ORIGINAL ARTICLE

Eliciting conditioned taste aversion in lizards:

Live toxic prey are more effective than scent and taste cues alone

Georgia WARD-FEAR[1,*], Jai THOMAS[2], Jonathan K. WEBB[3], David J. PEARSON[4], Richard SHINE[1]

[1]School of Life and Environmental Sciences, University of Sydney, NSW 2006, Australia, [2]School of Veterinary and Life Sciences, Murdoch University, Western Australia, [3]School of Life Sciences, University of Technology Sydney, Broadway, NSW 2007, Australia, [4]Western Australian Department of Parks and Wildlife, Waneroo, WA 6946, Australia

*_Correspondence_. Georgia Ward-Fear, School of Life and Environmental Sciences, University of Sydney, NSW 2006, Australia.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1749-4877.12226.

Email: georgia.ward-fear@sydney.edu.au

Tel: +61-408885720

Short running title: Cues for Conditioned Taste Aversion in Varanus

Abstract

Conditioned Taste Aversion (CTA) is an adaptive learning mechanism whereby a consumer associates the taste of a certain food with symptoms caused by a toxic substance, and thereafter avoids eating that type of food. Recently, wildlife researchers have employed CTA to discourage native fauna from ingesting toxic cane toads (*Rhinella marina* Linnaeus, 1758), a species that is invading tropical Australia. In this paper, we compare the results of two sets of CTA trials on large varanid lizards (‘goannas’, *Varanus panoptes* Storr, 1980). One set of trials (described in this paper) exposed recently-captured lizards to sausages made from cane toad flesh, laced with a nausea-inducing chemical (lithium chloride) to reinforce the aversion response. The other trials (in a recently-published paper, reviewed herein) exposed free-ranging lizards to live juvenile cane toads. The effectiveness of the training was judged by how long a lizard survived in the wild before it was killed (fatally poisoned) by a cane toad. Both stimuli elicited rapid aversion to live toads, but the CTA response did not enhance survival rates of the sausage-trained goannas after they were released into the wild. In contrast, the goannas exposed to live juvenile toads exhibited higher long-term survival rates than did untrained conspecifics. Our results suggest that although it is relatively easy to elicit short-term aversion to toad cues in goannas, a biologically realistic stimulus (live toads, encountered by free-ranging predators) is most effective at buffering these reptiles from the impact of invasive toxic prey.

Key words: Conditioned Taste Aversion, invasive species, *Rhinella marina*, *Varanus*, conservation, tropical Australia

INTRODUCTION

Conditioned Taste Aversion (CTA) is an adaptive learning mechanism whereby a consumer associates the taste of a certain food with symptoms caused by a toxic substance (Garcia *et al*. 1955; Yamamoto & Fujimoto 1991; Bures *et al*. 1998). Akin to a 'food poisoning' response, CTA has been used to mitigate human-wildlife conflicts by, for example, training predators not to attack domestic livestock (Gustavson *et al*. 1974). More recently, researchers using CTA have taught Australian native animals to avoid eating a highly toxic invasive species (see below). The continuing invasion of cane toads (*Rhinella marina* Linnaeus, 1758) across Australia has caused precipitous population declines in several wildlife taxa, because large predators such as lizards, snakes, crocodiles and quolls (carnivorous marsupials) are rapidly killed by the toad's powerful chemical defenses (Price-Rees *et al*. 2010; Shine 2010). Smaller predators are far less affected by toad invasion, because the small toads they attack do not contain enough toxin to kill them. However, the invasion front is dominated by very large (and thus very toxic) toads; hence, large predators are at great risk when the first toads arrive in an area (Shine 2010).

Given that we cannot prevent the toad invasion from spreading, can we use CTA to buffer the invader's impact on vulnerable native species? Studies to date are encouraging; survival rates in the wild were enhanced by CTA training in two endangered predator species – northern quolls (*Dasyurus hallucatus*: O'Donnell *et al*. 2010) and bluetongue lizards (*Tiliqua scincoides intermedia*: Price-Rees *et al*. 2011, 2013). Also, laboratory studies have shown that planigales (*Planigale maculata*: Webb *et al*. 2008), antechinus (*Antechinus flavipes*: Kaemper *et al*. 2013) and freshwater crocodiles (*Crocodylus johnstoni*: Somaweera *et al*. 2011) can rapidly learn toad-aversion. Thus, aversion learning successfully induces many types of native predators not to eat cane toads; and that shift in feeding responses may buffer imperiled taxa from the impact of the invasive anuran.

