Successful predatory-avoidance behaviour to lion auditory cues during soft-release from captivity in cheetah

Wemer, Nynke; Naude, Vincent N.; van der Merwe, Vincent C.; Smit, Marna; de Lange, Gerhard; Komdeur, Jan

Published in:
Ethology

DOI:
10.1111/eth.13261

Received: 25 June 2021 | Revised: 12 December 2021 | Accepted: 15 December 2021

DOI: 10.1111/eth.13261

RESEARCH ARTICLE

ethology WILEY

Successful predatory-avoidance behaviour to lion auditory cues during soft-release from captivity in cheetah

Nynke Wemer[1] | Vincent N. Naude[2] | Vincent C. van der Merwe[2,3] | Marna Smit[4] | Gerhard de Lange[5] | Jan Komdeur[1]

[1]Behavioral Physiology and Ecology Group, Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, the Netherlands

[2]Institute for Communities and Wildlife in Africa, University of Cape Town, Rondebosch, South Africa

[3]Endangered Wildlife Trust, Johannesburg, South Africa

[4]Ashia Cheetah Conservation, Paarl, South Africa

[5]Kuzuko Lodge Private Game Reserve, Greater Addo Area, South Africa

Correspondence
Nynke Wemer, Behavioral Physiology and Ecology Group, Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Nijenborgh 7, 9747 AG Groningen, the Netherlands.
Email: nynkewemer@gmail.com

Funding information
ASHIA Cheetah Conservation RF PTY LTD.

Abstract

Due to global biodiversity declines, conservation programmes have increasingly had to consider reintroducing captive animals into the wild. However, reintroductions often fail as captive individuals may be naïve to predators and do not recognise or respond appropriately to predatory cues, contributing to high mortality rates soon after release. This study evaluates differences in predator-response behaviours between individuals from three experimental groups, a captive population ($n = 13$), a semi-wild population (i.e. raised in captivity and successfully released; $n = 6$) and a wild population ($n = 2$) of cheetah (*Acinonyx jubatus*) to an artificially simulated auditory threat of lions (*Panthera leo*), a larger, natural predator in South Africa. Such comparisons improve our understanding of differences between captive and wild behaviours and provide an aspect to evaluating the relative success of reintroduction programmes. Changes in the proximal distance, the latency of approach and hesitation towards both control (African bush cricket, *Acanthoplus discoidalis*) and treatment (lion) auditory cues were observed for 29 cheetah from captive, semi-wild and wild populations in at least three trial replicates each. Overall, captive individuals consistently displayed poor predatory-response behaviours, approaching the treatment as often as the control, as well as spending more time near the stimulus (<10 m) and hesitating more often than semi-wild cheetah, which could distinguish between the control and treatment, consistently fleeing from the latter with little hesitation. Repeatability analyses indicated that these behavioural responses to predatory cues could not be explained by individual personality and between-trial learning comparisons showed no evidence of habituation. Our findings demonstrate how *a priori* testing for predator naïvety could inform future introductory decisions and thereby increase post-release survival rates, significantly improving the efficacy of reintroduction strategies. We, therefore, emphasise the importance of such research and screening in highly threatened species, such as cheetah, where reintroduction from captivity has become a necessary consideration.

KEYWORDS
Acinonyx jubatus, conservation, learned behaviour, metapopulation, *Panthera leo*, rewilding

1 | INTRODUCTION

Currently, many multifaceted conservation intervention programmes increasingly consider the value of captive (i.e. bred or held) individuals and focus on means of feasibly reintroducing them into the wild (Rowell et al., 2020). Reintroduction success is largely dependent on ecological factors such as prey availability (Berger-Tal et al., 2019), competition (Borrego et al., 2018; Briers-Louw et al., 2019), predation pressure (Buk et al., 2018) and reproductive potential (Bissett & Bernard, 2007; Komdeur & Hammers, 2014), as well as the release method itself. Using a "soft-release" method on captive individuals (as opposed to "hard-release") provides them with a gradual transition into the wild (De Milliano et al., 2016; Rowell et al., 2020). This allows previously captive individuals to learn and/or maintain behaviour that is associated with survival in the wild (e.g. predator avoidance, hunting experience and parental care), which is fundamental to the success of such conservation efforts (McPhee & Carlstead, 2010; Rowell et al., 2020). However, despite the global increase in reintroduction efforts, many are unsuccessful. Captive-bred individuals present especially high mortality rates after their release into the wild (Efrat et al., 2020; Greenspan et al., 2020; Jule et al., 2008), highlighting a major area of concern amongst captive-breeding and reintroduction programmes. Evaluation of multiple reintroduction programmes has revealed that a high proportion of reintroduction mortalities are due to individuals displaying detrimental behaviour in the wild (McPhee & Carlstead, 2010); including failure to adequately respond to predators, failure to locate food resources, and stress due to novel environmental exposure (Smith & Blumstein, 2013; Wells et al., 2004). If individuals that do survive and successfully establish themselves are unable to reproduce, the reintroduction is likewise considered unsuccessful (Komdeur & Hammers, 2014; McPhee, 2004).

Behavioural differences allow some individuals to better cope with reintroduction into the wild (Merrick & Koprowski, 2017; Schuett et al., 2010). For example, behavioural types that respond most beneficially to potentially risky, dangerous, and stressful situations are more likely to survive after being reintroduced. For instance, individual swift foxes (*Vulpes velox*) who died after being released, were considered "bolder" towards novel objects in captivity than those that survived (Bremner-Harrison et al., 2004). Such individuals may be risk-prone, endangering themselves through their behaviour, especially when facing new environmental challenges, such as competitors, predators and dangerous prey (Blaszczyk, 2017; Roy & Bhat, 2018). In contrast, releasing "timid" individuals also presents challenges, as these individuals generally experience lower reproductive success, which, in turn, leads to decreased fitness (Smith & Blumstein, 2013). Suitability of individuals for reintroduction, therefore, relies on a consideration of the match between the behaviour of the candidate and the environment into which it is intended to be introduced (Banks et al., 2001; Blumstein et al., 2019). Generally, animals kept in captivity have a higher chance of being preyed upon after reintroduction, becoming complacent or not having learned and retained the ability to recognise and respond adequately to environmental stimuli (DeGregorio et al., 2017; Ross et al., 2019; Shier & Owings, 2007). Captive-bred individuals, in particular, lose their anti-predatory behaviour within just a few generations (Jolly et al., 2018; Moseby et al., 2015; Rowell et al., 2020; Vilhunen, 2006). Therefore, it is necessary to closely monitor candidates for translocation both pre- and post-reintroduction to ensure that they are not only suitable candidates (Canessa et al., 2016; Kongsurakan et al., 2020; Rozhnov et al., 2011) but are also capable of thriving in these new habitats (Berger-Tal & Saltz, 2014; Blumstein et al., 2019). Despite being largely overlooked by the ecological rewilding community, animal behaviour may yet play a key role in conservation at the level of individually optimised success and site-specific tailoring for reintroductions (Bremner-Harrison et al., 2018; Merrick & Koprowski, 2017).

