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Not such silly sausages: Evidence suggests northern quolls exhibit aversion to toads after training with toad sausages

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Abstract The invasion of toxic cane toads (*Rhinella marina*) is a major threat to northern quolls (*Dasyurus hallucatus*) which are poisoned when they attack this novel prey item. Quolls are now endangered as a consequence of the toad invasion. Conditioned taste aversion can be used to train individual quolls to avoid toads, but we currently lack a training technique that can be used at a landscape scale to buffer entire populations from toad impact. Broad-scale deployment requires a bait that can be used for training, but there is no guarantee that such a bait will ultimately elicit aversion to toads. Here, we test a manufactured bait – a 'toad sausage' – in a small captive trial, for its ability to elicit aversion to toads in northern quolls. To do this, we exposed one group of quolls to a toad sausage and another to a control sausage and compared the quolls' predatory responses when presented with a dead adult toad. Captive quolls that consumed a single toad sausage showed a reduced interest in cane toads, interacting with them for less than half the time of their untrained counterparts and showing reduced Attack behaviour. We also quantified bait uptake in the field, by both quolls and non-target species. These field trials showed that wild quolls were the most frequent species attracted to the baits, and that approx. 61% of quolls consumed toad-aversion baits when first encountered. Between 40% and 68% of these animals developed aversion to further bait consumption. Our results suggest that toad-aversion sausages may be used to train wild quolls to avoid cane toads. This opens the possibility for broad-scale quoll training with toad aversion sausages: a technique that may allow wildlife managers to prevent quoll extinctions at a landscape scale.

Key words: bait uptake, *Bufo marinus*, conditioned taste aversion, *Dasyurus hallucatus*, invasive species, *Rhinella marina*.

INTRODUCTION

Invasive species are a major threat to biodiversity (Reaser *et al.* 2007; Woinarski *et al.* 2014). In Australia, species such as feral cats (*Felis catus*) (Legge *et al.* 2017), domestic dogs (*Canis familiaris*) (Doherty *et al.* 2017), foxes (*Vulpes vulpes*) (Short & Smith 1994; Risbey *et al.* 2000) and cane toads (*Rhinella marina*) (Burnett 1997; Letnic *et al.* 2008; Jolly *et al.* 2015) all have serious impacts on native species. Controlling these species at a landscape scale, however, has proved extremely difficult (Ziembicki *et al.* 2015; Tingley *et al.* 2017). Because of this, increasing attention is being paid to mitigating the impact of invasives, rather than supressing their populations (Simberloff *et al.* 2013).

Cane toads are a case in point. These invasive amphibians now occupy more than 1.5 million square kilometres of Australia, continue to spread (Urban *et al.* 2007; Tingley *et al.* 2013), and are extraordinarily difficult to control. The cane toads' defensive chemicals (bufadienalides and related toxins) are highly cardioactive and are unlike toxins possessed by native Australian animals (Hayes *et al.* 2009). As a result, many vertebrate predators, including varanid lizards, snakes and marsupial predators such as quolls, die after attacking or consuming toads (Covacevich & Archer 1975; Webb *et al.* 2005; Smith & Phillips 2006; Hayes *et al.* 2009; Shine 2010). Some reptilian predator populations have adapted to the presence of toads by evolving innate aversion to toads (Phillips & Shine 2005; Llewelyn *et al.* 2011). In the short term, some marsupial predators rapidly learn to avoid toads as prey (Webb *et al.* 2008, 2011; Ujvari *et al.* 2013). An obvious avenue for mitigating the impact of toads, then, is to train predators to avoid toads (Webb *et al.* 2008; Ward-Fear *et al.* 2016, 2017).

Such training can be achieved through conditioned taste aversion (CTA). Conditioned taste aversion is a powerful innate response found across all vertebrates; an evolved defence mechanism against poisoning (Sinclair & Bird 1984; Conover 1995; Cohn & MacPhail 1996; Bernstein 1999; Mappes *et al.* 2005; Page & Ryan 2005; Glendinning 2007). With CTA, animals acquire an aversion to a referent food as a

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result of a nauseating experience (Gustavson & Nicolaus 1987). Agriculturalists and wildlife managers have used conditioned taste aversion to reduce wildlife damage to crops, industry, or livestock (Gustavson *et al.* 1974; Ellins & Catalano 1980; Avery 1985; Provenza *et al.* 1990; Ternent & Garshelis 1999; Smith *et al.* 2000). CTA has also been used successfully to reduce predation on native or introduced wildlife (Nicolaus & Nellis 1987; Conover 1989; Nicolaus *et al.* 1989; Semel & Nicolaus 1992; Avery *et al.* 1995; Bogliani & Fiorella 1998; Cox *et al.* 2004), or ameliorate the impacts of invasive species (O'Donnell *et al.* 2010; Ward-Fear *et al.* 2016, 2017).