How can we best implement this approach? One fundamental question is the protocol used to induce CTA. Previous studies on the cane toad system largely have relied on captive-raised predators that are trained prior to release into the wild (O'Donnell *et al*. 2010), or have captured predators and brought them into captivity for training prior to re-release (Price-Rees *et al*. 2013). In both of those studies, predators were presented with lifeless or synthetic stimuli (toad flesh, or sausages made of toad flesh) and the effectiveness of CTA was enhanced by adding a nausea-inducing chemical to the stimulus. Alternatively, aversion can be induced simply by exposing the predator to a live toad too small to cause death but large enough to induce illness of the predator. Thus, for example, encounters with live toads have been reported to induce subsequent aversion in a wide range of native predators that usually attack and consume small anurans (including fishes, frogs, and reptiles: Shine *et al*. 2009; Greenlees *et al*. 2010; Nelson *et al*. 2010; Shine 2010). Which approach – toad sausage or live toad – is most likely to create an effective CTA response that is retained for long enough to affect the predator's responses during subsequent encounters with toads in the wild? We speculated that the more natural stimulus – a live toad – would be most effective in this respect. Live prey may not only appeal to a wider range of predators, but it could also strengthen the aversions required to prolong a predator's life. Exploring the options for CTA cues is particularly important when designing conservation strategies, aimed at mitigating the devastating impacts of cane toads on a suite of predators with varying hunting styles and physiological characteristics. The current paper compares the outcomes of two CTA experiments on the same predator species, to evaluate the prediction that CTA is more effectively induced by live toads than by artificial baits, in this instance.

Specifically, we trained varanid lizards ('goannas') either (1) using synthetic stimuli (toad-flesh sausages, plus a chemical additive to induce nausea) in captivity; or (2) using live small toads in the field. We then assessed whether or not the trials induced aversion to live adult toads; and subsequently radio-tracked goannas in the field to investigate the potential impact of CTA training on rates of survival. The results from the second of these trials (with live toads) have already been published (Ward-Fear *et al*. 2016), but the results of the former work (although conducted earlier) have not been described. In the current paper, we asked the following questions:

(1) Will goannas develop an aversion to cane toad baits (sausages)
(2) Will this aversion translate to an aversion to live cane toads (when tested in captivity)?
(3) Will toad-aversion induced by baits increase the survival of goannas in the wild?
(4) In light of these two studies, what method should managers use to buffer goanna populations from the impact of invasive cane toads?

MATERIALS AND METHODS

Study species and field site

The cane toad (*Rhinella marina*) is a large American anuran (exceptionally, to >1 kg) introduced into Australia in 1935 to control coleopteran pests of commercial sugarcane (Lever 2001). Since its introduction, the cane toad has rapidly dispersed across Australia, and has decimated populations of large native anurophagous predators (Shine 2010; Jolly *et al*. 2015). Lethal toxic ingestion is the primary mechanism of impact; but after decades of exposure, initially-vulnerable predator taxa can coexist with cane toads simply by deleting the toxic newcomer from their diets (Llewelyn *et al*. 2014).

The floodplain monitor (*Varanus panoptes*) is a very large (up to 7 kg) tropical lizard (‘goanna’) species. Prior to the cane toad invasion, the floodplain monitor was abundant and an important generalist predator in tropical ecosystems (Shine 1986). Populations of this apex predator have experienced 90% declines in many areas, leading to trophic cascades and imbalances across the fragile systems of northern Australia (Doody *et al*. 2009, 2013; Brown *et al*. 2011, 2013).

Our study using toad-based sausages to induce aversion was conducted in Kununurra (15°46’24”S, 128°44’21”E), a town in the east Kimberley region of Western Australia. At the time of the study (early 2011), the cane toad invasion was just arriving in the area. A few toads had been seen, male cane toads had been recorded calling for the first time, albeit in low numbers and large anurophagous predators (elapid snakes, crocodiles and goannas) were being found dead in places such as irrigation channels and around dams (J. Thomas, pers. obs.). Cane toads were still so scarce in the landscape that most predators had not encountered them, but it was clear that toad abundances were rapidly increasing (and so, animals such as goannas were under imminent threat).

We captured sexually mature (adult) goannas along ‘Packsaddle Road’ on the periphery of Kununurra using a long pole with a noose, and transported the lizards back to a secure holding facility nearby where trials were conducted. We recorded the mass, snout-to-vent length (SVL) and sex of all captured animals. They were held in captivity for at least two weeks prior to trials, and we fed them kangaroo mince three times a week, to allow them to acclimate. The lizards were housed in ambient conditions outside, in large plastic enclosures (110 x 110 x 60 cm). Larger animals were housed individually and small animals were paired, but with their enclosure separated by an opaque divider. A refuge shelter and bowl of water was provided for each goanna, and enclosures were cleaned weekly. The entire study was conducted between January and April of 2011, a time of year when goannas were most active.

Conditioned Taste Aversion experiment

We randomly allocated goannas to control and ‘trained’ groups (each consisting of eight individuals: six males, two females), and ensured there were no significant differences in body size or sex composition between the two groups. Each feeding trial began at 1100 h and any uneaten baits were removed after 1 h. On the first day of the trials all goannas were given a chicken neck. Two days later they were presented with a live cane toad (approx. 30 g, 55 mm snout-to-urostyle length) inside a mesh container (15 x 10 x 10 cm) for 1 h (the first CTA trial), such that they could see, smell and investigate the toad but could not eat it. Their response was filmed and later scored for behavioral variables (see below). Two day later, the control group of goannas were fed a chicken neck and a treatment group (hereafter 'trained') were fed a 25-g sausage made from minced toad flesh (minced legs stuffed into a toad stomach, to ensure that toad scent permeated the sausage casing as well as the contents). These sausages were dosed with the nausea-inducing compound lithium chloride at the rate of 8M 0.7 mL per kilogram mass of each lizard (based on Paradis & Cabanac [2004]). Adjusting the dose of lithium chloride for individual lizards standardized the aversion experience across the sample, and ensured that animals were treated humanely. Two days post-treatment, all goannas were again presented with a live toad in a mesh container and their responses filmed (the second CTA trial). At the end of 1 h, all goannas were fed a chicken neck to ensure that any toad-aversion was a specific response to the toad stimulus, rather than reflecting satiation or illness. The entire trial period ran for six days.

