Out of the frying pan: Reintroduction of toad-smart northern quolls to southern Kakadu National Park

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Abstract   Invasive species are a leading cause of native biodiversity loss. In Australia, the toxic, invasive cane toad *Rhinella marina* has caused massive and widespread declines of northern quolls *Dasyurus hallucatus*. Quolls are fatally poisoned if they mistakenly prey on adult toads. To prevent the extinction of this native dasyurid from the Top End, an insurance population was set up in 2003 on two toad-free islands in Arnhem Land. In 2015, quolls were collected from one of these islands (Astell) for reintroduction. We used conditioned taste aversion to render 22 of these toad-naïve quolls toad averse. Seven quolls received no taste aversion training. The source island was also predator-free, so all quolls received very basic predator-aversion training. In an attempt to re-establish the mainland population, we reintroduced these 29 northern quolls into Kakadu National Park in northern Australia where cane toads have been established for 13 years. The difference in survival between toad-averse and toad-naive quolls was immediately apparent. Toad-naive quolls were almost all killed by toads within 3 days. Toad-averse quolls, on the other hand, not only survived longer but also were recorded mating. Our predator training, however, was far less effective. Dingo predation accounted for a significant proportion of toad-smart quoll mortality. In Kakadu, dingoes have been responsible for high levels of quoll predation in the past and reintroduced animals are often vulnerable to predation-mediated population extinction. Dingoes may also be more effective predators in fire degraded landscapes. Together, these factors could explain the extreme predation mortality that we witnessed. In addition, predator aversion may have been lost from the predator-free island populations. These possibilities are not mutually exclusive but need to be investigated because they have clear bearing on the long-term recovery of the endangered northern quoll.

Key words: cane toad, northern quoll, reintroduction, survival, taste-aversion.

INTRODUCTION

The ecological impacts of invasive species are a major conservation concern and a leading cause of biodiversity loss (Mack *et al.* 2000; McGeoch *et al.* 2010). Typically, the management focus for invasive species is on reducing or eradicating populations. These direct approaches, however, are challenging to sustain and rarely successful on a landscape-scale (Myers *et al.* 2000). An alternative approach is to modify the responses of vulnerable taxa to render them more resilient to invasive species. By manipulating vulnerable taxa, we can improve anti-predator behaviours (Van Heezik *et al.* 1999; Moseby *et al.* 2012) or teach aversion to inappropriate prey (Ellins *et al.* 1977; Nicolaus *et al.* 1983; Conover 1990; O'Donnell *et al.* 2010). If learned behaviours are altered to provide resilience against an invader, these behaviours may be passed between individuals and through successive generations via cultural transmission (Galef & Giraldeau 2001) and such culturally transmitted behaviours can, in principle, persist indefinitely (Dawkins 1976). Mammals in particular often learn from their parents (Mirza & Provenza 1990; Griffin 2004), allowing rapid acquisition of novel behaviours. By exploiting these pathways of rapid behavioural exchange from one generation to the next, we may be able to effect beneficial lasting behavioural change in vulnerable taxa.

More than a quarter of Australia's native mammal species have been lost since European settlement (Short & Smith 1994; Woinarski *et al.* 2015). Some of these extinctions have been attributed to ineffective responses of natives to invasive predators (Banks & Dickman 2007; Salo *et al.* 2007; Carthey & Banks 2014). More recently, however, failure to respond appropriately to toxic invasive prey has further imperilled some native predators (Shine 2010). Since 1935, the spread of highly toxic cane toads *Rhinella marina* across Australia has caused severe declines in frog-eating predators via fatal ingestion (Shine 2010).

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Animals of Gondwanan origins have had no evolutionary history of exposure to toad toxins (*cf.* rodents; Cabrera-Guzmán *et al.* 2015), and these predators are extremely sensitive to them (Phillips *et al.* 2003; Smith & Phillips 2006). The cane toad invasion is responsible for massive population declines in blue-tongue lizards (Price-Rees *et al.* 2010), freshwater crocodiles (Letnic *et al.* 2008), varanid lizards (Doody *et al.* 2009; Jolly *et al.* 2016) and large elapids (Jolly *et al.* 2015), and has caused local extinctions of northern quolls *Dasyurus hallucatus* (Oakwood & Foster 2008; Woinarski *et al.* 2010, 2011). Prior to the arrival of toads, quoll populations had suffered gradual declines across northern Australia due to habitat clearing, changes in fire regimes and invasive predators (Braithwaite & Griffiths 1994). The arrival of toads has, however, caused dramatic declines; severe enough to list it as an endangered species under the Australian *Environment Protection and Biodiversity Conservation Act 1999*. Cane toads are abundant and since eradication is unfeasible for the foreseeable future, alternative means of reducing their impact should be explored.