One of the Australian species most strongly impacted by cane toads is the northern quoll, *Dasyurus hallucatus*. As toads have spread, they have caused numerous local extinctions of this native marsupial predator (Burnett 1997; Oakwood & Foster 2008). CTA training using small toads infused with the nausea inducing chemical thiabendazole (TBZ) elicits aversion to live toads in northern quolls (O'Donnell *et al.* 2010), suggesting the technique has promise as a management tool for mitigating toad impact. Capacity to elicit aversion is, however, only the first hurdle. To be effective as a management tool, CTA needs to meet two additional conditions. First, CTA training needs to be deliverable to a large number of individuals under field conditions. Second, prey aversion needs to occur in a large enough proportion of the population, and be behaviourally persistent for long enough (within and across generations), that population-level benefits are realized. In quolls, it is clear that CTA training in captivity can be used to elicit toad aversion, and that this aversion improves survival rates when animals are released into the field (O'Donnell *et al.* 2010). More importantly, parentage analyses demonstrated that some offspring of surviving 'toad smart' females also survived and reproduced (Cremona *et al.* 2017), suggesting that training a single generation could yield significant conservation benefits. The remaining challenge then is to effectively deliver CTA training to a large number of individuals under field conditions.

Recent studies by Ward-Fear *et al.* (2016) achieved CTA under field conditions in a species of monitor lizard, *Varanus panoptes*. Ward-Fear *et al.* (2017) also established that offering live 'teacher toads' induced CTA more successfully in this lizard than did baits made from cane toad flesh laced with lithium chloride. In captive quolls, by contrast, the use of live toads has been unsuccessful (J. Webb, unpub. data, 2017). Instead, CTA training is achieved by feeding individuals a small non-lethal-sized toad laced with the nausea-inducing chemical thiabendazole. Such a delivery mechanism is, however, not feasible at a large scale in a field setting. To achieve *in situ* training at scale requires use of a manufactured training bait. Any bait, of course, needs to fulfil the criteria we have identified above: elicits aversion to toads, has a high uptake rate; and effectively trains a high enough proportion of the population that population persistence is assured. An additional consideration is whether the bait is taken by non-target species. This is a major concern in lethal baiting campaigns (Sinclair & Bird 1984; Avery *et al.* 1995; Fairbridge *et al.* 2003; Glen & Dickman 2003; Claridge & Mills 2007; Jolley *et al.* 2012), but a smaller consideration in non-lethal baiting such as we envisage here. Non-target uptake remains important, however, because it can reduce target species' access to bait and so significantly increase the cost and complexity of the baiting effort. Because of this, it is important to understand non-target species uptake rates.

In this study, we assess the value of a manufactured bait ('toad aversion sausages'). We ask whether quolls generalize their CTA from the bait to toads, whether the bait is taken up by wild quolls (and non-target species), and whether it appears to elicit CTA under field conditions.

METHODS

Cane toad sausages

Cane toad sausages were made up of 15 g of minced skinned adult cane toad legs, 1 whole cane toad metamorph weighing <2 and 0.06 g of Thiabendazole (per sausage; dose rate less than 300 mg kg^{-1} adult quoll body weight, determined by the smallest – 200 g – adult seen at our study site) packed into a synthetic sausage skin and deployed fresh. In our captive trials, we used the same sausage composition, to accurately reflect our field scenario. Thiabendazole is an inexpensive, broad-spectrum anthelmintic and antifungal agent (Robinson *et al.* 1965). It is orally effective and regarded as relatively safe, producing low mammalian mortality: oral LD_{50} is 2.7 g kg^{-1} body weight (Dilov *et al.* 1981). It is fast acting and peak concentration occurs in the plasma 1 h after consumption (Tocco *et al.* 1966). Thiabendazole has produced strong aversions to treated foods in lab rats (Gill *et al.* 2000; Massei & Cowan 2002), wolves (*Canis lupus*) (Gustavson *et al.* 1983; Ziegler *et al.* 1983) and black bears (*Ursus americanus*) (Ternent & Garshelis 1999). Thiabendazole induces a robust CTA after a single oral dose (Nachman & Ashe 1973; O'Donnell *et al.* 2010) and is physically stable at ambient conditions in the bait substrate (Gill *et al.* 2000; Massei *et al.* 2003).