Goannas are predatory animals that possess a vomeronasal organ for the detection of olfactory cues (Cooper 1994; Pianka & King 2004). Nonetheless, detection of prey and subsequent attacks also can be triggered by movement (Thomson 1995; Losos & Greene 1988). Goannas actively hunt and are persistent in their physical attempts to access prey inside burrows (Pianka & King 2004). A goanna's level of interest in a prey item is readily identified by a set of actions that occur in quick succession (initial prey detection, then attempts to consume; see below). We filmed the feeding responses of goannas using a digital video camera (JVC Everio GZ-MG). Filming ran for 1 h with the first 4 min of footage being discarded to account for initial disturbance. We then scored the following behaviors:

(1) Latency: time taken for the goanna to investigate the toad in the container;

(2) Tongue-flicking: number of tongue-flicks directed towards the toad in the container;

(3) Nosing: number of times the goanna pushed its nose against the container in the direction of the toad; and

(4) Scratching: number of times the goanna scratched at the toad in the container.

We log-transformed all of these counts to achieve variance normality (confirmed with Shapiro-Wilk Goodness of Fit tests pre and post transformation), and subsequent homoscedasticity (verified with Bartlett's test for homogeneity). To test for differences in the biological significance of individual behaviors within the feeding response, we ran full factorial ANCOVAs on each trait. The independent variable was treatment and the covariate was an individual's behavioral score in Trial One; the dependent variable was the behavioral score in Trial Two. However, as these traits are not independent (a lizard with high scores on one trait also has high scores on another), we also combined these four variables in a Principal Components Analysis to produce a single integrated measure of the goanna's level of interest during its interactions with a toad. We then compared the scores on Principal Component Axis 1 (PC1) between trained versus control lizards, before versus after their treatment (exposure to cane toad sausage or chicken neck). For that comparison we used ANOVAs, with individual lizard ID included as a random factor to account for repeated measures on the same animal (Underwood, 1996).

Rates of survival in the field post-training

Following the CTA feeding trials, goannas were fitted with VHF radio transmitters (Holohil SI2, 40 x 11 mm, 11g) that were attached to the dorsal keel of the tail (see

Ward-Fear *et al*. 2016 for methodology) and the lizards were then released at their point of initial capture. Individuals were located at least once every two days thereafter, and the cause of any deaths ascertained. Predators are often killed before they fully ingest a toad; and if they do ingest it, often then regurgitate. Thus, toad-killed predators often do not contain toads in their alimentary tracts when examined post-mortem (Letnic et al. 2008). However, toad-induced mortalities are readily identified by the circumstances of the death. In our study, all goannas that died were found close to riparian zones (where toads are most likely to be encountered), exhibited contorted body postures with vomit present (indicative of poisoning) and had been seemingly healthy in previous observations right up until the time they were found dead. Thus, we attribute all of these mortalities to fatal toxic ingestion of cane toads (Fig. 1). Transmitters were removed from the remaining lizards at the end of the study (60 days), and the animals returned to the wild.

We compared rates of survival of the trained versus control groups using Kaplin-Meier survival analysis. The dependent variable was the number of days an animal was tracked alive, post-release. Following Kaplin-Meier methodology, we differentiated goannas that were killed by toads from goannas that left the longitudinal study for other reasons (e.g., transmitter loss) or that were still alive at the end of the study. This latter group of goannas is termed as being ‘censored’ (Kaplin & Meier 1958).

Using ANOVA, we also tested whether or not an individual’s PC1 score in its second CTA trial (irrespective of treatment group) influenced the length of time it was tracked in the wild, or its ultimate fate. All analyses for this study were conducted in JMP 11.1 (SAS Institute, Cary, North Carolina).

Ethics statement

This study abided by strict ethical protocols approved by the University of Sydney Animal Ethics Committee (Protocol number: L04/7-2009/3/5004, Scientific permit number: SF007673). These guidelines are in accordance with the international ‘Principles of Laboratory Animal Care’ as well as the ‘Australian Code for the Care and Use of Animals for Scientific Purposes’. Additionally, all care was taken to minimize any stress or suffering experienced by animals during this study.