This study explores predator-avoidance behaviour by measuring individual behavioural responses in cheetah (*Acinonyx jubatus*) from three experimental groups, a captive population, a semi-wild population (i.e. in the process of being rewilded through "soft-release" in larger reserves with exposure to predators), and a wild population. Cheetah are considered "vulnerable" on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, having disappeared from approximately 90% of their historical range in Africa (Durant et al., 2017; Menotti-Raymond & O'Brien, 1993) and present relatively low genetic diversity across the species range (Menotti-Raymond & O'Brien, 1993). The majority of remaining cheetah are found in Namibia, Kenya, Tanzania and South Africa (Durant et al., 2017; Marker, 2019). Cheetah reintroductions in South Africa intensified in the early 2000s, with most of the remaining population currently managed as a growing metapopulation comprising over 461 cheetah on 63 fenced reserves. These individuals are considered wild as they are required to hunt, are exposed to diseases and co-exist with competing predators (Buk et al., 2018), including lions (*Panthera leo*).

To inform and ultimately optimise release success in the South African cheetah metapopulation, we examine predator-avoidance behaviour in cheetah towards artificially simulated predatory cues of lion in captive, semi-wild and wild populations. Lions are amongst the primary causes of mortality in cheetah post-reintroduction (Buk et al., 2018), being highly territorial and opportunistic, they have been recorded actively pursuing and killing cheetah (Rostro-García et al., 2015). Cheetah were systematically exposed to auditory cues (pre-recorded lion roars) in captive, semi-wild and wild settings. We predicted that owing to the lack of predator interactions in captivity and the loss of anti-predatory behaviour after just a few generations, such individuals would not recognise and respond to these cues as a threat (Jolly et al., 2018; Moseby et al., 2015; Rowell et al., 2020; Vilhunen, 2006). We predicted that these predator-naïve cheetah will approach the artificial lion cues faster and more frequently than the semi-wild population, which are expected to recognise the lion cues as a threat and respond by more frequently moving away from the threat with less hesitation. Identifying which individuals in captivity express these maladaptive behaviours and intervening to limit such conduct before or exclude these cheetah from release could improve survival and establishment post-reintroduction.

2 | METHODS

2.1 | Study areas

Behavioural data were collected from two South African cheetah populations (Figure 1). The first, a captive population of 13 individuals (n_{male} = 7, n_{female} = 6) at Ashia Cheetah Conservation (ACC; 33°43'29.4"S 19°01'22.7"E) and the second, a semi-wild population of 6 adult individuals (n_{male} = 1, n_{female} = 5) and 8 cubs, as well as an additional wild population of 2 adult males at Kuzuko Lodge Private Game Reserve (KLPGR; 33°12'44.1"S 25°29'56.0"E). Due to the low "wild" population sample size (n = 2), any contrasts to these wild individuals should be seen as an additional exploratory analysis step rather than a directly comparable group throughout. ACC represents a fully captive population, KLPGR comprises semi-wild cheetah that have come from captivity but are in a three-stage "rewilding" process, whereby (1) individuals were kept in a small enclosure (2.5 km^2) with no predators and provisioned with food (similar to ACC), before (2) being moved to a larger area (4 km^2) with no predators, but which is stocked with prey species (Table S1) to encourage hunting behaviour, before being moved to stage (3) a large fully rewilded area (>150 km^2) either within KLPGR or another reserve, that is both stocked with prey and has natural predators, including lions (Figure S1). The time spent in each stage is approximately two weeks in stage 1 and, dependent on the space and current numbers of cheetah at KLPGR, approximately a year in stage 2, whilst stage 3 represents permanent placement. Whilst all individuals at KLPGR could hear and smell lions, only cheetah in stage 3 were in direct contact with them, and 3 were considered for this study. Stage 2 (the semi-wild population) consists of six cheetah that have been (partially) raised in captivity, some of them at ACC, whilst stage 3 (the wild population) consists of one previously captive cheetah (from ACC) and one wild-born cheetah. The previously captive cheetah from stage 3 was considered independent and 'wild' for over a year before these auditory response trials began. Individuals were monitored by direct observation at ACC, whereas at KLPGR, all individuals were fitted with VHF or IR-SAT tracking collars (African Wildlife Tracking; 148 MHz to 152 MHz).

