roars with the wildebeest ($Z_{3,53} = 1.43$, $P = 0.077$) and zebra ($Z_{3,53} = -1.37$, $P = 0.085$) alarm calls (Fig 4). Similarly, there was no significant difference in the vigilance of gemsbok comparing their responses to the wildebeest and zebra alarm calls ($Z_{3,53} = -1.09$, $P = 0.139$; Fig 4).

Next, we compared the herbivore species' mean vigilant responses to the different calls across the lion and lion-free sections. In response to the lion roars, zebra ($W_{4,27} = 405.5$, $P = 0.015$), red hartebeest ($W_{4,34} = 191$, $P = 0.002$) and wildebeest ($W_{4,79} = 510$, $P = 0.001$) within the lion section increased their vigilance more than conspecifics in the lion-free section (Fig. 4). Mean vigilance was 28% higher for zebra, 39% higher for red hartebeest, and 19% higher for wildebeest in response to the lion roars within the lion section compared to conspecifics in the lion-free section (Fig 4). Although, gemsbok in the lion section were 6% more vigilant in response to the lion roars than conspecifics in the lion-free section, this did not differ significantly ($W_{4,37} = 189$, $P = 0.222$; Fig. 4). In comparison, within the lion-free section, only zebra ($Z_{3,27} = 5.11$, $P < 0.001$) and gemsbok ($Z_{3,37} = 2.04$, $P = 0.004$) significantly increased their mean vigilance in response to the lion roars (increasing by 27% and 24%, respectively), while wildebeest and red hartebeest did not increase their vigilance compared to the control playback (Fig. 4).

In response to the herbivore alarm calls, gemsbok in the lion's section maintained similar vigilance levels in response to the zebra ($W_{3,26} = 156$, $P = 0.193$) and wildebeest ($W_{3,19}$

= 65, P = 0.904) alarm calls compared to conspecifics in the lion-free section (Fig 4). Likewise, zebra in response to the alarm calls (wildebeest calls; $W_{3,13} = 49$, P = 0.2381 and zebra calls; $W_{3,23} = 58$, P = 0.221) had similar vigilance levels compared to conspecifics in the lion-free section (Fig 4). In response to the alarm calls there was no significant difference in the mean vigilance responses of red hartebeest (wildebeest alarm call; $W_{3,28} = 55$, P = 0.636 and zebra call; $W_{3,19} = 153$, P = 0.115) across sections. Moreover, there was no significant difference in the vigilance responses of wildebeest in response to the call of conspecifics across sections ($W_{3,18} = 48$, P = 0.843; Fig. 4). In response to zebra alarm calls, wildebeest in the lion-free section responded more strongly than conspecifics in the lion section ($W_{3,86} = 646$, P = 0.029). Surprisingly, the baseline vigilant response to the control call for gemsbok ($W_{3,11} = 9$, P = 0.014), red hartebeest ($W_{3,20} = 17$, P = 0.015) and wildebeest ($W_{3,24} = 42$, P = 0.030) in the lion-free section was greater than conspecifics in the lion section (Fig. 4).

## Discussion

Comparing auditory cues as indicators of increased predation risk, several studies have highlighted that prey species react more strongly to conspecific and heterospecific alarm calls compared to predator sounds (Blumstein et al. 2008; Griffin et al. 2000; Hettena et al. 2014). However, some species seem to react more to cues given off by dangerous predators than alarm calls (Barrera et al. 2011; Rainey et al. 2004). We found that the vigilance responses of the different herbivores supported the prediction that the cues from a dangerous predator may indicate greater immediate risk than conspecific or heterospecific alarm calls. However, these responses were species-specific and varied significantly across the lion and lion-free sections.

We found that within the lion section, red hartebeest and zebra increased their vigilance more in response to lion roars (i.e. predator vocalization) compared to conspecific and/or heterospecific alarm calls. A similar increase was observed for wildebeest, although it was not

significantly different from their response to conspecific alarm calls. Although gemsbok significantly increased their vigilance in response to the lion roars within the lion section, they also responded strongly to the conspecific and heterospecific alarm calls. Specifically, gemsbok responded as strongly to the wildebeest and zebra alarm calls as they did to the lion roar. In the lion-free section, zebra and gemsbok increased their vigilance similarly in response to both the alarm calls and the lion roars. This was despite the likely periodic exposure to lion roars from the other side of the reserve. While, both zebra and gemsbok (preferred prey of lions; Hayward and Kerley 2005) significantly increased their vigilance in response to the lion roars, surprisingly, wildebeest (also preferred prey of lions) and red hartebeest did not.