RESULTS

Conditioned Taste Aversion experiments

All goannas acclimated to captivity, eating regularly prior to trials. All individuals consumed the non-laced baits (chicken necks) pre- and post-trials, confirming that rejection of prey (cane toads) during the second trial was a consequence of aversion rather than of illness or satiation. The ANCOVAs showed that training affected all behavioral traits (Latency: $F_{1,13} = 8$, $P = 0.014$; Tongue flicking: $F_{1,13} = 13.88$, $P = 0.003$; Nosing container: $F_{1,13} = 5.59$, $P = 0.034$; Scratching at container: $F_{1,13} = 5.43$, $P = 0.037$).

In the Principal Components Analysis, the first principal component (PC1) explained 72.5% of variation in the behavioral data recorded during trials with toads. The four variables were fairly equally represented in PC1, as seen by their loadings (Latency: -0.88, Tongue flicks: 0.92, Nosing: 0.94, Scratching: 0.63). An individual lizard's scores on the axis of Principal Component One provide a measure of the level of interest it exhibited when interacting with the cane toad in the container. Higher values of PC1 correlated with lower latency (lizards that were quicker to investigate the toad), and increased rates of tongue-flicking, nosing and more vigorous scratching of the container (Fig. 2). We interpret a high PC1 score as indicating a vigorous attempt to consume the toad. Lower values of PC1 correlated with higher latency (slower to investigate), fewer tongue-flicks, and less nosing and scratching of the container (i.e., lack of interest in the toad).

Our statistical analysis of these data revealed a significant interaction between treatment and toad exposure ($F_{1,14} = 13.63$, $P = 0.002$). During the first trial with the toads, when all goannas were naïve, interest was high and equal between the two groups. In the second trial with the toad (post-treatment), control goannas showed levels of interest only slightly lower than those manifested in pre-treatment trials. Conversely, the CTA-trained goannas showed far less interest in the toads during the second trial (Fig. 3). We interpreted this significant interaction term as evidence for the development of a behavioral aversion in the trained goannas.

Rates of survival in the field post-training

The longest duration of tracking was 60 days post-release; goannas known to be alive at this time were captured (at which time we removed the VHF tracker) and released. Over this 60-day period, seven goannas died as a result of ingesting cane toads, seven remained alive, and two could not be relocated (presumably due to emigration or equipment failure). Kaplin-Meier survival analysis revealed no significant difference in rates of survival between control versus CTA-trained goannas (*P* > 0.80; Table 1, Fig. 4). Furthermore, a goanna's PC1 score in its second trial with a toad (post-treatment) did not influence the length of time it was tracked post-release, or its ultimate fate (*P* > 0.90).

Trials using live toads as the CTA-inducing stimulus

In a follow-up study, we CTA-trained free-ranging *V. panoptes* (at a site 120 km from Kununurra) by offering them small (non-lethal) cane toads whilst the lizards foraged in the wild, then monitored their subsequent survival using radio telemetry (Ward-Fear *et al*. 2016). In striking contrast to the results described above, goannas not only rapidly learned toad-aversion, but retained that aversion for many months. By the end of the 18-month study, only one of 31 untrained lizards had survived longer than 110 days, compared to more than half (nine of 16) of trained lizards; the maximum known survival of a trained lizard in the presence of toads was 482 days (Ward-Fear *et al*. 2016).

DISCUSSION

In the laboratory, goannas developed an aversion to live cane toads after eating sausages made from cane toad flesh, and laced with the nausea-inducing compound lithium chloride. During the first trial with the toad, all lizards exhibited interest in the toad, demonstrating species-typical feeding behaviors and attempting to obtain access to the toad. During the second set of trials, after groups had either been fed the toad sausage or the control chicken neck, the behavioral responses of the two sets of lizards diverged significantly. The control group registered slightly less interest in the cane toad than they had in the initial trials, perhaps reflecting habituation to the test situation and their lack of success in accessing the toad in the previous trial. In contrast, the CTA-trained group registered almost no interest in the cane toad. All goannas consumed the chicken necks that were given subsequently, confirming that the lack of interest manifested by the CTA-trained individuals reflected an aversion to the toad rather than a disinclination to feed. The difference in these responses pre- and post-training was stark. For example, one goanna that was highly active in its pre-training trial (tongue-flicked 102 times, nosed the cage 235 times, and clawed at the cage 419 times within one hour) exhibited none of these activities in its second (post-training) trial.

Nonetheless, this strong aversion to a live cane toad in the laboratory apparently failed to translate to the lizards' responses to wild toads in the field. At least three of eight CTA-trained goannas consumed toads and died, within 60 days of release. Furthermore, there was no correlation between the PC1 scores of individuals in their second trial with the live cane toad (post-treatment) and their ultimate fate or length of survival post-release. Thus, CTA-training did not increase lizard survival (even in the short-term) after animals were released into the wild and they encountered adult toads. Why was this so? Why did goannas associate the sausage-induced illness with the live toad in laboratory trials, but fail to avoid live toads in the field?

