# Safeguarding the northern quoll.
# Can we mitigate cane toad impacts through conditioned taste aversion?



## Naomi Liza Indigo

https://orcid.org/0000-0002-0465-7849

# Abstract

Australia has the world's worst record of mammal extinctions. Changes to fire regimes, habitat loss and the impacts of invasive species are causal agents; but controlling invaders across landscapes is difficult. In northern Australia, the spread of toxic cane toads (*Rhinella marina*) has caused population declines of the northern quoll *(Dasyurus hallucatus*), a marsupial predator that dies from toad poisoning. Traditional methods have failed to halt the toad invasion, so we need new methods to reduce their impact.

In this thesis, I critically examined whether conditioned taste aversion (CTA) could reduce the impacts of cane toads on northern quolls. Trials on captive quolls confirmed that quolls fed toad meat infused with an emetic (of a substance causing vomiting) subsequently refused to attack live toads. This raised the question of whether we could use CTA *in situ* to train wild quolls to avoid cane toads. This thesis addresses that gap. Firstly, I analysed mark-recapture data to estimate quoll demographic parameters and used a population viability model to determine whether CTA could prevent quoll population extinction. The success of CTA in reducing the probability of extinction was strongly influenced by the baiting design and requires that a high proportion of the population can be trained prior to toad invasion. In addition, cultural transmission of food preferences from mothers to offspring is necessary for CTA to be a successful long-term mitigation strategy.

I developed a bait suitable for field deployment and demonstrated that captive quolls that ingested toad baits generalised their aversion to toads, and that wild quolls readily consumed baits. Importantly, bait uptake by non-target species was negligible. Next, using a BACI design, I carried out a replicated field experiment to determine whether baiting could mitigate the impact of toads. Surprisingly, at control and experimental sites, quoll populations went extinct following toad invasion. To understand this failure, I then determined the optimum level of bait deployment needed to reach my target species. I aimed to improve baiting design outcomes by employing a model that optimised the delivery of CTA, while accounting for the fact that some animals in the population could not be CTA trained.

Overall, my thesis highlights the challenges of implementing a novel technology in complex ecological systems and demonstrates the need for evidence-based data to guide decision makers. In a world experiencing rapid rates of mammalian extinction, novel conservation strategies such as CTA may help us mitigate threatened populations against the serious impacts of invasive species.

# Certificate of Original Authorship

I, Naomi Indigo declare that this thesis is submitted in fulfilment of the requirements for the award of the degree of Doctor of Philosophy in the School of Life Sciences, Faculty of Science, at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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# Preface

The main body of this thesis consists of 5 chapters, including four data chapters that I have, or intend to, submit to journals. I have therefore formatted each chapter according to the guidelines of the individual journals. This has caused some minor formatting differences between chapters and some unavoidable repetition in the species description, experimental methods and background information in some of the chapters.

At the beginning of each chapter, I have acknowledged all authors involved, in the same order as they appear in the manuscripts. In all the chapters, I am listed as the first author and was primarily responsible for conceiving, designing and implementing the research and writing the manuscripts.

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# Chapter contributions

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*Indigo N* Conceived and developed ideas, conducted data analysis and wrote manuscript.

*Smith J* Developed ideas, coordinated field-experiments, and assisted with manuscript preparation.

*Webb JK* Assisted in development of ideas, analysis and manuscript preparation.

*Phillips BL* developed ideas and contributed to experimental design, contributed to analysis, writing of the model and manuscript preparation.

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*Indigo N* Conceived and developed ideas, executed field experiments, conducted data analysis and wrote manuscript.

*Smith J* Developed ideas, assisted with coordination of data collection and assisted with manuscript preparation.

*Webb JK* Developed ideas and assisted with manuscript preparation.

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*Indigo N* Conceived and developed ideas, executed data analysis, writing of model, experiments and wrote manuscript.

*Kelly, E* Conceived and developed ideas, executed data analysis, experiments and wrote manuscript presented in the appendix.

*Smith J* Developed ideas, assisted with coordination and collection of data in the field, and assisted with manuscript preparation.

*Webb JK* Developed ideas and assisted with manuscript preparation.

*Phillips BL* developed ideas, contributed to experimental design, conceived and wrote data analysis model and assisted with manuscript preparation.

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*Smith J* Developed ideas, assisted with coordination and collection of data in the field, and assisted with manuscript preparation.

*Webb JK* Developed ideas and assisted with manuscript preparation.

*Phillips BL* developed ideas, contributed to experimental design, conceived and wrote data analysis model and assisted with manuscript preparation.

# Explanation of Terms

**Conditioned taste aversion** is the process whereby an animal associates the taste or smell of food with adverse gastrointestinal effects (such as malaise or emesis) post-ingestion, and subsequently avoids consuming that food (Garcia, Kimeldorf et al. 1955, Baker, Ellwood et al. 2005).

**Referent food** is the food item (prey) which an animal becomes averse to.

**Bait** is a mimic of the referent food paired with a noxious compound or CTA agent (Cohn and MacPhail 1996).

**CTA agent** is a toxic compound that evokes an adverse post-ingestion effect such as malaise or emesis. CTA agents are required to create a conditioned aversion to an odour or taste of a referent food (Baker et al. 2005, Cagnacci, Massei et al. 2005).

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# Chapter 1
# Introduction

## Conservation needs novel tools

**For** the past 200 years, Australia has had the world's worst record of mammal extinctions—with 34 mammals now officially recognised as extinct (Johnson 2006, IUCN 2019, Woinarski, Braby et al. 2019). Most recently, the Australian Bramble Cay melomys (*Melomys rubicola*) represents the first documented mammal extinction due to human-induced climate change (Waller, Gynther et al. 2017). Losses and range restrictions of mammals have been greatest in arid regions, and lowest in areas of high rainfall and low environmental change (McKenzie, Burbidge et al. 2007). Until recently, tropical savannahs retained a diverse mammal assemblage, though most populations have collapsed catastrophically. For instance Woinarski, Milne et al. (2001) observed that many small (35gm)- to medium-sized (<~5 kg) mammals had experienced a (1986-99) decline in the tropical savannah of Kakadu National Park, with a further decline occurring over the period 2001 to 2008 (Woinarski, Armstrong et al. 2010).

The precise cause of each wave of mammal declines has been the subject of much interest and discussion (Fisher, Blomberg et al. 2003, Johnson 2006, Woinarski, Legge et al. 2011). Several anthropogenic and ecological mechanisms have been hypothesised as causal agents of the declines (Johnson 1998, McKenzie et al. 2007). One driver of mammal decline in northern Australia that has received much attention is the impact of invasive species coupled with

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changed fire regimes (Woinarski et al. 2011, Woinarski, Burbidge et al. 2014, Legge, Murphy et al. 2017, Legge, Smith et al. 2019). Here, species such as feral cats (*Felis catus*) (Legge et al. 2017), livestock (Ziembicki, Woinarski et al. 2015, Legge et al. 2019), domestic dogs (*Canis familiaris*)(Doherty, Dickman et al. 2017), foxes (*Vulpes vulpes*) (Short and Smith 1994, Risbey, Calver et al. 2000) and cane toads (*Rhinella marina*) (Burnett 1997, Jolly, Shine et al. 2015) are all considered to have had negative impacts. Controlling any of these species at a landscape scale, however, has proved extremely difficult (Ziembicki 2015). Due to this, increasing attention is being paid to mitigating the impact of invasive species through novel strategies and translocation to fenced enclosures or offshore 'havens', rather than supressing their populations  (Myers, Simberloff et al. 2000, Legge, Woinarski et al. 2018).

Scientists expect further demise through habitat loss and serious range restrictions of mammalian biota in the future; particularly for restricted-range endemic species (McKenzie et al. 2007, Camacho, Doremus et al. 2010, Allek, Assis et al. 2018). Preventing or mitigating such declines is a significant and urgent challenge, to the extent that the effectiveness of traditional conservation tools are increasingly questioned (Thomas, Cameron et al. 2004, Camacho et al. 2010). The search for novel conservation strategies using new tools merits close and systematic attention. Indeed, novel conservation strategies may be our only hope for conserving some taxa (Johnson 2006, Camacho et al. 2010, Di Marco, Cardillo et al. 2012).

## Conditioned Taste Aversion- A Novel Strategy

One novel, yet promising management strategy – conditioned taste aversion (CTA) – is yet to receive examination under a conservation setting; although there is currently intense interest around using CTA as a management tool. Conditioned taste aversion (CTA) is a powerful form of one-trial learning that can be used to alter an animals predatory behaviour (Gustavson, Nicolaus et al. 1987). CTA harnesses a powerful innate response exhibited across all vertebrates; an evolved defence mechanism against poisoning (Sinclair and Bird 1984, Conover 1995, Cohn and MacPhail 1996, Mappes, Marples et al. 2005). CTA is the process whereby an individual is conditioned to associate the taste or smell of food with post-ingestion illness.  Consequently, conditioned animals avoid consuming that food (Garcia, Kimeldorf et al. 1955, Baker, Ellwood et al. 2005).  CTA has many practical applications, although prior to discussing these, it is important to appreciate the principles behind the idea.

## Principles Behind the Model of CTA

Predation is a behavioural sequence whereby an animal locates and captures prey to ingest. This may be divided into two components: attack and consumption. The 'attack' component of this sequence is dictated by visual, auditory and olfactory sensory information. The attack may be terminated by aversive stimuli such as bad odours, pain, alarming sights, sounds, and unpalatable flavours. Similarly to Pavlov's classical conditioning theory, these adverse events function as a 'punishment' for attack behaviour (Pavlov 1927, Garcia et al. 1955, Gustavson et al. 1987).

The second component of predatory behaviour is consumption, which is influenced by the flavour of a food. Familiar flavours are consumed readily, whereas novel flavours may be consumed with hesitation. If the flavour is unpalatable the food is likely to be rejected, if the effect of the meal is malaise, the value of the food is decreased, and the flavour of that meal may trigger an aversive response. Once this association has been made, the animal will avoid eating food with that taste again (Gustavson, Garcia et al. 1974, Gustavson, Kelly et al. 1976).

CTA is an innate response which represents a defence mechanism against poisoning (Sinclair et al. 1984, Conover 1995, Bernstein 1999, Mappes et al. 2005, Page and Ryan 2005, Glendinning 2007). This evolved to assess prey quality, allowing animals to avoid consuming noxious foods that are costly or lethal (Sinclair et al. 1984). CTA occurs widely in nature and is often associated with aposematism. Aposematic prey display signals such as bright coloured patterns, loud sounds and novel odours to signal the unprofitable outcome of consumption (Avery 1985, Conover 1995, Bogliani and Fiorella 1998, Mappes et al. 2005, Page et al. 2005, Skelhorn and Rowe 2006, Skelhorn, Griksaitis et al. 2008). Some species may mimic aposematic animals to avoid predation (Skelhorn et al. 2008) (Batesian mimicry (Brower 1969)). Mimics gain an advantage because after predators ingest toxic models, they generalise their aversion to similar-looking prey (Nicolaus, Carlson et al. 1983). Thus, palatable models gain a fitness advantage by looking similar to toxic models.

For CTA to be useful as a tool for wildlife conservation, we need to be able to deploy suitable baits to train predators to avoid certain prey types. For this approach to work, the predator must generalise its aversion from the bait to the

live prey. For example, to train coyotes to avoid attacking lambs, researchers fed coyotes lamb baits laced with lithium chloride that induced post-ingestion vomiting. The coyotes that became violently ill subsequently associated the taste and smell of lamb with illness, and refused to attack live sheep (Gustavson et al. 1974) . However, several factors, including the novelty of the flavour, the strength of the illness, and the time delay between ingestion and illness may affect the strength of CTA. Trials using baits to train predators can fail if the predators develop an aversion to the odour or taste of the emetic chemical rather than the bait. Moreover, if illness is mild, predators may not generalise their aversion of the bait to live prey (Conover and Kessler 1994, Conover 1995).

## CTA has Many Innovative Applications

Traditionally agriculture has benefited greatly from CTA (Gustavson et al. 1974, Avery 1985). Its application has successfully:

- decreased feather-pecking behaviour in domestic chickens (Harlander-Matauschek, Beck et al. 2009);
- reduced predation of seeding crops by birds (Avery 1985);
- livestock prevented from consuming toxic plants (Provenza, Burritt et al. 1990);
- and has offered an ethical, legal and ecological alternative for decreasing losses from stock predation (Ellins and Catalano 1980, Cowan and Tyndale-Biscoe 1997, Smith, Linnell et al. 2000).

In conservation, CTA has found some application in reintroduction programs by deterring introduced predators from preying upon vulnerable

individuals (e.g., Griffin, DT. et al. 2000, Moseby, Carthey et al. 2015, Scheibel, Dembkowski et al. 2016). For example, CTA has been used to reduce egg predation by crows (Bogliani et al. 1998) (Nicolaus, Herrera et al. 1989, Avery, Pavelka et al. 1995, Cox, Baker et al. 2004), mongooses (*Herpestes auropunctatus*) (Nicolaus and Nellis 1987), and racoons (*Procyon lotor*) (Conover 1989, Semel and Nicolaus 1992). Additionally, CTA has been used to reduce bait monopolisation when controlling dingoes (Gustavson, Gustavson et al. 1983) and foxes (Gentle, Massei et al. 2004).

More recently, CTA has been used to effectively train northern quolls (*Dasyurus hallucatus*) not to attack highly-toxic cane toads (O'Donnell, Webb et al. 2010). Cane toads are invading northern Australia, and they are highly toxic novel prey items to many predators in this region. In this regard, CTA has also shown promise to train blue-tongue lizards (*Tiliqua scincoides intermedia*) (Price-Rees, Webb et al. 2013) and the yellow-spotted monitor (*Varanus panoptes*) (Ward-Fear, Pearson et al. 2016) to avoid attacking toads. Given early encouraging results about the efficacy of CTA training to elicit toad aversion, it may be possible to train wild animals not to eat cane toads by deploying toad-aversion baits to wild predators before the toads invade (Webb, Brown et al. 2008). This approach could mitigate the impacts of toads on vulnerable taxa, and might prevent the catastrophic population declines of large predators (quolls, goannas) that occur following toad invasion(Shine 2010).

## The Practical Challenges of Applying CTA

CTA training shows much promise as a management tool, but its application in situations is relatively new, so there are many issues to resolve before this promise

can be met.  As well as the basic challenges of bait design and deployment,

challenges may also be generated by the complexity of an ecological system, or

potentially by a failure to appreciate that training might not last beyond the time

course of most of the initial trials, and logistical and ethical constraints may halt

progress and hinder its success. Surprises are inevitable, particularly in applied

ecology, and good translational research process should elicit them early. In the

following sections I will describe some major challenges for applying CTA as a

management tool.

## Developing a Lasting CTA Association to Referent Food

In a study by Gustavson et al. (1974) CTA was employed to reduce predation of

coyotes on domestic sheep. This was achieved in two ways: by feeding coyotes

mutton laced with an emetic (lithium chloride, LiCl), and by administering lithium

chloride via intraperitoneal injection after captive coyotes consumed mutton.

Coyotes that became violently ill subsequently avoided live lambs or refused to

attack them. Injections of LiCl produced stronger aversions in coyotes than did the

bait containing LiCl, but injections were not logistically feasible for application in

the field (Gustavson et al. 1974). Thus, carcass baits were distributed throughout

the landscape, laced with LiCl, with the expectation that some of the coyotes that

consumed the carcasses would become ill, develop an aversion to the taste of

mutton, and cease killing live sheep. The field tests produced mixed and

controversial results (Gustavson et al. 1976, Sterner and Shumake 1978, Lehner

and Horn 1985). Despite the initial promise of pen trials (above), it was proposed

that CTA towards treated carcass baits did not transfer to the killing of live

animals; because the carcasses were not are similar enough to live sheep, or

because coyotes became averse to LiCl rather than the taste of meat (Conover et al. 1994, Conover 1995).

It has been argued that conditioned taste aversion may be more successful in species that rely primarily on visual information for hunting, such as avian species (Skelhorn et al. 2008). For these animals, the visual properties of their prey is the most salient cue (compared to taste) when paired with illness (Brett, Hankins et al. 1976, Bogliani et al. 1998). This was true for Buteo hawks (Brett et al. 1976). On the contrary, when salient cues for inducing CTA were analysed separately Clarke, Westbrook et al. (1979) concluded that aversion to the taste of prey is stronger than a visual aversion. More recently, Kelly, Phillips et al. (2018) observed CTA in northern quolls (*Dasyurus hallucatus*) and determined taste and smell to be the most salient cues, compared with visual cues. The predominance of taste as a cue might be generally true for mammalian predators which attack with their mouth, thereby tasting and smelling their prey (Brett et al. 1976).

## Persistence and Transference of a CTA Lesson

While CTA offers a plausible management intervention and has found some application already in reintroduction programs (e.g., Griffin et al. 2000, Moseby et al. 2015, Scheibel et al. 2016), the rate at which aversion decays is a key variable in these and future applications. There is currently intense interest in training native Australian predators (ranging from quolls to reptiles) to avoid cane toads using CTA, (Webb et al. 2008, O'Donnell et al. 2010, Webb, Legge et al. 2015, Ward-Fear et al. 2016, Jolly, Kelly et al. 2017, Kelly and Phillips 2017, Ward-Fear, Thomas et al. 2017), but knowledge about the rate at which CTA training decays is non-existent for most taxa.