Captive trials

The uptake of toad aversion sausages by *D. hallucatus* and their subsequent response to toads was observed in captive northern quolls previously collected from toad-free areas of Astell Island, and then housed at the Territory Wildlife

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Park, Northern Territory. Animals (nine male and nine female) were randomly allocated treatment ($n = 9$) or control ($n = 9$) sausage groups. Treatment sausages were exactly as described previously. Control sausages were comprised of store purchased beef sausages. These were selected as a control sausage as it was an item that animals are also not familiar with to control for hunger differences and any possible neophobic responses.

To measure individual responses to cane toads following ingestion of sausage, each individual was presented with a dead adult cane toad the following evening. The dead adult toad was secured in a 15 × 15 × 10 cm wire cage, so that animals could come into close proximity to see and smell the prey item but not access it. The experiment was run over three nights. Experiments began at sunset and ran for on average 2 h. The response was filmed using a GoPro Hero 3 White camera (GoPro Inc, San Mateo, CA, USA).

Field trials

Study area

The field study was conducted between May 2016 and February 2017 at Mornington Wildlife Sanctuary, a 300 000 ha property in the central Kimberley region of Western Australia managed for conservation by the Australian Wildlife Conservancy (17°01′S, 126°01′E; Fig. 1). The area is characterized by savanna woodland dissected by sandstone gorges of varying topographic complexity. On average, this area receives 788 mm of rain annually, most of which falls during the wet season from November to April.

We worked at four sites on the property; Site 1 (SJ) was at Sir John Gorge (17°31.780S, 126°13.080E) along the Fitzroy River. Site 2 (KP) (17°31′43.032, 126°13′11.050) was approx. 2 km upstream from Site 1 in the same gorge. Site 3 (TC) (17°30′37.213, 126°14′4.092) was 5 km upstream from Site 2 in a narrow rocky gorge that feeds into Sir John Gorge. Site 4 (RP) (17°35′12.119, 126°19′21.959) was a narrowly incised sandstone gorge following a watercourse within rocky range country approx. 9 km north-east of Site 1. Sites were selected based on the detection of quolls in the Australian Wildlife Conservancy's fauna surveys (J. Smith, AWC, unpub. data, 2017). At the time of the study, toads were yet to arrive at our sites; they subsequently arrived by March 2017.

CTA sausage field trials

In this study, 'site' is the location where an experiment took place. 'Bait station' is a location within a site where sausage bait was offered. A 'session', is a time interval when bait stations were active. A total of four sessions were conducted approx. 5 months apart. Sessions recorded up to