In 2003, insurance populations of northern quolls were established on two toad-free islands in Arnhem Land – Astell and Pobassoo Islands, Northern Territory – with the hope that 1 day they could help repopulate the mainland (Rankmore *et al.* 2008). Since effective landscape-level control of toads is currently impossible, an alternative approach is to make quolls 'toad-smart' and reintroduce these behaviourally modified individuals (O'Donnell *et al.* 2010). O'Donnell *et al.* (2010) showed that toad-aversion training improved the quolls' short-term (10 days) survival. A subsequent reintroduction of 22 'toad-smart' female quolls to East Alligator region of Kakadu National Park in 2010 showed that not only did some 'toad-smart' females survive long-term and reproduce, but their offspring also did not consume cane toads (Webb *et al.* 2015; Cremona *et al.* 2017b). However, the presence of wild male quolls at the reintroduction site made it unclear whether young quolls learnt to avoid toads via cultural learning (by mimicking their mothers), via genetic inheritance of toad avoidance traits from their fathers, or from the ingestion of small non-lethal toads that induce aversion to live toads (Webb *et al.* 2008).

To determine whether toad aversion training alone can provide long-term benefits by facilitating population recovery, we reintroduced wild quolls to Mary River district of Kakadu National Park, a site where quolls were locally extinct. We collected 68 quolls from Astell Island in 2015. Most of these animals were set aside for breeding, but 29 were reintroduced into Kakadu in a wild-to-wild reintroduction. Prior to reintroduction, we rendered most of these quolls toad averse by modifying their feeding behaviour using conditioned taste aversion training. We gave each 'toad-smart' quoll a small, sub-lethal metamorph toad laced with the nausea-inducing chemical thiabendazole (O'Donnell *et al.* 2010) and then tested whether this resulted in aversion to subsequent exposures to toads. A small ($n = 7$) control group received no taste aversion training. Since Astell Island is also mammalian predator-free, all quolls received predator scent aversion training. By monitoring the survival of our reintroduced quolls and their offspring, at a site where quolls were believed locally extinct, we hoped to determine whether conditioned taste aversion training can be passed on to future generations and facilitate population re-establishment in an area where the species had previously gone extinct. The experiment failed to achieve this aim, not because toad aversion training was ineffective, but because dingo predation caused a substantial decline in our release population. To test whether dingoes were depredating live quolls and not scavenging toad-killed quolls, we compared the time taken for us to locate toad-killed quolls to that of dingoes to discover rat carcasses.

METHODS

Study species

Northern quolls are a cat-sized, native marsupial predator. They are generalist carnivores that consume insects, amphibians, reptiles and mammals (Oakwood 1997; Pollock 1999). Northern quolls are the largest semelparous marsupials (Oakwood *et al.* 2001), with both sexes maturing at 11 months and males typically dying soon after reproduction (Dickman & Braithwaite 1992; Oakwood 2000; Oakwood *et al.* 2001). Male quolls seldom live longer than 14 months in the wild and nearly three quarters of females do not survive to produce a second litter (Oakwood 2000). Quolls are nocturnal, and in savanna woodlands they shelter during the day in hollow logs, dense grass, termite mounds, burrows and rock crevices (Oakwood 1997).

Study site

We introduced northern quolls to Ferny Gully (13°33′54″ S, 132°17′30″E), in the Mary River region at the southern border of Kakadu National Park, where quolls were common prior to the arrival of toads (Woinarski *et al.* 2010). After the invasion of toads in 2004, northern quoll populations across Kakadu National Park suffered massive declines (Woinarski *et al.* 2010). Monitoring of the Mary River region's quoll population before and during the arrival of toads (Oakwood & Foster 2008; Woinarski *et al.* 2010) and recent exhaustive camera trap surveys (Department of Environment and Natural Resources, unpubl. data, 2016), confirmed this once healthy population of quolls to be locally extinct. The study area is on the south-western edge of the Arnhem escarpment, a sandstone massif that extends from the eastern side of the Kakadu National Park into Arnhem

Land to the west. The area consists of continuous and isolated sandstone outcrops bisected by Ferny Gully Creek and surrounded by dry savanna woodland (Fig. 1).

Toad aversion training

After capture from Astell Island, quolls were housed in individual enclosures at the Territory Wildlife Park, Berry Springs, Northern Territory. We assigned quolls to one of two experimental groups: quolls trained to avoid toads via conditioned taste aversion (CTA), henceforth 'toad-smart' quolls ($n = 22$; 11 males and 11 females) and those that received no such training, henceforth 'toad-naïve' quolls ($n = 7$; all females). Prior to release, we trained the 'toad-smart' group to avoid eating cane toads by feeding each a small (<2 g), dead metamorph toad coated with the nausea-inducing chemical thiabendazole (Sigma Aldridge, Sydney, Australia) at a dose rate of 400 mg kg^{-1} quoll mass (O'Donnell et al. 2010). Thiabendazole-laced toads were presented in the evening with no other food and were checked the following morning. If the CTA toads were uneaten they were presented again the following night until they were consumed (max. three nights). To test the efficacy of the trained CTA, we video recorded (GoPro HERO; Woodman Labs, San Mateo, California, USA) the response of 'toad-smart' quolls to a small