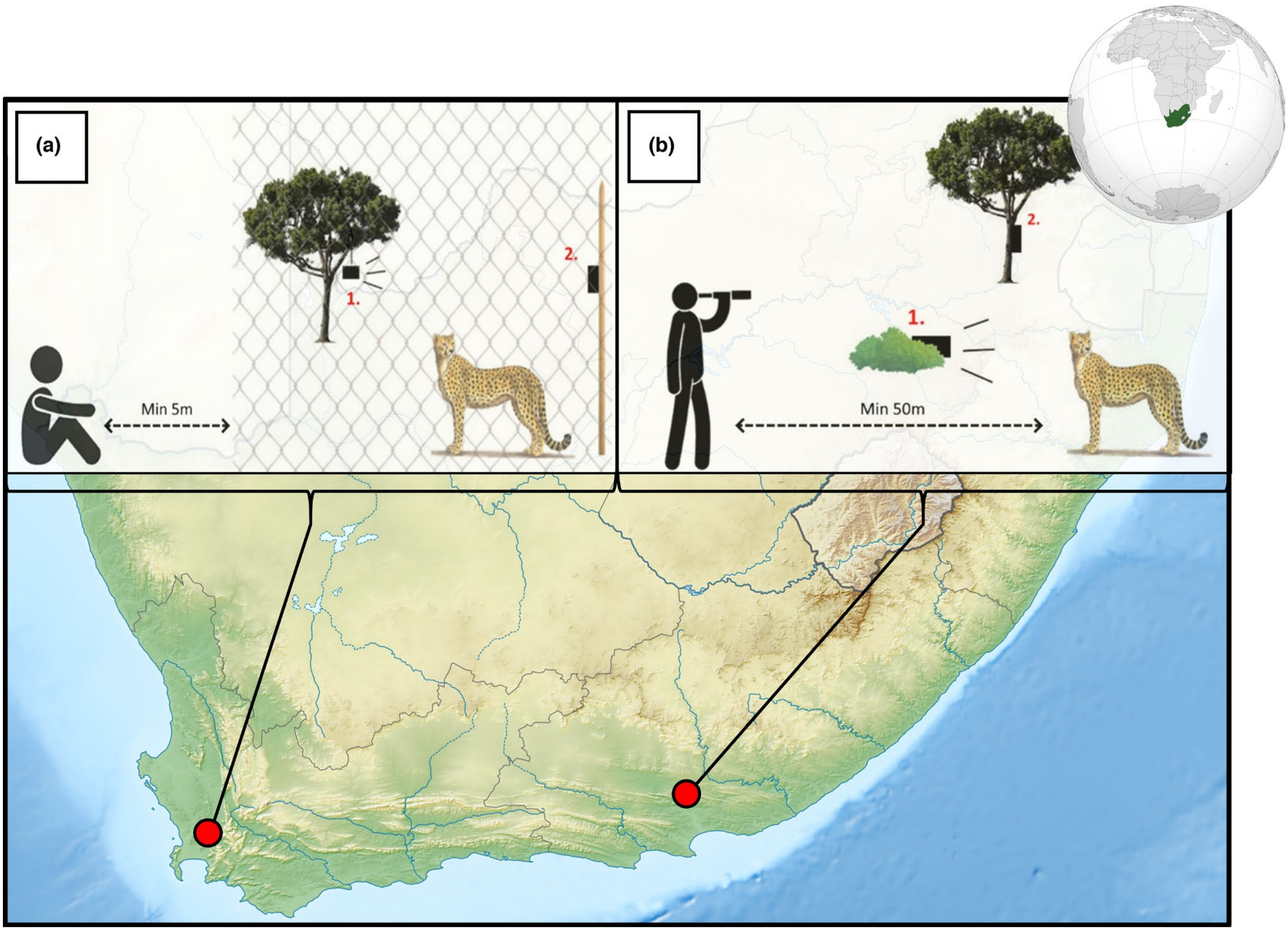

FIGURE 1 The two predatory-response trial study areas in South Africa. At Ashia Cheetah Conservation (ACC), trials were observed ≥5 m from the enclosure fence (a), whilst at Kuzuko Lodge Private Game Reserve (KLPGR), trials were observed from ≥50 m (b). Indicated in both are the strategic auditory cue (1) and camera trap (2) placement for each trial

2.2 | Data collection

All cheetah were exposed to artificially simulated predatory cues to determine the effect of captivity on both recognition and response to these stimuli that would otherwise represent potentially dangerous situations for cheetah in the wild (Figure 1). Trials consisted of systematic exposure to auditory cues (Table S2), which would simulate either naturally ambient sound (control) or sounds indicating the presence of natural predators in the area (Chame, 2003; Piñeiro & Barja, 2012). Each individual (Table 1), underwent a minimum of three randomised trials and control, with the same individual being tested at least four days apart to prevent habituation. As both cheetah and lion activity levels decline in response to midday heat in the wild (Hetem et al., 2019), no trials were conducted when ambient temperatures exceeded 25°C (Figure S2). This resulted in average daily trial windows of 06:00–10:00 and 16:00–19:00 over a dedicated monitoring period of three weeks at each location (ACC: 03/02/2020–20/02/2020; KLPGR: 27/02/2020–17/03/2020). Trials were conducted by a single observer, even when multiple cheetah were present (with a maximum coalition size of two). Individual cheetah were identified by unique facial markings and verified by GPS collar data (where available).

2.3 | Auditory cues

Auditory cues (Table S2) comprised one of three lion roar recordings randomly selected per trial and a control recording of African bush crickets (*Acanthoplus discoidalis*). In the captive population (ACC), enclosures (2 km^2) were cleaned daily (06:00–09:00), allowing for the safe, random placement of the auditory cue station. Stations included a speaker (MIFA A1 outdoor wireless speaker, MIFA INNOVATIONS LLC) inside a camouflaged protective cage (155 × 50 mm) positioned in a tree at ≥1 m above the ground (Figure 1a). Each trial began when the individual(s) re-entered the enclosure and ended after 15 min of behavioural observation from >5 m distance. This relatively short distance was unlikely to affect the trials as captive cheetah were habituated to human presence. Observations were recorded verbally (Philips DVT 1110 Digital VoiceTracer Audio Recorder, 750 Hz–18 KHz, PHILIPS) and by trail camera video (Foxelli Oak's Eye 2, 20MP 1080P HD, Motion Activated Night Vision, 120° Wide Angle Lens, 42 IR LEDs, IP66, FOXELLI BV™). In the semi-wild and wild populations (KLPGR), a long-range speaker with remote control was used (FOXPRO Spitfire Electronic Predator Call speaker, FOXPRO inc.) to account for the substantial difference in enclosure size and reduce the influence of human presence. The speaker and trail camera were placed on foot at random within the landscape (Figure 1b). Each trial began when an individual came within 40m of a station, followed by 15 min of behavioural observation (voice and video recordings) from ≥50m using binoculars (Bresser, Condor | 10 × 42, 154 × 133 × 52 mm, 669 g, Bresser GmbH). Once the individual(s) had left the area and it was determined safe to do so, stations were disassembled and reset for the next trial.

2.4 | Behavioural responses

Recognised behaviour observed and recorded in response to auditory trials included grooming, vocalisation, resting, feeding, locomotion, rapid locomotion, social interaction with conspecifics, exploration and aggression, as well as territorial, abnormal and other behaviours. For each of these behaviours, the relative frequency and time spent (in seconds) were measured throughout the trial period (15 min) for each individual. As locomotion, rapid locomotion and resting were the only measured behavioural types that occurred often and consistently amongst individuals, these were focal to the study. When observing different predatory responses to auditory cues, the difference between end and start distances of each individual to the auditory station were considered per trial (i.e. proximal distance). When comparing the three populations, two focal behavioural response categories were considered: (1) proximal distance categories from the station (>20 m, 10–20 m and <10 m) and (2) hesitation, defined as the number of distance category transitions, allowing at least seven seconds to exclude pacing behaviour when approaching the station (i.e. every time an individual takes longer than seven seconds and crosses a distance category, the hesitation score increases). All behavioural responses were analysed using Behavioural Observation Research Interactive Software (BORIS; Friard & Gamba, 2016).

