

Recognition of reptile predator scent is innate in an endangered lizard species

Authors: Daniell, Tara L., Hutchinson, Mark N., Ainsley, Phil, and

Gardner, Michael G.

Source: Australian Journal of Zoology, 68(2): 76-84

Published By: CSIRO Publishing

URL: https://doi.org/10.1071/ZO20064

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Australian Journal of Zoology, 2020, **68**, 76–84 https://doi.org/10.1071/ZO20064

Recognition of reptile predator scent is innate in an endangered lizard species

Tara L. Daniell ^{DA}, Mark N. Hutchinson ^{A,B}, Phil Ainsley ^C and Michael G. Gardner ^{DA,B,D}

Abstract. Chemical cues can alert prey to the presence of predators before the predator is within visual proximity. Recognition of a predator's scent is therefore an important component of predator awareness. We presented predator and control scents to wild, wild-born captive, and predator-naive captive-born pygmy bluetongue lizards to determine (1) whether lizards respond to reptile chemical cues differently from controls, (2) whether captive lizards respond more strongly to a known predator than to other predatory reptiles, (3) whether captive-born lizards recognise predators innately, whether captive-born lizards have reduced predator recognition compared with wild lizards and whether time spent in captivity reduces responses to predators, and (4) whether the avoidance response to predator detection differs between naive and experienced lizards. There was no significant difference in the number of tongue flicks to predator scent among wild, wild-born and captive-born lizards, suggesting that predator detection is innate in the pygmy bluetongue lizard and time in captivity did not reduce predator recognition. The number of tongue flicks directed towards brown snake scent was significantly higher than that to the novel and water controls for all lizard origins. Lizards of all origins continued to bask in the presence of predator scents, suggesting that chemical cues alone may be insufficient to instigate an avoidance response and other cues may be required.

Keywords: behaviour, captive management, conservation, scent, predation, olfactory cues, predator recognition, lizard.

Received 14 July 2020, accepted 5 May 2021, published online 9 June 2021

Introduction

When prey species are isolated from predators, such as in captive environments or predator-free islands or reserves, predator avoidance behaviours that have evolved over time can be relaxed or lost (DeGregorio et al. 2017; Jolly et al. 2018; Muralidhar et al. 2019). Therefore, captive-born animals that have no prior exposure to predators may lack the ability to recognise and respond to predator threats if released into the wild. A major concern for captive conservation projects that intend to release captive-born animals for reintroduction purposes is therefore the potential for reduced survival due to reduced predator avoidance. Unsustainable predation is a major cause of mortality in translocation or reintroduction of captive animals (Jule et al. 2008; Aaltonen et al. 2009; Moseby et al. 2011). Hence, it is important to understand the effect of the captive environment on predator avoidance so as to improve survival of animals released back into the wild.

Prey species have evolved to recognise predator presence and respond with anti-predator behaviour to help avoid predation attempts (Apfelbach *et al.* 2005; Atkins *et al.* 2016). One way in which prey can achieve this is through the detection of predator chemical cues, which can alert a prey

species to the presence of a predator. Such chemical cues can be utilised independently of or in conjunction with visual and auditory cues (Apfelbach *et al.* 2005; MacLean and Bonter 2013). Many taxa are known to recognise and respond to chemical stimuli from predators, including reptiles, amphibians, mammals, fish, birds, crustaceans and invertebrates (Kats and Dill 1998; Apfelbach *et al.* 2005). Recognition of predator cues can be threat sensitive, as predicted by the threat-sensitive hypothesis, whereby prey can differentiate between predators based on the degree of threat posed (Helfman 1989; Stapley 2003; Lloyd *et al.* 2009; Cornelis *et al.* 2019). Alternatively, predator recognition can be generalised via avoidance of multiple species cues (Blumstein 2006; Webb *et al.* 2009, 2018).

Squamates tongue flick to transport chemical cues to the vomeronasal organ (VNO) so as to detect stimuli that assist in avoiding predators, communicating with conspecifics and foraging (Cooper 1994). The family Scincidae, including the subfamily Egerniinae, is known to have advanced vomerolfactory abilities (Bull *et al.* 1999; Cooper 1994). A member of the Egerniinae, the pygmy bluetongue lizard (*Tiliqua adelaidensis*), is an endangered species rediscovered

Journal compilation © CSIRO 2020 Open Access CC BY-NC

www.publish.csiro.au/journals/ajz

^ACollege of Science and Engineering, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia.

^BSouth Australian Museum, Adelaide, SA 5000, Australia.

^CAdelaide Zoo, Adelaide, SA 5000, Australia.

^DCorresponding author. Email: michael.gardner@flinders.edu.au

in 1992, that is found in native grasslands in mid-northern South Australia (Armstrong and Reid 1992; Hutchinson et al. 1994). These lizards inhabit burrows constructed by spiders, which provide shelter from temperature extremes, a site to ambush prey, and refuge from predators such as eastern brown snakes and various bird species (Hutchinson et al. 1994; Milne et al. 2003; Fenner et al. 2008). Pygmy bluetongue lizards use vomerolfactory cues for social signalling, that is, to communicate burrow ownership to conspecifics and locate mates by following female scent trails, and, as such, are suitable subjects to study predator scent response (Ebrahimi et al. 2014; Fenner and Bull 2011). Pygmy bluetongues are threatened by climate change, habitat loss and fragmentation, and a captive population was established as a potential source for translocations that will be required to conserve the species (Smith et al. 2009; Fordham et al. 2012; Delean et al. 2013).