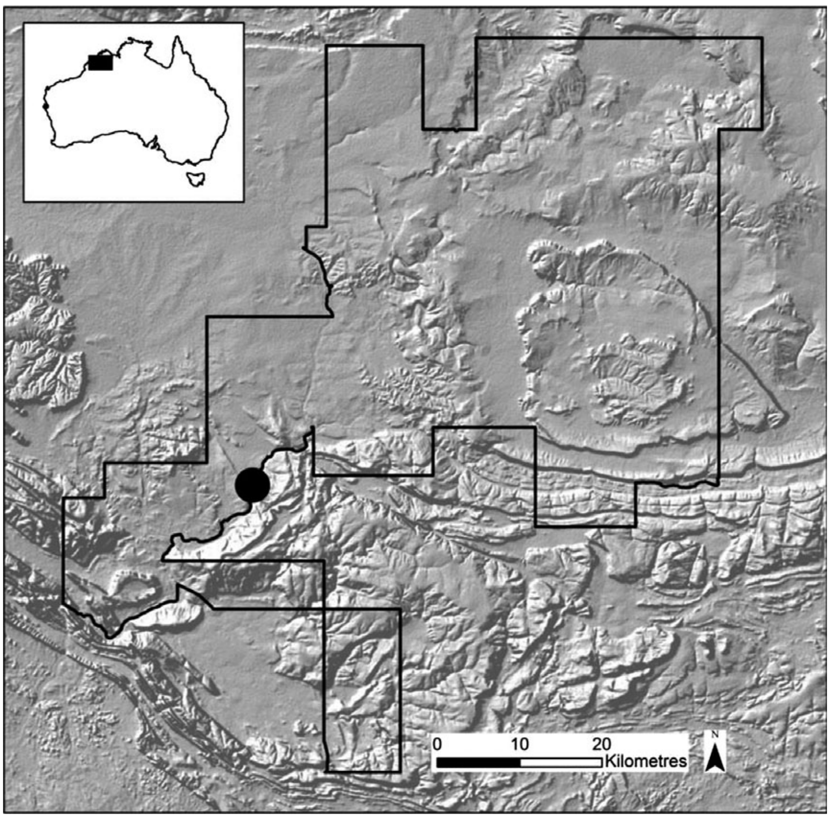

Fig. 1. Location of the study area within Australian Wildlife Conservancy's Mornington Wildlife Sanctuary, in the central Kimberley, Western Australia.

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Table 1. Conditioned taste aversion sessions and bait events,† denotes empty cells

Site name	Session year	Session month	No. bait events (BE)	BE 1-date	BE 2-date	BE 3-date	BE 4-date	No. of bait stations
KP	2016	November	1	31/10/16	1/11/16	2/11/16	†	20
RP	2017	February	1	3/2/2017	†	†	†	20
RP	2016	May	3	10/5/16	13/5/16	21/5/16	†	20
RP	2016	September	3	15/9/16	16/9/16	17/9/16	†	20
SJ	2017	February	1	3/2/2017	†	†	†	20
SJ	2016	May	3	10/5/16	13/5/16	21/5/16	†	20
SJ	2016	September	4	15/9/16	16/9/16	17/9/16	19/9/16	20
TC	2017	February	1	3/2/2017	†	†	†	33
TC	2016	May	3	10/5/16	13/5/16	21/5/16	†	20
TC	2016	September	3	15/9/16	16/9/16	17/9/16	†	20

Bait events occurred at the same time within each site. KP was baited only once in November to expand the sample size and CTA train quolls prior to cane toad arrival.

four 'bait events'. Bait Events are defined as an occasion when new bait was placed unsecured at a bait station and (if still existing) the old bait removed.

Each site contained 20 bait stations placed 50–80 m apart in a linear transect along a gorge wall where the presence of *D. hallucatus* was previously confirmed (J. Smith, AWC, unpub. data, 2017). Bait stations consisted of one cane toad sausage placed under a single camera trap (White flash and Infrared Reconyx Motion Activated, HP800, U.S.A). Cameras were secured to trees or rocky ledges approx. 1 m from the ground and aligned to face directly downwards (Diete *et al.* 2016). Cameras were set to take five consecutive photographs for each trigger with no delay between triggers. Each cane toad sausage was placed inside a ring of powdered insecticide (Coopex) to protect from ant spoilage. Each session's bait stations were rebaited up to three times (for a maximum of four bait events within any given session) whereby bait stations were rebaited with fresh CTA bait and the old bait removed (Table 1). A total of 513 individual cane toad sausages were deployed over the period of study.

Data analysis

Captive trials

Videos were scored by the same observer who was blind to the quoll's treatment or control group. Following Kelly and Phillips (2017), we separated the time that quolls spent exhibiting various predatory behaviours into three categories: 'Sniff', 'Investigate' and 'Attack'. Sniff was defined as when quolls were visibly twitching their nose in the direction of the toad, 'Investigating' behaviour was defined as the quoll being engaged with the cage containing the toad, exhibiting scent marking or digging around the outside of cane toad enclosure and 'Attack' behaviour was defined as quolls exhibited pawing or licking or biting behaviour to toads cages. We summed all of these to measure the total time spent interacting with a toad. We converted each of these variables to a proportion of time spent in each of these activities, where the denominator was the total time that the animal was observable on camera. These response variables were not normally distributed, and could not be made to conform to normality through transformation. Because of this, we used bootstrapping to obtain confidence intervals for the mean time engaged in each behaviour, and to test the null hypothesis that there was no difference between treatments in mean time spent in each activity. The perception that animals exhibit a lower propensity towards attacking a prey item following ingestion and subsequent malaise during CTA training is non-controversial (Gustavson *et al.* 1974, 1976, 1983; Gustavson 1982; Gustavson & Basche 1983; Ziegler *et al.* 1983; Gustavson & Nicolaus 1987; Nicolaus 1987; Nicolaus & Nellis 1987; Nicolaus *et al.* 1989; Schneider & Pinnow 1994; Smith *et al.* 2000; Riley & Freeman 2004; Sevelinges *et al.* 2009; O'Donnell *et al.* 2010; Thornton & Raihani 2010; Thornton & Clutton-Brock 2011). More relevant to this study is the outcomes of previous trails by O'Donnell *et al.* (2010) and Kelly and Phillips (2017), where quolls exhibited less interest in prey items after consuming a toad metamorph laced with thiabendazole. Based on these previous results, we had a strong *a priori* expectation that animals could either be unaffected or only become less interested in toads after ingestion of cane toad sausages. Thus, we employed a one-tailed test, with the alternative hypothesis that the mean time spent Investigating and Attacking toads will be lower in the treatment group. This analysis was performed using R (R Core Team 2017).