Rainey et al. (2004) proposed the 'information precision hypothesis' in support of the stronger anti-predator response to predator vocalizations, which suggests that in contrast to alarm calls, the information contained in predator cues provides more accurate spatial information on predator location. An alarm signal may represent the signaller's perception of risk, rather than the listeners, whereas, a predator call provides accurate information on the exact type and location of a predator (Rainey et al. 2004). Therefore, for many species, direct cues on predator location can be more useful, and thus reduce predation risk more than an indirect cue (Rainey et al. 2004; van der Veen 2002). For example, Gil-da-Costa et al. (2003) found that howler monkeys (*Alouatta palliate*) rapidly responded to the calls of recently reintroduced harpy eagles (*Harpia harpyja*), increasing the mean amount of time they spent vigilant compared to the alarm calls of other bird species (Gil-da-Costa et al. 2003). In addition, in response to the playback calls of red-tailed hawks (*Buteo jamaicensis*), Zenaida doves (*Zenaida aurita*) had higher levels of vigilance compared to the alarm signals of conspecifics (Barrera et al. 2011). Across these systems, these predators presented a significant predation risk to these prey species and therefore the prey showed strong responses to cues from these predators.

Although not one of the aims of the study, comparing the strength of the herbivores' vigilant responses to the different alarm calls revealed an interesting interaction between zebra and wildebeest within the lion section. Both fall within the preferred prey class of lion (Hayward and Kerley 2005). However, zebra increased their vigilance in response to both alarm calls, with a slightly stronger vigilance response to the conspecific alarm call. In contrast, wildebeest only increased their vigilance in response to conspecific alarm calls. Similarly, a study by Meise et al. (2018) investigated species-specific dependencies on heterospecific alarm calls in an African savanna and found that wildebeest had a lower probability of responding to the alarm calls of zebra than zebra had to wildebeest alarm calls. Overall, in their study, wildebeest had a lower probability of responding to heterospecific calls from multiple herbivore species compared to zebra. One possibility is that wildebeest employ anti-predator behaviours that improve their early predator detection (i.e. improved hearing, smell; Schmitt et al. 2014), such that zebra rely on cues from wildebeest as reliable sources of anti-predatory information (Schmitt et al. 2014).

If wildebeest do indeed employ improved predator detection behaviours, then they may respond more to the alarm calls of conspecifics. Alternatively, the information conveyed in wildebeest alarm calls could provide more accurate and reliable information on predation risk than zebra calls, with both wildebeest and zebra (often occurring within mixed herds) relying on these alarm calls to manage potential predation risk (Meise et al. 2018). Moreover, prey species' responses to heterospecific alarm calls have been shown to increase with a greater predator overlap. Thus, prey species that are preferentially targeted by the same predators may respond more strongly to each other's alarm calls as indicators of shared risk (Meise et al. 2018). In contrast to our findings, Palmer and Gross (2018), found that wildebeest responded more strongly to zebra alarm calls, than either wildebeest or impala (*Aepyceros melampus*) calls within Pilanesberg National Park, possibly due to zebra calls as greater indicators of risk.

These differences may reflect site-specific prey preferences of lions between Pilanesberg National Park, South Africa (Palmer and Gross (2018) and Tswalu (our study), and thus potentially reflect differences in the reliability of conspecific and heterospecific alarm calls. This, however, requires further investigation.

In the absence of predators, prey species can lose previously adaptive anti-predator behaviours over time (Blumstein 2006). However, some species retain these anti-predator behaviours and therefore respond quickly and appropriately to cues from these predators (Blumstein et al. 2009; Chamaillé-Jammes et al. 2014). These innate anti-predator behavioural responses may be due to interactions with other extant predators on the landscape (Blumstein 2006). For example, yellow-bellied marmots retained their recognition of wolf cues (an extinct predator) due to extant predation risk from red foxes (*Vulpes vulpes*) and coyotes (Canis latrans; Blumstein et al. 2009). Alternatively, the loss of these behaviours may only occur after extended periods of isolation from the predator (Sih et al. 2010). The results of our study indicated that red hartebeest, wildebeest and zebra responded to the lion roars more strongly within the lion section than the same herbivores species living without lions. This suggests that the effect of lion presence on the landscape coupled with spatial information obtained from the lion roar provided a greater indicator of potential risk than the call in the absence of the predator. Similarly, Berger (2007) found that elk (*Cervus canadensis*) and bison (*Bison bison*) displayed increased vigilant responses to wolf (*Canis lupus*) calls in areas where wolves had been reintroduced compared to wolf-free areas.