(<2 g), live metamorph toad housed within an open topped glass jar. 'Toad-smart' quolls were given 2 h to investigate the live toad, if the quoll appeared within the frame but did not consume the live toad they were considered to be toad-averse. If the live toad was consumed, quolls were presented with another thiabendazole-laced toad (max. three times) until the training was successful. As a procedural control, toad-naïve quolls were presented with a live metamorph inside a jar covered with mesh (so they could see and smell the toad but not attack it). From previous work on CTA of toads in northern quolls we anticipated that males would be more difficult to train, and that trained males were more likely than trained females to consume a live toad (O'Donnell et al. 2010). In addition, males move greater distances, have larger home ranges (Oakwood 2000) and were much more likely to leave the reintroduction area (Cremona 2015). Since most, if not all, wild male northern quolls die prior to young leaving their mother's pouch, males are also unlikely to play any part in cultural transmission in this species. It was obvious early in the study that quolls that were untrained rapidly died from poisoning due to cane toad ingestion (see Results). For ethical reasons, we assigned males only to the 'toad-smart' group. This had the added benefit of potentially increasing the likelihood that some males would survive within the study area until the breeding season.
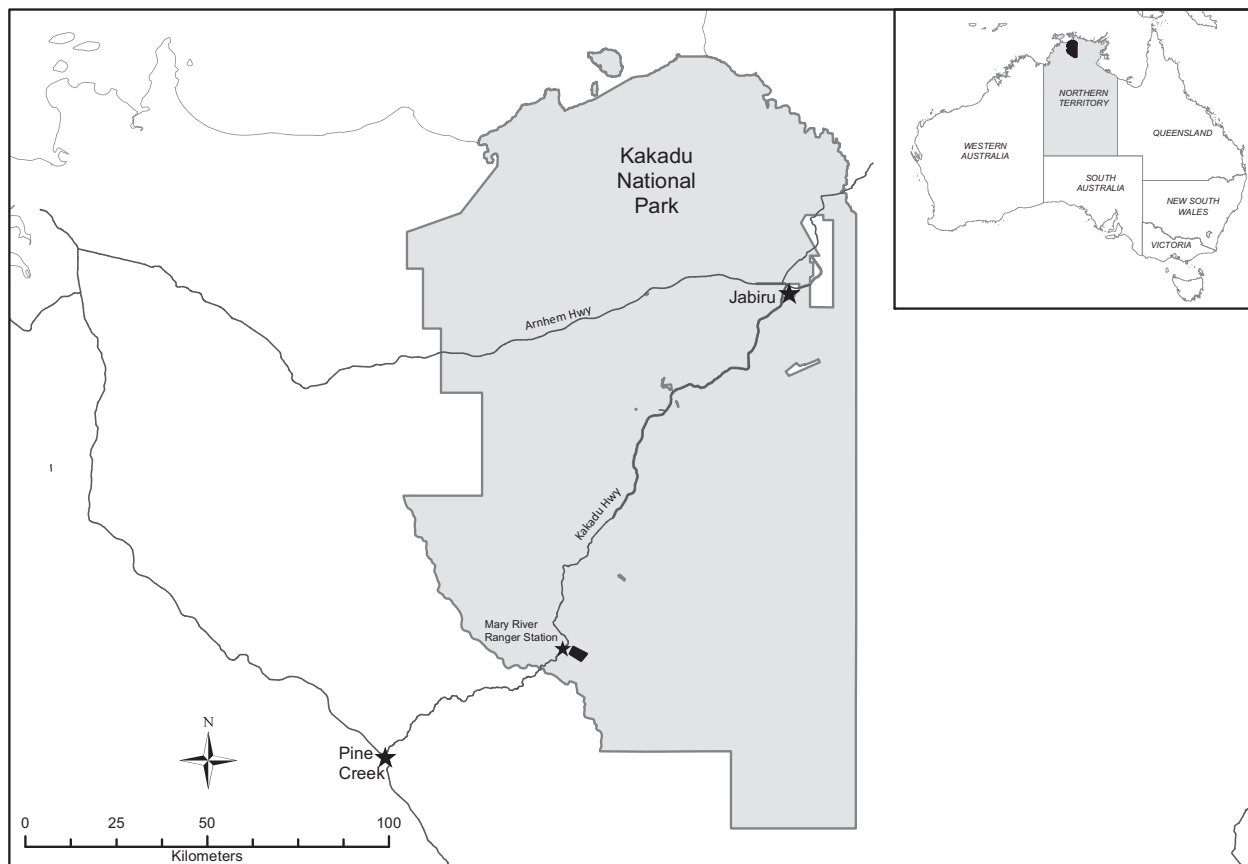

Fig. 1. Ferny Gully (■) reintroduction site (13°33′54″S, 132°17′30″E) located near Mary River Ranger Station within Kakadu National Park, Northern Territory, Australia.

Predator aversion training

Since this project was primarily invested in determining whether toad-aversion training improved the long-term survival of northern quolls after reintroduction, predator aversion training was added as a procedure that was not controlled for (i.e. all reintroduced quolls received predator training). Astell Island is mammalian predator-free island; which northern quolls were introduced to in 2003 (Rankmore *et al.* 2008). Camera trapping at and around our study site at Ferny Gully in Kakadu National Park revealed that both dingoes and feral cats were present (DENR-Parks Australia, unpubl. data, 2016). Since the Astell Island quoll population had no prior exposure to these predators for up to 12 generations, we made the *a priori* assumption that they were predator-naïve and would require some form of aversion-training against both dingoes and cats. Prior to release, a commercially available rat-trap (housed within a plastic box) was baited and set within each quoll's enclosure. When quolls triggered the trap, it shut creating a loud snap while elevating a picture of the face of either a dingo or a cat. Simultaneously, the catapult mechanism flung either dingo or cat fur into the quoll's face. Each quoll received both the dingo and cat aversion trap on different nights in the week prior to their release.