Field trials

Images from bait stations were collated and tagged by pass, session, site, bait-event, species and activity. A 'pass' was defined as when a new species entered the frame or when images that were at least 5 min between when the previous detection of the same species passed. This reduced any likelihood of individuals of the same species being overlooked during analysis. 'Activity' was hierarchical, with the highest activity being 'Bait taken'; this was defined as either photographic evidence of animal eating bait or bait being taken from the bait station. 'Bait investigated' was defined as when bait was Sniffed but not consumed or taken. 'Bait area investigated with no bait available' was defined as when no bait was

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available at a bait station, but the animal was still visiting or Investigating the bait station.

We analysed data using two levels of observation to determine (i) which species were attracted to bait, and (ii) which species took bait. A frequency distribution (*n* times each species was recorded) was calculated and the proportion of bait takers in each species was estimated. Passes in which we were unable to identify the species were pooled and removed from further analysis. Additionally, if a species total number of visits was less than 10, we removed that species from the analysis. Additionally *Varanus tristis*, *V. panoptes*, *V. mitchelli* and *V. mertensi* were pooled into '*Varanus* other species' due to small sample sizes.

We identified individual *D. hallucatus* that visited bait stations by their unique spot patterns (Hohnen *et al.* 2013) to determine visitation rate and bait uptake of individuals. To do this, we employed Wild ID (Version 1.0, January 2011) (Bolger *et al.* 2011) to extract distinctive image features in animals spot patterns, the program calculates a matching score that characterizes the goodness of fit between two images. These matching scores were then used to rank and select matches to each focal image. We also conducted manual checks with all photographs and compared them to those already identified to determine whether a new individual had been recorded. Quolls were identified to individual within each session, and we treat each session (separated by a minimum of 4 months) as independent with regard to quoll ID and behaviour. This decision was made for logistic reasons (difficulty of identifying individuals using spot ID), but supported by exploratory analysis of first pass uptake rates showing that these do not vary systematically with session (see Results). It is likely, therefore, that any training is forgotten within the 4–5 month window between sessions.

RESULTS

Captive trials

Of the treatment animals, seven (77%) consumed all or part of a cane toad sausage and eight (88%) control animals consumed beef sausages. Treatment had no significant effect on whether the initial sausage was consumed, ($\chi^2 = 0.0$, d.f. = 1, $P = 1$). In our video trials, quolls spent an average of only 0.6% of the total time on camera interacting with the toad (mean = 60.58 s, SE = 13). Control animals, however, spent more than twice as much time interacting with the toad than treatment animals (control = 0.95%; treatment = 0.42%, bootstrap *P*-value = 0.022). When we break this down by specific types of interaction, control animals spend approx. 60 times longer Investigating (control = 0.15%; treatment = 0.00024%, bootstrap *P*-value = 0.051); twice as much time Sniffing (control = 0.70%; treatment = 0.35%, bootstrap *P*-value = 0.044); and twenty times more time Attacking (control = 0.03%; treatment = 0.0015%, bootstrap *P*-value = 0.036) toads when compared with the control (Table 2; Fig. 2).