However, despite generally lower levels of vigilance in the lion-free section in response to the playback calls, we found that zebra and gemsbok significantly increased their vigilance in response to the lion roar. This suggests that although lions are absent from the landscape, their auditory cues still indicated potential risk to these herbivores. This observation may reflect a spill over effect, with calling lions (± 8km call distance; Sunquist and Sunquist 2017) in the

lion section being heard by these ungulates within the lion-free section and thus predator recognition was potentially maintained over time even in the absence of the actual predator and direct mortality. This would suggest that habituation did not occur, despite the lack of actual predation risk. Thus, our study shows that they have retained their anti-predator responses for this dangerous ambush predator (Creel et al. 2014). Similarly, Dalerum and Belton (2014), found that both naïve and lion exposed populations of impala, wildebeest and warthog (*Phacochoerus africanus*) responded to auditory calls of lion by increasing their vigilance. This is similar to mule deer (*Odocoilus hemionus*) in the East River Valley, USA, that responded to wolf vocalizations despite the fact that wolves had been absent from the area for over 100 years. Therefore, mule deer retained their anti-predator behaviours to cues from wolves possibly due to predation risk from coyotes and puma (*Puma concolor*; Hettena et al. 2014). In contrast, black-tailed deer (*Odocoilus hemionus sitkensis*) showed an innate anti-predator response to an absent dangerous predator (wolf cue) by strongly modifying their threat-sensitive foraging behaviour more so than in response to a black bear cue (*Ursus americanus* – less dangerous present predator). Therefore, prey can also retain recognition of and respond to absent predators for several generations, even when closely related predator species are absent (Chamaillé-Jammes et al. 2014).

Although wildebeest and red hartebeest fall within the preferred weight range of lion (Clements et al. 2014), neither species significantly increased their vigilance in response to the lion roar within the lion-free area. This was particularly surprising for wildebeest as based on a multi-site analysis they are a highly preferred prey of lion (Hayward and Kerley 2005). This lower observed vigilant response to the lion roar by wildebeest and red hartebeest is in part due to the high vigilance observed for these species in response to the control call within the lion-free section. Their vigilance responses to the black cuckoo calls suggested that they maintain a higher baseline level of vigilance compared to conspecifics in the lion's section. This was

unexpected as wildebeest are largely avoided by cheetah and wild dogs, while red hartebeest are taken relative to availability by cheetah and avoided by wild dog (Hayward et al. 2006a; Hayward et al. 2006b). However, based on Hayward et al. (2006b), Jacobs' Index of prey preferences from a multi-site analysis of wild dog kill data, wildebeest (-0.70) and red hartebeest (-0.56) are more preferred by wild dogs than either zebra (-0.88) or gemsbok (-1.0). This potentially provides an explanation for the higher baseline level of vigilance of wildebeest and red hartebeest as these species likely perceive wild dogs to be a greater threat at waterholes than either zebra or gemsbok. This does not, however, provide an explanation for why gemsbok maintained higher vigilance in response to the control call in the lion-free section than conspecific in the lion's section.  A higher baseline of vigilance should have been observed in the lion section for all four species as they are more preferred prey of lion (Hayward and Kerley 2005). Nevertheless, this was not the case. The factors driving this observation are unclear.

Potentially, greater responses to the lion roars may be due to the fact that the lion's calls were played at 100db, compared to the alarm calls (75db) and control call (65db), whereby herbivores were responding to the greater call volume rather than the increased immediate risk coded for in the call. However, as herbivore responses to alarm calls and the control call were as great or greater than their response to the lion roars for some of the species, this suggests that prey species responded to the perceived risk associated with each call and not the call volume.

While most studies have pointed to alarm calls as indicators of greater risk than predator calls (Magrath et al. 2015; Shriner 1998), our study suggests that auditory cues from dangerous predators can reflect high levels of predation risk, thus prompting prey species to react by employing anti-predator behaviours. Moreover, this can extend to prey species that no longer live with these key predators (Blumstein 2006; Sih et al. 2010). Specifically, the predation risk associated with a cue from an absent but dangerous predator can trigger a strong innate anti-

predator response in prey species that are preferentially targeted by the predator (for olfactory cues see; Chamaillé-Jammes et al. 2014). Alternatively, these anti-predator behavioural responses to dangerous but absent predators can be retained in prey populations when other extant predators are present on the landscape (i.e. cheetah and wild dogs in this study; Blumstein 2006). Therefore, with the return of predators into systems, prey species are potentially able to recognize predator cues as indicators of risk and adjust their anti-predator behaviours accordingly to minimize risk.