2.5 | Statistical analyses

All statistical analyses were conducted in R studio v3.5.1 (R Core Team, 2018). Observed differences in predator-response behaviour were categorised according to (1) proximal distance (control versus treatment), (2) presence at <10 m, (3) time spent at <10 m, as well as (4) presence and, if any, (5) frequency of hesitation. A within-individual comparison was implemented in the “lme4” package v1.1–27.1 (Bates et al. 2015) to analyse the difference between proximal distances to auditory stations between the three populations. Generalised linear mixed-effects models (GLMER; $\alpha = 0.5$) were used to analyse presence at <10 m from the set-up, with evidence of hesitation following a Binomial distribution, whilst the frequency of hesitation was modelled following a Poisson distribution. Time spent at <10 m was log-transformed due to non-normality before a linear mixed-effects model (LMER; $\alpha = 0.5$) was implemented. Herein, parameters 2–5 were response variables per population. To control for conspecific learning, female-male behavioural differences (e.g. when females have cubs) and relative activity levels, we used partnership, sex and ambient temperature as explanatory variables, including individual identity as a random factor. Proximal distance and hesitation were found to

TABLE 1 Number of individual cheetah and trials run per experimental group

	Captive		Semi-wild		Wild	
	Individuals	Trials	Individuals	Trials	Individuals	Trials
N_{total} (time <10m)	13 (10)	30 (18)	6 (3)	26 (3)	2 (2)	8 (2)
$N_{control}$ (time <10m)	10 (10)	16 (9)	6 (1)	6 (1)	2 (0)	2 (0)
N_{lion} (time <10m)	10 (10)	14 (9)	6 (2)	20 (2)	2 (2)	6 (2)

Note: Indicated are the overall number and (the subset of individuals that came within 10 m of the auditory cue and could, therefore, be tested for both proximity and hesitation).

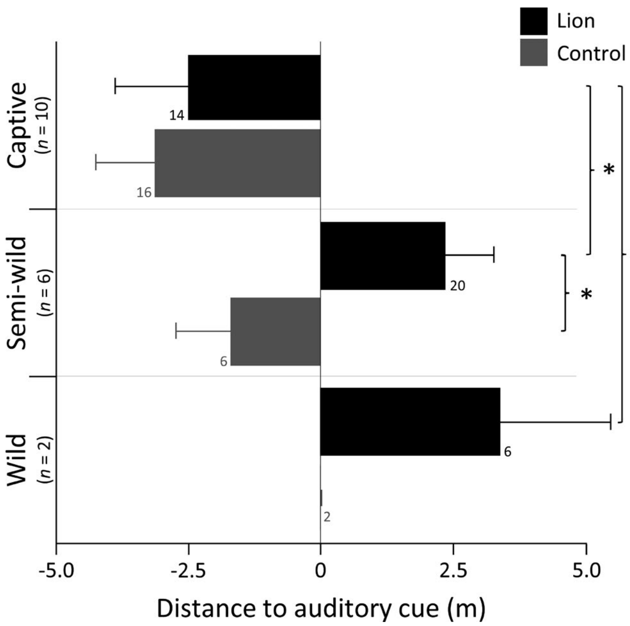

FIGURE 2 Changes in captive (top; $n = 10$), semi-wild (middle; $n = 6$) and wild (bottom; $n = 2$) cheetah mean distance (m) from auditory cues of both predator (lion; black) and control (African bush cricket; grey) sounds after 15 min of observation post-stimulus. Here negative values indicate that the cheetah moved towards the stimulus, whereas positive values indicate movement away from the auditory cue. The number of trials per test are indicated per bar; error bars = SE; ● $p \le .10$; * $p \le .05$

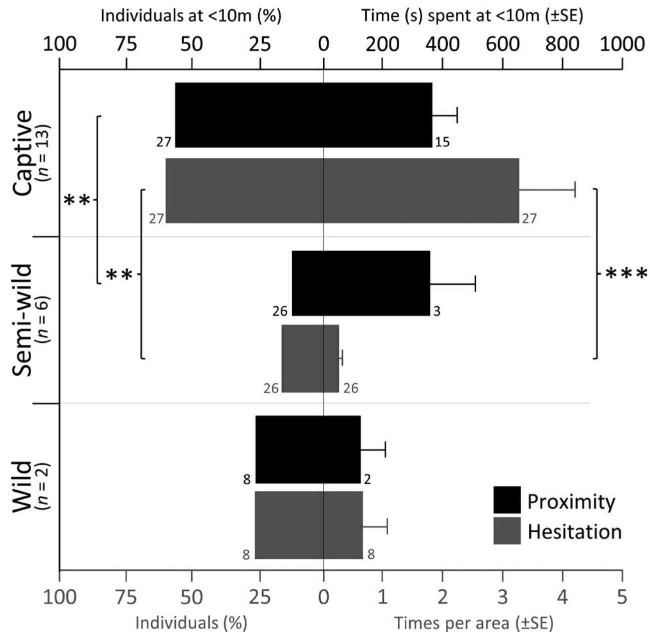

FIGURE 3 Mean proportion (%) of all cheetah, in captive (top; $n = 13$), semi-wild (middle; $n = 6$) and wild (bottom; $n = 2$), that moved to <10 m from the lion auditory cue (black; left), and the mean time (s) that these individuals spent <10 m from the lion auditory cue (black; right), as well as the mean proportion (%) of all cheetah that showed evidence of hesitation (grey; left) and the mean number of times these individuals hesitated per area (grey; right). The number of trials per test are indicated per bar; error bars = SE; ** $p \le .01$; *** $p \le .001$

be independent of sex, having a partner and ambient temperature, thus justifying pooling all data per population in subsequent analyses. Repeatability was calculated using the "rptR" package v0.9.22 (Stoffel et al., 2017) to test whether the variables can be consistently associated with differences in individual cheetah behaviour. In animal personality research the repeatability score (R) specifies the amount of between-individual variation in comparison to the total variation within the population for duplicated measures (R > 0.37) of the same behavioural trait (Dingemanse et al., 2010; MacKay & Haskell, 2015). Individual learning ability was also determined for all individuals using both a GLMER ($\alpha = 0.5$) and a LMER ($\alpha = 0.5$) implemented in the "lme4" package v1.1–27.1 (Bates et al. 2015). A correlation matrix was used to determine the possible interactions between parameters 2–5 (Figure S3), where the resulting significant autocorrelation between parameters required that each parameter be modelled independently.

3 | RESULTS

The proximal distance to the auditory cue for each parameter comparing all three experimental groups is presented (Figure 2). Captive cheetah showed no predator recognition in response to auditory cue trials, consistently approaching both the control (African bush cricket) and treatment (lion). Semi-wild cheetah showed significant ($Z = -2.18$, $p < .05$) predator recognition, approaching the control but fleeing from the treatment. Wild individuals did not approach either cue, instead, these two individuals seemed indifferent to the control, but fled from the treatment (lion), though there was no significant difference in the behavioural responses within this group. Semi-wild and wild cheetah showed no significant difference in their proximal distance to the auditory control cue, instead, both populations (semi-wild: $Z = -2.28$, $p < .05$ and wild: $Z = -1.78$, $p = .076$) moved further away from the lion treatment than captive individuals.