What is certain, for a CTA lesson to remain sustainable over multiple generations, animals must develop a bias that is passed on genetically or culturally (Lindström, Alatalo et al. 1999). Culturally acquired information may be transmitted though learning (by watching demonstrators; (Kuan and Colwill 1997, Thornton and Clutton-Brock 2011). Alternatively, if an individual is genetically predisposed to avoid a particular food this might be expressed though the exhibition of avoidance, or neophobic behaviour towards that prey item (Lindström et al. 1999, Greggor, Clayton et al. 2014). Despite the existing evidence that food preferences are culturally transmitted (Birch 1999, Lindström et al. 1999, Greggor et al. 2014),  little research exists examining the possibility that CTA aversion can be culturally transmitted (Kuan et al. 1997, Jing, Zhou et al. 2014).  On the other hand, populations often exhibit variation in prey preference, so innate (genetic) aversion may often exist in a subset of any large population (Arnold 1981, Burghardt, Layne et al. 2000, Aubret, Burghardt et al. 2006).

## Selecting a Suitable CTA Agent

A CTA agent is any noxious compound that evokes an adverse post-ingestion effect such as malaise or emesis. CTA agents are required to create a conditioned aversion to a referent odour or taste (Baker et al. 2005, Cagnacci, Massei et al. 2005).  Learned aversions may vary with the strength of the nominated CTA agent (Conover 1995, Gill, Whiterow et al. 2000, Massei, Lyon et al. 2003).  For field use, a CTA agent should not cause chronic illness or long-lasting detrimental effects to target and non-target species (Belcher 1998, Fairbridge, Anderson et al. 2003, Glen, Gentle et al. 2007) and it should be physically stable at ambient conditions in

the bait substrate (Gill et al. 2000, Massei et al. 2003). Route and ease of administration, availability, and cost of CTA-agent must also be considered.

Many CTA agents are detectable (via taste or smell) at doses producing CTA, with some agents harder to detect than others (Conover 1989, Nicolaus et al. 1989, Conover 1995). Low detectability is vital to the success of CTA, as avoidance to a particular food or prey item can only be expected when the CTA agent remains unnoticeable (Conover 1995).  Detectable doses of CTA agents may produce discrimination against the bait rather than avoidance of the referent food (Baker et al. 2005). For instance, LiCl is an effective agent for producing CTA which can be successfully applied at undetectable doses (Nachman and Ashe 1973, Gustavson et al. 1974, Gustavson et al. 1976). Although trials by Burns and Connolly (1980) and Hansen, Bakken et al. (1997) on canids resulted in the taste of the LiCl being associated with illness rather than the referent food. Subsequently animals avoided baits, but continued to consume live prey (Semel et al. 1992).

One alternative to LiCl is thiabendazole. This compound induces robust CTA behaviour and meets all of the above-mentioned criteria (Gill et al. 2000, Massei and Cowan 2002). This compound is traditionally used as a broad-spectrum anthelmintic and antifungal agent (Robinson, Stoerk et al. 1965). It is relatively safe, orally-effective and has low toxicity in mammals; oral $LD_{50}$ is 2.7g/kg body weight (Dilov, Chaleva et al. 1981). It is fast acting and peak concentration occurs in the plasma one hour after consumption (Tocco, Rosenblum et al. 1966). Additionally, it is relatively inexpensive. Thiabendazole has been successfully trailed in small samples of wild carnivorous populations of species such as wolves

(*Canis lupus*) (Gustavson et al. 1983, Ziegler, Gustavson et al. 1983) and black bears (*Ursus americanus*) (Ternet and Garshelis 1999).

## Administration of a CTA-agent

There are numerous modes of delivery to induce illness in target species post-food ingestion. For CTA to be used effectively as a wildlife management tool, CTA-agents must be induced orally via a single-dose (Gill et al. 2000, Massei et al. 2002). One way to achieve this is to add the CTA agent into the referent-food. The use of live referent food is not always feasible or desirable as referent food may become damaged through sampling (tasting). A possible solution to this is to deliver CTA via a bait made up of referent food and a single dose of CTA-agent.

## Designing a Suitable CTA Bait

The success of inducing CTA in the field relies upon maximising the number of individuals of the target species consuming a CTA bait (Conover 1995, Cagnacci et al. 2005). Some vertebrates may seldom consume baits if they are neophobic, bait shy, or uninterested because their usual diet is abundant (Mappes et al. 2005). Designing a CTA bait for operational use that is attractive, palatable, shares close association in taste and odour of the referent food, and induces an aversion after consuming a single portion is, thus, surprisingly challenging.

Further considerations include reducing cost of the production of bait, constraints of handling requirements and bait longevity. Developing an artificial bait which is less expensive, has a longer shelf-life and can be stored at room temperature may provide one solution to this problem (Kimball, Stelting et al. 2016). It is possible to develop a CTA bait that has high operational success, this

could be achieved by borrowing trialled techniques from feral animal eradication campaigns. Mechanisms found to increase bait attractiveness during such campaigns include incorporating various triglycerides to mimic the chemo-sensory qualities produced by live animals (Kimball et al. 2016), manipulating the size of the bait so that target animals consume the entire treatment in one bite (Marks, Johnston et al. 2006), careful placement of baits, and the use of auditory or olfactory lures (Moseby, Read et al. 2011). Bait shelf-life can also be increased by adding food grade stabilisers (Johnston, Bould et al. 2014), drying of baits (Risbey, Calver et al. 1997) or storing baits in specialised bait dispensing units (Lapidge, Wishart et al. 2012).

To ensure wildlife managers can apply a CTA in the most ecological and economically effective manner, field testing of baits must be conducted; results with captive animals may not always apply to field conditions (Kimball et al. 2016). For example, the rate of bait consumption and bait longevity will typically be a function of environmental conditions (Algar, Angus et al. 2007), and non-target species may interfere with bait delivery. Because of this, basic field data on bait uptake, bait longevity, and non-target uptake are imperative to designing an efficient and successful baiting program.

## Mitigating Non-Target Species Impacts

Managing the impacts of non-target species is a critical component of CTA baiting operations. Non-target uptake may reduce target species' access to bait and significantly increase the cost and complexity of the baiting effort (Sinclair et al. 1984, Avery et al. 1995, Fairbridge et al. 2003, Glen and Dickman 2003, Claridge and Mills 2007, Glen et al. 2007, Jolley, Campbell et al. 2012). In some cases, baiting

may present an unacceptable risk to non-target species (Avery et al. 1995, Jolley et al. 2012).

The interest and uptake of bait by non-target species can be difficult to predict and quantify. Evidence of non-target impacts can be collected by quantifying interest, uptake (Thomson and Kok 2002) and susceptibility of species that inhabit the study area (Glen et al. 2007). This can be achieved through the use of camera traps (Belcher 1998, 2003, Glen et al. 2003), hair sampling tubes (Ruibal, Peakall et al. 2010), sand plots (Thomson et al. 2002), radio-tracking (King 1989) and the inclusion of a biomarker like Rhodamine B in baits (Fisher 1999, Fairbridge et al. 2003, Claridge et al. 2007). All of these methods vary in terms of labour, effect, reliability and accuracy although all should detect non-target activity in association with bait (Fisher 1999, Purdey, Petcu et al. 2003, Körtner 2007). If these data are absent or unobtainable, wildlife managers should adopt a precautionary approach to minimize potential risks for non-target animals (Glen et al. 2003). Precautionary measures include considering the choice and dose rate of CTA agent, reducing the number of baits used, careful placement of baits in the environment (Glen et al. 2003), and the removal of uneaten baits (Thomson et al. 2002).

## Application of CTA Baits

In principle, CTA can be an inexpensive management technique, because it does not require complex tools or abilities (Avery et al. 1995). Although expense and efficacy clearly depend upon the methods employed to deliver baits. Aircraft such as helicopters may provide an efficient and fast means of distributing baits over large or otherwise inaccessible tracts of country (Thomson 1986, thThomson et al.

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2002), although removing the ability to control exact bait placement. The optimal mode of delivery will depend on the particulars of any system: the behaviour of target-species, bait longevity and robustness, the need for careful placement or retrieval of baits, and so on.

Individual behavioural responses to bait delivery mode may be a particularly important consideration: a lack of information on individual behavioural responses may have limited the practical development of CTA for managing wildlife in the past (Gustavson et al. 1976, Baker et al. 2005). For instance, in a study on CTA baiting for badgers, individual animals were more likely to consume dispersed single baits than multiple baits at fixed stations (Cagnacci, Massei et al. 2007). Determining if target-species would take baits in differing microhabitats will also help ensure that CTA can be applied on a landscape scale in the most effective manner (Jacob, Ylönen et al. 2003).

## Northern Quolls and Cane Toads- Background

When native predators are naïve to novel invasive prey, they may lack innate or learned behaviours to respond appropriately to that novel prey. As a consequence, prey defences may impose significant injury or mortality during their first encounters (Webb, Pearson et al. 2011). This is particularly true when the novel species is a highly toxic organism that shares similarities to an existing prey type (Webb et al. 2008). The highly toxic cane toad (*Rhinella marina*) was introduced in 1935 and has since rapidly expanded across the north of Australia (Phillips, Brown et al. 2007). Bufadienolides—the major component of toad defensive secretions—are unlike toxins possessed by native Australian animals. As a result, many vertebrate predators (such as northern quolls (*Dasyurus hallucatus*), varanid

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lizards, and some snakes), die after attacking or consuming toads (Covacevich and

Archer 1975, Oakwood 2004, Webb, Shine et al. 2005, Smith and Phillips 2006,

Hayes, Crossland et al. 2009, Shine 2010).

Northern quolls—a native marsupial predator (Figure 1-1, Appendix 1)—are

now listed as federally endangered, and the primary reason for this is the toad

invasion: abundant anecdotal evidence tells of rapid declines in quolls upon the

arrival of toads (Burnett 1997), and several radiotelemetry studies demonstrate

very high mortality rates of quolls that have ingested toads (Oakwood 2004,

O'Donnell et al. 2010, Cremona, Spencer et al. 2017, Jolly et al. 2017).

Unfortunately, meaningful control of toads at a landscape scale in the wet-dry

tropics is, to date, an impossible proposition (Tingley, Ward-Fear et al. 2017).

Cane toads are expected to invade the entirety of northern quoll's geographic

range within the next twenty years (Urban, Phillips et al. 2007, Kearney, Phillips et

al. 2008). Research and wildlife management has shifted focus toward developing

methods to mitigate cane toad impacts. Significant actions to protect the northern

quoll included moving quolls to island or 'ark' stronghold populations, and

establishing captive breeding programs to maintain genetic diversity (Woinarski,

Rankmore et al. 2007, Rankmore, Griffiths et al. 2008). Some reptilian predator

populations have adapted to the presence of toads by evolving innate aversion to

toads (Phillips & Shine 2005; Llewelyn *et al.* 2011). This rapid adaptation has also

occurred in a small number of quoll populations (Kelly and Phillips 2019), but in

the short term, marsupial predators can also rapidly learn to avoid toads as prey

(Webb et al. 2008, Webb et al. 2011). An obvious avenue for mitigating the impact

of toads, then, is to train predators to avoid toads (Webb et al. 2008).

**Figure 1-1**: Northern quoll consuming chicken neck (Jonathan Webb)

## CTA – Quolls as Ideal Candidates

In 2008, following trials in which planigales—mouse-sized relatives of quolls—learned to avoid cane toads (Webb et al. 2008), the idea emerged that it might be possible to train quolls to avoid toads, and so mitigate the toads' impact on quoll populations (O'Donnell et al. 2010). Early trials on captive quolls confirmed that, after consuming a small dead toad coated with TBZ, quolls exhibited single-trial conditioned taste aversion, and avoided attacking live toads immediately afterwards (O'Donnell et al. 2010). Radiotelemetry of trained quolls in a toad infested landscape confirmed that at least some of these trained animals survived four months thereafter in the presence of toads (O'Donnell et al. 2010). Follow-up radiotelemetry studies in which control (untrained) groups were also released,

confirmed a very large positive effect of training on survival in the weeks following release (Jolly et al. 2017). A longer-term reintroduction and mark-recapture study also showed that some trained captive reared animals survived in the wild and reproduced (Cremona et al. 2017).

## Current Gaps in Knowledge

Previous work raised the possibility of *in situ* training of northern quolls to avoid cane toads (O'Donnell et al. 2010), but *in situ* training methods (such as baiting) had not been developed. A noteworthy feature of the above-mentioned CTA studies is that animals were either raised or trained under captive conditions. The techniques required to apply CTA to northern quolls at a landscape level have never been examined. This thesis addresses that gap, investigating the efficacy of CTA at a landscape scale and whether we can use this method to safeguard populations of an endangered mammalian species.

Ideally, we would teach populations of northern quolls to avoid attacking toads prior to the toads' arrival, by deploying low-toxicity baits to induce taste-aversion learning (Webb et al. 2008). This would buffer quoll populations from the initial impact of toads but requires the method to be scaled up substantially (captive learning trials are not a feasible option). Capacity to elicit aversion has been demonstrated in captive quolls but translating this result to the field remains a challenge. To be effective as a management tool, CTA needs to meet two additional conditions. First, CTA training needs to be deliverable to many individuals under field conditions, prior to the arrival of cane toads. 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 realised.

## Thesis Objectives

Throughout this thesis, I answer fundamental questions about how northern quoll populations respond to cane toads and whether toad aversion baiting can mitigate the toads' impact. In order to align the timing of my experiments, I monitored the position of the cane toad front for six months prior to the start of my candidature (Figure 1-2). I began my candidature when the location of the toad front was approximately 30kms from my field sites.
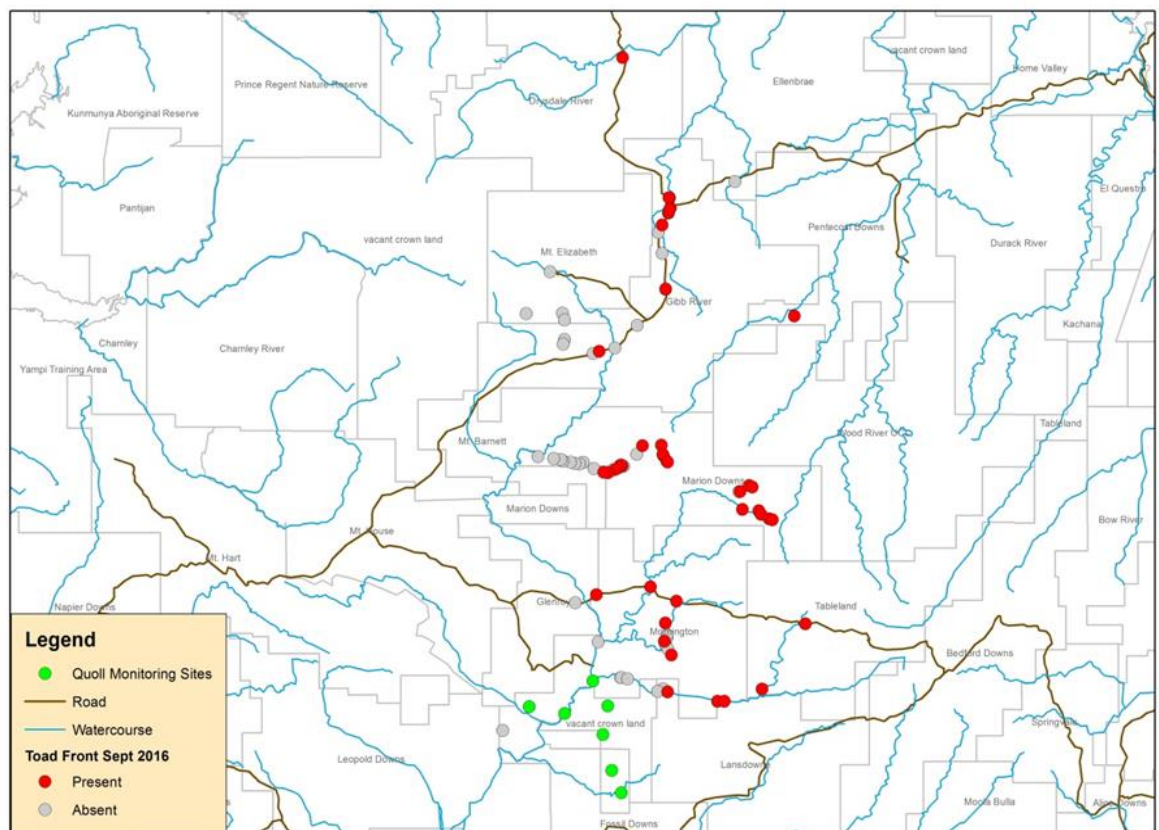


**Figure 1-2**: The best-estimate of the known location of the toad front in the central Kimberley region. This information was current as of the September 2016- 9 months into my candidature, the toad invasion front was <5km from my study sites. This map was developed using targeted camera surveys, waterway searches,

incidental sightings during fauna surveys, and communication with other landholders in the area.