Table 2. Mean time (seconds) and corresponding standard errors (SE) that treatment and control animals spent exhibiting specific types of interactions with toads e.g. sniffing, investigating and attacking during captive trial

	Overall time with toad (s)	Time sniffing toad (s)	Time investigating toad (s)	Time attacking toad (s)
Treatment				
Mean	37.33	31.22	0.22	0.11
SD	36.15	30.90	0.63	0.31
SE	12.05	10.30	0.21	0.10
Control				
Mean	91.56	66.56	14.78	2.89
SD	54.26	41.72	26.22	3.90
SE	18.09	13.91	8.74	1.30

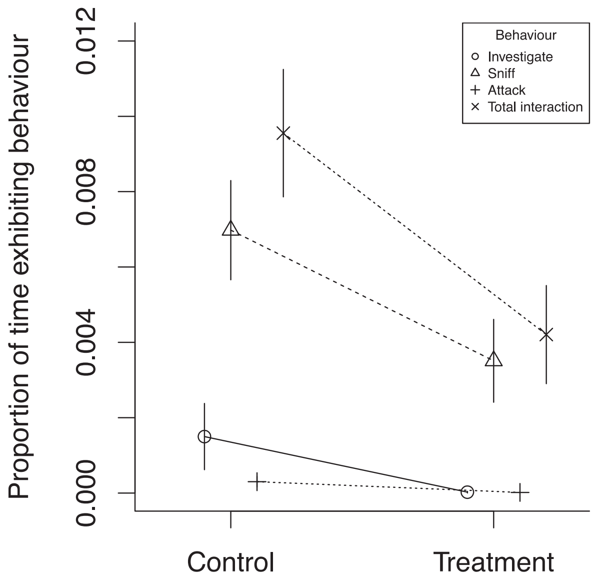

Fig. 2. Mean proportion of active time that quolls spent directed towards toads. Behaviours are split into categories and across control and treatment groups. Error bars represent bootstrap standard errors.

Field trials

Target and non-target uptake

A total of 26 species were captured on camera traps visiting bait stations. For eleven of these species, there were sufficient data to compare their response to bait uptake. The most frequent visitors to the bait stations were quolls, with $n = 345$ passes (Fig. 3). Almost all bait removal was executed by quolls that took 65 baits of the 90 baits removed. Other species

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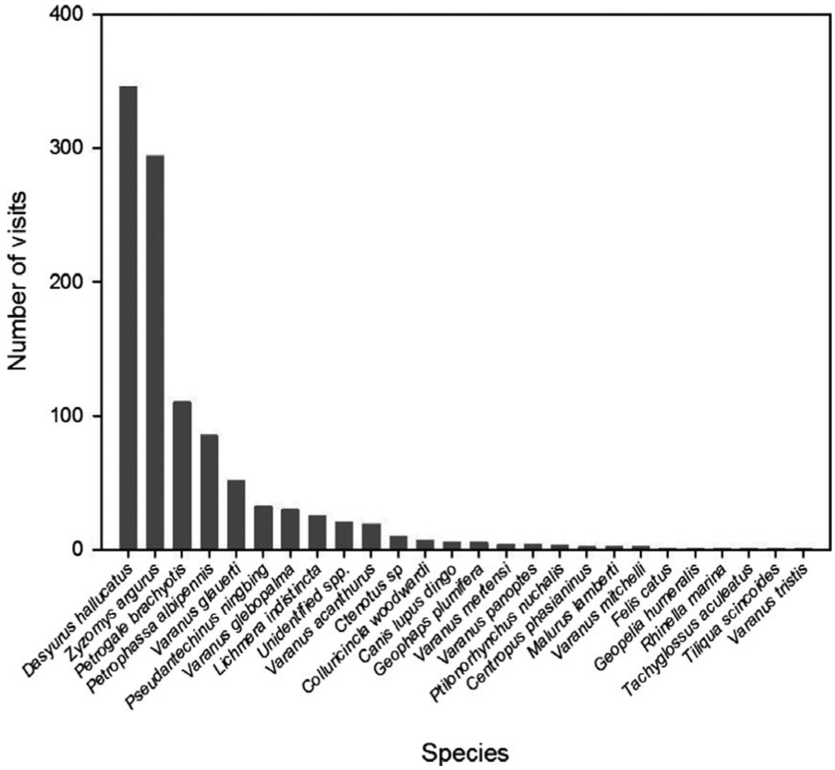

Fig. 3. Frequency of visits to conditioned taste aversion bait stations by each species. Unidentified species group comprises unidentified rodents, birds and frogs.

took far fewer: *Zyzomys argurus*, 9; *Ctenotus* spp., 2; *Pseudantechinus ningbing*, 2; *Varanus glauerti*, 2; and *Varanus glebopalma*, 2.

Target uptake and training rates

First pass uptake responses to the bait did not vary systematically across sessions ($\chi^2 = 1.7$, d.f. = 4, $P = 0.79$; Fig. 4). We thus treated individuals as independent across sessions with regard to behaviour.