Release and radio monitoring of reintroduced quolls

Prior to release, all animals were weighed, treated for internal and external parasites and PIT tagged (under their skin, anterior to shoulder blade; Mini Microchip, Troven Ltd, UK). All quolls were fitted with a radio-collar containing a mortality sensor (Holohil Systems Ltd, RI-2DM, 8.0 g, Ontario, Canada). We released 22 'toad-smart' (treatment) and seven 'toad-naïve' (control) quolls in three groups, balanced as far as possible by training treatment, over a 4-week period between April and May 2016 (Table 1). We transported quolls from the Territory Wildlife Park (TWP) to the study site in the hide boxes they had been using in captivity in the weeks prior to their release. At dusk (18.00–19.00 hours) on release night, we placed quolls contained within their hide boxes in suitable rock crevices at 100 m intervals on either side of Ferny Gully Creek. The entry hole of each box was covered by paper towel to allow the quolls to leave their boxes of their own volition.

To monitor quoll survival, we tracked each individual to its diurnal shelter. This was achieved within the first hour or two of light each morning. We re-located quolls daily until they were either found deceased, went missing or until the conclusion of the study (21 weeks post-release (*n* = 2)). Because the collars were fitted with a mortality sensor (activated with the cessation of movement), we could prioritize locating deceased quolls and assess each quoll's fate with minimal disturbance to carcasses. Since symptoms of toad toxicity are overt (no sign of physical injury, but with bleeding gums, mouth and/or ears; Roberts *et al.* 2000; Sakate & Oliveira 2000; Reeves 2004; O'Donnell *et al.* 2010) toad-induced mortality was readily identifiable. Another major cause of mortality was quolls being consumed by dingoes (see Results). All quolls that were recorded to have been predated by a dingo were tracked to a dingo or to convincing signs of a dingo. In all cases, the dingo consumed the

Table 1. Release dates of Astell Island northern quolls to Ferny Gully, Kakadu National Park to assess their survival after reintroduction to a toad-infested landscape

Release date	N	Toad-smart		Toad-naïve	
		Males	Females	Males	Females
21/04/2016	9	0	5	0	4
28/04/2016	10	2	5	0	3
12/05/2016	10	9	1	0	0

quoll and the collar, and the dingo was then tracked for up to 3 days. On two occasions a dingo was sighted, but most of the time a dingo was suspected before it could be confirmed because of the atypical movements of the radio-collar (i.e. moving long distances and staying >50 m out of reach of the observer throughout the entire day; moving through savanna grassland rather than outcrops). On three occasions, collars were retrieved after being regurgitated by a dingo, along with quoll hair and bone. To reduce the number of 'uncertain fate' quolls, we searched for radio signals of unaccounted animals by helicopter every week.

Carcass removal experiment

Although dingoes are a known predator of quolls (Oakwood 2000; Cremona 2015; Cremona *et al.* 2017a) and we suspected they were hunting our reintroduced quolls, there was no way for us to confirm with absolute certainty that dingoes were hunting live quolls and not scavenging the carcasses of toad-killed quolls. We could, however, design an experiment to test whether dingoes were more efficient at scavenging carcasses than we were at finding dead quolls. Cane toads are nocturnal amphibians that emerge from their diurnal refuges at dusk (Zug & Zug 1979). Since we located each quoll as soon as possible each morning, we assumed the longest they could have been dead was from sunset the previous day until the time when we recovered their body the next morning. For comparison, we placed 10 large (>500 g) lab rat carcasses around the study site at dusk on two separate nights (*n* = 20) with a week between trials. Each rat was placed within 20 m of where a quoll had previously been found fatally poisoned and was recorded by camera trap (Bushnell Trophy Cam HD 119466, Kansas City, MO, USA) to determine how long it took for scavengers to remove these carcasses.

Data analysis

Our data were interval censored for various reasons: at best, we could identify mortality time to a 24-hour window, but often animals were lost for periods of time, before being found dead (a larger window), or were lost altogether (right censored). Thus, we treated all our data as interval censored with right-censored data being treated as an infinite bound on the right-hand side. We used Cox proportional hazards models with a constant hazards baseline to test for the effect of toad-aversion training on survival time (Cox

1972). We used likelihood ratio tests to identify the best model and to evaluate factor significance (Smith & Blumstein 2010). All statistical analyses were conducted in R (R Core Team 2016). We conducted our analysis on various subsets of the data and under varying treatments of the mortality data, detailed below.