The overall goal of this research was to investigate how northern quoll populations respond to cane toads and determine whether CTA can mitigate their impact; ultimately, I aimed to slow the decline of the northern quoll using CTA. To investigate the feasibility of CTA baiting, I first had to develop an approach that allowed me to monitor quoll populations closely to measure the short-term, immediate and long-term impacts of the toad arrival. This methodology allowed me to determine if CTA treatment has been successful. With this in mind, my specific aims were:

**Aim 1:** Gather baseline demographic data about the study species and use population viability analysis to determine how CTA influences extinction probability of adults following cane toad arrival.

**Aim 2**: Develop toad-aversion baits that produce the desired aversion response in northern quolls and are attractive to wild quolls.

**Aim 3:** Deliver aversion training at a large scale to northern quoll populations at control and treatment sites on Mornington Sanctuary (an Australian Wildlife Conservancy property located in the central Kimberley region of western Australia- Appendix 1).

**Aim 4:** Gauge the effectiveness of CTA treatment via population monitoring across seven populations of northern quolls before and after the arrival of cane toads.

**Aim 5:** Develop a predictive model which can inform each step of applying CTA at a landscape level which is cost effective and at densities sufficient to train large populations of northern quolls.

## Thesis Structure

In this thesis I examine conditioned taste aversion as a conservation tool. In **Chapter 2**, I gather baseline data about the study species at one primary study site in the central Kimberley, WA. I then use a population viability model, designed by Kelly & Phillips (2019) to address questions about the implementation of conditioned taste aversion as a mitigation strategy, to conserve a population of northern quolls in the Central Kimberley. We use this model to investigate the efficacy of CTA at preventing extinction. Broadly, for CTA to be a viable strategy requires a high proportion of the population being CTA trained *in situ,* prior to toad invasion. This requires a training technique that can be used at a landscape scale. This requires a bait that will ultimately elicit aversion to toads. In **Chapter 3**, I use a small captive trial to assess the value of a manufactured bait ('toad aversion sausages'). I ask whether quolls generalise their CTA from the bait to toads. Additionally, I assess whether the bait is taken up by wild quolls and non-target species, and whether it appears to elicit CTA under field conditions.

I then place these ideas in a real-world context in **Chapter 4**. Given the lack of robust long-term study to explore the efficacy of toad aversion training, I executed a robust test of the baiting methodology in a before-after-control-impact (BACI) framework. Throughout the rest of the thesis I assess conditioned taste aversion baiting as a practical and viable management tool. A successful baiting program is one that is cost-effective and results in a large proportion of target individuals

taking bait, thus in **Chapter 5** I refine the technique of baiting. I aimed to optimise

bait delivery by determining the fewest baits required to achieve a given

proportion of the population baited.  Finally, In **Chapter 6**, I provide my general

conclusions on the applicability of delivering conditioned taste aversion on a

landscape scale, to mitigate the impacts of cane on northern quolls. In this chapter

I provide a critical summary of my results and discuss some direction for future

research.

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# Cultural and genetic transmission of a conditioned taste aversion lesson can interact to prevent extinction: Cane toads and northern quolls in Australia.

Indigo, N., J. Smith, J. Webb, and B. Phillips.

## Abstract

Population viability analysis (PVA) is a useful tool for conservation practitioners for predicting the likelihood that a population will persist under various scenarios. PVA outputs may identify management strategies that have a higher likelihood of achieving a particular outcome. In northern Australia, the endangered northern quoll (*Dasyurus hallucatus*) has undergone significant range reductions in the last 40 years and extant populations have contracted to rocky areas within the former range. The recent invasion of highly toxic cane toads (*Rhinella marina*) has contributed to local extinctions of quolls across the Top End because quolls are fatally poisoned when they attack cane toads. One novel, yet promising management strategy, the deployment of 'toad aversion baits' ahead of the toad invasion front to train wild quolls to avoid toads before the toads invade, has received widespread interest, but we do not know whether this approach can prevent population extinction.

Here we use a population viability model, designed by Kelly & Phillips (2019) to address questions about the implementation of conditioned taste aversion as a mitigation strategy to conserve a population of northern quolls in the Central Kimberley.  We used this model to investigate whether CTA could preventing extinction under scenarios in which we varied: (1) the efficacy of the initial CTA training (i.e. the proportion of the population that was successfully trained to avoid toads); and (2) the rate of cultural transmission of the CTA lesson (i.e., the ability of females to pass the lesson on to their offspring). We used long-term mark-recapture data to generate population estimates and demographic parameters for a population of quolls at Sir John Gorge (Mornington Wildlife Sanctuary). Using these parameters, we modelled the probability of extinction given varying levels of transmission and efficacy of the conditioned taste aversion lesson. Our results showed that toad-aversion baiting could reduce the probability of extinction provided that efficacy of baiting was higher than 0.5 and transmission of the CTA lesson was > 0.7. Not surprisingly, in the absence of any management intervention, quoll populations were likely to go extinct unless there was moderate heritability ($h^2 > 0.3$) for toad-aversion traits in toad-naïve populations.  Our results demonstrate that managers need to develop baiting designs to ensure that a high proportion of the population is trained prior to toad invasion. In addition, our results suggest that cultural transmission of food preferences from mothers to offspring is necessary for conditioned taste aversion to be a successful long-term mitigation strategy.

# Introduction

Projecting the population trajectory of an endangered species is a useful tool for conservation practitioners. Population viability models are particularly useful for predicting the likelihood that a population will persist into arbitrary lengths of time under various scenarios (Boyce 1992, Brook, O'Grady et al. 2000). Modelling can provide guidance for successful delivery of management techniques in complex biological systems (Restif et al., 2012). Quantitatively synthesizing existing life history information and comparing outcomes of certain management activities can guide conservation decision making (e.g Bode and Brennan, 2011). For instance, when we have a good handle of population demography, such model systems allow us to test critical assumptions and assess the effect of various management levers without the need for significant investments of time and money (Beissinger and Westphal 1998). Additionally, model systems may allow us to avoid pursuing techniques that are unlikely to result in demonstrably significant conservation outcomes for threatened species recovery given the potential expense of investment (McLane et al., 2011).

Gathering accurate demographic data needed for PVA can be achieved relatively easily for common species by accessing the published literature, but it is more difficult for threatened or declining species. For the latter, information on population size are often gleaned from estimates derived from live trapping or camera traps (Belcher 1998, 2003, Glen et al. 2003), or indirect indices such as hair sampling tubes (Ruibal et al. 2010) and sand plots (Thomson et al. 2002). All of these methods vary in terms of labour, effect, reliability and accuracy (Fisher 1999, Purdey et al. 2003, Körtner 2007). However, obtaining accurate estimates of

demographic parameters for threatened or endangered species is necessary to adequately model population persistence. For instance, a lack of capture-recapture data may lead to inappropriate estimates of survival rates being used, which reduce the precision of parameter estimates (Brook, O'Grady et al. 2000, Coulson, Mace et al. 2001). For PVA models to have biological value and utility, parameters must be estimated from the data on the biological population of interest (Heppell, Caswell et al. 2000, White, Franklin et al. 2002, Link and Barker 2005).

The endangered northern quoll (*Dasyurus hallucatus*) belongs to the family Dasyuridae. This family includes 51 individual species located within Australia and 15 in New Guinea (Flannery 1995, Strahan and Conder 2007). The northern quoll is the smallest of six members of the genus *Dasyurus,* with adults weighing up to 1100g (Oakwood 1997, Strahan et al. 2007, Van Dyck and Strahan 2008). At the time of European settlement the species was widespread across northern Australia, but it suffered a precipitous decline in geographic range over the period 1977 to 1991 (Braithwaite and Griffiths 1994). The species contracted to rocky areas within its former broad geographic range, potentially because those sites offered protection from putative threatening processes such as predation by introduced carnivores (Woinarski et al. 2014), grazing and changed fire regimes (Fisher et al. 2003, Legge, Murphy et al. 2011). More recently, quoll populations across the Top End crashed due to the invasion of the highly toxic cane toad *Rhinella marina* (Burnett 1997, Woinarski et al. 2010, Woinarski, Burbidge et al. 2015). Quolls lack immunity to toad toxins (Ujvari, Oakwood et al. 2013) , and consequently, ingestion of toads is typically fatal for quolls (Covacevich and Archer 1975, O'Donnell et al. 2010).

The complex rocky habitats in the Kimberley region of northern Australia is one of the last strongholds for *D. hallucatus* populations (Braithwaite et al. 1994). Furthermore, the population in this region is genetically distinct from the populations of quolls located in other bioregions such as the Pilbara (WA) and NT/Queensland (Westerman and Woolley 2016), and hence should constitute separate management units. However, the continuous spread of the cane toads across the north of Australia has caused, and is expected to cause, further major declines or local extinctions of northern quoll populations in the Kimberley over the coming decade (Phillips, Chipperfield et al. 2008). Unfortunately, meaningful control of toads at a landscape scale in the wet-dry tropics is, to date, an impossible proposition (Tingley et al. 2017). However, in 2008, research showed that planigales (*Planigale maculata*)– mouse-sized relatives of quolls – could rapidly learn to avoid cane toads provided that they ingested a non-lethal sized toad (Webb et al. 2008). Thus, it should be possible to use conditioned taste aversion (CTA) to train quolls to avoid consuming toads, and so mitigate the toads' impact on quoll populations (O'Donnell et al. 2010). Captive-reared quolls fed small non-lethal toads laced with a nausea-inducing chemical subsequently refused to attack toads, and had higher survival in the wild following reintroduction (O'Donnell et al. 2010). Follow up radiotelemetry and mark-recapture studies demonstrated that some toad-trained female quolls survived and reproduced, and their offspring also survived in toad-infested landscapes (Cremona et al. 2017, Jolly et al. 2017). Theoretically, it should be possible to deploy toad-aversion baits ahead of the toad invasion front to train wild quolls not to eat cane toads. CTA therefore offers a novel method for mitigating toad impacts on quoll populations (O'Donnell et al. 2010, Webb et al. 2015).

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For deployment of toad-aversion baits to be effective, we need to know whether training a cohort of quolls can prevent local populations from going extinct. Previous population viability studies on northern quolls from the Pilbara and Kakadu National Park utilised baseline demographic data collected from field monitoring programs to show that increases in mortality rates of juveniles can drive populations towards extinction (Cremona, Crowther et al. 2017, Moro, Dunlop et al. 2019). Anecdotal studies, and radio-telemetry studies have demonstrated that cane toads significantly increase mortality rates. Using a PVA framework, Kelly and Phillips (2019) showed that populations would begin to collapse when the probability of attacking a toad for each individual was greater than 68% – thus 32% of the population would need to express a rapid shift in toad-smart behaviour to survive the cane toad invasion. To date, no one has used this modelling framework to predict the likelihood of persistence and population viability following the application of CTA in the landscape.

Here we use these data on quoll life history and the full evolutionary model taken directly from Kelly et al. (2019) to determine whether CTA can enable quolls to persist following the cane toad invasion. This study comprised two main components – each provides complementary approaches to understanding factors that may influence the persistence of a northern quoll population. Firstly, we undertake a demographic analysis of historic mark and recapture records from 2011-2017, in a geographically isolated location on Mornington Wildlife Sanctuary WA, to answer fundamental questions about northern quoll survival rates in this population. Second, we examine the robustness of CTA for preventing population extinction under scenarios in which we varied: 1) the efficacy of the initial CTA

training (i.e. the proportion of the population that was successfully trained to avoid toads) and 2) the transmission of the CTA lesson (i.e., the ability of females to pass the lesson on to their offspring.

## Methods

### *Mark and Recapture*

To provide an additional estimate of survival rates of quolls in the field, we used a long-term mark-recapture dataset from the central Kimberley, in Western Australia. The field study was conducted between October 2011 and August 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). The data were collected from one site on the property; Sir John Gorge (17°31.780S, 126°13.080E) along the Fitzroy River. 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 making this one of the drier locations that quolls have been studied. Most rain falls during the wet season (from November to April). The site was selected based on the detection of quolls in the Australian Wildlife Conservancy's fauna surveys (AWC unpub. data). At the time of the study, toads were yet to arrive at the site; they had subsequently arrived by March 2017.

Live trapping and mark-recapture techniques were used to estimate demographic parameters. Trapping occurred over a minimum of four consecutive nights within 24 distinct trapping sessions. At least 4 trapping sessions were conducted each year with at least 4930 trap-nights recorded. Traps were placed in suitable locations 50m apart (under ledges, beside crevices) in the late afternoon,

baited with a mixture of peanut butter, mackerel, honey and oats, and checked within 2 hours of sunrise the following morning. Captured quolls were injected with a sub-dermal microchip for individual identification. Sex, mass, reproductive status, and age were also recorded. When recaptured, the microchip number was read and recorded. All individuals were immediately released after capture and processing.

The Cormack–Jolly–Seber (CJS) (Cormack 1989) method was used to derive apparent survival estimates from the mark-recapture data using program MARK v9.9 (White and Burnham 1999). Sixteen candidate models were compared where survival ($\Phi$) and recapture (p) were sex specific (sex), time dependent (t) or constant (.). Closed population size estimates were obtained for 24 trapping sessions with mark-recapture data using the Robust Design with Huggins' p and c, in program MARK v9.9 (White et al. 1999) . The Robust Design incorporates open sampling events called "primary periods", within which are a number of closed "secondary periods" (Pollock, Nichols et al. 1990). Closure is assumed within primary periods but not between them. In this study, we used the robust design to calculate population estimates within secondary periods but not between them. A Robust Design procedure was selected to calculate population estimates for the 24 secondary periods, as it had the most flexibility in setting sampling occasions, parameters and incorporating unequal time intervals between sampling (White et al. 1999). For the purpose of our PVA we selected the CJS results that calculated daily survival for the entirety of the sampling period (2011-17) (Kendall, Nichols et al. 1997).

*Population Viability Model*

For population viability analysis, we used the discrete-time, individual-based population viability model described in Kelly et al. (2019). The complete description of the model is available in Kelly et al. (2019), all code is provided in the corresponding supplementary materials– available at https://github.com/elkelly/TGFquolls. The model is motivated by empirical observations that a proportion of the quoll population are innately toad smart, and that this trait has been shown to be heritable (Kelly and Phillips 2018). Given this heritability and initial proportion of toad smart quolls, the modelled population evolves in response to the arrival of toads; increasing the proportion of toad smart individuals in the population. Despite the population's capacity to evolve, it often goes extinct before adaptation is complete; a result echoed in wild quoll populations exposed to toads (Woinarski et al. 2010). Here, we extend this model to incorporate the capacity for additional quolls in the population to learn toad avoidance, through the deployment of toad-aversion baits prior to toad invasion.

The model incorporates baseline survival rates that differ by sex and age collected by previous studies (Begg 1981, Schmitt, Bradley et al. 1989, Braithwaite et al. 1994, Oakwood 2000). Recent survival estimates published by Hernandez-Santin, Dunlop et al. (2019) were not included in our PVA model, on the basis that individuals from the Pilbara bioregion are genetically distinct, reproduction occurs at a different time of year and semelparity is not complete, when compared with populations in other bioregions. The input values along with the information source for the model are provided in Table A-II-1, (in Appendix II). To these data, we also add our CJS survival values estimated from the Kimberley.

Our extension of Kelly and Phillips' (2019) model incorporates two extra parameters to describe the efficacy with which the one-trial CTA lesson is imparted to wild quolls, $E$ (describing the proportion of the toad-susceptible population that is effectively trained) and the probability of cultural transmission of the CTA lesson—from mother to offspring—$T$ (Figure 2-1). We examine a carrying capacity ($K$) of 100 individuals for the Kimberley bioregion and initiated our scenarios with an initial fitness ($\overline{W}_0$) of 0.38 as the proportion of animals that would not take a cane toad sausage and thus likely to exhibit an innate aversion to consuming cane toads (see Indigo, Smith et al. (2018). Heritability of toad-smart traits is determined by the animal's genotype and environmental variation, and is unknown, though as with many behavioural traits, likely lies somewhere between 0.1 to 0.3 (Roff 2012, Kelly et al. 2019). In the results presented here, we explore outcomes across a range of heritability's between 0 and 0.5.