The success of our field-based trials, where we trained goannas using small live toads rather than chemical-laced sausage baits (Ward-Fear *et al*. 2016) hints that the answer lies in the lower effectiveness of training that (a) occurs in the laboratory not the field, and/or (b) relies upon the lizard's response to a live toad being formed by an aversion to toad-based sausages rather than to live toads. Clearly, a live toad offers a wide range of cues unavailable from a sausage. Most obviously, a live toad moves around in ways that may trigger a predator's attack; and the scent cues emanating from a sausage or dead toad may differ in many ways from those that are emitted by an intact live toad. The success of toad-flesh and sausage-based CTA-training in quolls and bluetongue lizards (O'Donnell *et al*. 2010; Price-Rees *et al*. 2013) shows that some types of predators can indeed forge a strong aversion to live toads based upon encounters with a lifeless or synthetic bait. However, the contrasting result of our two CTA-training studies with floodplain monitors suggest that for these animals, training based on a live toad provides a more long-lasting deterrent to feeding on toads.

Our study highlights the need to develop conservation strategies based on a detailed understanding of the biology of the species involved. A method that works for one taxon may be ineffective with another. Although goannas developed aversions to the cues used in both of our CTA experiments (small live toads and the toad-sausages), one of those training regimes translated to the field whereas the other did not. We do not know why laboratory-based training failed to enhance survival rates of goannas in the field. Potentially, captivity may have confounded the results. However, the conditions were relatively natural (in outside enclosures), lizards were acclimated and feeding for two weeks prior to trials, and individuals developed short-term aversion to live toads post-trial. Rather, we speculate that the differences in cue association between the two studies relates to the foraging and feeding ecology of these large lizards.

The foraging behavior of varanids is linked to tongue morphology (Cooper 1994). The varanid tongue, having lost its prey-handling function, is a purely sensory apparatus that works in conjunction with the vomeronasal organ to detect olfactory signals important in food acquisition and social contexts (Cooper 1994). However, cue association based on scent alone may be over-ridden by other (visual) stimuli (Pianka & King 2004). Varanid attacks are often triggered by prey movement (Thompson 1995; Losos & Greene 1988; G. Ward-Fear Pers. Obs.), potentially explaining why naïve goannas consume non-native cane toads (because toads resemble frogs visually but perhaps not in the scent cues they provide). Although prey-location is mostly carried out vomeronasally, subsequent feeding behaviors are driven by visual as well as olfactory cues. Thus it is not surprising that goannas need visual reinforcement to ensure cue association between a nausea-inducing 'bait' and a live toad (as experienced when the baits were small live toads). Furthermore, the speed at which associations are recalled is important in this context; the formidable chemical defenses of cane toads mean that even seizing a toad briefly might be enough to cause death of the predator (Shine 2010). For CTA to protect a goanna in the wild, then, the lizard must immediately recognize the potential meal as a toad. A sausage may simply not be toad-like enough to serve that role.

One of our motivations for trialing the use of baits to induce toad-aversion was the ease with which such baits potentially can be deployed on a landscape scale ahead of the toad invasion. Wildlife authorities already spread baits to control feral cats and dingos using this method (Thomson 1986; Short *et al.* 1997), further facilitating uptake of the technology if it was effective. However, our results suggest that baits may not be a useful way to buffer populations of this large predatory varanid lizard from the impact of cane toad invasion. Fortunately, the other method we trialed – with far more success – is also readily deployed on a broad spatial scale. Releasing small live toads in advance of the main invasion front, or just as the main invasion front arrives, should provide opportunities for predators to learn to avoid the toxic newcomers (Ward-Fear *et al*. 2016).

Overall, our results confirm the potential value of CTA training as a management tool to mitigate the impact of cane toads on native predators (O'Donnell *et al*. 2010). A wide range of potentially vulnerable anurophagous predators clearly are capable of learning to avoid cane toads as long as their first experience is with a small (non-lethal) toad. Because we currently lack any methods capable of controlling or eradicating the cane toad, we need to develop innovative strategies to minimize invader impact. Managers need to field-trial a diversity of methods in order to identify the techniques most effective with specific vulnerable taxa, or applicable to specific habitats.

ACKNOWLEDGEMENTS

Our study was funded by the Australian Research Council. Logistical assistance was provided by the Western Australian Department of Parks and Wildlife (especially Corrin Everett), Bill Stewart and Craig Mills. Permits to collect animals from the field and conduct research in Western Australia were obtained from the University of Sydney and the Western Australian Department of Parks and Wildlife.

REFERENCES

Brown GP, Phillips BL, Shine R (2011). The ecological impact of invasive cane toads on tropical snakes: field data do not support predictions from laboratory studies. *Ecology* **92**, 422–431.

Brown GP, Ujvari B, Madsen T, Shine R (2013). Invader impact clarifies the roles of top-down and bottom-up effects on tropical snake populations. *Functional Ecology* **27**, 351–361.

Bures J, Bermúdez-Rattoni F, Yamamoto T (1998). *Conditioned Taste Aversion: Memory of a Special Kind*. Oxford University Press, Oxford, UK.

Cooper WE (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.

Doody JS, Green B, Rhind D, Castellano CM, Sims R, Robinson T (2009). Population-level declines in Australian predators caused by an invasive species. *Animal Conservation* **12**, 46–53.

Doody JS, Castellano CM, Rhind D, Green B (2013). Indirect facilitation of a native mesopredator by an invasive species: are cane toads re-shaping tropical riparian communities? *Biological Invasions* **15**, 559–568.