Following identification of individual quolls within sessions, it became apparent that bait stations were visited by a total of 70 'individual' quolls over the period of the study. Unfortunately, 24 of these individuals visited bait stations when there was no bait available. Considering only individuals that encountered a bait ($n = 46$), and counting only their first encounter with the bait, the bait was taken initially by 28 individuals and rejected (bait investigated but not taken) by 18 individuals. Thus, the total bait uptake rate at first encounter was 61% (SE = 7.2%). From the 18 individuals which initially did not take bait upon first encounter, three later returned to bait stations to take a bait.

Ten of these animals ultimately consumed baits on more than one occasion within a session (32%, SE = 8.3%). Clearly, these individuals were not effectively trained, failing to even exhibit aversion to the bait. We have two ways of estimating the conversion rate (from untrained to trained, given bait consumption). Placing an upper bound, we could consider all individuals that took a bait but were not observed to take a second bait (20 of 31 = 68%) as

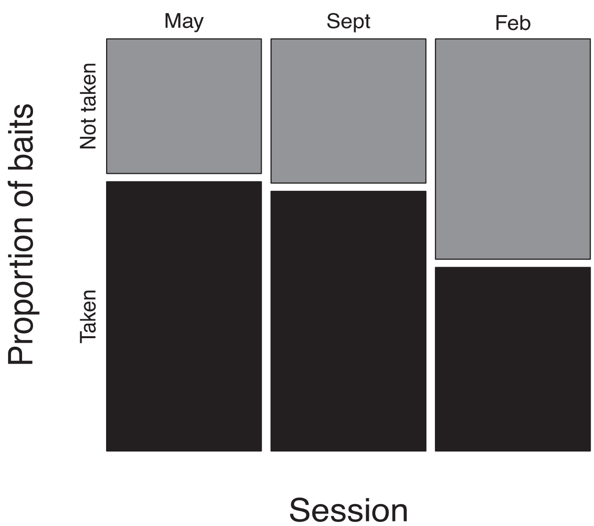

Fig. 4. First pass behavioural responses of northern quolls to the bait each session. 'Sept' includes the one November session also.

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trained. For a lower bound, we could take the conservative approach and consider only those known to have consumed a bait and then seen to approach and reject a bait as trained (7 of 17 = 41%). Thus, somewhere between 41% and 68% of animals consuming a bait appear to have been trained.

DISCUSSION

Captive trials

Our captive trials indicate that training a quoll using a thiabendazole-laced toad sausage changes their behaviour towards toads. Although our sample sizes were modest and not all of our treatment animals fully consumed the bait, it was apparent that sausage-trained animals spent less time interacting with a toad – between one half to one sixtieth of the time as control animals. This behavioural shift is reflected across all prey acquisition behaviours: Investigating, Sniffing, and Attacking. Indicating captive quolls generalized their acquired aversion from the bait to a real toad.

Field trials

The field trials show that the toad sausages are attractive to quolls. Although 26 species encountered the baits, quolls were the most frequent visitors to the bait at our study sites, and were far and away the most likely species to consume the bait. Thus, non-target uptake is relatively modest, compared with the high level of uptake of baits by non-target animals observed in other lethal-baiting studies (Cowled *et al.* 2006; Dundas *et al.* 2014). It is more difficult to estimate the rate of successful training in the field, but it is likely that between 41% and 68% of animals consuming a bait in the field have been successfully trained. The apparent independence of quoll behaviour to bait uptake across sessions also suggests that, in the absence of further reinforcing stimulus (i.e. cane toads), CTA training potentially only elicits aversion for a limited time (<4 months).

The TBZ dose of <300 mg kg^{-1} animal body weight in our cane toad sausages was relatively low compared to earlier work (400 mg kg^{-1} in (O'Donnell *et al.* 2010; Cremona *et al.* 2017; Jolly *et al.* 2017; Kelly & Phillips 2017) but was set low by regulators (Australian Pesticides and Veterinary Medicines Authority (APVMA)) to allow for potential multiple bait uptake, sub-adult target, and non-target species. Given the LD_{50} of TBZ is more than nine times higher than our dose rate; the delivered dose is very conservative. Our results suggest, however, that it is still effective. Regulators (APVMA) also limited the number of treatment baits available at a site at any one time to 30 baits per hectare. It is clear from our study that, at this density of baits, many quolls are simply not encountering the bait; arriving at the bait station after baits have been taken; this in a relatively low density quoll population, and despite multiple bait events at each site. Thus, to effectively bait a large proportion of the quolls at a site (particularly a high density site), a greater density of baits will be required.