We explore CTA under several scenarios. Firstly, we examined CTA efficacy-with no transmission between generations (cultural transmission) ($E$ = 1, $T$ = 0). Following this we explored the possibility of CTA with differing levels of $T$ from 0-1 (with 0 being no transference and 1 being perfect transference) in increments 0.1; and $E$ from 0-1 in increments of 0.1, over 50 generations. We then investigate how these parameters influence extinction probability (estimated over 200 simulations per parameter set). In each scenario; we recorded whether the population went extinct or not over 50 years following the in arrival of cane toads. Model simulations were implemented in R (R Core Team 2019).
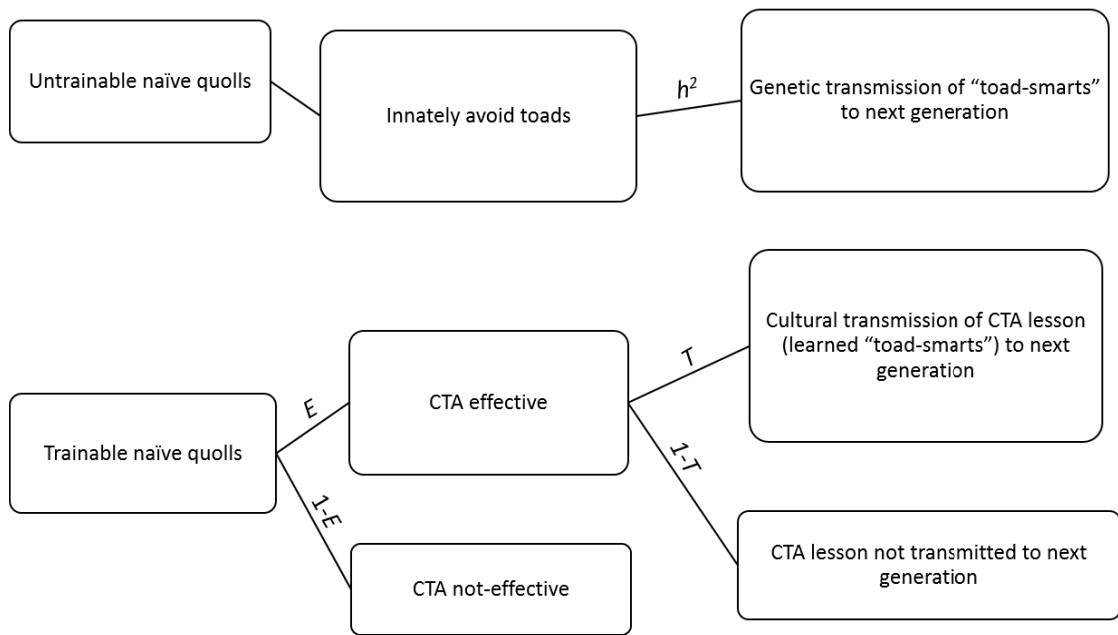
**Figure 2-1**: Flow diagram which demonstrates the capacity for quolls to learn toad avoidance, through CTA using two extra modelled parameters (E and T), added to the Kelly and Phillips (2019) model. Extra parameters describe the efficacy with which the one-trial CTA lesson is imparted to wild quolls, E and the probability of cultural transmission of the CTA lesson—T. Bifurcation in the chart occurs when trainable northern quolls effectively learn a CTA lesson (E) or when CTA training is not effective (1-E). The second bifurcation for trainable northern quolls occurs when the CTA lesson is transmitted culturally (T) or when cultural transmission is not achieved (1-T). Untrainable northern quolls innately avoid toads, and this is influenced by heritability ($h^2$) of toad-smart traits. The heritability of toad smart traits is determined by the animal's genotype and environmental variation. This parameter ($h^2$) governs evolutionary dynamics; and determines the speed with which the population responds to selection. Trainable northern quolls are subject to the same evolutionary process. The innate tendency to avoid toads is heritable, for all animals. Although CTA can modify the innate tendency of toad-naïve individuals and may be transmissible (culturally) to offspring of toad-naïve individuals.

# Results

*Mark and Recapture*

The results of the CJS analyses in MARK identified a single model with ΔAICc < 2.0, and this was the only model that was well supported by the data (See Appendix: Table A-II-2)(Burnham and Anderson 2002). The best-supported model was $\Phi(Sex) p(t)$ in which survival was affected by sex (independent of age) and recapture was time dependent. In this model, annual survival rates were significantly higher in females (0.249 ± 0.005) than males (0.062 ± 0.001, Table 2-1). The number of individual northern quolls estimated to be alive during each secondary sampling period ranged from 1.37- 14.66 individual females and 0-16.65 individual males (Figure 2-2; See Appendix II: Table A-II-3).
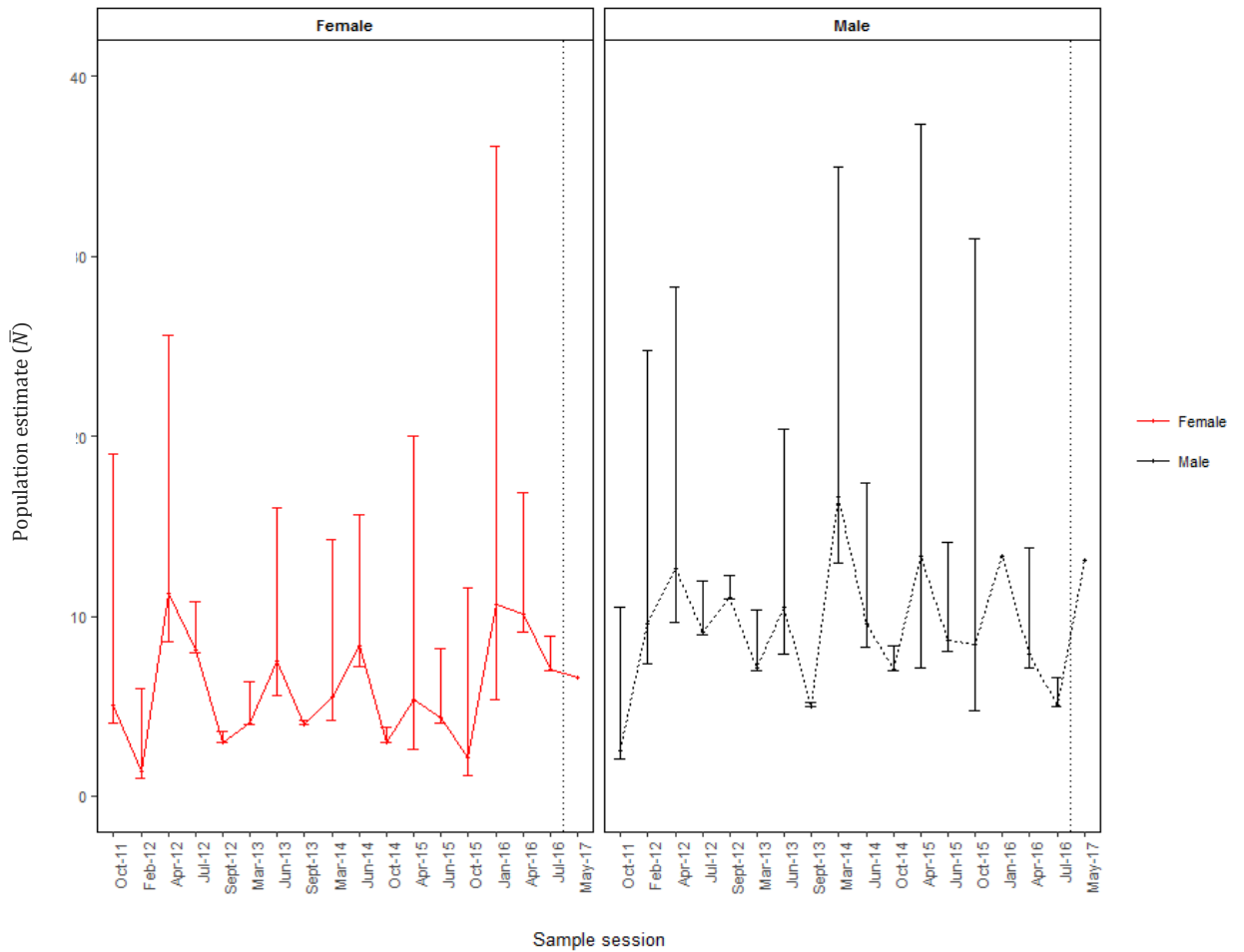
**Figure 2-2**: Population estimates ($\overline{N}$) for the northern quoll population (*Dasyurus hallucatus*) at Sir John Gorge, within Australian Wildlife Conservancy's Mornington Wildlife Sanctuary, in the central Kimberley, Western Australia. Estimates were derived using Pollock's Robust Design in program MARK. Data points presented are from one primary session each month– multiple sessions within a month were omitted, see Table. A-II-2 (See Appendix II) for a full list of primary occasion population estimates. Upper and Lower confidence intervals for the final session in May-17 were omitted from the figure as these were too large to plot– this is a result of small sample sizes captured during these sampling periods; which correspond with the arrival of the cane toads and subsequent reduction in capture rates compared with other sessions. The timing of cane toad arrival is indicated by

**Table 2-1**: Estimates of daily and yearly survival ($\Phi$) and recapture rates (p) derived from the best-supported CJS model [ $\Phi$ (Sex) p(t) ] in program MARK.

|  | Rate | Estimate | SE | LCI | UCI |
|---|---|---|---|---|---|
| **Female Survival** | Daily | 0.9961 | 0.0005 | 0.9949 | 0.9971 |
| **Female Survival** | Yearly | 0.2488 |  |  |  |
| **Male Survival** | Daily | 0.9924 | 0.0011 | 0.9898 | 0.9943 |
| **Male Survival** | Yearly | 0.0621 |  |  |  |
| **Recapture** | Daily | 0.6093 | 0.0410 | 0.5265 | 0.6862 |

*Population Viability Model*

Extinction probability decreased rapidly as genetic heritability ($h^2$) increased (Fig. 2-3). Conditioned taste aversion reduced the probability of extinction when the efficacy of training ($E$) was greater than 0 and the transmissibility ($T$) of the lesson between generations was greater than 0.7 (Fig. 2-3; b–d). When $E > 0.5$ and $T > 0.7$ the probability of extinction begins to fall below the reference case of E = 0 (Fig. 2; a). In the absence of CTA intervention, quoll populations are likely to go extinct unless there is moderate heritability ($h^2 > 0.3$) for toad-aversion traits in toad-naïve populations.
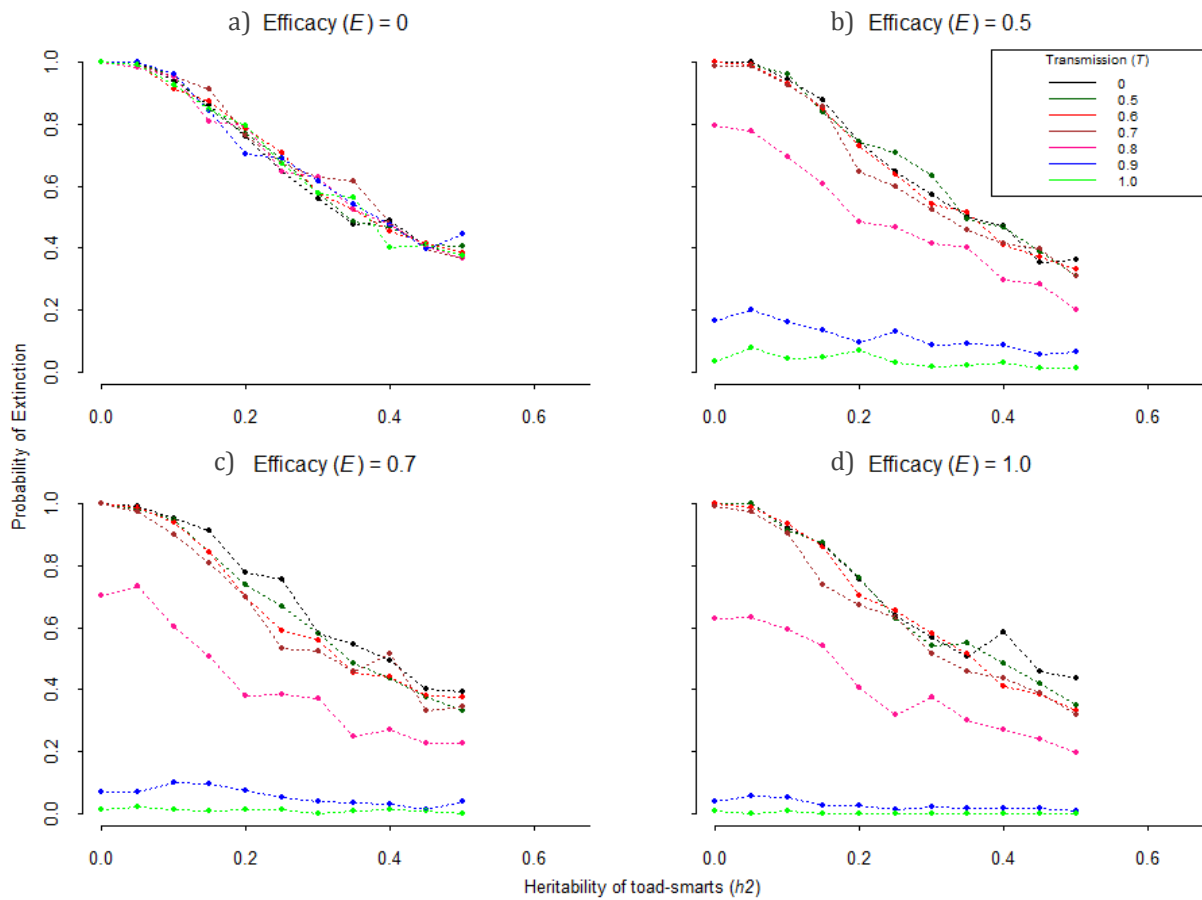
**Figure 2-3**: Relationship between the efficacy of conditioned taste aversion (E) and the proportion of individuals in the population that are toad-smart (heritability; $h^2$). When efficacy is zero (baiting fails to train quolls), population viability depends only on the heritability of innate toad-avoidance in the population (a). By contrast, when E ≥ 0.5, the probability of extinction is influenced by the transmission of toad-smart information from mother to offspring (T) and heritability ($h^2$; (b)). Even with low heritability of toad-avoidance ($h^2 = 0.2$) a high level of social transfer of toad-smart information (T > 0.7) can substantially reduce the likelihood of population extinction (panel: c & d).

## Discussion

Confronted with rapid environmental change, many populations go extinct before they can adapt. Here we ask whether CTA might provide an additional, potentially faster, mechanism for adaptation, beyond that provided by evolution. Our analysis using the population viability model, designed by Kelly & Phillips (2019)

demonstrates that managers need to develop baiting designs to ensure that a high

proportion of the population is trained prior to toad invasion. In addition, our

results suggest that cultural transmission of food preferences from mothers to

offspring is necessary for conditioned taste aversion to be a successful long-term

mitigation strategy.

The results reported here present the first systematic assessment of

conditioned taste aversion as a management strategy using population viability

analysis techniques. These findings are specifically relevant to the persistence of a

geographically isolated population of endangered northern quolls in the central

Kimberley, WA. This study comprised two main components – demographic

analysis of historic mark and recapture records, and population viability analysis

under assumptions about CTA efficacy and transmission. These provide

complementary approaches to understanding factors that influence persistence of

this northern quoll population.

Our mark-recapture results are broadly consistent with previous studies

(Cremona et al. 2017), but also reveal separate insights.  For instance, sex-based

survival estimates derived from our CJS analysis revealed that the annual survival

rate of northern quolls in the central Kimberley region of WA; for females (0.249)

and male survival ((0.062); see Table 2-1); are very similar to those reported from

other studies in differing bioregions (See Appendix II: Table A-II-3) (Begg 1981,

Schmitt et al. 1989, Braithwaite et al. 1994, Oakwood 2000). Robust design closed

population estimates suggested that quoll population size changes markedly

throughout the year; this is consistent with male die-off following the annual

breeding season recorded elsewhere (Oakwood, Bradley et al. 2001). Data on

variation in population size and survival are rarely available for endangered or threatened species. Thus our estimates are valuable for increasing precision when modelling northern quoll populations under different PVA scenarios in the future (White et al. 2002, Gerber, Wielgus et al. 2007).

The results of our population viability analysis revealed a new insight in the context of CTA application. If complete efficacy (*E*) of CTA training is achieved in the first generation but no cultural transmission (*T*) of the CTA lesson occurs, population viability is dependent on the proportion of innately toad-averse quolls in the population (Figure 2-3; a). That is, without cultural transmission of toad-smart behaviour, toad-aversion baiting has no impact on decreasing the probability of population extinction (Figure 2-3; a). Further scenarios were also explored varying the efficacy (*E*) of the initial CTA training and the rate of cultural transmission (*T*) of the CTA lesson. Importantly, when *E = >0.5* and *T* = >0.7 the probability of extinction decreased substantially (Figure 2-3; b). At high levels of T (>0.8), the probability of extinction was zero, and was unaffected by the heritability of toad-averse traits. At lower levels of *T*, and with low heritability ($h^2$ (0.2)), even moderate levels of efficacy of CTA (*E*) significantly decreased the extinction probability. That is, training 50% of wild quolls to avoid toads decreased extinction probability. Hence, our population viability analysis results presented here has identified that high levels of cultural transmission (*T*) of a CTA lesson along with moderate efficacy (*E > 0.5*) can increase the long-term population viability under this management strategy.

Cultural transference of information occurs in many species, and can enable species to adapt rapidly to environmental change (Whitehead, Rendell et al. 2004,

Thornton and Raihani 2008, 2010). Cultural transference of an acquired lesson –

such as a food preference and avoidance – is transmitted though peripheral cues to

conspecifics and offspring. This occurs by either observing demonstrators

(Thornton et al. 2011, Hoppitt and Laland 2013) or through active teaching, where

a demonstrator may jeopardise its own fitness to facilitate the learning of a naïve

animal (McComb, Moss et al. 2001, Thornton et al. 2010).  An animal relies heavily

upon peripheral cues during active teaching (Gustavson et al. 1974, Brett et al.

1976) and these cues develop as a result of early learning, experience, interactions

with the environment, genetic predispositions, food availability and the feeding

preferences of adults (Birch 1999, Lindström et al. 1999, Francis, Szegda et al.

2003, Mappes et al. 2005, Sheppard, Marshall et al. 2018).  Although offspring may

readily learn from others which foods to eat, it is much less clear that they learn

from others which foods to avoid eating (Galef 1989, Galef and Giraldeau 2001,

Dewar 2004). Rigorous experimental data to support or reject the possibility that a

CTA lesson might be passed on to offspring is extremely challenging to collect.