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**Conflict of interest** The authors declare that they have no conflict of interest.

**Data Availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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**Table 1.** The total number of playback calls the herbivore species were presented with at waterholes, including mean time spent at waterholes (minutes ± SE) across the lion and lion-free sections.

| Species | Playback Call | Lion | Lion-free |
|---|---|---|---|
| Gemsbok | Black cuckoo | 7 | 10 |
| | Lion | 30 | 10 |
| | Wildebeest | 9 | 15 |
| | Zebra | 15 | 24 |
| | Mean time (mins ± SE) | 11.1 ± 1.4 | 14.4 ± 1.6 |
| Zebra | Black cuckoo | 9 | 10 |
| | Lion | 23 | 25 |
| | Wildebeest | 19 | 20 |
| | Zebra | 17 | 15 |
| | Mean time (mins ± SE) | 6.26 ± 0.7 | 6.45 ± 1.1 |
| Red hartebeest | Black cuckoo | 7 | 14 |
| | Lion | 8 | 28 |
| | Wildebeest | 8 | 12 |
| | Zebra | 8 | 28 |
| | Mean time (mins ± SE) | 7.3 ± 1.8 | 8.9 ± 0.9 |
| Wildebeest | Black cuckoo | 17 | 10 |
| | Lion | 11 | 57 |
| | Wildebeest | 17 | 15 |
| | Zebra | 27 | 67 |
| | Mean time (mins ± SE) | 12.3 ± 1.7 | 7.8 ± 0.6 |

**Figure 1**. Location of waterholes (black stars) used for the playback experiments within the Tswalu-Kalahari Game Reserve, South Africa. Grey lines denote boundary fences separating the two predator sections. The straight black line between the sections is the district road running through the 60-metre-wide buffer zone.

**Figure 2**. Example of the auditory playback experimental design with speakers placed 100 m away from the waterhole. The observer was positioned 100 m away in a hide with a clear view of the entire waterhole. Dark spots are bush and tree clumps.

**Figure 3**. Representative spectrograms of the wildebeest and zebra alarm calls, lion roars, and black cuckoo territorial calls that were played at waterholes to herbivore species groups in Tswalu Kalahari Reserve. Spectrograms were obtained through the Macaulay Library Cornell Lab of Ornithology, Ithaca, USA).