Lizards that lack the ability to recognise and avoid predators, or in which avoidance behaviour has been relaxed because of the lack of predation pressure, are at greater risk of predation, potentially reducing the success of translocation. Therefore, we investigated the use of chemical cues for predator avoidance in captive and wild pygmy bluetongue lizards. Our aims were to determine (1) whether lizards respond to reptile chemical cues differently from controls, (2) whether pygmy bluetongue lizards respond more strongly to a known predator, the eastern brown snake (Pseudonaja textilis), than to other potentially predatory reptiles, (3) whether captive-born pygmy bluetongue lizards recognise predators innately, whether captive-born lizards have reduced predator recognition compared with wild lizards and whether time spent in captivity reduces response to predators, and 4) whether the avoidance response to predator detection differs between naive and experienced lizards.

Materials and methods

Study populations

Our study incorporated three treatments groups, namely, wild lizards, wild-born captive lizards and captive-born lizards.

Monarto Safari Park

We used a captive population of 37 pygmy bluetongue lizards at Monarto Safari Park, South Australia. The population was composed of nine potentially predatorexperienced wild-born adults, which had been captive since spring 2014, and 28 predator-naive captive-born offspring born in either 2016 or 2017, including 12 immatures and 16 juveniles respectively. Lizards were housed singly in sections of raised enclosures divided into thirds and situated within two 15-m-diameter circular caged areas that had a netted roof and wire-mesh sides to exclude predators. Lizard enclosures were 0.65 m high, 2.4 m long, 1.2 m wide and were filled with sandy loam to a depth of 0.4 m. Each enclosure was divided into three sections to house lizards individually, with six artificial burrows per section spaced ~30 cm apart. Artificial burrows were constructed from wooden dowel, with a circular hole drilled through the centre; adult burrows were 300 mm long, 30 mm in diameter with a 20-mm hole; juvenile and immature burrows were 200 mm long, 28 mm in diameter with an 18-mm

hole. Four native grasses (*Austrostipa* sp. and *Austrodanthonia* sp.) per section provided shade and potential retreats for lizards venturing on the surface.

Burra

The wild population for this study (20 lizards), was located at Tiliqua Reserve, a Nature Foundation property ~10 km from Burra, in mid-northern South Australia. The site consists of native grassland and exotic weeds usually grazed by sheep. The Burra area experiences hot, dry summers and cool, moist winters, with a mean annual rainfall of 421.4 mm for the years 1961-2019 (Commonwealth of Australia Bureau of Meteorology 2020). At the time of scent trials, no grazing was occurring as vegetation levels were low following several years of below-average rainfall, namely, 259 mm in 2018 and 220.6 mm in 2019 (Commonwealth of Australia Bureau of Meteorology 2020). One paddock of the site was searched to locate 20 natural burrows, which we confirmed were occupied by pygmy bluetongue lizards, by using an optic fibre scope (Medit Inc. FI4-2BDP-1850, Canada; Milne and Bull 2000) and the burrows were then marked by numbered plastic pin markers for the study duration.

Scent treatments

Our study tested five scent treatments, including three reptile species' scents, a novel control and an odourless water control applied to absorbent paper towel. The first reptile species was the eastern brown snake (*Pseudonaja textilis*), an active forager known to predate the pygmy bluetongue (Hutchinson *et al.* 1994). The second species was the eastern bluetongue lizard (*Tiliqua scincoides*), a omnivorous species that predates on small lizards and is sympatric but is not known to predate pygmy bluetongues (Pelgrim *et al.* 2014). Last, was the Rosenberg's monitor (*Varanus rosenbergi*), a species known to predate on smaller lizards but not found within the pygmy bluetongue's current range of distribution (King and Green 1979; Smith *et al.* 2007). The two controls were a novel odour control of 1:10 diluted eucalyptus oil (Stapley 2003) and an odourless distilled-water control.

All reptile scent was collected by zoo keepers from Adelaide Zoo, South Australia. Scent was collected from one eastern brown snake only. In the case of both the Rosenberg's monitors and eastern bluetongue lizards, individuals were housed in groups so that the corresponding scent donor to each sample could not be determined but the samples were likely to be mixtures of different individuals. Clean gloves were worn to place absorbent paper towels, dampened with distilled water, in reptile enclosures for 48 h to absorb scent. Paper towel was then removed and stored in ziplock bags in a freezer (–20°C) until use (Bourke *et al.* 2017). Storage time between scent collection and trials did not exceed 3 weeks.

Scent trial

Scent trials were conducted over a 5-day period at both Monarto and Burra. Each individual lizard was exposed to each of the five scents separately, with a single scent being presented 1 day at a time until each scent had been presented

over the 5-day period. The order of scent presentation was randomised among lizards.