Comparing survival of 'toad-smart' and 'toad-naïve' female quolls

Initially, males and females were ear-marked for assignment to both 'toad-smart' and 'toad-naïve' treatments, however, after substantial losses of 'toad-naïve' females it was decided, for a few reasons (see Methods), that no more quolls would be released without being trained to avoid toads. Therefore, only female quolls were assigned to both 'toad-smart' (treatment; $n = 11$) and 'toad-naïve' (control; $n = 7$) groups. Analyses were conducted after all males were removed to ensure differences in survival probability between the training treatments were not driven by sex differences (see O'Donnell et al. 2010).

Comparing survival of 'toad-smart' and 'toad-naïve' female quolls with dingo predation removed

Since dingo predation was the major cause of mortality in 'toad-smart' quolls, and what we were most interested in determining was whether toad-training improved long-term survival, we examined only our female quolls and removed dingo predation as a cause of death. We re-ran the analysis assuming that female quolls lost to dingo predation were instead part of the cohort of quolls that were simply lost from the study (right censored). This approach gives us a better estimate of the magnitude of the toad training effect on survival, in the absence of other sources of mortality (i.e. dingoes). In addition, this gives us an estimate of the survival predictions if we can resolve dingo predation. We interpreted this difference by looking at the risk of toad induced mortality associated with each treatment in a risk ratio analysis.

Survival of probability of quolls regardless of sex, treatment or cause of death

Since overall survival of reintroduced quolls was extremely poor, we used Kaplan–Meier survival analysis (Kaplan & Meier 1958) to model the survival of all quolls regardless of sex, treatment or cause of death to examine the overall pattern of survival in our quolls.

Carcass removal experiment

For our experimental observations of scavenging rate, we compared the mean time taken by us to retrieve toad-killed quolls (assuming, conservatively, they were killed at dusk the previous night; $n = 10$) with the time taken by any scavenger ($n = 9$) to encounter a rat (two-tailed t-test)

and time taken by dingoes only ($n = 2$) to encounter a rat (two-tailed t-test).

RESULTS

Fate of quolls after release

Quolls were tracked daily until they either died ($n = 19$), disappeared from the study area ($n = 7$) or were caught and had their collars removed at the conclusion of the study ($n = 2$). Six of the seven 'toad-naïve' quolls (control) were killed by toads; whereas only 4 of the 22 toad averse quolls were confirmed to have been killed by toads. All known toad mortality happened within 4 days of release. One 'toad-naïve' quoll survived for 12 days before being consumed by a dingo. Six 'toad-smart' quolls were confirmed to have been killed and were consumed by dingoes. The fate of seven 'toad-smart' quolls, six males and one female, is uncertain and was recorded as 'fate unknown', though it is possible that some of these were also killed by dingoes and their transmitter destroyed as a consequence. A helicopter was employed on four separate occasions to locate these quolls, but was unsuccessful. Three of the 'toad-smart' males whose fate we are uncertain of dropped their collars from 0 to 21 days after release (Table 2). At the conclusion of the study, there were two 'toad-smart' female quolls surviving at the study site (21-weeks post-release). Furthermore, camera-trapping at the study site identified an additional male quoll at Ferny Gully. This male was identified as a 'toad-smart' male that removed its collar (chew marks on retrieved collar). He was also seen on camera mating with a 'toad-smart' female (Fig. 2).

Effect of toad-aversion training on survival of reintroduced quolls

*Females only compared between treatment*

Whether a female quoll was trained to avoid toads or not significantly predicted individual survival with trained quolls far less at risk than 'toad-naïve' quolls ($\chi^2 = 7.81$, d.f. $= 1$, $P = 0.005$; Fig. 3). 'Toad-smart' quolls not only had longer median survival (16.48 days, $CI_{95\%}$ 0–745 days) than that of untrained females (0.36 days $CI_{95\%}$ 0–16.34 days), but also were observed mating (camera trap footage; Fig. 2).

Females only compared between treatment with dingo predation removed

To more accurately estimate the effect of toad training on mortality from toads, we assumed that we

Table 2.   Fate of northern quolls reintroduced to Ferny Gully, Kakadu National Park

Treatment	Sex	Number	Killed by toads	Killed by dingoes	Cause of death unknown	Fate uncertain	Known to be alive
Toad-smart	Male	11	2	2	0	6	1
Toad-smart	Female	11	2	4	2	1	2
Toad-naïve	Female	7	6	1	0	0	0
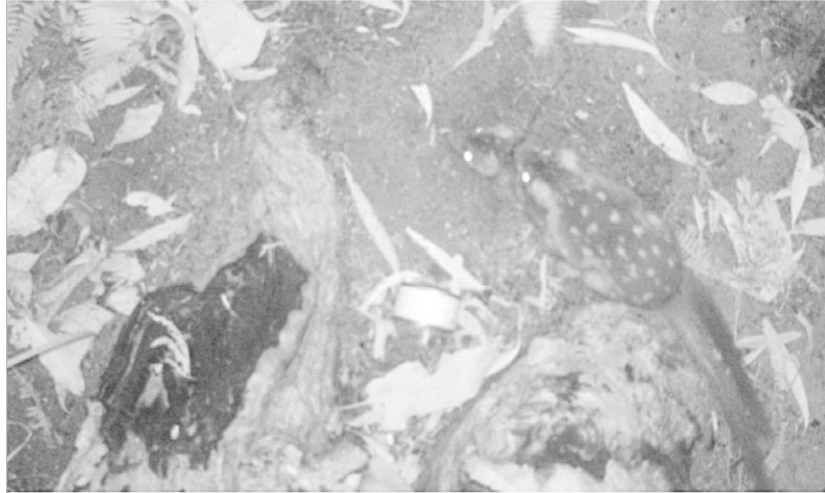
Fig. 2.   Uncollared male northern quoll mating with radio-collared 'toad-smart' female northern quoll on baited camera trap at Ferny Gully, Kakadu National Park (Parks Australia/KNP).