Obtaining this information for a non-model and endangered species such as the

northern quoll renders this an exceptionally difficult challenge indeed.  For a CTA

lesson to remain sustainable over multiple generations, animals must first learn a

CTA lesson, this can be achieved via an efficient CTA strategy (represented in our

study by $E$). Secondly animals must develop a bias that is passed on to the next

generation culturally ($T$).

If an individual is genetically predisposed to avoid a particular food this is

often expressed though the exhibition of neophobic behaviour to novel prey items

(Greggor et al. 2014). This neophobic response or 'innate' food avoidance was

demonstrated within a quoll population by Kelly et al. (2017). This is further

evidenced by quoll populations persisting in toad infested landscapes >30 years, as

reported in the Mackay area of Queensland by Pollock (1999). The development of

feeding variation (or cane toad-smarts) among surviving individuals persisting in

these regions may have initially arisen genetically, following this, toad aversions

may be passed to each generation culturally through learning from parents and

conspecifics (Mappes et al. 2005, Kelly et al. 2017).

It is possible that adult quolls that receive CTA training can teach their

offspring to also avoid eating cane toads. This socially induced food aversion would

occur indirectly as a result of socially induced preferences as described above

(Mirza and Provenza 1990, Read, Peacock et al. 2016). That is, it is likely that

juvenile quolls learn what to eat, and what not to eat, by copying the food

preferences of their mothers (Galef and Giraldeau 2001). It was suggested by

Ujvari, Oakwood et al. (2013) that social transmission of feeding preferences is an

unlikely explanation for persistence of northern quolls in already cane-toad

infested landscapes. This view stemmed from the assumption that quolls are

solitary and therefore unable to culturally transmit toad avoidance (Oakwood

2002). However, juvenile quolls spend long periods foraging with their mothers

(Cremona, unpublished data 2017), and like other mammals, would learn to eat

palatable foods and avoid unpalatable foods simply by observing their mothers'

food choices (Mirza et al. 1990, Galef et al. 2001). In a study by Cremona et al.

(2017), female quolls subjected to CTA treatment and reintroduced into toad-

infested areas survived and reproduced. Parentage genetic analyses determined

that animals persisting in this landscape were the offspring of CTA-trained

females; suggesting that aversion to consuming toads was passed onto offspring, though whether genetic or cultural transmission (or both) remains unclear (Webb et al. 2015, Cremona et al. 2017).

Researchers have been unable to determine the mechanism whereby quolls acquire an aversion to toads. This is because there is difficulty differentiating between a genetic or cultural acquisition of a feeding lesson (Jing et al. 2014, Kelly et al. 2017). One hypothesis on the likelihood that an individual acquires information culturally, in regard to conditioned taste aversion, suggests that offspring may learn from others which foods to eat, but not which foods to avoid eating (Galef 1989, Galef et al. 2001, Dewar 2004).  Animals may learn what to eat, where to eat, and how to eat typically by observing parents (Galef et al. 2001). Subsequently, an individual will avoid a food that parents don't eat. In this way, an aversion to toads can be transferred simply because parents don't eat that food type. The collection of a systematic, cohesive body of evidence to support or reject this hypothesis likely requires an experiment that captures the initiation of a conditioned taste aversion lesson, observations on the subsequent maintenance of an aversion and the cultural transference of this lesson onto offspring. Obtaining a robust amount of this information for an endangered species such as the northern quoll is logistically difficult (Galef et al. 2001).

In conclusion, our PVA highlighted that the deployment of toad-aversion baits could help to mitigate the impact of cane toads on northern quolls but would require high cultural transmission and a moderate efficacy of CTA training. Our findings should sound a note of caution regarding deployment of CTA in the landscape; we propose careful attention should be paid to ensure that high CTA

training efficacy (i.e. bait uptake by a high proportion of the population) is possible

to achieve, as a first step.  By focusing on a parameter ($E$) that researchers can

manipulate through baiting density, CTA management strategies should have a

higher likelihood of succeeding.

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# Chapter 3

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

## 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 approximately 61% of quolls consumed toad-aversion baits when first encountered.

Between 40-68% 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.

## Introduction

Invasive species are a major threat to biodiversity (Reaser, Meyerson et al. 2007, Woinarski, Burbidge et al. 2014). In Australia, species such as feral cats (*Felis catus*) (Legge, Murphy et al. 2017), domestic dogs (*Canis familiaris*) (Doherty, Dickman et al. 2017), foxes (*Vulpes vulpes*) (Short and Smith 1994, Risbey, Calver et al. 2000) and cane toads (*Rhinella marina*) (Burnett 1997, Letnic, Webb et al. 2008, Jolly, Shine et al. 2015) all have serious impacts on native species. Controlling these species at a landscape scale, however, has proved extremely difficult (Ziembicki, Woinarski et al. 2015, Tingley, Ward-Fear et al. 2017).

Because of this, increasing attention is being paid to mitigating the impact of invasives, rather than supressing their populations (Simberloff, Martin 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, Phillips et al. 2007, Tingley, Phillips 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, Crossland 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 and Archer 1975, Webb, Shine et al. 2005, Smith and 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 and Shine 2005, Llewelyn, Phillips et al. 2011). In the short term, some marsupial predators rapidly learn to avoid toads as prey (Webb, Brown et al. 2008, Webb, Pearson et al. 2011, Ujvari, Oakwood 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, Pearson et al. 2016, Ward-Fear, Thomas et al. 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 and Bird 1984, Conover 1995, Cohn and MacPhail 1996, Bernstein 1999, Mappes, Marples et al. 2005, Page and Ryan 2005, Glendinning 2007). With CTA, animals acquire an aversion to a referent food as a result of a nauseating experience (Gustavson and

Nicolaus 1987). Agriculturalists and wildlife managers have used conditioned taste aversion to reduce wildlife damage to crops, industry, or livestock (Gustavson, Garcia et al. 1974, Ellins and Catalano 1980, Avery 1985, Provenza, Burritt et al. 1990, Ternent and Garshelis 1999, Smith, Linnell et al. 2000). CTA has also been used successfully to reduce predation on native or introduced wildlife (Nicolaus and Nellis 1987, Conover 1989, Nicolaus, Herrera et al. 1989, Semel and Nicolaus 1992, Avery, Pavelka et al. 1995, Bogliani and Fiorella 1998, Cox, Baker et al. 2004), or ameliorate the impacts of invasive species (O'Donnell, Webb et al. 2010, Ward-Fear et al. 2016, Ward-Fear et al. 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 and 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 realised. 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, Spencer 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). 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 et al. 1984, Avery et al. 1995, Fairbridge, Anderson et al. 2003, Glen and Dickman 2003, Claridge and Mills 2007, Jolley, Campbell 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 generalise 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 15g of minced skinned adult cane toad legs, 1 whole cane toad metamorph weighing <2g, and 0.06g of Thiabendazole (per sausage; dose rate less than 300mg/kg adult quoll body weight, determined by the smallest – 200g – 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, Stoerk et al. 1965). It is orally-effective and regarded as relatively safe, producing low mammalian mortality: oral $LD_{50}$ is 2.7g/kg body weight (Dilov, Chaleva et al. 1981). It is fast acting and peak concentration occurs in the plasma one hour after consumption (Tocco, Rosenblum et al. 1966). Thiabendazole has produced strong aversions to treated foods in lab rats (Gill, Whiterow et al. 2000, Massei and Cowan 2002), wolves (*Canis lupus*) (Gustavson, Gustavson et al. 1983, Ziegler, Gustavson et al. 1983), and black bears (*Ursus americanus*) (Ternent et al. 1999). Thiabendazole induces a robust CTA after a single oral dose (Nachman and Ashe 1973, O'Donnell

et al. 2010) and is physically stable at ambient conditions in the bait substrate (Gill et al. 2000, Massei, Lyon et al. 2003).

<u>*Captive trials*</u>

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 Park, Northern Territory. Animals (9 male and 9 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 15x15x10cm 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 3 nights. Experiments began at sunset and ran for on average 2 hours. The response was filmed using a GoPro Hero 3 White camera (GoPro Inc, San Mateo, California, USA).

<u>*Field trials*</u>
*Study area*
The field study was conducted between May 2016- 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; Figure 3-1).  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 approximately 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 approximately 9 km north-east of Site 1. Sites were

selected based on the detection of quolls in the Australian Wildlife Conservancy's

fauna surveys (AWC unpub. data).  At the time of the study, toads were yet to arrive at our sites; hey subsequently arrived by March 2017.
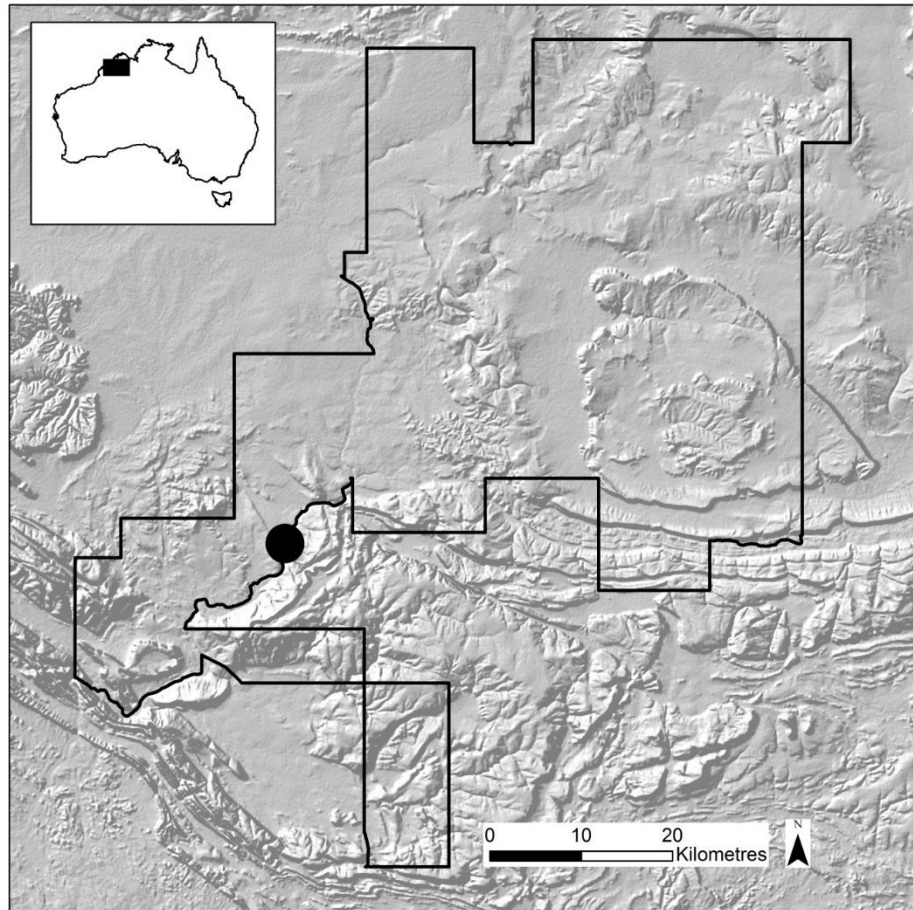


**Figure 3-1**: Location of the study area within Australian Wildlife Conservancy's Mornington Wildlife Sanctuary, in the central Kimberley, Western Australia.

*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 approximately five months apart. Sessions recorded up to 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 50-80m apart in a linear transect along a gorge wall where the presence of *D. hallucatus* was previously confirmed (AWC, unpub. data). 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 approximately 1m from the ground and aligned to face directly downwards (Diete, Meek 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 4 bait events within any given session) whereby bait stations were rebaited with fresh CTA bait and the old bait removed (Table 3-1). A total of 513 individual cane toad sausages were deployed over the period of study.

**Table 3-1:** CTA sessions and bait events, † denotes empty cells. 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.

| 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 | Nov | 1 | 31/10/17 | 1/11/17 | 2/11/17 | † | 20 |
| RP | 2017 | Feb | 3 | 3/2/2017 | † | † | † | 20 |
| RP | 2016 | May | 3 | 10/5/16 | 13/5/16 | 21/5/17 | † | 20 |
| RP | 2016 | Sept | 3 | 15/9/16 | 16/9/16 | 17/9/17 | † | 20 |
| SJ | 2017 | Feb | 1 | 3/2/2017 | † | † | † | 20 |
| SJ | 2016 | May | 3 | 10/5/16 | 13/5/16 | 21/5/17 | † | 20 |
| SJ | 2016 | Sept | 4 | 15/9/16 | 16/9/16 | 17/9/17 | 19/9/16 | 20 |
| TC | 2017 | Feb | 1 | 3/2/2017 | † | † | † | 33 |
| TC | 2016 | May | 3 | 10/5/16 | 13/5/16 | 21/5/17 | † | 20 |
| TC | 2016 | Sept | 3 | 15/9/16 | 16/9/16 | 17/9/17 | † | 20 |

_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, Gustavson, Kelly et al. 1976, Gustavson 1982, Gustavson

and Basche 1983, Gustavson et al. 1983, Ziegler et al. 1983, Gustavson et al. 1987,

Nicolaus 1987, Nicolaus et al. 1987, Nicolaus et al. 1989, Schneider and Pinnow

1994, Smith et al. 2000, Riley and Freeman 2004, Sevelinges, Mouly et al. 2009,

O'Donnell et al. 2010, Thornton and Raihani 2010, Thornton and Clutton-Brock

2011). More relevant to this study is the outcomes of previous trails by O'Donnell

et al. (2010) and Kelly et al. (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, 2016).

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 minutes 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 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 1) which species were attracted to bait, and 2) 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, Ashby 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, Vance 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 four months) as independent with regard to quoll ID and behaviour. This decision was made for logistical reasons (difficulty of identifying individuals using spot ID) but is 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, df = 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 ($\bar{x}$ = 60.58 seconds, 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 approximately sixty 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 3-2; Figure 3-2).

**Table 2-2:** Mean ($\bar{x}$) time 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 (sec) | Time sniffing toad (sec) | Time investigating toad (sec) | Time attacking toad (sec) |
|---|---|---|---|---|
| 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 |
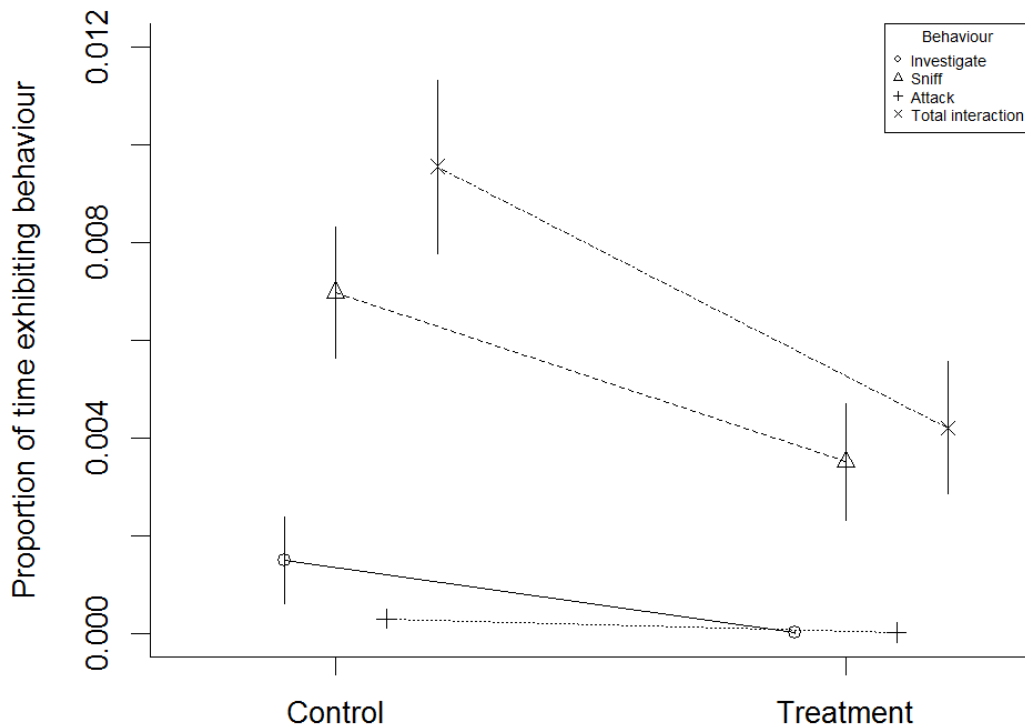
**Figure 2-2:** Mean proportion of active time that quolls spent directed towards toads. Behaviours are split into categories and across control and treatment groups. Error bars represent bootstrap standard errors.