The response of each population to the lion treatment and its effect on proximity and hesitation is presented (Figure 3 and Figure S4). A significantly ($Z = 3.11$, $p < .01$) higher proportion of captive cheetah were found within <10 m of the auditory cue than in semi-wild cheetah (Figure 3); however, there was no significant difference in the time spent within <10 m. There was also a significantly lower proportion ($Z = 3.10$, $p < .01$) and frequency ($Z = 4.09$, $p < .001$) of hesitation amongst semi-wild cheetah relative to captive individuals.

Repeatability was assessed to determine if these behavioural responses to predatory cues could be explained by individual personality, rather than overall life history. For all parameters, repeatability scores were lower than the average value of 0.37 and, therefore, non-significant for all behavioural traits (Table 2). Individual learning ability was tested to control for habituation between auditory trials for all parameters. No positive significant response differences between subsequent trials were observed (Figure S5).

4 | DISCUSSION

Reintroduction efforts are often unsuccessful with captive individuals dying soon after being released into the wild (McPhee & Carlstead, 2010; Smith & Blumstein, 2013). These individuals may fail to recognise and respond to predatory cues, resulting in the performance of maladaptive behaviours such as not seeking refuge from or aggression towards a larger predator (Magno De Faria et al., 2018; McPhee, 2004). Our study showed that when exposed to artificial auditory cues, semi-wild cheetah (i.e. captive-raised and rewilded) showed predatory-response behaviours more similar (albeit non-significant) to that of wild individuals, compared to the captive individuals. Captive cheetah had difficulty differentiating between control and treatment, showing no difference in behavioural response between these cues. One captive individual did distinguish between the control and lion cue by expressing aggressive behaviour towards the threat. Such maladaptive behaviours often occur in captivity due to isolation from and consequent lack of predatory encounters (Griffin et al., 2000; Magno De Faria et al., 2018; Schetini de Azevedo et al., 2012). Semi-wild and wild individuals do differentiate between auditory cues as opposed to captive individuals. When presented with the lion roar, semi-wild and wild cheetah end further away from the station by the end of each trial window than where they had started, whilst semi-wild individuals approached the control and the two wild individuals behaved indifferently towards these African bush cricket sounds. This corroborates concerns over a lack of predator encounters in captivity leading to maladaptive behaviours, where similar outcomes were observed in captive-born greater rheas (*Rhea Americana*; Azevedo et al. 2012) and collared peccary (*Pecari tajacu*; Magno De Faria et al., 2018). One captive cheetah in this study approached the predatory cue and expressed aggressive behaviour towards this "larger predator," suggesting a lack of danger recognition, an inappropriate response that is at least partially the cause of high mortality after many reintroductions (Edwards et al., 2020; Rowell et al., 2020).

TABLE 2 Repeatability (R) calculated for all parameters to test whether these variables are consistently associated with differences in individual cheetah behaviour (repeatability score >0.37), rather than the behaviour of the population as a whole

	Proximal distance	Individuals at <10 m	Time spent at <10 m	Hesitation present	Total hesitation
Behaviour					
$N_{captive}$ Individuals	13	13	10	13	13
$N_{captive}$ Trials	30	30	18	30	30
$N_{semi\text{-}wild}$ Individuals	6	3	6	6	6
$N_{semi\text{-}wild}$ Trials	26	3	26	26	26
N_{wild} Individuals	2	2	2	2	2
N_{wild} Trials	8	2	8	8	8
Mean ($\Sigma x/n$)	9.59	0.36	149.35	0.36	1.61
SE ($SD/\sqrt{n}$)	0.59	0.062	35.76	0.062	0.46
CV (SD/mean)*100	47.87	134.25	187.00	134.25	223.93
Repeatability					
R	0.20	0.13	0.023	0.087	0.03
SE	0.13	0.12	0.079	0.11	0.069
Cis	0; 45	0; 0.40	0; 0.26	0; 0.36	0; 0.26
p-value	0.058	0.15	0.49	0.23	0.16

Note: Repeatability specifies the amount of between-individual variation in comparison to the total variation within the population for duplicated measures of the same behavioural trait.

Captive individuals approached the auditory cue significantly more often than semi-wild cheetah. However, of those that approached (<10 m a radius of the station), the time spent there was equal. This is likely explained by the comparatively small sample size of wild cheetah and the fact that semi-wild individuals, whilst less predator naïve than captive individuals, still have not perfected their predator-avoidance behaviour (De Milliano et al., 2016; Tetzlaff et al., 2019). In addition, semi-wild cheetah showed significantly less hesitation compared to captive individuals, approaching the auditory station significantly more often when the lion recording was played. Captive individuals thus showed significantly more maladaptive behaviour, in both proximal distance and overall hesitation, compared to semi-wild cheetah, which fled the predatory cue without hesitation. These findings match anti-predatory responses in Oldfield mice (*Peromyscus polionotus subgriseus*), where generations of captivity lowered good anti-predatory behaviour (McPhee, 2004). These captive mice failed to identify predatory cues as danger and took significantly more time to find refuge after being exposed to a predator when compared to wild mice. These results suggest that pre-release training and maintaining some wild behaviours in captivity can help avoid the expression of maladaptive behaviour post-release (Blumstein et al., 2019; De Milliano et al., 2016; Rostro-García et al., 2015; Rowell et al., 2020). Pairing a predatory cue with a negative consequence for the individual may teach it to associate predators with an adverse outcome (Blumstein et al., 2019). Many pre-release training experiments have been conducted with this in mind, for instance, fright responses were observed in captive-reared rufous hare-wallabies (*Lagorchestes hirsutus*) by exposing them to predator models to mimic the encounters they might experience after release (Griffin et al., 2000; McLean et al., 1996). However, most research on the effect of pre-release training on increased survival has been done in marine systems, leaving the efficacy of such training in terrestrial vertebrates poorly understood (Edwards et al., 2020; Rowell et al., 2020). Additionally, many of these experiments never released the individuals after exposing them to pre-release training, rendering the long-term success of this training unclear (Edwards et al., 2020).