simply lost track of female quolls that were killed by other sources of mortality. Under this assumption, the survival probability of 'toad-smart' quolls remained constant at around 0.75 and from <15 days post-release 'toad-smart' quolls were 4.71 times more likely to survive than 'toad-naïve' quolls (CI$_{95\%}$ 1.25–9.04; Fig. 4).

Survival of probability of quolls across all categories

Although 'removing' dingo predation as a source of mortality substantially increased the survival probabilities of 'toad-smart' female quolls (Fig. 4), the overall survival probability of quolls reintroduced to southern Kakadu Nation Park was extremely low (median: 8.14 days CI$_{95\%}$ 0.0003–1512.61 days; Fig. 5).

Comparing scavenger and human carrion removal

On average (±SE), we were able to locate quolls that were fatally poisoned by toads far more rapidly (14.39 ± 0.33 h) than scavengers were able to find similar-sized deceased lab rats (49.0 ± 9.22 h; two-tailed *t*-test; $t_{18} = 2.608$, $P = 0.02$; Fig. 6). Four species of scavenger (Torresian crows, dingoes, feral cats and northern quolls) were captured on camera after being lured to dead rats. Of these, only Torresian
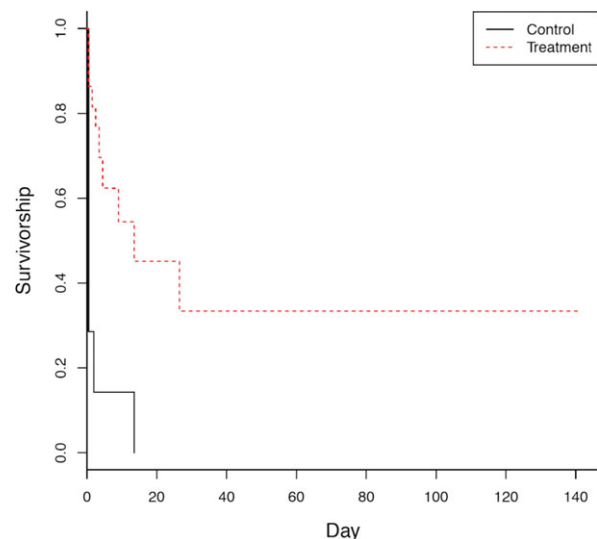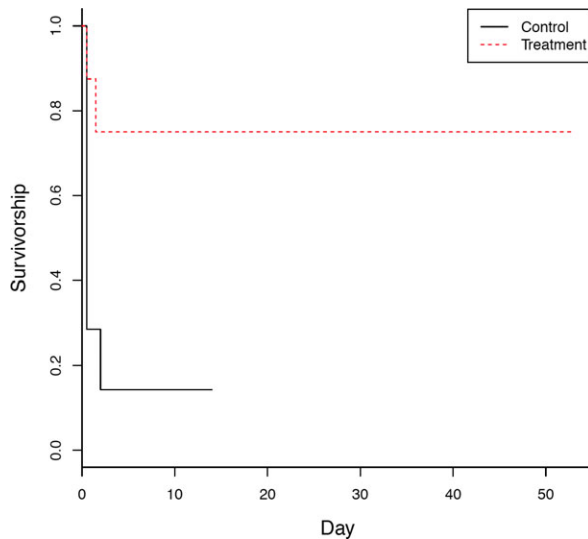

Fig. 3.   Kaplan–Meier survival curves comparing survival of female 'toad-smart' (treatment) *versus* 'toad-naïve' (control) quolls following reintroduction to a toad infested landscape. Deaths from both toads and dingoes are included here.

crows actually removed rat carcasses. When mean time taken for us to locate toad-killed quolls was compared only to the mean (±SE) time taken by dingoes to find rat carcasses (53.47 ± 22.28 h), we

located quolls far more rapidly (two-tailed *t*-test; $t_9 = 4.73$, $P = 0.001$; Fig. 7). In addition, only a single scavenger located a rat within the conservatively estimated time it took for us to locate a deceased quoll (min. time to locate rat: 16.12 h *vs.* max. time for us to locate a quoll: 16.75 h). The only rat to be discovered in under 24 h was found by a Torresian crow that was physically incapable of moving the rat from where it was placed. The fastest a dingo located a rat was in 31.18 h.