*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 (Figure 3-3). Almost all bait removal was executed by quolls that took 65 baits of the 90 baits removed. Other species took far fewer: *Zyzomys argurus,* 9;

*Ctenotus spp.*, 2; *Pseudantechinus ningbing*, 2; *Varanus glauerti*, 2; and *Varanus glebopalma*, 2.
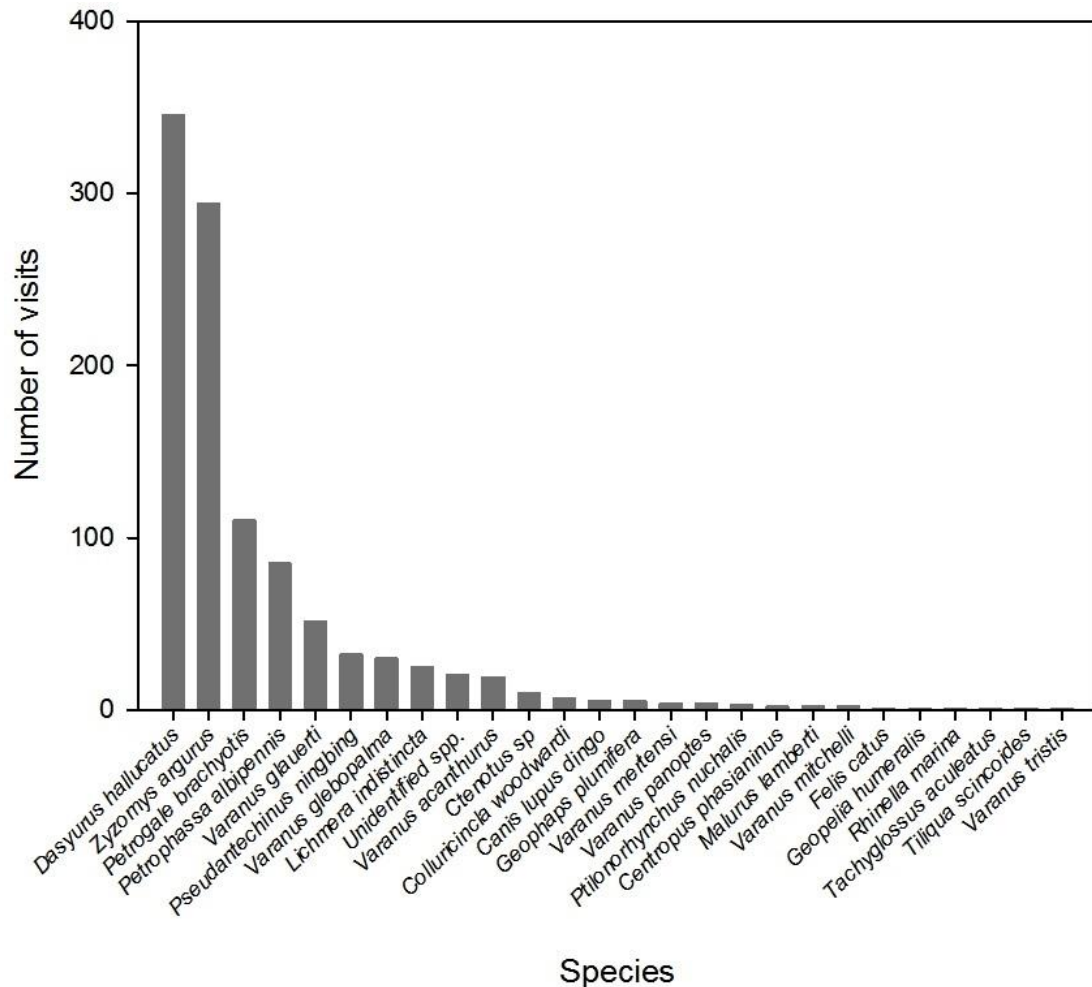


**Figure 3-3**: Frequency of visits to CTA bait stations by each species. Unidentified species group comprises unidentified rodents, birds, and frogs.

*Target uptake and training rates*

First pass uptake responses to the bait did not vary systematically across sessions ($\chi^2$=1.7, df=4, p=0.79; Figure 4-4).  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 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.
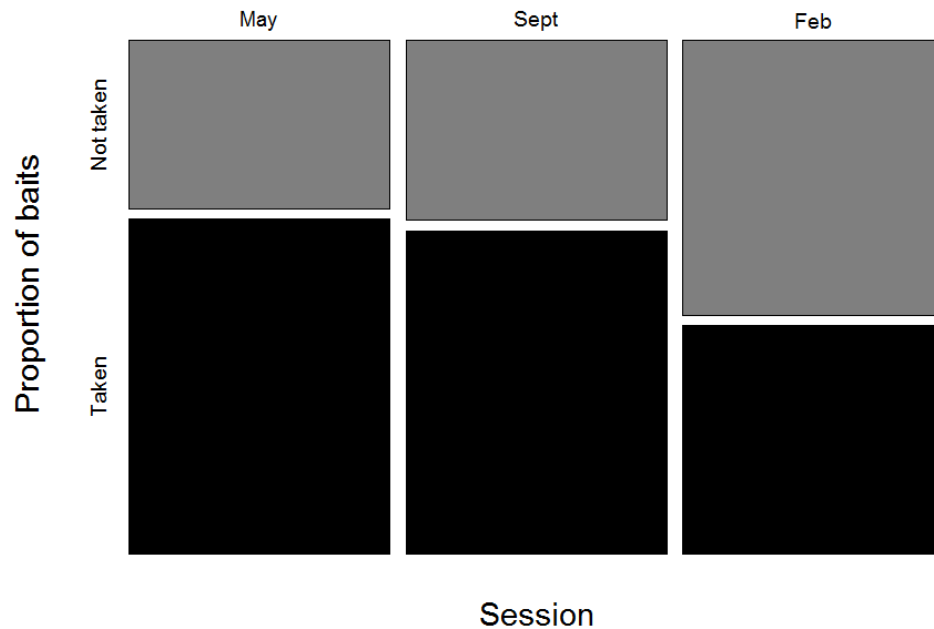
**Figure 4-4**: Frequency of visits to CTA bait stations by each species. Unidentified species group comprises unidentified rodents, birds, and frogs.

## 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 generalised 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, Gifford et al. 2006, Dundas, Adams et al. 2014). It is more difficult to estimate the rate of successful training in the field, but it is likely that between 41-68% 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 <300mg/kg animal body weight in our cane toad sausages was relatively low compared to earlier work (400mg/kg in (O'Donnell et al. 2010, Cremona et al. 2017, Jolly, Kelly et al. 2017, Kelly et al. 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, 0.06g of TBZ in each sausage was calculated to provide 300mg/kg to the smallest adult quoll at our site; a 200g female.  Long-term trapping at the site (AWC, unpub. data) suggests that adult quolls in this population can reach more than 815g 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.

*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 generalised 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 occur in wild quolls as it has in captive quolls, thus will, as a consequence, be less inclined to attack cane toads. 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 five 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.

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# Chapter 4

# Unpleasant surprises: a preliminary field study testing the efficacy of a promising conservation action suggests it will not work.

Indigo, N., J. Smith, J. Webb, and B. Phillips

## Abstract

Toxic cane toads (*Rhinella marina*) are spreading across northern Australia and severely impacting naïve predator populations as they arrive. A decade of research effort has consistently shown that training predators to avoid toads using conditioned taste aversion (CTA) is both achievable and can significantly improve survival rates of predators in a toad-infested landscape. This research is most strongly advanced for a native marsupial predator, the northern quoll.

To determine whether large scale toad aversion baiting could effectively reduce toad impacts on northern quolls, we deployed training baits to seven quoll populations. Following the arrival of toads, we observed a 65% mean reduction in population size at our four control sites and a 94% mean reduction in our three treatment sites. These are precipitous declines, in line with evidence of toad impact from elsewhere. Unexpectedly, however, our results also clearly indicate a failure of aversion training to mitigate the impact of toads.

There are a range of possibilities why our trial failed, however, the most likely explanation for this failure is that aversion training decays rapidly, such that most animals have forgotten their lesson within 120 days post training. The result emerges from two underappreciated complexities in the system: decay of aversion, and individual variation in innate tendency to consume toads. These complexities have masked each other, and may have been unearthed through captive trials, but logistical and well-meaning ethical constraints prevented this from occurring. Our results call into question the utility of toad-aversion training at a landscape scale.

## Introduction

The journey from scientific idea to practical outcome is long and often bedevilled by surprises. In medicine, translational science – the theory and practice of taking an idea from bench to bedside – is now a distinct field, with some effort to formalise the steps in the journey so as to minimise risk, and monitor unexpected outcomes (Woolf 2008). In ecology, there is still a much more ad hoc approach to the translation from basic to applied research. This no doubt reflects the greater complexity of ecological systems, but also a greater tolerance to risk in applying new ideas. Nevertheless, because of this complexity, we can expect that outcomes will sometimes be unpredictable. When such outcomes occur, however, it is worth reflecting on whether they could, or should, have been elicited earlier in the process. Here we report the outcome of a large field trial designed to test an idea that had passed several important tests and, until now, looked to be a particularly promising strategy for conservation of an endangered species.

In Australia, cane toads (*Rhinella marina*) were introduced to Queensland in 1935; and have since rapidly expanded across the north of Australia (Phillips,

Brown et al. 2007). The toads carry with them a suite of defensive toxins –
Bufadienolides – that are unlike toxins possessed by native Australian animals. As
a result many of the vertebrate predators that prey on native anuran species,
including northern quolls (*Dasyurus hallucatus*), die after attacking or consuming
toads (Watson and Woinarski 2004, Smith and Phillips 2006, Woinarski, Oakwood
et al. 2008, Hayes, Crossland et al. 2009, Shine 2010). Northern quolls – a 520-
1000 g marsupial predator – are now listed as endangered under the EPBC Act
(Act 2011), and the primary reason for this is the toad invasion: abundant
anecdotal evidence tells of rapid declines in quoll populations upon the arrival of
toads (Burnett 1997), and several radiotelemetry studies demonstrate very high
mortality rates of quolls in the presence of toads (O'Donnell, Webb et al. 2010,
Cremona, Spencer et al. 2017, Jolly, Kelly et al. 2017). Unfortunately, meaningful
control of toads at a landscape scale in the wet-dry tropics is, to date, not possible
(Tingley, Ward-Fear et al. 2017).

In 2008, following trials in which planigales (*Planigale maculata*) – mouse-
sized relatives of quolls – learned to avoid cane toads (Webb, Brown et al. 2008),
the idea emerged that it might be possible to train quolls to avoid toads, and so
mitigate the toads' impact on quoll populations (O'Donnell et al. 2010). Early trials
on captive quolls confirmed that, by associating consumption of a small toad with a
nauseating experience (using a tasteless drug called Thiabendazole, TBZ), quolls
exhibited single-trial conditioned taste aversion (CTA), and avoided toads
immediately afterwards (O'Donnell et al. 2010). Radiotelemetry of 31 CTA trained
quolls in a toad-infested landscape confirmed that two mortalities occurred due to
cane toad consumption at least some of these trained animals survived four

months thereafter in the presence of toads. Monitoring intervals following this period were somewhat limited for technical and logistical reasons. (O'Donnell et al. 2010). Follow-up radiotelemetry studies in which control (untrained) groups were also released, confirmed a very large positive effect of training on survival (Jolly et al. 2017). A longer-term reintroduction and mark-recapture study also showed that some trained captive reared animals survived and reproduced, though a lack of control group (for logistical and ethical reasons) as well as an unexpected still-extant quoll population at the release site complicated interpretation (Cremona et al. 2017). Nonetheless, all initial tests were strongly encouraging, and efforts were then directed to applying the idea to train wild quolls at a landscape level. To do this required an effective CTA strategy that successfully trains at least 50% of the quoll population to avoid toads (Indigo, CJ et al. 2019), and a bait that could deliver a nauseating experience associated with salient toad-cues. Research showed that taste and smell of the meat were the salient cues in quolls (Kelly, Phillips et al. 2018), and a sausage containing minced toad and TBZ was shown to elicit conditioned-taste aversion to toads during pen-trials (Indigo, Smith et al. 2018). The toad sausage was also shown to be attractive to quolls in the field, and to generate single-trial conditioned taste aversion for 41-68% of quolls that took a bait (Indigo et al. 2018). Again, all results looked very promising.

Cane toads are continuing to spread rapidly across northern Australia (Urban, Phillips et al. 2007, Tingley et al. 2017), and, in 2016,toads were observed at our field site. Given the lack of robust long-term studies that explore the efficacy of toad aversion training, and given the technique looked to be very promising, we

took this opportunity to test our CTA baiting methodology in a before-after-control-impact (BACI) framework (Smith, Orvos et al. 1993). We quantify the change in population size from November 2015- November 2017; across seven independent populations of northern quoll, before and after cane toad arrival in four replicate control sites (where no cane toad sausages were offered), and three replicate treatment sites (where cane toad sausages were delivered). We had a strong expectation that our treatment sites would be buffered from impact and so retain many more quolls than our control sites. We expected our study would provide not only unequivocal support for the method, but also some of the first robust impact-controlled population-level evidence that quolls are severely impacted by toads.

## Methods

### *Study area*

Our field study was conducted between November 2015- November 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 (AWC (17°31'S, 126°12'E; Figure 4-1)). The sites are dominated by sandstone boulders creating varying topographic complexity along the perennial Fitzroy River (Figure. A-I-2 and Figure A-I-3). The rocky habitat is suitable for northern quolls short eared rock wallabies (*Petrogale brachyotis*), common rock rats (*Zyzomys argurus*) and dingoes (*Canis lupus dingo*). Spinifex grass (*Triodia epactia*) and *Eucalyptus brachyandra, Terminalia hadleyana, Vitex acuminata* and *Cochlospermum fraseri* are common along the gorge. On average, this area receives 788-900 mm of rain annually, most of which falls from November to April.

On average, this area receives 788 mm of rain annually, most of which falls from November to April.

We worked at seven sites on the property along the Fitzroy River and its main tributaries (Figure 4-1); sites were separated by >5km and selected based on the detection of quolls during AWC fauna surveys (AWC unpub. data, 2017).  At the beginning of the study, toads were yet to arrive at our sites; they subsequently arrived at all sites by March 2017.
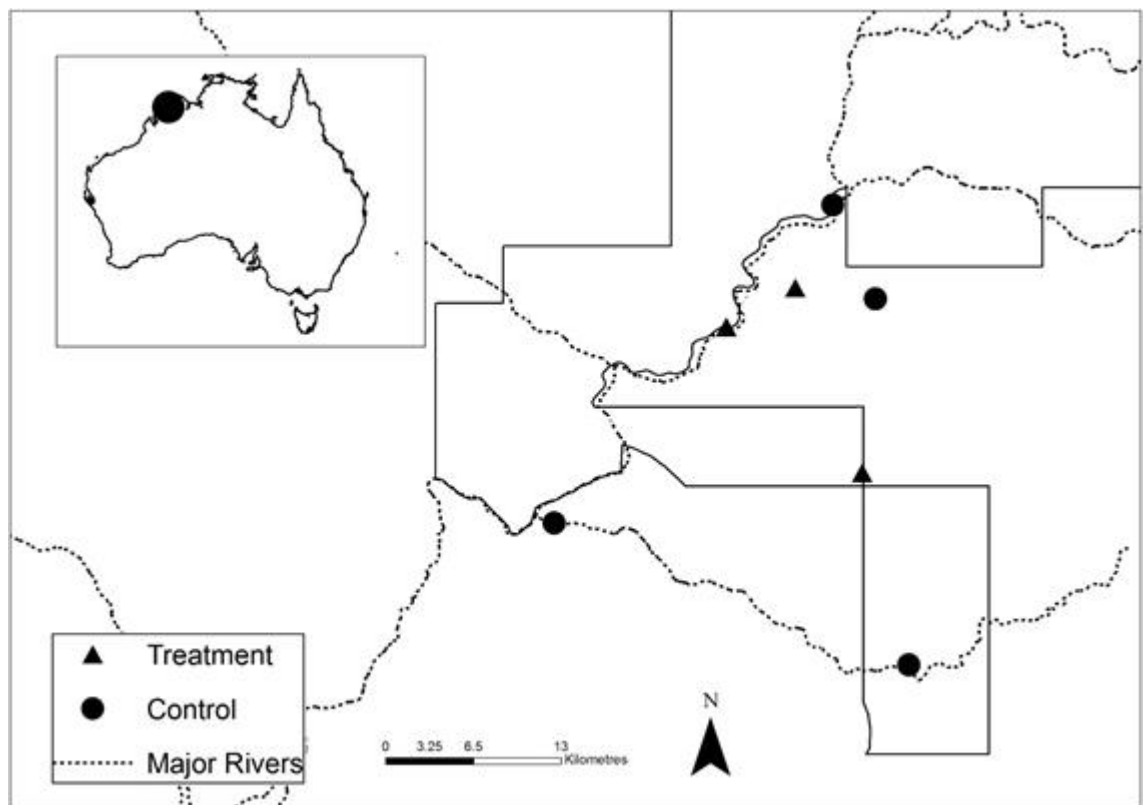


**Figure 4-1:** Location of control and Treatment monitoring sites within and adjacent to AWC's Mornington Wildlife Sanctuary, in the central Kimberley, Western Australia.

This study comprised two main components – population monitoring and CTA baiting sessions. These provide complementary approaches to determine whether large scale toad aversion baiting could reduce toad impacts on northern

quolls. In this study, "site" is defined as the location where we deployed an array of baits and cameras. "Bait station" is a location within a site where bait was offered. A "session" is a time interval when bait stations were active. Three CTA baiting sessions were conducted on treatment sites, approximately five months apart (Indigo et al. 2018). CTA baiting sessions recorded up to 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. Bait events occurred every three days as sausages decay after this, and at the same time within each site.