Filming was conducted once lizard activity was observed in the late morning or early afternoon between the hours of 1000 hours and 1500 hours at Monarto between 30 September and 4 October 2019 and at Burra between 11 and 15 October 2019. Filming took place when the temperature was between 16.8°C and 30.1°C and there was no rain. There was a 6-day gap between the Monarto and Burra trials because weather was unfavourable. We used a combination of five GoPro cameras and 15 Movii cameras, with an external power bank placed 30 cm from the lizard burrow. The Monarto lizards were divided into two groups for filming, one group in the morning and one in the afternoon in a randomised order, because of a limited number of cameras. We placed the cameras at ground level and close to burrows, so as to provide clear vision of tongue flicking behaviour, or on short tripods when placing on the ground was not practical owing to surrounding vegetation. Scent papers were placed approximately 5 cm from lizard burrows and pinned down with nails to hold in place. Clean disposable gloves were worn when handling scent papers and were changed for each scent treatment. Lizards were filmed for 30 min, after a 30-min acclimation period after camera setup, each treatment day to record the number of tongue flicks directed towards scent paper and minutes spent basking per lizard.

Statistical analysis

Primer v7/PERMANOVA+ was used to conduct univariate multifactorial repeated-measures PERMANOVA tests based on Euclidean distance matrices and PERMANOVA pair-wise tests. PERMANOVA can be used to analyse data that are not normally distributed, as was the case with our data. The behavioural parameters tested were the number of tongue flicks directed towards scent papers (Aims 1-3) and minutes spent basking (Aim 4) at the burrow entrance for each individual lizard and each scent treatment. Lizard origin (captive-born, wild-born and wild) and treatment were between-subject factors, and day was a within-subject factor. Day was included as a factor to account for potential habituation to the treatment presentation over the trial. Temperature (°C) during filming was obtained from the Commonwealth of Australia's Bureau of Meteorology (BoM) from the nearest weather stations to Monarto and Burra, namely, Pallamana station 11 km away, and Clare station 37 km away respectively. We analysed temperature (°C) as a covariate in the tongue flicking and basking models because it may influence lizard tongue flicking and basking.

We tested each of our aims in the following manner:

Aims 1–3 were addressed by comparing tongue flick responses to the five scent treatments, so as to determine whether they differed between reptile and control scents, and how lizards with varying levels of experience with predators of different origins responded.

For Aim 4, we compared basking time in the presence of the five scent treatments between predator-naive captive-born lizards and potentially experienced wild-born and wild lizards, to investigate whether the predator avoidance response differs.

Results

Differential response to scent treatments (Aims 1 and 2)

The number of tongue flicks lizards directed towards scent papers differed significantly among scent treatments (Table 1, Fig. 1). The mean number of tongue flicks towards brown snake scent papers was 24.07 ± 4.93 s.e., towards bluetongue lizard it was 14.33 ± 2.83 s.e., towards Rosenberg's monitor it was 12.81 ± 4.04 s.e., with tongue flicks to the novel control being 5.81 ± 1.73 s.e. and 5.42 ± 1.59 s.e. towards the water control. Response to the brown snake treatment differed significantly from both the water control (PERMANOVA pair-wise test P = 0.005) and novel control (PERMANOVA pair-wise test P = 0.022). There was no significant difference among other treatments.

Innate predator recognition and the effect of captivity on predator recognition (Aim 3)

Naive captive-born lizards had a slightly lower total number of tongue flicks than did wild-born and wild lizards, but this difference was not significant, and wild-born lizards were comparable to wild lizards (Fig. S1, Supplementary Material). The number of tongue flicks varied among lizard origin and day but the variation was not significant (Fig. S2). Temperature, analysed as a covariate, was found to have a significant effect on the mean number of tongue flicks among days of the trial (Table 1, Fig. 2). Temperature varied among days, and tongue flicks were variable but generally lower at lower and also higher temperatures.

Behavioural response to predator detection (Aim 4)

There was no significant effect of treatment, lizard origin or day of trial on pygmy bluetongue basking time. Lizards basked for a similar amount of time in the presence of all scent treatments (Fig. 3). There was variation in basking time in the presence of the five scent treatments for captive-born, wild-born and wild lizards, but this was not significant (Fig. S3). However, there was a significant interaction effect of lizard origin and day on basking time (Table 1). Basking time varied among lizard origin and day of trial, with a general trend of basking time decreasing over the 5 days for captive-born lizards and fluctuating for both wild-born and wild lizards (Fig. S4).

Discussion

The ability to recognise and respond to predator chemical cues in the natural environment provides prey species the opportunity to become aware of the presence of predators and undertake antipredator behaviours to minimise risk of predation. Prey species can display species-specific recognition of predator cues, or generalised predator recognition. Hence, an important question is what type of predator recognition a species displays, because this will determine how the species responds to predators they encounter. Our study had the following four main findings: (1) pygmy bluetongue lizards tongue flicked towards the brown snake predator's scent more than towards the scents in the control treatments; (2) tongue flicking behaviour in

Table 1. PERMANOVA results for the variables: tongue flicks, temperature covariate (tongue flicks), basking time and temperature covariate (basking time)

Data not transformed. Origin, lizard origin; wild, wild-born and captive-born; treatment, to the five scents presented to lizards; day, trial day; d.f., degrees of freedom; SS, sum of squares; MS, mean sum of squares; pseudo-*F*, *F*-value by permutation; *P*(perm), *P*-values based on >9000 permutations; perms, number of permutations. Bold text indicates a significant *P*-value