Fig. 4. Kaplan–Meier survival curves comparing survival of female 'toad-smart' (control) *versus* 'toad-naïve' (treatment) quolls following reintroduction to a toad infested landscape assuming that quolls killed by dingoes would have otherwise survived.
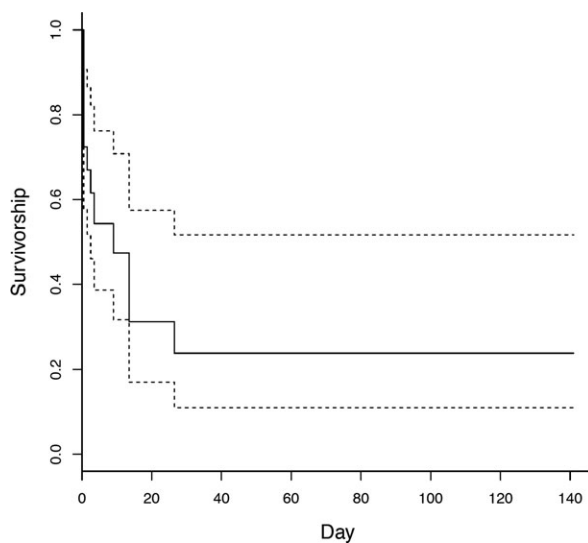

Fig. 5. Kaplan–Meier curve of survival probability ($CI_{95\%}$) of all quoll regardless of sex, treatment and cause of death following reintroduction to Kakadu National Park.

DISCUSSION

Our work supports previous studies showing cane toads pose a severe threat to 'toad-naïve' northern quolls, and that eliciting conditioned taste aversion to toads can vastly reduce toad-induced mortality in the wild (O'Donnell *et al.* 2010; Fig. 3). Our survival analysis showed trained individuals survived significantly longer than untrained individuals (Fig. 3). Overall, however, our analysis showed survival to be very low (Fig. 5). In fact, the proportion of females that survived (2 of 11) was similar to the previous reintroduction at East Alligator, where only 7 of 22 females survived ($\chi^2 = 0.69$, $P = 0.41$; Cremona *et al.* 2017a). Since we located individuals daily, we were often able to determine causes of mortality (Table 2). Dingoes were likely predating live quolls (Figs 6,7) and this predation pressure is suspected to have driven our population to extinction within weeks (Fig. 4). In the absence of dingo predation, survival of trained quolls would have been ≈0.8 over the course of the study (Fig. 4), instead survival was closer to 0.2 (Fig. 3). For 'toad-smart' quolls the main source of mortality was dingo predation (Table 2), and although dingoes are known predators of northern quolls in Kakadu (Oakwood 2000) and predator-caused mortality is the leading cause of reintroduction failure (Moseby *et al.* 2015), we had not anticipated the speed with which dingoes would imperil our reintroduced population.

Due to generally high rates of mortality (Fig. 5) we were unable to achieve our *a priori* aim of determining whether the offspring of 'toad-smart' quolls also avoid toads. There is still a possibility that the females surviving may produce young (Fig. 2). However, in the absence of a control (the survival of a control group makes them by definition 'toad-smart' and no longer a control group) it will be impossible to know whether the offspring's survival in a toad
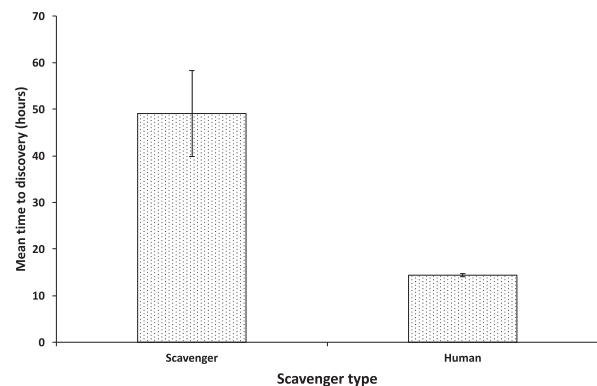

Fig. 6. Mean time (hours ± SE) taken to discover quolls killed by toads (by humans; *n* = 10) *versus* time for scavengers to find similar-sized dead rats (all scavenger species, *n* = 9).
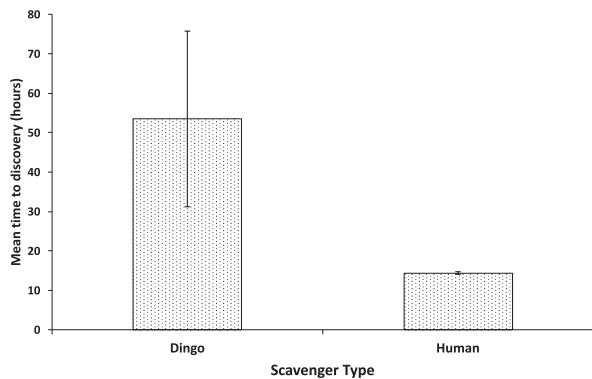
Fig. 7. Mean time (hours ± SE) taken to discover quolls killed by toads (by humans; *n* = 10) *versus* time for dingoes to find similar-sized dead rats (*n* = 2).

infested landscape is a result of: (i) cultural transmission of toad-aversion from their mothers; (ii) juveniles consuming a small toad and teaching themselves toad-aversion; (iii) an innate aversion which is inherited or (iv) some combination of these factors. Since there is no control group to compare trained quolls against, this question of cultural transmission may only be assessed in captivity. Nonetheless, there remains an important question: can 'toad-training' of a single generation facilitate population recovery and have long-term conservation benefits, or does training need to be effected every generation?