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 approximately 1m from the ground and aligned to face directly downwards (Hohnen, Ashby et al. 2013, Diete, Meek et al. 2016). Each cane toad sausage was placed inside a ring of powdered low toxicity insecticide (Coopex) to protect from ant spoilage. Currently no evidence exists to suggest ill effect or reduced interest in baits from quolls from the application of Coopex (Petit and Waudby 2013). Each session's bait stations were baited in 4 separate bait events within any given session (Table 4-2).

Cane toad sausages (bait) were made of 15g of minced skinned adult cane toad legs, 1 whole cane toad metamorph (weighing <2g), and 0.06g of TBZ (per sausage; dose rate less than 300mg/kg adult quoll body weight, determined by the smallest – 200g – adult seen at our study site), all packed into a synthetic sausage skin and deployed fresh. Bait uptake data collation methodology and results from the CTA baiting sessions can be found in Indigo et al. (2018).

*CTA Baiting Session*

Of the seven sites selected for the study, four were designated as control sites; where no cane toad sausages were deployed. The remaining three treatment sites were selected for CTA bait deployment (see Table 4-1). Sites were not assigned to treatments randomly: because Mornington is a conservation reserve, and the technique showed such promise, managers wanted sites with high quoll density to be treated, with less important sites relegated to the control group.  Cane toad sausages were delivered to treatment sites prior to the arrival of cane toads, at bait stations over three sessions from May 2016- February 2017. Each site contained 20-33 bait stations placed 50-80m apart in a linear transect along a gorge wall. Timing of baiting events are listed in Table 4-2.

**Table 4-1**: Timing of monitoring sessions. Number of consecutive nights that bait stations were active, month and year that that bait stations were open during the study.  Cane toad presence is denoted by 0 or 1; whereby the occurrence of cane toads = 1 and the absence of toads = 0. The number of nights each bait station was active, and the number of bait stations remained the same within sites, although differed between sites.

| Type | Site Number | Month | Year | Toads present | # Consecutive nights bait stations active | # Bait stations |
|---|---|---|---|---|---|---|
| Control | 1 | Nov | 2015 | 0 | 7 | 20 |
| | | Nov | 2016 | 0 | 32 | 20 |
| | | May | 2017 | 1 | 9 | 20 |
| | | Nov | 2017 | 1 | 12 | 20 |
| Treatment | 2 | Nov | 2015 | 0 | 7 | 20 |
| | | May | 2016 | 0 | 7 | 20 |
| | | Sept | 2016 | 0 | 74 | 20 |
| | | Nov | 2016 | 0 | 35 | 20 |
| | | May | 2017 | 1 | 39 | 20 |
| | | Nov | 2017 | 1 | 12 | 20 |
| Treatment | 3 | May | 2016 | 0 | 8 | 20 |
| | | May | 2017 | 1 | 10 | 20 |
| | | Nov | 2017 | 1 | 10 | 20 |
| Control | 4 | Nov | 2015 | 0 | 42 | 20 |
| | | Nov | 2016 | 1 | 38 | 20 |
| | | May | 2017 | 1 | 9 | 20 |
| | | Nov | 2017 | 1 | 15 | 20 |
| Treatment | 5 | Nov | 2015 | 0 | 40 | 20 |
| | | May | 2016 | 0 | 7 | 20 |
| | | Sept | 2016 | 0 | 40 | 20 |
| | | Nov | 2016 | 0 | 12 | 20 |
| | | May | 2017 | 1 | 9 | 20 |
| | | Nov | 2017 | 1 | 15 | 20 |
| Control | 6 | Nov | 2015 | 0 | 7 | 6 |
| | | Nov | 2015 | 0 | 10 | 6 |
| | | May | 2017 | 1 | 8 | 6 |
| Control | 7 | Nov | 2015 | 0 | 10 | 6 |
| | | May | 2017 | 1 | 8 | 6 |

**Table 4-2**: Table replicated from Indigo et al. (2018). CTA baiting sessions and bait events, † denotes empty cells.  Bait events occurred at the same time within each site. % of animals treated in each session (Indigo et al. 2018) was calculated according to: bait uptake/$N_s$.

| Site Number | Session Year | Session Month | No. Bait Events (BE) | BE 1- date | BE 2- date | BE 3- date | BE 4- date | No. of bait stations | % of animals treated |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 2016 | May | 3 | 10/5/16 | 13/5/16 | 21/5/16 | † | 20 | 34.3 |
| 2 | 2016 | Sept | 3 | 15/9/16 | 16/9/16 | 17/9/16 | † | 20 | 26.3 |
| 2 | 2017 | Feb | 1 | 3/2/2017 | † | † | † | 20 | † |
| 3 | 2016 | May | 3 | 10/5/16 | 13/5/16 | 21/5/16 | † | 20 | 43.3 |
| 3 | 2016 | Sept | 4 | 15/9/16 | 16/9/16 | 17/9/16 | 19/9/16 | 20 | † |
| 3 | 2017 | Feb | 1 | 3/2/2017 | † | † | † | 20 | † |
| 5 | 2016 | May | 3 | 10/5/16 | 13/5/16 | 21/5/16 | † | 20 | 45.6 |
| 5 | 2016 | Sept | 3 | 15/9/16 | 16/9/16 | 17/9/16 | † | 20 | 19.5 |
| 5 | 2017 | Feb | 1 | 3/2/2017 | † | † | † | 33 | † |

*Population Monitoring Session*

All sites were monitored at the same time within each session. Monitoring was conducted either with 20 or 6 bait stations (Table 4-1). Bait stations were active for seven to eleven nights (Table 4-1). Bait consisted of peanut butter (Kraft, Australia) and mackerel in brine (Homebrand. Australia Ltd, Australia) (Austin *et al.* 2017) smeared onto a surface under a single camera trap (White flash and Infrared Reconyx Motion Activated, (HP800, U.S.A). Cameras were set in the same manner as described previously (CTA baiting session). We conducted monitoring sessions seasonally, in the dry and wet season; both pre-and post-cane toad

arrival. Twenty-seven monitoring sessions were conducted over the period of study.

<u>Statistical Analysis</u>

During both CTA baiting and monitoring sessions we identified individual quolls that visited bait stations by their unique spot patterns (Hohnen et al. 2013) to determine visitation rate of individuals. To do this we employed Wild ID (V1.0, January 2011) (Bolger, Vance et al. 2011), the program calculates a matching score that characterizes the goodness of fit between two images, these scores are then ranked. Two independent observers conducted manual checks of each match. Quolls were identified to individual within each site and each session, and we treated each site (separated by 5km) as independent regarding quoll ID and behaviour. This decision was made for logistical reasons (difficulty of identifying individuals using spot ID across sessions) but is supported by exploratory analysis of first pass uptake rates showing that these do not vary systematically with session (see Chapter 3 Results).

Our observations consist of a sighting history for each observed individual over the number of nights for each camera-trapping session at the site. We denote the number of individuals at site $s$ at session $k$; $N_{sk}$. To estimate $N_{sk}$ we used a closed population mark-recapture analysis in which each individual (denoted $i$) was either observed, or not, according to a Bernoulli distribution:

$$O_{istk} \sim \text{Bernoulli}(d_t)$$

Detection rate of individuals declines within seven days (Smith, Legge et al. 2017), previous experience suggests that this is because quolls rapidly habituate to

novel items in their environment (Diete et al. 2016, Smith et al. 2017). Thus, we assumed detection probability ($d_t$ driven by attraction to bait stations) declines over time, $t$, within each trapping session (Otis, Burnham et al. 1978). We assumed that all individuals had an equal initial detection probability, and that this declined over time according to:

$$\text{logit } (d_t) = \mu_d + \beta t$$

Where $d_t$ denotes the expected detection probability at time $t$ within a baiting session, and $\mu_d$ is the expected detection probability at $t = 0$, $\beta$ is the change in log odds of detection over time.

We used the "data augmentation" method (Tanner and Wong 1987, Royle, Dorazio et al. 2006, Royle, Dorazio et al. 2007, Kery and Schaub 2011) in combination with this detection probability, to estimate $N_{sk}$ for each site. Under this approach, the data are 'padded' by adding an arbitrary number of zero-only encounter histories of 'potential' unobserved individuals. The augmented dataset is modelled as a zero-inflated model (Royle et al. 2006, Royle et al. 2007) and changes the problem from estimating a count, to estimating a proportion. This was executed by adding a latent binary indicator variable, $R_{isk}$, to classify each row in the augmented data matrix as a 'real' individual or not, where $R_{isk} \sim$ Bernoulli($\Omega_{sk}$). The parameter $\Omega_{sk}$ is estimated from the data, and $N_{sk} = \sum_{i(sk)} R_{isk}$.

We then made $\Omega_{sk}$ a function of toad presence/absence, $r_c$, and baiting treatment $b_j$, (cane toad sausages, or none):

$$\text{logit } (\Omega_{sk}) = \mu_p + r_c + b_j + \gamma_{cj} + w_d + x_h$$

where $\gamma_{cj}$ represents the interaction between toad presence/absence and treatment (and is the main effect of interest in our analysis). We also expected that population size would differ between seasons, $w_d$. This was considered as a two factor level fixed effect, to account for the marked fluctuation in quoll population size as a result of die-off of male northern quolls following breeding (Oakwood, Bradley et al. 2001). The two seasons were defined as running from January-July and August-December. Finally, population size would also differ between years, so we included this as a random effect $x_h$.

As well as these model effects, we estimated the percent change in mean population size between the two treatments. This is representative of the percent reduction in mean population size at control and treatment sites, rather than absolute change. This proportion (denoted by $a$) was calculated within each treatment level according to:

$$a = (\frac{\bar{N}_2 - \bar{N}_1}{\bar{N}_1}) \times 100$$

Where $\bar{N}_2$ = the across-site mean number of individuals alive following the arrival of cane toads and $\bar{N}_1$, is the before-toad equivalent.

The model was fitted using Bayesian Markov Chain Monte Carlo (MCMC) methods within the package JAGS (Plummer 2016) using Program R (R Core Team 2019). We used minimally informative priors (available in Table 4-3) except for the variables $\mu_d$ and $\beta_d$. For these variables we had prior information from Indigo et al. (2018), on initial detection probability ($\mu_d$) and the effect of time on detection probability ($\beta_d$). This prior information came from a near-identical camera trap

array methodology executed on a population of quolls 180 km to the north of the current study site.

Parameter estimates were based on 100,000 iterations with a thinning interval of 5 following a 10,000-sample burn-in. Three MCMC chains were run, and model convergence assessed by eye, and using the Gelman-Rubin diagnostic (Gelman and Rubin 1992, 1992) .

**Table 4-3:** Model parameters and their priors including prior distributions, standard deviation, estimated posterior means and their 95% credible intervals. † denotes empty cells. N denotes normal probability distribution; U denotes uniform probability distribution.

| Model Parameters | | | | |
|---|---|---|---|---|
| **Name for parameter** | **Parameter** | **Distribution (Priors, SD)** | **Posterior mean** | **95% CI** |
| Intercept for detection | $\mu_d$ | N (-0.43, 38.77) | 0.23 | 0.04, 0.29 |
| Intercept for Omega | $\mu_P$ | N (0,1000) | -2.26 | -3.41, -1.89 |
| Slope of time effect on detection | $\beta_d$ | N (-0.23, 644.5) | -0.26 | -0.30, -0.25 |
| Toads [present] | $r_c$ | N (0, 1000) | -1.24 | -2.30, -0.88 |
| Treatment [baited] | $b_j$ | N (0, 1000) | 1.94 | 0.76, 2.31 |
| Interaction [toads present * treatment] | $\gamma_{cj}$ | N (0,1000) | -2.34 | -3.53, -0.41 |
| Season (majority female population) | $w_d$ | N (0, 1000) | -0.07 | -0.62, 0.11 |
| Variation Site | $\sigma^2_s$ | Gamma (0.001, 0.001) | 0.51 | 0.022, 0.58 |

# Results

A total of 513 individual cane toad sausages were deployed over the period of study. Bayesian mark-recapture population estimates ($N_{sk}$) and associated credible

intervals (CI = 95%) for each site are shown in Figure 4-2 (and listed in Table 4-4). Post hoc estimation of our baiting effectiveness, suggest we delivered baits to between 19-43% of quolls at each site over two sessions in the months before toads arrived (Table 4-2). Despite this effort, and our expectation that this would improve quoll survival, a strong and unambiguously negative interaction, -2.34 (95% credible intervals -3.89, -1.82), indicated that the impact of toad arrival was greater at the baited (treatment) sites.  This was true in both absolute and relative senses, with a 65% (21-77%) decline observed in control sites compared with a 94% (86-96%) decline at treatment sites (Figure 4-2).
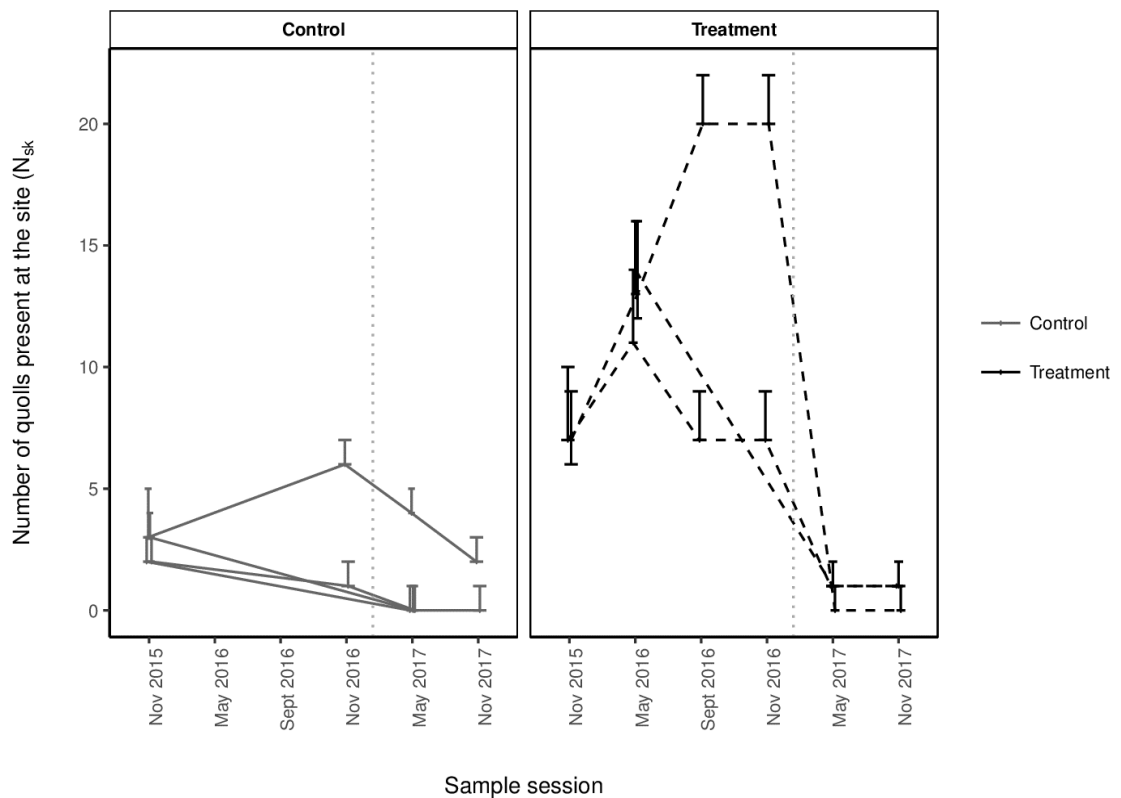


**Figure 4-2:** Posterior mean population sizes ($N_{sk}$) and 95% credible intervals for treatment and control populations of northern quolls at Mornington Wildlife Sanctuary before and after toad arrival (vertical dotted line). Estimates assume closure of the population within each session.