Garcia J, Kimeldorf DJ, Koelling RA (1955) Conditioned aversion to saccharin resulting from exposure to gamma radiation *Science* **122**,157–158

Greenlees M, Phillips BL, Shine R (2010). Adjusting to a toxic invader: native Australian frog learns not to prey on cane toads. *Behavioral Ecology* **21**, 966–971.

Gustavson CR, Garcia J, Hankins WG, Rusiniak KW (1974). Coyote predation control by aversive conditioning. *Science* **184**, 581–583.

Jolly CJ, Shine R, Greenlees MJ (2015). The impact of invasive cane toads on native wildlife in southern Australia. *Ecology and Evolution* **5**, 3879–3894.

Kaemper W, Webb JK, Crowther MS, Greenlees MJ, Shine R (2013). Behaviour and survivorship of a dasyurid predator (*Antechinus flavipes*) in response to encounters with the toxic and invasive cane toad (*Rhinella marina*). *Australian Mammalogy* **35**, 136–143.

Kaplin EL, Meier P (1958). Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association* **53**, 457–481.

Letnic M, Webb JK, Shine R (2008). Invasive cane toads (*Bufo marinus*) cause mass mortality of freshwater crocodiles (*Crocodylus johnstoni*) in tropical Australia. *Biological Conservation* **141,** 1773-1782.

Lever C (2001). *The Cane Toad. The History and Ecology of a Successful Colonist.* Westbury Academic Publishing, Otley, West Yorkshire, UK.

Llewelyn J, Schwarzkopf L, Phillips BL, Shine R (2014). After the crash: how do predators adjust following the invasion of a novel toxic prey type? *Austral Ecology* **39**, 190–197.

Losos JB, Greene HW (1988). Ecological and evolutionary implications of diet in monitor lizards. Biological Journal of the Linnaean Society **35**, 379-407.

Nelson D, Crossland MR, Shine R (2010). Indirect ecological impacts of an invasive toad on predator-prey interactions among native species. *Biological Invasions* **12**, 3363–3369.

O'Donnell S, Webb JK, Shine R (2010). Conditioned taste aversion enhances the survival of an endangered predator imperilled by a toxic invader. *Journal of Applied Ecology* **47**, 558–565.

Paradis S, Cabanac M (2004). Flavor aversion learning induced by lithium chloride in reptiles but not in amphibians. *Behavioural Processes* **67**, 11–18.

Pianka E, King D (2004). *Varanoid Lizards of the World.* Indiana University Press, Bloomington, USA.

Price-Rees SJ, Brown GP, Shine R (2010). Predation on toxic cane toads (*Bufo marinus*) may imperil bluetongue lizards (*Tiliqua scincoides intermedia,* Scincidae) in tropical Australia. *Wildlife Research* **37**, 166–173.

Price-Rees S, Webb JK, Shine R (2011). School for skinks: Can conditioned taste aversion enable blue-tongue lizards (*Tiliqua scincoides*) to avoid toxic cane toads (*Rhinella marina*) as prey? *Ethology* **117**, 749–757.

Price-Rees SJ, Webb JK, Shine R (2013). Reducing the impact of a toxic invader by inducing taste-aversion in an imperilled native reptile predator. *Animal Conservation* **16**, 386–394.

Shine R (1986). Food habits, habitats and reproductive biology of four sympatric species of varanid lizards in tropical Australia. *Herpetologica* **42**, 346–360.

Shine R (2010). The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. *Quarterly Review of Biology* **85**, 253–291.

Shine R, Greenlees MJ, Crossland MR, Nelson D (2009). The myth of the toad-eating frog. *Frontiers in Ecology and the Environment* **7**, 359–361.

Short J, Turner B, Risbey D, Carnamah R (1997). Control of feral cats for nature conservation. II. Population reduction by poisoning. *Wildlife Research* **24**, 703–714.

Somaweera R, Webb JK, Brown GP, Shine R (2011). Hatchling Australian freshwater crocodiles rapidly learn to avoid toxic invasive cane toads. *Behaviour* **148**, 501–517.

Thompson GG (1995). Foraging patterns and behaviours, body postures and movement speed for goannas, Varanus gouldii (Reptilia: Varanidae), in a semi-urban environment. *Journal of the Royal Society of Western Australia* **78**, 107–114.

Thomson PC (1986). The effectiveness of aerial baiting for the control of dingoes in north-western Australia. *Australian Wildlife Research* **13**, 165–176.

Ward-Fear G, Pearson DJ, Brown GP, Balanggarra Rangers, Shine R (2016). Ecological immunization: *In situ* training of free-ranging predatory lizards reduces their vulnerability to invasive toxic prey. *Biology Letters* **12**, 20150863.

Webb JK, Brown GP, Child T, Greenlees MJ, Phillips BL, Shine R (2008). A native dasyurid predator (common planigale, *Planigale maculata*) rapidly learns to avoid toxic cane toads. *Austral Ecology* **33**, 821–829.