**Figure 3**. Mean proportion of time spent vigilant by a) gemsbok, b) red hartebeest, c) zebra and d) wildebeest at waterholes within the two predator treatments (i.e. lions, lion-free). Vigilance levels of these species are shown after playbacks of a black cuckoo (control), lion roars, and wildebeest and zebra alarm calls (e.g. snorts). Bars represent SE. Mean proportion vigilance values sharing letters are not significantly different, as assessed by the Dunn's *post-hoc* comparison tests.
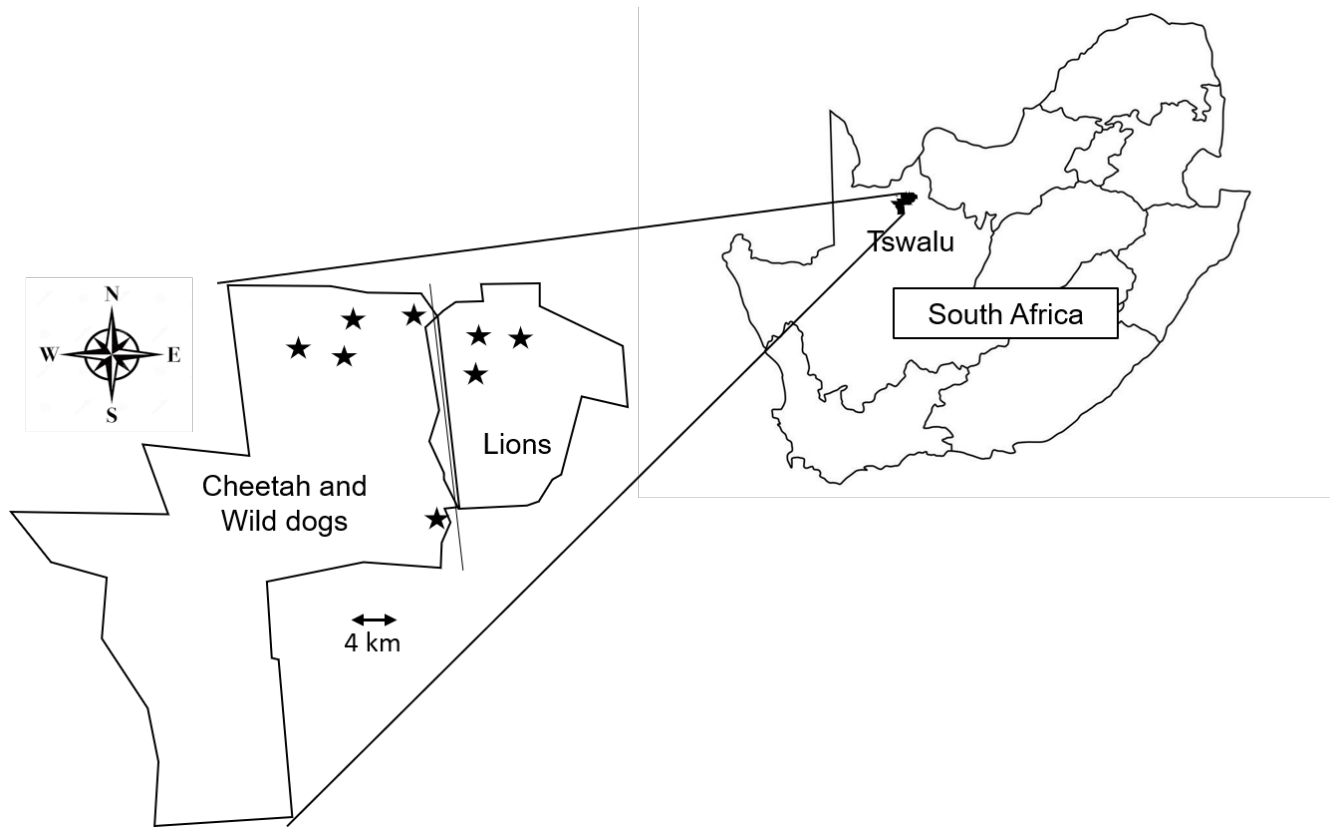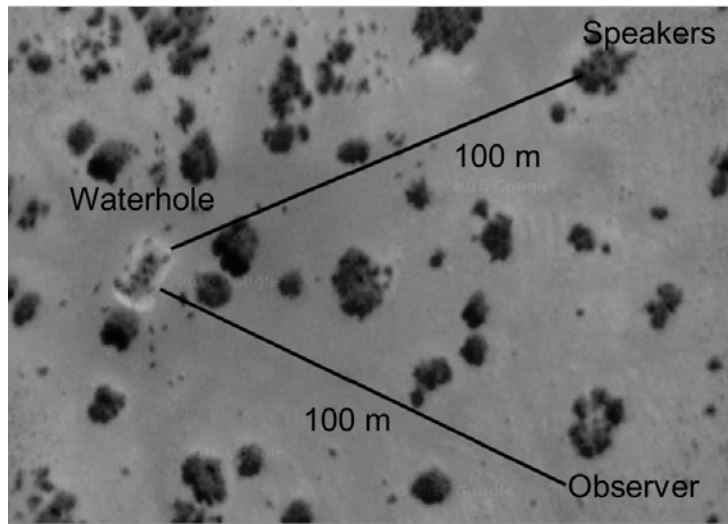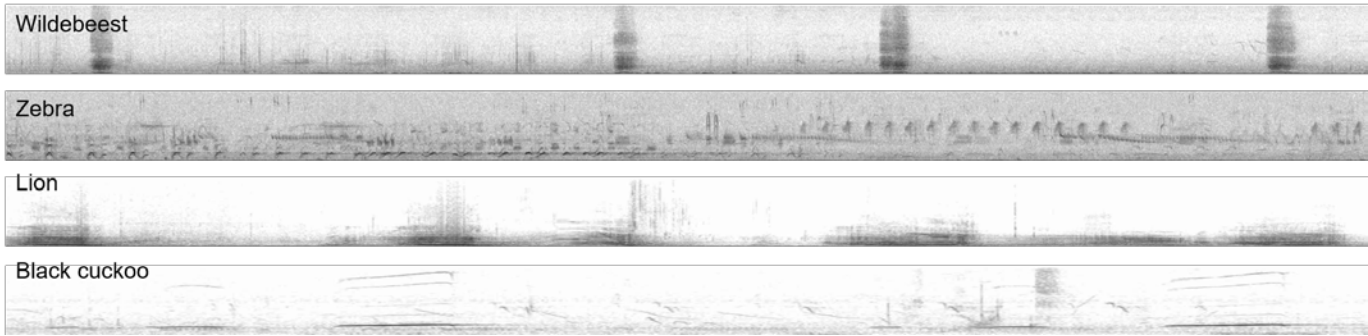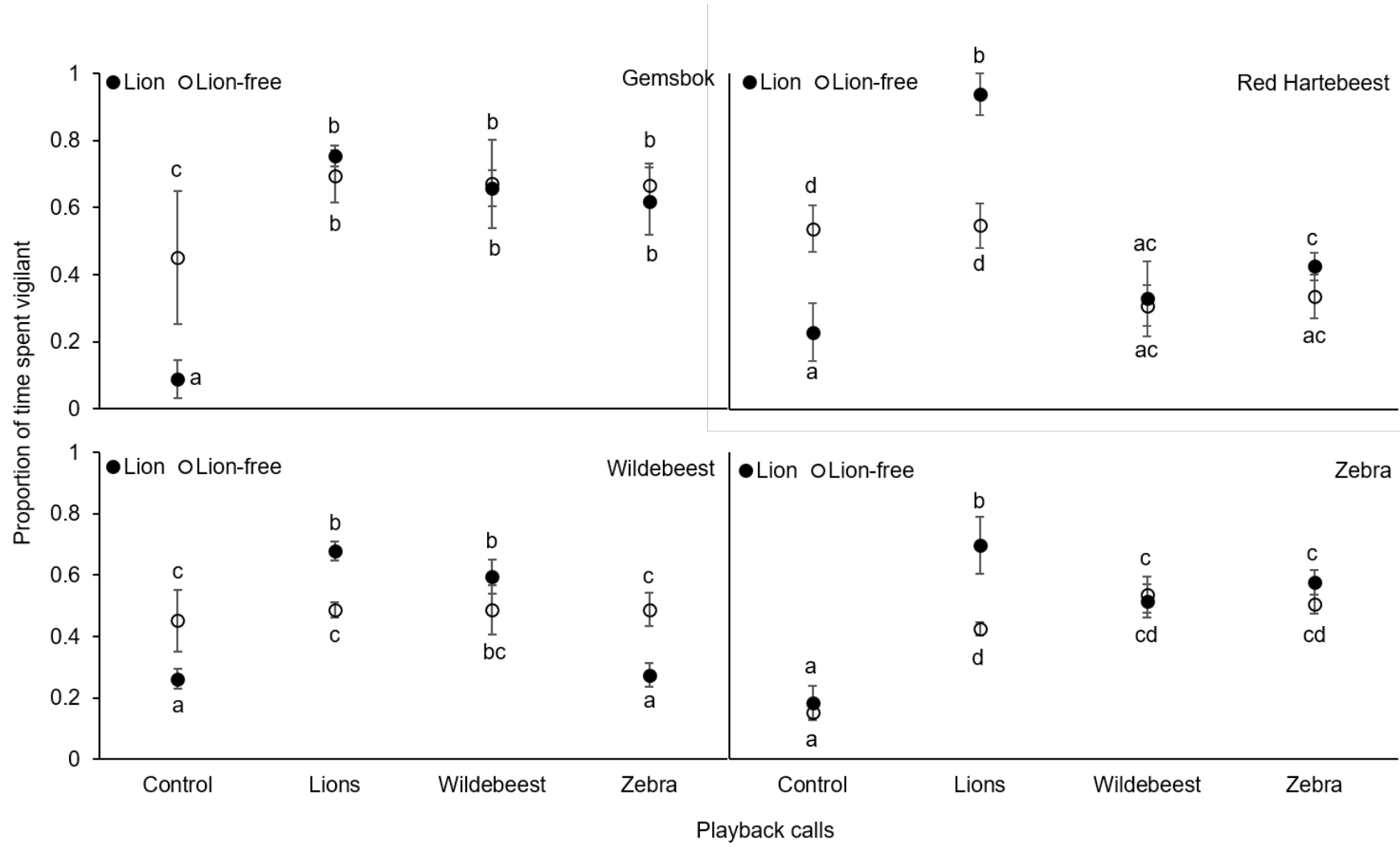
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