Parameter	d.f.	SS	MS	Pseudo-F	P(perm)	Perms
Tongue flicks						
Origin	2	1265.4	632.69	1.17	0.313	9960
Day	4	4345.6	1086.4	2.00	0.108	9952
Treatment	4	8382.4	2095.6	3.86	0.018	9953
Origin × Day	8	4354.4	544.29	1.00	0.414	9947
Origin × Treatment	8	5017.7	627.21	1.15	0.328	9937
Day × Treatment	16	8629.1	539.32	0.99	0.448	9921
Origin \times Day \times Treat	23	13 843	601.86	1.11	0.327	9910
Res	219	118 930	543.08			
Total	284	175 700				
Tongue flicks temperature c	ovariate					
Temperature	1	2532.3	2532.3	4.65	0.035	9955
Origin	2	583.35	291.67	0.54	0.580	9962
Day	4	5548.1	1387	2.55	0.043	9953
Treatment	4	13 091	3272.8	6.00	< 0.001	9939
Origin \times Day	8	5075.6	634.45	1.16	0.327	9933
Origin × Treatment	8	5945.3	743.16	1.36	0.224	9942
Day × Treatment	16	10 125	632.82	1.16	0.301	9916
Origin \times Day \times Treat	23	13 973	607.54	1.11	0.334	9922
Res	218	118 830	545.08			
Total	284	175 700				
Basking						
Origin	2	391.46	195.73	1.95	0.146	9946
Day	4	446.43	111.61	1.11	0.361	9958
Treatment	4	622.97	155.74	1.55	0.190	9958
Origin × Day	8	5467.6	683.45	6.8	< 0.001	9936
Origin × Treatment	8	868.46	108.56	1.08	0.376	9928
Day × Treatment	16	2376.7	148.54	1.50	0.104	9919
Origin \times Day \times Treat	23	2286	99.39	0.99	0.481	9909
Res	219	22 011	100.51	0.,,,	001	,,,,,
Total	284	34 397	100.01			
Basking temperature covaria		2.37,				
Temperature	1	13.00	13.00	0.13	0.718	9933
Origin	2	928.92	464.46	4.81	0.008	9952
Day	4	853.66	213.42	2.21	0.070	9961
Treatment	4	247.27	61.82	0.64	0.639	9960
Origin × Day	8	6050	756.25	7.83	< 0.001	9935
Origin × Treatment	8	627.49	78.44	0.81	0.582	9947
Day × Treatment	16	2636.6	164.79	1.71	0.046	9918
Origin \times Day \times Treat	23	1989.5	86.5	0.90	0.593	9909
Res	218	21 051	96.56	0.70	0.575	7707
Total	284	34 397	90.50			
10141	204	JT J7 /				

captive-born lizards was comparable to that in wild-born and wild lizards, suggesting that recognition of predator chemical cues is innate; (3) there was no difference in tongue flicking towards predator scent among lizards that were captive-born, wild-born or wild, which suggests that lizards recognise predators regardless of prior experience and that time spent in captivity did not reduce predator recognition; and (4) unexpectedly, the presence of predator scent did not result in reduced basking or an increase in lizards retreating to their refuges, suggesting that predator chemical cues

alone are not enough to prompt predator avoidance behaviour in this species.

We found that pygmy bluetongue lizards tongue flicked towards eastern brown snake scent significantly more than they did towards the water and novel odour controls. Pygmy bluetongue lizards also tongue flicked more towards the eastern brown snake scent than towards the eastern bluetongue and Rosenberg's monitor scents, although this difference was not significant. Eastern brown snakes are a known predator of the pygmy bluetongue lizard (Hutchinson *et al.* 1994), which

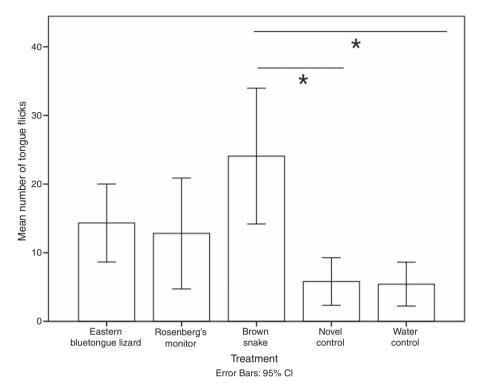


Fig. 1. Mean number of tongue flicks (95% CI) made by pygmy bluetongue lizards of all origins (captive-born, wild-born and wild) over a 30-min period for the following five scent treatments: eastern bluetongue lizard, Rosenberg's monitor, eastern brown snake, novel control and water control. Asterisk indicates treatments that were significantly different in pairwise comparisons.

could explain the higher rate of tongue flicks than in other scent treatments. The antipredator response of prey species can be threat sensitive or generalised to a range of predators. The threat-sensitive hypothesis predicts that prey should assess and respond according to the level of threat posed by the specific predator (Helfman 1989; Forester *et al.* 2019). We found that the pygmy bluetongue reacted strongest to a known predator scent, which may suggest that this species can distinguish among predators. However, caution in interpreting this result is required because the reaction between known and potential predator scents was not significantly different.