Yamamoto T, Fujimoto Y (1991). Brain mechanisms of taste aversion learning in the rat. *Brain Research Bulletin* **27**, 403–406.

Underwood AJ (1996) *Experiments in ecology: Logical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge.

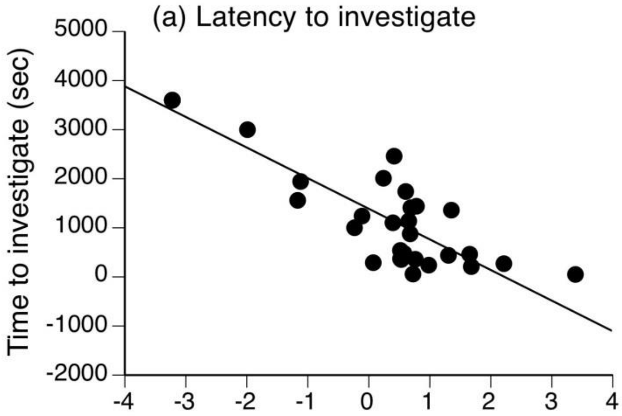
Table 1 Survival rates of radio-tracked control and CTA-trained lizards by the end of study (60 days post-release)

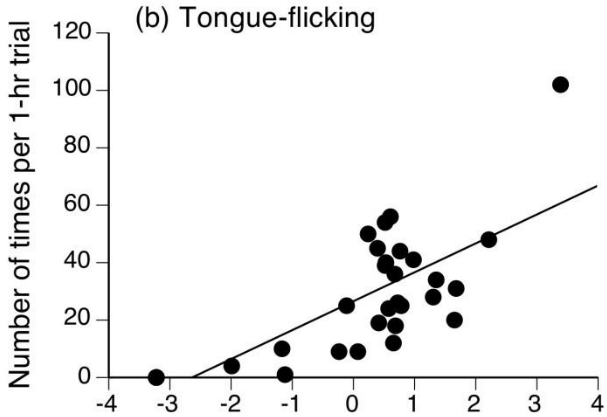
Lizards	Dead	Alive	Unknown fate
Control	4	3	1
CTA-trained	3	4	1

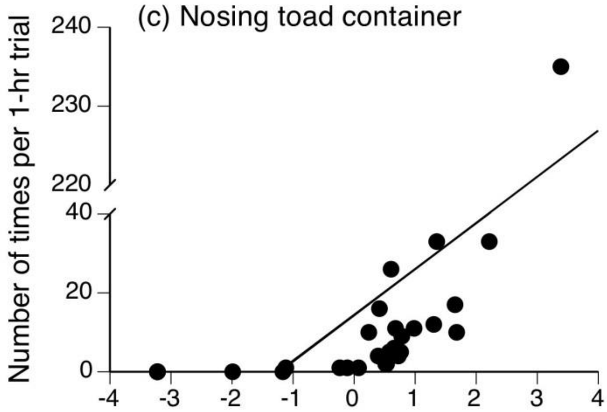
FIGURE LEGENDS

Figure 1 (a) Floodplain monitor (*Varanus panoptes*) equipped with radio-transmitter for field tracking study. (b) Floodplain monitor found dead in the field after being fatally poisoned by ingesting a cane toad. Photographs by J. Thomas (a) and G. Clarke (b).

Figure 2 Relationship between the first principal component (PC1) and the four behavioral variables scored in trials with live cane toads. Behavioral variables were scored as (a) the time taken in seconds for an individual to investigate the container (latency), and the number of times an individual (b) tongue-flicked towards the toad in the container, (c) nosed the container, and (d) scratched the container.


(a) Latency to investigate
Time to investigate (sec)
5000
4000
3000
2000
1000
0
-1000
-2000
-4
-3
-2
-1
0
1
2
3
4


(b) Tongue-flicking
Number of times per 1-hr trial
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
3
4


(c) Nosing toad container
Number of times per 1-hr trial
240
230
220
40
20
0
-4
-3
-2
-1
0
1
2
3
4

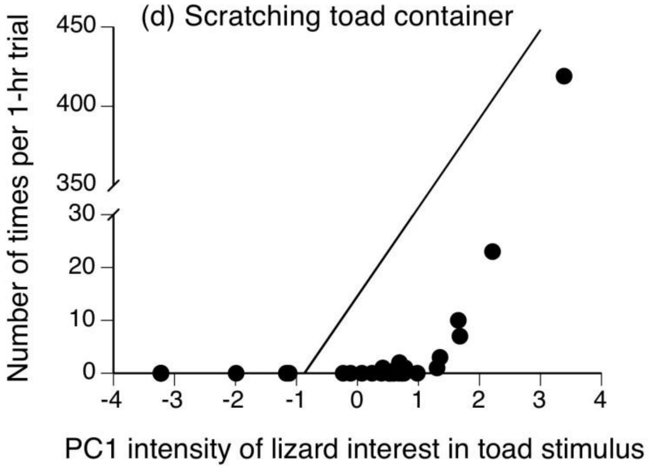

(d) Scratching toad container
Number of times per 1-hr trial
450
400
350
30
20
10
0
-4
-3
-2
-1
0
1
2
3
4
PC1 intensity of lizard interest in toad stimulus

Figure 3 Level of interest exhibited by captive floodplain monitors (*Varanus panoptes*) in response to exposure to a live cane toad (*Rhinella marina*), as a function of whether or not the lizards were given Conditioned Taste Aversion (CTA) training between the two exposure episodes. Untrained control lizards showed a modest decline in interest level, whereas CTA-trained goannas exhibited an abrupt decline in interest. The lizard's response to the stimulus was quantified by calculating the individual's score on the first axis of a Principal Components Analysis that incorporated multiple measures of interaction intensity.