Behavioural studies carry inherent biases and limitations that must be considered when interpreting results. Throughout this study, learning behaviour was tested to avoid habituation between trials; however, there was no evidence to support such behaviour, which would likely have occurred over a longer period of trial exposure (Magno De Faria et al., 2018). Repeatability was also tested for all parameters to determine if behavioural responses could be linked to individual personality (Bell et al., 2009; Stoffel et al., 2017; Myers and Young 2018). However, low repeatability scores (<0.37) showed that predator-avoidance behaviour could not be assigned with certainty to consistent individual personality differences. This was likely the result of low sample sizes (both for individuals and trials) or due to high consistency both within and between individuals (Bell et al., 2009; Dingemanse et al., 2010; MacKay & Haskell, 2015). Such low sample size effects are especially evident in the wild population having a greater but non-significant response to lion predatory cues than captive and semi-wild populations. Additionally, only cheetah that are successful at predator-avoidance and, therefore, survive in the wild are sampled in such trails, whereas those in captivity or semi-wild conditions are not naturally selected against. Therefore, non-wild cheetah are likely to present greater variability in predator-avoidance and other behaviours, making observing wild-like behaviour in semi-wild cheetah even more compelling. Opportunities to perform longitudinal monitoring of predator-avoidance behaviour are rare. Nevertheless, this pilot study shows significant differences between captive and semi-wild predator-avoidance behaviour and clear differences between the captive and wild populations.

Semi-wild populations recognise and respond to the lion auditory cue in a more behaviourally beneficial manner than those in captivity. This is an important finding for reintroduction programmes as it provides novel evidence to support previously described differences in predator-response behaviour between captive and wild populations, as well as highlighting the relative success of a "soft-release" approach, as the semi-wild individuals were raised in captivity. Reintroductions and methods of staged release as a conservation intervention for many threatened species remain a fairly recent and experimental field that has been met with varying success (Adania et al., 2016; de Milliano et al., 2016; Richardson et al., 2013). Whilst the absence of predators in many current reintroduction sites certainly increases the overall reintroduction success of cheetah, it is important to have wild cheetah populations coexisting with large predatory species. This would maintain ecological integrity and allow cheetah to inhabit a much larger range (Boast et al., 2018; Buk et al., 2018; Hetem et al., 2019; Hunter, 2007; Lindsey et al., 2011; Rostro-García et al., 2015). As animals rely on a suite of senses to recognise and respond to predators (Cornhill & Kerley, 2020; Edwards et al., 2020; Hubel et al., 2016), it should be mentioned that an auditory stimulus is just one aspect of predatory response and that in isolation, such a pre-recorded roar may not of itself sufficiently warrant a predatory threat (Moseby et al., 2015). Testing captive cheetah with visual and olfactory stimuli in addition to auditory cues will likely provide further insight into these aversive behaviours. Many captive cheetah are currently not tested for predator-response behaviour prior to reintroduction, which may result in high mortality soon after release, low reproductive success and poor parental care (Blumstein et al., 2005; Cornhill & Kerley, 2020; Crane & Mathis, 2011; Rostro-García et al., 2015). Determining which individuals are likely to have a higher chance of survival and reproduction after release will increase the success of reintroductions by artificially selecting for their overall fitness. Such a method could be crucial to securing individual reintroduction success and, therefore, future range rehabilitation of many threatened species such as the cheetah.

ACKNOWLEDGEMENTS

This research was supported by grants from the University of Groningen, the Christine Buisman grant and the Jo Kolk grant. We thank the big cat conservation organisation Felida Big Cat Centre (Grindweg 22, 8422 DN Nijeberkoop, Friesland, the Netherlands) for providing information on the current problems in big cat conservation. Additionally, we thank Prof.dr.ir. H.H. de Iongh for providing us with lion recordings to use in our trials. Furthermore, we thank Ashia Cheetah Conservation, Kuzuko Lodge Private Game Reserve and their staff for opening up their sanctuaries for this research and their assistance in the field.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Supplementary data to this article can be found online at https://doi.org/10.1002/10.25375/uct.14839575

ORCID

Nynke Wemer https://orcid.org/0000-0001-6402-5555
Jan Komdeur https://orcid.org/0000-0002-9241-0124

REFERENCES

Adania, C. H., de Carvalho, W. D., Rosalino, L. M., de Cassio Pereira, J., & Crawshaw, P. G. (2016). First soft-release of a relocated puma in South America. *Mammal Research*, *62*, 121–128. https://doi.org/10.1007/s13364-016-0302-0

de Azevedo, C. S., Lima, M. F., da Silva, V. C., Young, R. J., & Rodrigues, M. (2012). Visitor influence on the behavior of captive greater rheas (Rhea americana, Rheidae Aves). *J Appl Anim Welf Sci.*, *15*(2), 113–25. https://doi.org/10.1080/10888705.2012.624895

Banks, P. B., Norrdahl, K., & Korpimaki, K. (2001). Mobility decisions and the predation risks of reintroduction. *Biological Conservation*, *102*, 133–138. https://doi.org/10.1016/S0006-3207(01)00110-0

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss

Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. *Animal Behavior*, *77*, 771–783. https://doi.org/10.1016/j.anbehav.2008.12.022

Berger-Tal, O., Blumstein, D. T., & Swaisgood, R. R. (2019). Conservation translocations: a review of common difficulties and promising directions. *Animal Conservation*, *23*, 121–131. https://doi.org/10.1111/acv.12534

Berger-Tal, O., & Saltz, D. (2014). Using the movement patterns of reintroduced animals to improve reintroduction success. *Current Zoology*, *60*, 515–526. https://doi.org/10.1093/czoolo/60.4.515

Bissett, C., & Bernard, R. T. F. (2007). Habitat selection and feeding ecology of the cheetah (Acinonyx jubatus) in thicket vegetation: is the cheetah a savanna specialist? *Journal of Zoology*, *271*, 310–317.

Blaszczyk, M. B. (2017). Boldness towards novel objects predicts predator inspection in wild vervet monkeys. *Animal Behavior*, *123*, 91–100. https://doi.org/10.1016/j.anbehav.2016.10.017

Blumstein, D. T., Holland, B., & Daniel, J. C. (2005). Predator discrimination and "personality" in captive Vancouver Island marmots (*Marmota vancouverensis*). *Animal Conservation*, *9*, 274–282. https://doi.org/10.1111/j.1469-1795.2006.00033.x

Blumstein, D. T., Letnic, M., & Moseby, K. E. (2019). In situ predator conditioning of naive prey prior to reintroduction. *Philosophical Transactions of the Royal Society B*, *374*, 1–8.

Boast, L. K., Chelysheva, E. V., van der Merwe, V., Schmidt-Küntzel, A., Walker, E. H., Cilliers, D., Gusset, M., & Marker, L. (2018). Cheetah translocation and reintroduction programs: past, present, and future. In P. Nyhus (Eds.),*Cheetahs: biology and conservation: biodiversity of the world: conservation from genes to landscapes*. Elsevier pp. 275–289.