The ability to distinguish between predators and nonpredators and risk levels have been found in reptile and primate species (Buchanan-Smith et al. 1993; Stapley 2003; Lloyd et al. 2009; Cisterne et al. 2014; Cornelis et al. 2019). The pygmy salamander (Desmognathus wrighti) could distinguish between chemical cues from a specialist snake predator and two generalist predators, namely, beetle and salamander species (Forester et al. 2019). Some prey species display a generalised response to predators when antipredator behaviours are not costly or multiple species are potentially dangerous (Blumstein et al. 2006; Webb et al. 2009). For example, two Australian lizard species were found to recognise and respond to chemical cues of both native and invasive predator species, which could be due to either generalised predator recognition or rapid evolution or learned behavioural response to invasive predators (Webster et al. 2018) and fawn-footed mosaic-tailed rats (Melomvs

cervinipes) recognised but did not discriminate between venomous sympatric and non-venomous non-sympatric snake species (Paulling *et al.* 2019). The recognition of predators and the response to the recognition of predators appears variable among species.

We found no significant difference in responses among the captive-born pygmy bluetongue lizards and wild or wild-born adults, which suggests that the recognition of predator chemical cues is innate in this lizard species. The lack of difference in responses between captive-born juveniles and potentially experienced wild-born adults suggests that there is not a learned component in predator recognition of potential versus non-potential predators in this species. Additionally, in the case of wild-born adults, time in captivity did not alter the lizard's ability to recognise predator scent. These results contrast those from other species. For example, concealment behaviour of translocated ratsnakes (Pantherophis obsoletus) decreased the longer the snakes were in captivity, likely increasing vulnerability to predation (DeGregorio et al. 2017). Although the exact timeframe required for prey species to lose predator avoidance behaviours is not known and is likely to be variable among species, recent studies have shown that robins reduced antipredator behaviour towards predators within 3 years (Muralidhar et al. 2019) and quolls lost recognition and avoidance behaviours in 13 generations (Jolly et al. 2018). Although our study found that predator recognition was not reduced after 5 years in captivity, pygmy bluetongues did not show avoidance behaviour and may have other altered

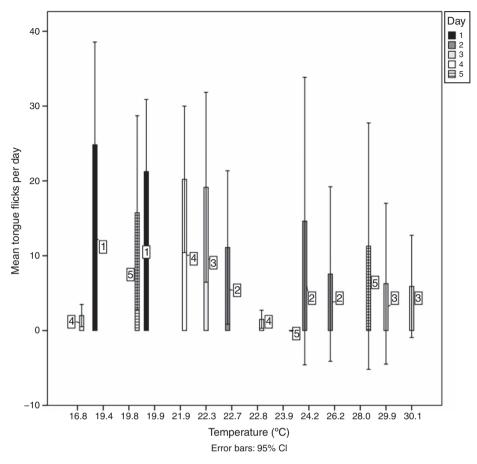


Fig. 2. Mean number of tongue flicks (95% CI) directed towards scent paper across the filming temperature range, °C by day of trial. Numbered labels on boxplots indicate day of trial.

behaviours that may reduce survival that were not investigated in the present study, such as spending more time exposed on the surface.

Our findings suggest that, like many other species, pygmy bluetongue lizards have an innate ability to recognise predator chemical cues. For example, cotton-top tamarin monkeys (Saguinus Oedipus; Buchanan-Smith et al. 1993), and leopard geckos (Eublepharis macularius; Landová et al. 2016), can innately recognise predator chemical cues, regardless of whether they are wild or captive-born. However, juvenile Baltic sturgeon (Acipenser oxyrinchus) was found to lack the innate ability to recognise predator chemical cues (Cámara Ruiz et al. 2019). For some species, there is a learned component in predator recognition; for example, captive-born Iberian wall lizards (Podarcis hispanicus) can innately recognise predator scent, but experienced wild-born adults can also distinguish between sympatric and non-sympatric predators (Martín et al. 2015). However, this is not the case in all species, because our study did not find evidence for a learned component in the pygmy bluetongue lizard.

Pygmy bluetongue lizards did not reduce their basking time in the presence of predator scent compared with when presented with control scents, despite our finding of these lizards being able to recognise predator scent. This lack of reduction in basking suggests that chemical cues alone are not

enough to elicit the predator avoidance behaviour of retreating into the burrow generally displayed by the pygmy bluetongue lizard when threatened (Tara Daniell (TD), pers. obs.). Perhaps these lizards show a threat-sensitive response to risk from predator cues, because scent alone was not enough to elicit an avoidance response. Lizards are quite safe when basking at the burrow entrance because they can rapidly retreat into burrows if threatened and choose burrows with diameters close to their head width, which would block predators from entering the burrow and snakes are not able to open their mouths to bite the lizards when inside a burrow (Milne and Bull 2000). Therefore, the presence of predator scent may increase vigilance, although further cues, i.e. visual, may be required to elicit avoidance behaviour. We have anecdotal evidence from video recordings of wild pygmy bluetongue lizards retreating into burrows when magpies walked near burrows and when an eastern brown snake partially entered a burrow in a predation attempt (Tara Daniell (TD), pers. obs.). Therefore, visual cues or a combination of chemical, visual and auditory cues may be required for these endangered lizards to display avoidance behaviours. Chemical cues can remain in the environment for long periods, but this may not be a useful predator cue in the case of active foraging predators (Head et al. 2002). We do not believe that our use of frozen scent was the reason for the lack of avoidance response, because frozen scent has been