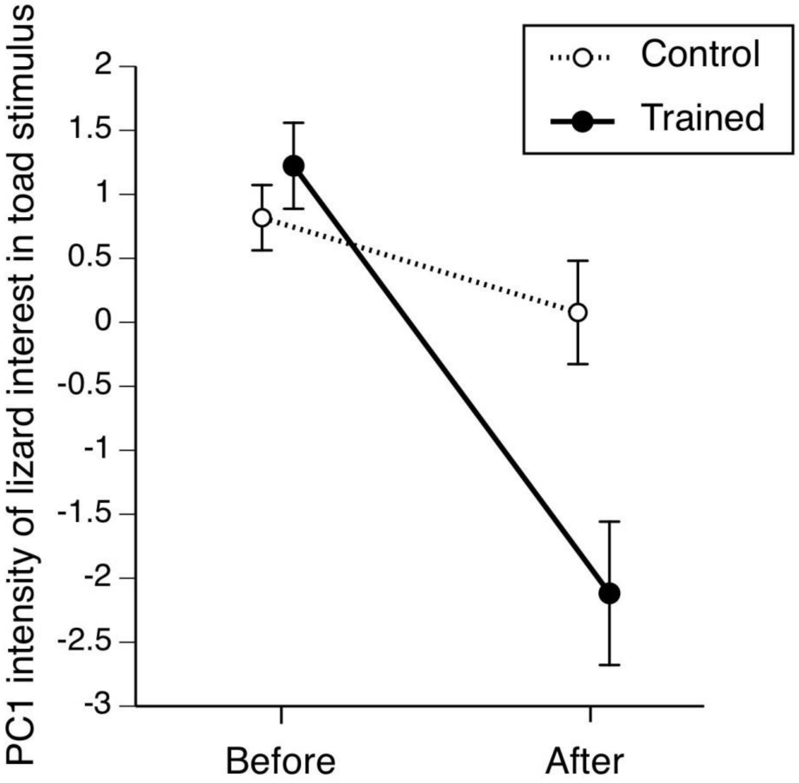
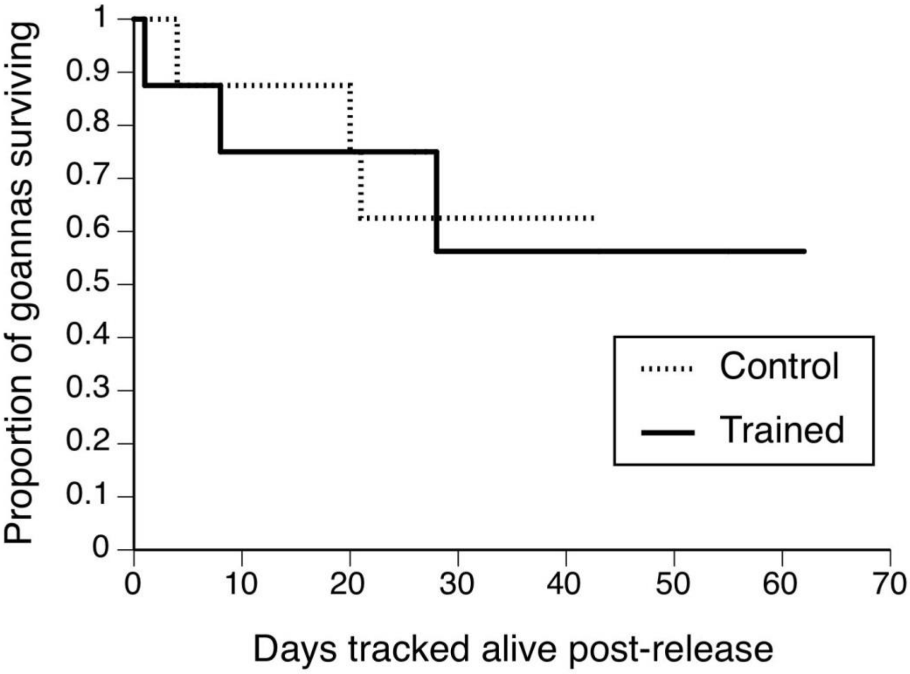


Figure 4 Kaplin-Meier survival curves showing rates of survival in the wild of control versus Conditioned Taste Aversion-trained ‘at risk’ floodplain monitors (*Varanus panoptes*). There was no significant difference in rates or length of survival between the two groups.


Proportion of goannas surviving
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
Days tracked alive post-release
Control
Trained