In addition to the high visitation rate of individual quolls to bait stations, some individual quolls took baits on multiple occasions. Of the 70 'individual' quolls that visited bait stations throughout study period, ten individuals consumed a cane toad sausage on more than one occasion within a session. Why did these individuals manifestly fail to train? One possibility is the low dose rate, 0.06 g of TBZ in each sausage was calculated to provide 300 mg kg^{-1} to the smallest adult quoll at our site; a 200 g female. Long-term trapping at the site (J. Smith AWC, unpub. data, 2017) suggests that adult quolls in this population can reach more than 815 g in weight. Thus, large animals could receive less than one quarter of the dose ingested by small animals. As a consequence, we could expect larger animals (typically males) to be harder to train with a fixed-dose bait. Another possibility is that these individuals were unhealthy for other reasons (e.g. males in the process of annual die-off) and so were willing to risk poisoning in order to acquire food, although such a mechanism would presumably cause changes in uptake rate across sessions, so seems unlikely.

Our results also hint strongly that individuals lose their acquired aversion over the 4–5 month window between our baiting sessions. There was no evidence that first pass rates of bait uptake declined over time across sessions. Whether this aversion would decline in the presence of ongoing stimulus (i.e. continuous baiting, or the presence of toads) is unknown, but long-term mark-recapture studies of CTA-trained quolls released into toad-infested landscapes suggest that aversion can be long-held in the presence of reinforcing stimulus (Cremona *et al.* 2017). Nonetheless, our finding should sound a note of caution with regard to deployment of CTA. Training prior to toad arrival will need to be delicately timed: too early, and trained animals may lose their aversion before toads arrive. This need for precision timing is complicated by inevitable uncertainty with regard to where the toad invasion front is, and when it will arrive at the site (with spread rate also being contingent on the unpredictable timing of the wet season in northern Australia). Thus, any baiting campaign will need to dedicate effort to predicting the date of toad arrival at the site.

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Implications for CTA application in the wild

Overall, our study is encouraging with regard to the use of toad sausages as a vehicle for large-scale CTA training of quolls. Our results suggest that captive quolls generalized their aversion from cane toad sausages to actual cane toads, and in the field, will readily consume cane toad sausages. We infer that aversion would also likely occur in wild quolls, and so quolls consuming sausages in the field will be less inclined to attack cane toads thereafter. This opens the possibility for broad scale application of CTA as a management technique for mitigating the impact of toads on quolls.

While many questions remain about optimal bait design, delivery, and timing, the present study suggests that CTA training using toad sausages is likely a viable tool for land managers seeking to protect quoll populations. Given that quoll populations in the Kimberley will likely be completely overrun by toads within the next 5 years, this is a tool that is urgently needed. We propose an adaptive management approach towards developing a broad-scale baiting program. A structured, iterative process of delivering baits to quoll populations with aim to reduce uncertainty over time, via ongoing rigorous system monitoring. This is particularly relevant given the uncertainty of the impact baiting may have on non-target species in other regions and the apparent short term nature of aversion training. In addition, to be effective, adaptive management efforts will require incorporated knowledge, support and cooperation among stakeholders.

This study directly contributes to the feasibility of undertaking an adaptive management approach to baiting. More generally, however, our work joins a growing list of studies demonstrating that the impact of invasive species can be mitigated not only by controlling the invasive species, but also – or instead – by manipulating its mechanism of impact.

AUTHORS' CONTRIBUTIONS

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing or revision of the manuscript.

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COMPLIANCE WITH ETHICAL STANDARDS

The study was conducted within Mornington Wildlife Sanctuary in accordance with Wildlife Conservation Regulation 17 (Permit number: SFO10584). The area is jointly managed by traditional landowners and the Australian Wildlife Conservancy. The research was approved by the University of Melbourne Animal Ethics Committee (Protocol: 1413369.2) and the University of Technology Sydney Animal Care and Ethics Committee (Protocol: 2012-432A) and Department of Parks and Wildlife Animal Ethics Committee (Protocol: DPaW AEC 2016_50 and Protocol 2013_37). Additionally this study was conducted in accordance with the approved outline submitted to the AVPMA by the Investigating team (Permit to allow the possession and supply for research use of an unregistered Agvet chemical product. Permit number: PER92262).

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