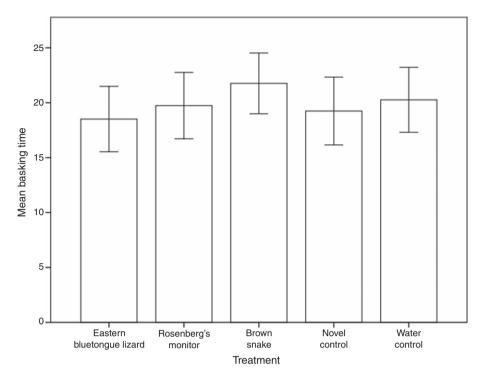


Fig. 3. Mean basking time (95% CI) by pygmy bluetongue lizards during 30-min filming sessions, in the presence of the five scent treatments across all lizard origins.

used effectively previously (Bourke et al. 2017) and our lizards did show increased tongue flicking towards reptile scent compared with controls. In other species, predator avoidance behaviours can be elicited by chemical, visual or auditory cues alone, or a combination of cues and these may be context or habitat specific. For example, wall lizards (*Podarcis* muralis) did not respond more strongly when presented with a combination of visual and chemical cues than with chemical cues alone, possibly because visual cues are less useful in low-visibility conditions present in refuges (Amo et al. 2006). The three-spined stickleback (Gasterosteus aculeatus) responded strongest to visual predator cues, whereas responses to chemical cues were weaker, and behavioural responses differed between visual and chemical cues (Landeira-Dabarca et al. 2019). Adult southern water skinks (Eulamprus heatwolei) showed no response to chemical cues from a known predator, the red-bellied black snake (Pseudechis porphyriacus), possibly owing to chemical cues being ineffective in detecting the presence of the snake because it is an active forager (Head et al. 2002). Australian house geckos (Gehyra dubia) did not avoid shelters with chemical cues from predatory snake species, which was interpreted as a threatsensitive response and that chemical cues alone were not threatening enough to cause avoidance (Cisterne et al. 2014).

Our study found that lizards tongue flicked more towards the known predator, the brown snake, than in other reptile or control treatments, that predator scent recognition is innate in the pygmy bluetongue and time in captivity did not reduce predator recognition, and that the presence of predator scent did not result in reduced basking behaviour. In summary, our results suggest that although the pygmy bluetongue lizard can innately recognise predator scent, chemical cues alone are not enough to elicit predator avoidance behaviour. Furthermore, we cannot confirm that captive lizards released into the wild would display predator avoidance behaviour. Future studies should use multiple individuals from each species as scent donors to provide chemical cues, to avoid the possibility that an individual is not representative of the species. Further research is required focusing on visual cues before being able to confirm captive lizards would be able to avoid predators if released into the wild. Our research further highlighted how reactions to predator cues differ among species and contexts, and the need to study predator recognition and avoidance towards multiple cue types, particularly for endangered species that are subject to captive breeding and release programs. Ecological traits of predator and prey species, such as active foraging behaviour of predators, and prey species that are associated with safe refugia, can provide insight and help drive hypotheses for testing of predator cues most likely to elicit predator avoidance.

Ethics statement

This study was conducted according to the approval of the Wildlife Ethics Committee, Project number 28/2017.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of funding

We thank Nature Foundation SA, Field Naturalists of South Australia and the Australian Research Council (LP120100426

83

to MNH and MGG), which provided funding. Tara Daniell received support from the Australian Government RTP scholarship and Flinders University Research Scholarship.

Acknowledgements

We thank Zoos SA for providing access to their captive pygmy bluetongue lizard colony, Daniel Saliba who collected the reptile scent from Adelaide Zoo lizards, and the Nature Foundation SA for access to their property at Burra. We would like to thank C. Michael Bull for his years of thorough research on the pygmy bluetongue that led to this work.