Borrego, N., Ozgul, A., Slotow, R., & Packer, C. (2018). Lion population dynamics: do nomadic males matter? *Behavioral Ecology*, *29*, 660–666. https://doi.org/10.1093/beheco/ary018

Bremner-Harrison, S., Cypher, B. L., Van Horn Job, C., & Harrison, S. W. R. (2018). Assessing personality in San Joaquin kit fox in situ: efficacy of field-based experimental methods and implications for conservation management. *Journal of Ethology*, *36*, 23–33. https://doi.org/10.1007/s10164-017-0525-9

Bremner-Harrison, S., Prodohl, P. A., & Elwood, R. W. (2004). Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (Vulpes velox). *Animal Conservation*, *7*, 313–320. https://doi.org/10.1017/S1367943004001490

Briers-Louw, W. D., Verschueren, S., & Leslie, A. J. (2019). Big cats return to Majete Wildlife Reserve, Malawi: evaluating reintroduction success. *African Journal of Wildlife Research*. *49*:34–50.

Buk, K. G., van der Merwe, V. C., Marnewick, K., & Funston, P. J. (2018). Conservation of severely fragmented populations: lessons from the transformation of uncoordinated reintroductions of cheetahs (Acinonyx jubatus) into a managed metapopulation with self-sustained growth. *Biodiversity and Conservation*, *27*, 2293–3423.

Canessa, S., Genta, P., Jesu, R., Lamagni, L., Oneto, F., Salvidio, S., & Ottonello, D. (2016). Challenges of monitoring reintroduction outcomes: insights from the conservation breeding program of an endangered turtle in Italy. *Biological Conservation*, *104*, 128–133. https://doi.org/10.1016/j.biocon.2016.05.003

Chame, M. (2003). Terrestrial mammal feces: a morphometric summary and description. *Memorias do Instituto Oswaldo Cruz*, *98*, 71–94. https://doi.org/10.1590/S0074-02762003000900014

Cornhill, K. L., & Kerley, G. I. H. (2020). Cheetah communication at scent-marking sites can be inhibited or delayed by predators. *Behavioral Ecology and Sociobiology*, *74*, 1–10. https://doi.org/10.1007/s00265-020-2802-9

Crane, A. L., & Mathis, A. (2011). Predator-recognition training: a conservation strategy to increase postrelease survival of hellbenders in head-starting programs. *Zoo Biology*, *30*, 611–622. https://doi.org/10.1002/zoo.20358

de Milliano, J., Di Stefano, J., Courtney, P., Temple-Smith, P., & Coulson, G. (2016). Soft-release versus hard-release for reintroduction of an endangered species: an experimental comparison using eastern barred bandicoots (*Perameles gunnii*). *Wildlife Research*, *43*, 1–12. https://doi.org/10.1071/WR14257

DeGregorio, B. A., Sperry, J. H., Tuberville, T. D., & Weatherhead, P. J. (2017). Translocating ratsnakes: does enrichment offset negative effects of time in captivity? *Wildlife Research*, *44*, 348–448. https://doi.org/10.1071/WR17016

Dingemanse, N. J., Kazem, A. J. N., Ré, D., & Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution*, *25*, 81–89. https://doi.org/10.1016/j.tree.2009.07.013

Durant, S., Mitchell, N., Groom, R., Pettorelli, N., Ipavec, A., Jacobson, A. P., Woodroffe, R., Böhm, M., Hunter, L. T. B., Becker, M. S., Broekhuis, F., Bashir, S., Andresen, L., Aschenborn, O., Beddiaf, M., Belbachir, F., Belbachir-Bazi, A., Berbash, A., de Matos, B., … Young-Overton, K. (2017). The global decline of cheetah Acinonyx jubatus and what it means for conservation. *PNAS*, *114*, 528–533.

Edwards, M. C., Ford, C., Hoy, J. M., Fitzgibbon, S., & Murray, P. J. (2020). How to train your wildlife: a review of predator avoidance training. *Applied Animal Behaviour Science*, *234*, 1–7. https://doi.org/10.1016/j.applanim.2020.105170

Efrat, R., Hatzofe, O., Miller, Y., & Berger-Tal, O. (2020). Determinants of survival in captive-bred Griffon Vultures Gyps fulvus after their release to the wild. *Conservation Science and Practice*, 1–9.

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*, 1325–1330.

Greenspan, E., Giordano, A. J., Nielsen, C. K., Sun, N. C. M., & Pei, K. J. C. (2020). Evaluating support for clouded leopard reintroduction in Taiwan: insights from surveys of indigenous and urban communities. *Human Ecology*, 1–15. https://doi.org/10.1007/s10745-020-00195-9

Griffin, A. S., Blumstein, D. T., & Evans, C. S. (2000). Training captive-bred or translocated animals to avoid predators. *Conservation Biology*, *14*, 1317–1326. https://doi.org/10.1046/j.1523-1739.2000.99326.x

Hetem, R. S., Mitchell, D., De Witt, B. A., Fick, L. G., Maloney, S. K., Meyer, L. C. R., & Fuller, A. (2019). Body temperature, activity patterns and hunting in free-living cheetah: biologging reveals new insights. *Integrative Zoology*, *14*, 30–47.

Hubel, T. Y., Shotton, J., Wilshin, S. D., Horgan, J., Klein, R., McKenna, R., & Wilson, A. M. (2016). Cheetah reunion—the challenge of finding your friends again. *PLoS One*, *11*, 1–8. https://doi.org/10.1371/journal.pone.0166864

Hunter, L. T. B. (2007). The behavioural ecology of reintroduced lions and cheetahs in the Phinda Resource Reserve, KwaZulu-Natal, South Africa. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.630.5217&rep=rep1&type=pdf

Jolly, C. J., Webb, J. K., & Phillips, B. L. (2018). The perils of paradise: an endangered species conserved on an island loses antipredator behaviours within 13 generations. *Biology Letters*, *14*, 1–4. https://doi.org/10.1098/rsbl.2018.0222

Jule, K. R., Leaver, L. A., & Lea, S. E. G. (2008). The effects of captive experience on reintroduction survival in carnivores: a review and analysis. *Biological Conservation*, *141*, 355–363. https://doi.org/10.1016/j.biocon.2007.11.007

Komdeur, J., & Hammers, M. (2014). Failed introductions: finches from outside Australia. In H. H. T. Prins, & I. J. Gordon (Eds.), *Invasion biology and ecological theory: insights from a continent in transformation*. Cambridge University Press, pp. 324–350.

Kongsurakan, P., Chaiyarat, R., Nakbun, S., Thongthip, N., & Anuracpreeda, P. (2020). Monitoring body condition score of reintroduced banteng (Bos javanicus D'Alton, 1923) into Salakphra Wildlife Sanctuary, Thailand. *Peer-reviewed Journal*, *8*, 1–17.