For now, however, we can be confident that conditioned taste aversion is a powerful tool for mitigating toad mortality in quolls. There can, of course, be no long-term benefits of this strategy if dingoes exterminate reintroduced populations (Cremona *et al.* 2017a). This leads us to question why dingo predation so rapidly caused our reintroduced quoll population to decline. Northern quolls have existed in sympatry with dingoes for at least 3500 years (Letnic *et al.* 2014), and they are still sympatric in areas where quolls naturally persist (Schmitt *et al.* 1989; Woinarski *et al.* 2008; Hernandez-Santin *et al.* 2016). Concomitant with their role as top predators, dingoes likely control populations of mesopredators such as quolls (Glen *et al.* 2007; Letnic & Dworjanyn 2011). Certainly, prior to the arrival of cane toads in Kakadu, dingoes were a significant predator of northern quolls, and accounted for 36% of adult mortality per year (Oakwood 2000). It is, therefore, no surprise that dingoes accounted for some mortality. Nonetheless, the rate of predation we observed here was more rapid than we had anticipated.

Recent studies have identified that the wet-dry tropics of northern Australia has experienced a rapid and severe decline in native mammal species, with catastrophic declines of natives attributed to too frequent burning and cat predation in Kakadu National Park (Woinarski *et al.* 2011; Ziembicki *et al.* 2015).

Improper fire regimes have resulted in a reduction in the availability and variety of food plants for native fauna, as well as a reduction in shelter such as ground cover vegetation, fallen logs, and hollow bearing trees (Ziembicki *et al.* 2015). Carnivores, such as raptors, feral cats and dingoes, are more successful in habitats that have been opened up by fire (McGregor *et al.* 2015). Therefore, the general state of the landscape we introduced our animals into may have favoured high canid predation.

In addition to quolls being vulnerable to dingo predation under normal circumstances, quolls isolated on predator-free Astell Island may have become predator-naïve in the absence of dingoes (Blumstein 2002; Carthey & Banks 2014). In 2003, 45 quolls were released on Astell Island with the hope that 1 day they would be used to repopulate the mainland (Rankmore *et al.* 2008). Islands by definition are isolated, and animals confined to islands are likely to only encounter a subset of the selective pressures they evolved with. On the mainland, quolls would have evolved with predation pressure from various mammalian, reptilian and avian predators (Oakwood 2000), which would have selected for vigilant, predator-averse animals. Quolls on remote, predator-free islands are instead limited by competition for finite food resources. Although twelve generations may seem brief, a shift in the selection regime faced by quolls between the mainland and Astell island may have resulted in island quolls being unsuitable for reintroduction to a burnt landscape where predators are abundant. Inevitably, antipredator behaviours of island quolls would be predicted to follow that of other species that find themselves isolated from the predators with which they evolved on islands (Blumstein 2002; Blumstein & Daniel 2005); anti-predator behaviours progressively being lost over time. Anti-predator behaviours are often costly in terms resource acquisition if expressed unnecessarily (Hunter & Skinner 1998), and behaviours that are maintained via experience, or by watching conspecifics, can be lost from predator-free populations very rapidly (Griffin *et al.* 2000; Blumstein 2002). If predator-aversion and other adaptive traits are rapidly lost from populations maintained in isolation, future studies must address whether such sanctuaries are an effective long-term conservation measure. Further study is required to determine whether the quolls of Astell Island have become naïve to the threat of dingo predation to determine whether they are suitable for reintroduction to the mainland. Seemingly, our attempt to train predator aversion using visual and olfactory cues to elicit anti-predator behaviour was unsuccessful. Future attempts to train predator aversion may be improved by replacing cue-based training protocols that have a poor record of success (Moseby *et al.* 2015) with aversion learning via actual

or simulated predation with live predators (West *et al.* 2017).

The success of our taste-aversion training may actually inform how we minimize the impact of dingo predation on quolls, as well as having broader implications on how to reduce/ameliorate the impact that invasive predators have on native prey. Since naivety in vulnerable natives is fundamental to the devastating impacts of evolutionary novel predators (Cox & Lima 2006; Carthey & Banks 2014), taste-aversion training could be used to alter the feeding behaviour of invasive predators (Nicolaus *et al.* 1983; Nicolaus & Nellis 1987). For example by introducing 'Trojan' quolls carrying taste-aversion chemicals in collars (Read *et al.* 2015), we may be able to harness this technique to alter the feeding behaviour of dingoes so that they become averse to preying on reintroduced quolls. In addition, if quolls are typically vulnerable to dingo predation, their recovery may be markedly improved via simply increasing the propagule pressure by releasing more 'toad-smart' quolls (Cremona 2015). In conclusion, taste aversion is a powerful, but currently under-exploited tool for conservation (Greggor *et al.* 2014), which may improve our ability to ameliorate the impacts of invasive species.

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