References

- Aaltonen, K., Bryant, A. A., Hostetler, J. A., and Oli, M. K. (2009).
 Reintroducing endangered Vancouver Island marmots: survival and cause-specific mortality rates of captive-born versus wild-born individuals. *Biological Conservation* 142, 2181–2190. doi:10.1016/j.biocon.2009.04.019
- Amo, L., López, P., and Martín, J. (2006). Can wall lizards combine chemical and visual cues to discriminate predatory from non-predatory snakes inside refuges? *Ethology* 112, 478–484. doi:10.1111/j.1439-0310. 2005.01170.x
- Apfelbach, R., Blanchard, C. D., Blanchard, R. J., Hayes, R. A., and McGregor, I. S. (2005). The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neuroscience* and Biobehavioral Reviews 29, 1123–1144. doi:10.1016/j.neubiorev. 2005.05.005
- Armstrong, G., and Reid, J. (1992). The rediscovery of the Adelaide pygmy bluetongue *Tiliqua adelaidensis* (Peters, 1863). *Herpetofauna* **22**, 3–6.
- Atkins, R., Blumstein, D. T., Moseby, K. E., West, R., Hyatt, M., and Letnic, M. (2016). Deep evolutionary experience explains mammalian responses to predators. *Behavioral Ecology and Sociobiology* 70, 1755–1763. doi:10.1007/s00265-016-2181-4
- Blumstein, D. T. (2006). The multipredator hypothesis and the evolutionary persistence of antipredator behavior. *Ethology* 112, 209–217. doi:10.1111/j.1439-0310.2006.01209.x
- Blumstein, D. T., Bitton, A., and Da Veiga, J. (2006). How does the presence of predators influence the persistence of antipredator behavior? *Journal of Theoretical Biology* **239**, 460–468. doi:10.1016/j.jtbi.2005.08.011
- Bourke, G., Matthews, A., and Michael, D. R. (2017). Can protective attributes of artificial refuges offset predation risk in lizards? *Austral Ecology* 42, 497–507. doi:10.1111/aec.12469
- Buchanan-Smith, H. M., Anderson, D. A., and Ryan, C. W. (1993). Responses of cotton-top tamarins (*Saguinus oedipus*) to faecal scents of predators and non-predators. *Animal Welfare* 2, 17–32.
- Bull, C. M., Griffin, C. L., and Johnston, G. R. (1999). Olfactory discrimination in scat-piling lizards. *Behavioral Ecology* 10, 136–140. doi:10.1093/beheco/10.2.136
- Cámara Ruiz, M., Espírito Santo, C., Mai, A., Gessner, J., and Wuertz, S. (2019). Can juvenile Baltic sturgeon (*Acipenser oxyrinchus*) smell the enemy? *Journal of Applied Ichthyology* 35, 835–842. doi:10.1111/ jai.13934
- Cisterne, A., Vanderduys, E. P., Pike, D. A., and Schwarzkopf, L. (2014). Wary invaders and clever natives: sympatric house geckos show disparate responses to predator scent. *Behavioral Ecology* 25, 604–611. doi:10.1093/beheco/aru031
- Commonwealth of Australia Bureau of Meteorology (2020) Climate data online: Burra community school. Available at: http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_nccObsCode=139&p_display_type=dataFile&p_startYear=&p_c=&p_stn_num=021077
- Cooper, W. E. (1994). Chemical discrimination by tongue-flicking in lizards: a review with hypotheses on its origin and its ecological and phylogenetic relationships. *Journal of Chemical Ecology* 20, 439–487. doi:10.1007/BF02064449

- Cornelis, J., Nordberg, E. J., and Schwarzkopf, L. (2019). Antipredator behaviour of invasive geckos in response to chemical cues from snakes. *Ethology* 125, 57–63. doi:10.1111/eth.12826
- DeGregorio, B. A., Sperry, J. H., Tuberville, T. D., and Weatherhead, P. J. (2017). Translocating ratsnakes: does enrichment offset negative effects of time in captivity? *Wildlife Research* 44, 438–448. doi:10.1071/WR17016
- Delean, S., Bull, C. M., Brook, B. W., Heard, L. M. B., Fordham, D. A., and Fitzpatrick, M. (2013). Using plant distributions to predict the current and future range of a rare lizard. *Diversity & Distributions* 19, 1125–1137. doi:10.1111/ddi.12050
- Ebrahimi, M., Godfrey, S. S., Fenner, A. L., and Bull, C. M. (2014). Mating behaviour in pygmy bluetongue lizards: do females 'attract' male lizards? *Australian Journal of Zoology* 62, 491–497. doi:10.1071/ZO14055
- Fenner, A. L., and Bull, C. M. (2011). Responses of the endangered pygmy bluetongue lizard to conspecific scats. *Journal of Ethology* **29**, 69–77. doi:10.1007/s10164-010-0225-1
- Fenner, A. L., Bull, C. M., and Hutchinson, M. N. (2008). Injuries to lizards: conservation implications for the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*). Wildlife Research 35, 158–161. doi:10.1071/WR07103
- Fordham, D. A., Watts, M. J., Delean, S., Brook, B. W., Heard, L. M. B., and Bull, C. M. (2012). Managed relocation as an adaptation strategy for mitigating climate change threats to the persistence of an endangered lizard. *Global Change Biology* 18, 2743–2755. doi:10.1111/ j.1365-2486.2012.02742.x
- Forester, J. D., Forester, D. C., and Matkowski, J. M. (2019). Making the best of a bad situation: differential predator avoidance in a diminutive woodland salamander. *Animal Behaviour* 148, 169–181. doi:10.1016/ j.anbehav.2018.12.009
- Head, M. L., Keogh, J. S., and Doughty, P. (2002). Experimental evidence of an age-specific shift in chemical detection of predators in a lizard. *Journal of Chemical Ecology* 28, 541–554. doi:10.1023/A:101459 2028693
- Helfman, G. (1989). Threat-sensitive predator avoidance in damselfishtrumpetfish interactions. *Behavioral Ecology and Sociobiology* 24, 47–58. doi:10.1007/BF00300117
- Hutchinson, M. N., Milne, T., and Croft, T. (1994). Redescription and ecological notes on the pygmy bluetongue, *Tiliqua adelaidensis* (Squamata: Scincidae). *Transactions of the Royal Society of South Australia* 118, 217–226.
- Jolly, C. J., Webb, J. K., and Phillips, B. L. (2018). The perils of paradise: an endangered species conserved on an island loses antipredator behaviours within 13 generations. *Biology Letters* 14, 20180222. doi:10.1098/rsbl.2018.0222
- Jule, K. R., Leaver, L. A., and Lea, S. E. G. (2008). The effects of captive experience on reintroduction survival in carnivores: a review and analysis. *Biological Conservation* 141, 355–363. doi:10.1016/j. biocon.2007.11.007
- Kats, L. B., and Dill, L. M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5, 361–394. doi:10.1080/11956860.1998.11682468
- King, D., and Green, B. (1979). Notes on diet and reproduction of the sand goanna, *Varanus gouldii rosenbergi. Copeia* 1979, 64–70. doi:10.2307/1443730
- Landeira-Dabarca, A., Näslund, J., Johnsson, J. I., and Álvarez, M. (2019). Cue recognition and behavioural responses in the three-spined stickleback (*Gasterosteus aculeatus*) under risk of fish predation. *Acta Ethologica* **22**, 209–221. doi:10.1007/s10211-019-00324-8
- Landová, E., Musilová, V., Polák, J., Sedlácková, K., and Frynta, D. (2016).
 Antipredatory reaction of the leopard gecko *Eublepharis macularius* to snake predators. *Current Zoology* 62, 439–450. doi:10.1093/cz/zow050
- Lloyd, R., Alford, R. A., and Schwarzkopf, L. (2009). Chemical discrimination among predators by lizards: responses of three skink species to the odours of high- and low-threat varanid predators. *Austral Ecology* 34, 50–54. doi:10.1111/j.1442-9993.2008.01881.x