Lindsey, P., Tambling, C. J., Brummer, R., Davies-Mostert, H., Hayward, M., Marnewick, K., & Parker, D. (2011). Minimum prey and area requirements of the vulnerable cheetah acinonyx jubatus: implications for reintroduction and management of the species in South Africa. *Oryx*, *45*, 587–599.

MacKay, J. R. D., & Haskell, M. J. (2015). Consistent individual behavioral variation: the difference between temperament, personality and behavioral syndromes. *Animals*, *5*, 455–478.

Magno De Faria, C., De Souza Sá, F., Costa, D. D. L., da Silva, M. M., da Silva, B. C., Young, R. J., & de Azevedo, C. S. (2018). Captive-born collared peccary (Pecari tajacu, Tayassuidae) fails to discriminate between predator and non-predator models. *Springer*, *21*, 175–184.

Marker, L. (2019). Cheetahs race for survival: ecology and conservation. In: *Wildlife population monitoring*. InTechOpen, pp. 51–65.

McLean, I. G., Lundie-Jenkins, G., & Jarman, P. J. (1996). Teaching an endangered mammal to recognise predators. *Biological Conservation*. *75*, 51–62.

McPhee, M. E. (2004). Generations in captivity increases behavioral variance: Considerations for captive breeding and reintroduction programs. *Biological Conservation*, *115*, 71–77.

McPhee, M. E., & Carlstead, K. (2010). Effects of captivity on the behavior of wild mammals. *ResearchGate*, 303–313.

Menotti-Raymond, M., & O'Brien, S. J. (1993). Dating the genetic bottleneck of the African cheetah (DNA rmgerprint/mtDNA). *Proceedings of the National Academy of Sciences of the United States of America*, *90*, 3172–3176. https://doi.org/10.1073/pnas.90.8.3172

Merrick, M. J., & Koprowski, J. L. (2017). Should we consider individual behavior differences in applied wildlife conservation studies? *Biological Conservation*, *209*, 34–44. https://doi.org/10.1016/j.biocon.2017.01.021

Moseby, K., Carthey, A., & Schroeder, T. (2015). The influence of predators and prey naivety on reintroduction success: current and future directions. In: D. Armstrong, M. Hayward, M. Dorian & S. Phillip J. (Eds.), *Advances in reintroduction biology of Australian and New Zealand Fauna* (pp. 29–42). CSIRO.

Myers, P. J., & Young, J. K. (2018). Consistent individual behavior: evidence of personality in black bears. *Journal of Ethology*, *36*(2), 117–124. https://doi.org/10.1007/s10164-018-0541-4

Piñeiro, A., & Barja, I. (2012). The plant physical features selected by wildcats as signal posts: an economic approach to fecal marking. *Naturwissenschaften*, *99*, 801–809. https://doi.org/10.1007/s00114-012-0962-9

R Core Team (2018). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Richardson, K., Castro, I. C., Brunton, D. H., & Armstrong, D. P. (2013). Not so soft? Delayed release reduces long-term survival in a passerine reintroduction. *Oryx*, *49*, 535–541. https://doi.org/10.1017/S0030605313001014

Ross, S. R., Hansen, B. K., Hopper, L. M., & Fultz, A. (2019). A unique zoo-sanctuary collaboration for chimpanzees. *American Journal of Primatology*, *81*, e22941. https://doi.org/10.1002/ajp.22941

Rostro-García, S., Kamler, J. F., & Hunter, L. T. B. (2015). To kill, stay or flee: the effects of lions and landscape factors on habitat and kill site selection of cheetahs in South Africa. *PLoS One*, *2*, 1–20. https://doi.org/10.1371/journal.pone.0117743

Rowell, T. A. A. D., Magrath, M. J. L., & Magrath, R. D. (2020). Predator-awareness training in terrestrial vertebrates: progress, problems and possibilities. *Biological Conservation*, *252*, 1–13.

Roy, T., & Bhat, A. (2018). Repeatability in boldness and aggression among wild zebrafish (Danio rerio) from two differing predation and flow regimes. *Journal of Comparative Psychology*, *4*, 349–360. https://doi.org/10.1037/com0000150

Rozhnov, V. V., Hernandez-Blanco, J. A., Lukarevskiy, V. S., Naidenko, S. V., Sorokin, P. A., Litvinov, M. N., Kotlyar, A. K., & Pavlov, D. S. (2011). Application of satellite collars to the study of home range and activity of the Amur tiger (*Panthera tigris altaica*). *Biological Bulletin*, *38*, 834–847. https://doi.org/10.1134/S1062359011080073

Schetini de Azevedo, C., John Young, R., & Rodrigues, M. (2012). Failure of captive-born greater rheas (Rhea americana, Rheidae, Aves) to discriminate between predator and nonpredator models. *Acta Ethologica*, *15*, 179–185.

Schuett, W., Tregenza, T., & Dall, S. R. X. (2010). Sexual selection and animal personality. *Biological Reviews*, *85*, 217–246. https://doi.org/10.1111/j.1469-185X.2009.00101.x

Shier, D. M., & Owings, D. H. (2007). Effects of social learning on predator training and postrelease survival in juvenile black-tailed prairie dogs, *Cynomys ludovicianus*. *Animal Behavior*, *73*, 567–577. https://doi.org/10.1016/j.anbehav.2006.09.009

Smith, B. R., & Blumstein, D. T. (2013). Animal personality and conservation biology; the importance of behavioral diversity. *Animal personalities: behavior, physiology, and evolution*. Chicago Scholarship Online, pp. 381–413.

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *8*, 1639–1644. https://doi.org/10.1111/2041-210X.12797

Tetzlaff, S. J., Sperry, J. H., & DeGregorio, B. A. (2019). Effects of antipredator training, environmental enrichment, and soft release on wildlife translocations: a review and meta-analysis. *Biological Conservation*, *236*, 324–331.

Vilhunen, S. (2006). Repeated antipredator conditioning: a pathway to habituation or to better avoidance? *Journal of Fish Biology*, *68*, 25–43. https://doi.org/10.1111/j.0022-1112.2006.00873.x

Wells, A., Terio, K. A., Ziccardi, M. H., & Munson, L. (2004). The stress response to environmental change in captive cheetahs (*Acinonyx jubatus*). *Journal of Zoo and Wildlife Medicine*, *35*, 8–14. https://doi.org/10.1638/02-084

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Wemer, N., Naude, V. N., van der Merwe, V. C., Smit, M., de Lange, G., & Komdeur, J. (2022). Successful predatory-avoidance behaviour to lion auditory cues during soft-release from captivity in cheetah. *Ethology*, 128, 247–256. https://doi.org/10.1111/eth.13261