MacLean, S. A., and Bonter, D. N. (2013). The sound of danger: threat sensitivity to predator vocalizations, alarm calls, and novelty in gulls. *PLoS One* 8, e82384. doi:10.1371/journal.pone.0082384

84

- Martín, J., Ortega, J., and López, P. (2015). Experience may allow increasing accuracy of the innate chemosensory recognition of snake predators by Iberian wall lizards. *Behavioral Ecology and Sociobiology* 69, 1565–1572. doi:10.1007/s00265-015-1968-z
- Milne, T., and Bull, C. M. (2000). Burrow choice by individuals of different sizes in the endangered pygmy blue tongue lizard *Tiliqua* adelaidensis. Biological Conservation 95, 295–301. doi:10.1016/ S0006-3207(00)00040-9
- Milne, T., Bull, C. M., and Hutchinson, M. N. (2003). Use of burrows by the endangered pygmy blue-tongue lizard, *Tiliqua adelaidensis* (Scincidae). Wildlife Research 30, 523–528. doi:10.1071/WR02087
- Moseby, K. E., Read, J. L., Paton, D. C., Copley, P., Hill, B. M., and Crisp, H. A. (2011). Predation determines the outcome of 10 reintroduction attempts in arid South Australia. *Biological Conservation* 144, 2863–2872. doi:10.1016/j.biocon.2011.08.003
- Muralidhar, A., Moore, F. L., Easton, L. J., Jamieson, I. G., Seddon, P. J., and van Heezik, Y. (2019). Know your enemy? Conservation management causes loss of antipredator behaviour to novel predators in New Zealand robins. *Animal Behaviour* 149, 135–142. doi:10.1016/j.anbehav. 2019.01.014
- Paulling, K., Wilson, D., and Rymer, T. L. (2019). Olfactory recognition of snake cues by fawn-footed mosaic-tailed rats *Melomys cervinipes*. Behaviour 156, 1235–1253. doi:10.1163/ 1568539X-00003563

- Pelgrim, K., Fenner, A. L., Schofield, J. A., and Bull, C. M. (2014).
 Dynamics of a temperate grassland reptile community in the mid-north of South Australia. *Transactions of the Royal Society of South Australia* 138, 257–266. doi:10.1080/03721426.2014.11649013
- Smith, W., Scott, I. A. W., and Keogh, J. S. (2007). Molecular phylogeography of Rosenberg's goanna (Reptilia: Varanidae: *Varanus rosenbergi*) and its conservation status in New South Wales. *Systematics and Biodiversity* 5, 361–369. doi:10.1017/S147720000 7002460
- Smith, A. L., Gardner, M. G., Fenner, A. L., and Bull, C. M. (2009). Restricted gene flow in the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*) in a fragmented agricultural landscape. *Wildlife Research* 36, 466–478. doi:10.1071/WR08171
- Stapley, J. (2003). Differential avoidance of snake odours by a lizard: evidence for prioritized avoidance based on risk. *Ethology* 109, 785–796. doi:10.1046/j.1439-0310.2003.00920.x
- Webb, J. K., Du, W. G., Pike, D. A., and Shine, R. (2009). Chemical cues from both dangerous and nondangerous snakes elicit antipredator behaviours from a nocturnal lizard. *Animal Behaviour* 77, 1471–1478. doi:10.1016/j.anbehav.2009.02.020
- Webster, C., Massaro, M., Michael, D. R., Bambrick, D., Riley, J. L., and Nimmo, D. G. (2018). Native reptiles alter their foraging in the presence of the olfactory cues of invasive mammalian predators. *Royal Society Open Science* 5, 180136. doi:10.1098/rsos.180136

Handling Editor: Janine Deakin