**Table 4-4**: Posterior mean population sizes ($N_s$ for each sampling session) and 95% credible intervals, assuming closure of the population during the time of the study.

| Posterior estimates | | | | |
|---|---|---|---|---|
| **Site #** | **Month** | **Year** | **$N_s$ (95% CI)** | **Toads present** |
| 2 | Nov | 2015 | 3.34 (3.0, 5.0) | 0 |
| 2 | Nov | 2016 | 6.21 (6.0,7.0) | 0 |
| 2 | May | 2017 | 4.08 (4.0,5.0) | 1 |
| 2 | Nov | 2017 | 2.07 (2.0,3.0) | 1 |
| 3 | Nov | 2015 | 7.66 (7.0,10.0) | 0 |
| 3 | Nov | 2016 | 7.46(7.0,9.0) | 0 |
| 3 | May | 2017 | 1.02(1.0,1.0) | 1 |
| 3 | Nov | 2017 | 1.01 (1.0,1.0) | 1 |
| 3 | May | 2016 | 11.60 (11.0,14.0) | 0 |
| 3 | Sept | 2016 | 7.46 (7.0,9.0) | 0 |
| 4 | May | 2016 | 13.83 (13.0,16.0) | 0 |
| 4 | May | 2017 | 1.03 (1.0,2.0) | 1 |
| 4 | Nov | 2017 | 1.03 (1.0,2.0) | 1 |
| 7 | Nov | 2015 | 2.10 (2.0,3.0) | 0 |
| 7 | Nov | 2016 | 1.03 (1.0,2.0) | 1 |
| 7 | May | 2017 | 0.04 (0.0,1.0) | 1 |
| 7 | Nov | 2017 | 0.03 (0.0,1.0) | 1 |
| 6 | Nov | 2015 | 6.95 (6.0,9.0) | 0 |
| 6 | Nov | 2016 | 20.39 (20.0,22.0) | 0 |
| 6 | May | 2017 | 0.04 (0.0,1.0) | 1 |
| 6 | Nov | 2017 | 0.03 (0.0,1.0) | 1 |
| 6 | May | 2016 | 13.14 (12.0,16.0) | 0 |
| 6 | Sept | 2016 | 20.40 (20.0,22.0) | 0 |
| 1 | May | 2017 | 0.04 (0.0,1.0) | 1 |
| 1 | Nov | 2015 | 2.15 (2.0,3.0) | 0 |
| 5 | Nov | 2015 | 3.13 (3.0,4.0) | 0 |
| 5 | May | 2017 | 0.05 (0.0,1.0) | 1 |

# Discussion

We monitored seven northern quoll populations pre- and post-cane toad arrival. Three of these populations were repeatedly treated with TBZ-laced cane toad sausages, which have been shown to elicit strong toad-aversion in captive quolls (Indigo et al. 2018). Sausages were delivered at a rate of roughly one to two sausages per hectare, over two to three bait-events, in the seven months prior to toad arrival, and our post-hoc estimates suggest that between 19-43% of the quolls at our sites took baits within each baiting session. We expect between 41-68% of these animals to have developed an aversion and so the impact of toads to be mitigated at the treatment sites (Indigo et al. 2018). We monitored these sites for an additional two sessions post toad arrival. Our data clearly demonstrate a decline in quolls associated with the arrival of toads, and also that the training did not effectively mitigate toad impact as expected (Indigo et al. 2018). Increasing population viability in the context of CTA was investigated by Indigo et al. (2019), it was predicted that CTA must be effective at high levels – with at least 50% of the population CTA trained – to have an influence on decreasing the probability of extinction post-cane toad arrival (see Chapter 2). Our post-hoc estimates of 19-43% of quolls taking baits suggests that our trial may have failed just by not effectively reaching enough animals. Further modelling, presented in Chapter 5, suggest there is competition for baits at high density sites. Our model indicated that a higher density of baits relative to quoll density was needed (Indigo, Smith et al. 2018).

Although we effectively baited 19-43% of the target animals at our treatment sites, these sites experienced a larger decline in both absolute and

percentage terms compared with control sites. We measured a 65% decline at control sites and a 94% decline at treatment sites. These are staggering, rapid declines, but are consistent with anecdotal reports of quoll population extinctions upon toad arrival elsewhere (Burnett 1997), as well as results from several studies showing that individual quolls typically die within a few days to a few months of sharing habitat with toads (Oakwood 2004, 2008, Woinarski et al. 2008, Jolly et al. 2017). Although our results, with regard to toad impact, are expected, ours is arguably the first study to robustly demonstrate population-level decline in quolls following the arrival of toads.

The unexpected result, however, is that our treatment sites appeared to fare worse upon toad arrival than the control sites. The interaction between treatment and toads is non-zero, but it is in the opposite direction to that expected. It is possible that the interaction is an artefact of the non-random assignment of sites to treatment and control groups – treatment sites started with more animals (Figure 4-2), and if both sets of sites end near zero, then there is an interaction – but the larger relative decline in the treatment sites argues against this being the sole effect. We conclude that baiting did not assist these populations, and, if anything, it had a negative impact.

Given that every relevant study before this one painted an encouraging picture of the efficacy of CTA (Webb et al. 2008, O'Donnell et al. 2010, Webb, Legge et al. 2015, Cremona et al. 2017, Jolly, Kelly et al. 2017, Kelly et al. 2018). We can exclude one obvious explanation – that the baits themselves kill quolls – because this dose of toad meat + TBZ has been given to more than 112 quolls (O'Donnell et al. 2010, Cremona, Crowther et al. 2017, Jolly et al. 2017, Indigo et al. 2018) with

no observed mortality. The $LD_{50}$ of TBZ in northern quolls was subject to investigation by the Australian Pesticides and Veterinary Medicines Authority (Permit: PER82262). In addition to this, the $LD_{50}$ of TBZ in rodents is more than an order of magnitude higher than the dose we use (Dilov, Chaleva et al. 1982). There is very little toad toxin present in the muscle tissue used to prepare these baits: indeed quolls have been fed the back legs of toads including the skin (where highest toxin concentrations are found) without ill effect (Kelly and Phillips 2018). Finally, there is no signal of decline in our populations between baiting sessions.

Another possibility is that our dose rate of TBZ was, in fact, too low. We administered 0.06 g of TBZ into each sausage. This was calculated to provide 300 mg/ kg to the smallest adult quoll at the site; a 200 g female; a dose that very effectively generates CTA in quolls (Webb et al. 2015, Indigo et al. 2018). Long-term trapping at the site (J. Smith AWC, unpublished. data, 2017) shows that adult quolls in this population can reach up to 815 g in weight. Thus, large animals could receive less than one quarter of the dose ingested by small animals. Consequently, it is possible that larger animals (typically males) were not effectively trained with this fixed-dose bait. For field use, a CTA agent should induce a robust CTA after a single oral dose (Nachman and Ashe 1973), and learned aversions may vary with the strength of the nominated CTA agent (Massei, Lyon et al. 2003). Animals receiving a dose that is too small may not be adequately trained. Designing a CTA bait for operational use that is attractive, palatable, shares a close association in taste and odour of the referent food, is safe for non-target species and induces an aversion across a range of body sizes after consuming a single portion is, of course, challenging (Conover 1995, Cagnacci, Massei et al. 2005, Mappes, Marples et al.

2005). Insufficient TBZ dose remains a possibility here, though we would expect that most females (the smaller, longer lived sex and the one most relevant to population growth) were dosed sufficiently to induce an aversion.

Another likely explanation for the negative outcome observed here, in our opinion, is that animals lose their learned aversion, and that this loss of aversion typically occurs within 120 days of training. We advance three lines of evidence for this. First, bait uptake rates do not appear to decline across baiting sessions. This was first noticed in the initial trials and deployment of the baits ((Indigo et al. 2018); this study) where bait sessions 3-4 months apart had very similar bait uptake rates. Although there was evidence that baited individuals would not come back for a second bait within sessions (Indigo et al. 2018), there was no evidence that the level of aversion to baits was higher (at the population level) when the site was re-baited 3-4 months later. If aversion was maintained for longer than 3-4 months, we would expect lower bait uptake rates in the second bait deployment, but these were not observed. Our second line of evidence comes from a small captive trial that due to very small sample sizes – 5 animals – has not been reported to date. Because of its importance here (and the difficulty of acquiring more data) we report on that trial in an appendix to this paper. In summary, 5 male quolls were taught, using TBZ-laced baits, to avoid an arbitrary referent novel food item (see Appendix III for details). For some weeks thereafter, the animals were offered the referent food and we recorded whether they consumed it. If we treat the time to consumption as a simple survival model, our best inference suggests that the proportion of individuals that remained averse decayed to zero by around 120 days post-training (Appendix III). Our third line of evidence is

highlighted by the timing of our baiting events and the arrival of cane toads to our sites. Cane toad sausages were delivered over three bait-events to treatment sites, with the last baiting deployment occurring in September 2016. Cane toads arrived at these sites between late December 2016/ early January 2017 (N. Indigo personal obs. 2017) leaving approximately 105-110 days between CTA sausage deployment and cane toad arrival. Thus, two independent lines of evidence suggest that aversion decays within 120 days, and our bait deployment occurred around 110 days prior to toad arrival, at a time when we now expect almost all individuals to have lost their aversion.

Decay and eventual extinction of a lesson is defined as decline in the frequency or intensity of a conditioned response following the withdrawal of reinforcement (Pavlov 1927). Such decay is expected, and occurs as a result of competition of complex neurobiological mechanisms that control behaviour (Berman, Hazvi et al. 2003). Reacquisition of a CTA lesson is a reconditioning process (Gustavson, Garcia et al. 1974): the original lesson may be renewed and recovered rapidly (Bouton 2004); either spontaneously (Pavlov 1927), or through reinstating of the training regime (Bouton 1993). An implicit assumption of conditioned taste aversion training via sausage baits; is that the arrival (exposure) of cane toads to a population would constitute reinstating the reconditioning process.  Additionally, decay may also occur faster in field situations when food is scarce: Gustavson et al. (1974), for example, found coyotes began to attack referent food to which they had previously demonstrated an aversion. This was contradictory to results from pen trials, where such rapid decay had not been observed, suggesting the increased decay rate was due to limited food resources in

the field. If our contention is correct – that the lesson is lost by quolls within 120 days – then it also must be true that the arrival of toads does not constitute an appropriate re-training opportunity.  Indeed, our population-level results suggest that, if anything, previous toad-aversion training may render an individual more susceptible to the arrival of toads.

There is much evidence that conditioned-taste-aversion (CTA) is a powerful tool for animal training (Conover 1995, Bernstein 1999, Mappes et al. 2005, Glendinning 2007), but its application in the field can clearly yield different outcomes relative to captive trials. In our case, if learned aversion to toads is rapidly lost, and if toads do not constitute a re-training opportunity, then the efficacy and value of CTA for mitigating the impact of toads on quolls becomes questionable in a landscape-level deployment.  Given this, we suggest that re-training (with baits) would need to occur regularly (1-2 monthly intervals). Although such re-training also increases the risk that animals simply learn not to eat the bait, and dissociate baits from toads (Webb et al. 2008).  To avoid disassociation timing of training needs to be precise regarding 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.

While CTA offers a plausible management intervention and has found some application already in reintroduction programs (e.g., Griffin, DT. et al. 2000, Moseby, Carthey et al. 2015, Scheibel, Dembkowski et al. 2016), it is clear that the rate at which aversion decays is a key variable in these and future applications.

There is currently intense interest in training native Australian predators (ranging from quolls to reptiles) to avoid cane toads using CTA, (O'Donnell et al. 2010, Webb et al. 2015, Ward-Fear, Pearson et al. 2016, Jolly et al. 2017, Kelly and Phillips 2017, Tingley et al. 2017, Ward-Fear, Thomas et al. 2017), but knowledge about the rate at which CTA training decays is non-existent for most taxa. Ours is the first study to identify decay of aversion as a critical factor in these efforts.

We must now turn to why decay of aversion was not thoroughly considered before now. One possibility is that the time course of decay is beyond the time course of most of the captive and field experiments conducted. All of the published captive trials involve assessing aversion only days after training (O'Donnell et al. 2010, Cremona et al. 2017, Jolly et al. 2017, Indigo et al. 2018). Because the training was so successful at improving survival of quolls, the radiotelemetry studies only needed to follow individuals for a period of 10-42 days to demonstrate a very large positive effect of training on survival (O'Donnell et al. 2010). Apparent survival analysis demonstrated a significant difference between control and treatment groups. Trained toad smart animals survived twice as long as toad naïve females and toad-smart males survived five times longer than toad-naïve males (O'Donnell et al. 2010). In addition, anecdotal reports from park managers observed reintroduced toad smart northern quolls breeding at Mary River Park, NT (J. Webb, Pers. Comm. 2018).

The one long-term study, a mark and recapture study of CTA-trained quolls released into a toad-infested landscape, suggested that trained animals can survive for many months, and contribute offspring to the next several generations (Cremona et al. 2017). This study had no control (untrained) group. The lack of

control was not an oversight, but was a decision made on the strong evidence from previous studies that untrained animals would die, the inevitably small number of animals available, and the fact that this was an endangered species. Nonetheless, 30% of the trained female quolls in that study survived, and 42% reproduced, demonstrating that some captive quolls trained with small dead toads, and tested with a live toad stimulus retained their aversion to live toads for long time periods. Additional observations of radio-tracked quolls, and camera trap images, showed that quolls encountered toads during nocturnal foraging (J. Webb, Pers. Comm. 2018). Thus, it is possible that intermittent and brief encounters with toads prolonged the aversion (Haselgrove and Pearce 2003, Bouton 2004). Alternatively, quolls may have ingested smaller non-lethal toads that produce nausea, further reinforcing the aversion to large toads.  By contrast, in our field experiment, there were no conditioned stimuli that could prevent extinction of the aversion prior to the arrival of cane toads.

In earlier studies, CTA training was achieved using live toads (O'Donnell et al. 2010, Cremona et al. 2017, Jolly et al. 2017). This training CTA technique is in fact, quite different from our field study. For instance, in captivity, animals that were not successfully trained on the first occasion were offered additional training opportunities, until they were deemed to be trained (J. Webb, Pers. Comm. 2018). Thus, it is likely that individuals paired visual cues of a live toad with odour cues and associated this with the onset of illness (Bogliani and Fiorella 1998).  Animals trained with live toads may have developed cues that result in avoidance of prey at a distance (Clarke, Westbrook et al. 1979). Additionally, those captive trained were

released into a habitat where they encountered live toads daily, thereby reinforcing the aversion.

If an individual is genetically predisposed to avoid a particular food this is expressed through the exhibition of neophobic behaviour to novel prey items (Greggor, Clayton et al. 2014). Intriguingly, recent trials on toad-naïve quolls observed around 30% of untrained, toad-naïve quolls will not eat a toad in captivity, and that this natural aversion to cane toads has a genetic basis (Kelly et al. 2018). Thus, the results of previous studies (Cremona et al. 2017, Indigo et al. 2018) may have unwittingly revealed this innate variation in toad aversion in the population, rather than an effect of CTA training. Determining the proportion of innate variation in toad aversion behaviour that exists across the northern quoll population needs more attention in future studies.

Our study demonstrates a failure of a very promising technique – CTA training – to mitigate the impact of cane toads on northern quolls. Such unanticipated results highlight the challenges faced when testing a novel technique, particularly in complex ecological systems. On reflection, the surprise in this instance has been generated partly by the complexity of the system; partly by a failure to appreciate that training might not last beyond the time course of most of the initial trials; and partly by the logistical and ethical constraints attendant to working on an endangered species. Unexpected outcomes are inevitable, particularly in applied ecology, and future careful process should elicit them early. This study shows that translational process can be compromised for very real logistical, and ethical reasons in applied ecology, and this can be costly: causing surprises to lurk for longer than they should.

## Management Implications

Our study demonstrates a dramatic decline across seven populations of quolls associated with the arrival of toads. Additionally, we highlight three major suggestions that may increase the efficacy of CTA baiting to >0.5 during future baiting programs (Indigo et al. 2019). They are: 1) increasing the TBZ dose rate in each bait from 300mg/kg (dosed for a 200 g female);  to account for individuals within the population possessing a higher body weight 2) increase precision of timing with regards to delivering CTA baits; to 1-2 months prior to the arrival of the cane toad front line. 3) Increase the density of baits delivered to greater than 1-2 baits per hectare implemented here – this should result in almost all baitable individuals consuming a bait (Indigo et al. 2018).

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# Chapter 5

# Bangers and cash: Baiting efficiency in a heterogeneous population

## Abstract

The uptake of baits is a key variable in management actions aimed at the

vaccination, training, or control of many vertebrate species. Increasingly, however,

it is appreciated that individuals of the target species vary in their likelihood of

taking baits. To optimise a baiting program then, we require knowledge not only

on the rate of bait uptake, and how this rate changes with bait availability, but also

knowledge on the proportion of the target population that will take a bait.

The invasive cane toad (*Rhinella marina*) is a major threat to northern

quolls (*Dasyurus hallucatus*), which are poisoned when they attack this novel toxic

prey item. Conditioned taste aversion baits (cane toad sausages) can be delivered

in the field to train northern quolls to avoid toads.

Here we report on a large-scale field trial across eleven sites across one

large property in Western Australia. Camera trapping and statistical modelling was

used to estimate the proportion of baitable animals in the population, their encounter rate with baits, and the survival rate of baits in the environment.

Population estimates varied at each site from 2. 5– 16.8 quolls per site, resulting in a range across sites of 0.7– 4.6 baits available per individual. Approximately 64% of individual quolls were bait-susceptible and both encounter rate, and bait survival were very low, resulting in our baiting regime treating <30% of the bait-susceptible population.  Using our model parameters, we estimate that we would need to increase our bait density six-fold to treat all bait-susceptible individuals in the population. Without accounting for heterogeneity in bait-susceptibility, our model would suggest we would need a ten-fold increase in bait density. Thus, accounting for heterogeneity provides a more efficient baiting design.  Data and models such as ours provide wildlife managers with information critical to informed decision making and are fundamental to estimate the cost-efficiency of any baiting campaign.

## Introduction

Globally, many populations of wildlife are intensively managed, and the delivery of baits is an important management tool in this context (Bomford and O'Brien 1995). The uptake of doses of toxic or non-toxic compounds in baits is a necessity in the vaccination (Henning, Giorgi et al. 2017), training (Gentle, Massei et al. 2004, Cagnacci, Massei et al. 2005), control (Kirkpatrick and Turner 1985)  and eradication (Moseby, Read et al. 2011, Dundas, Adams et al. 2014, Johnston, Bould et al. 2014, Kimball, Stelting et al. 2016) of many vertebrate species.

A successful baiting program is one that is cost-effective and results in a large proportion of target individuals taking bait (Thomson 1986). Significant progress has been made with the technical aspects of delivering a baiting program, such that baiting can typically be achieved inexpensively and without complex tools or training (Avery, Pavelka et al. 1995). Aircraft, for example, can provide an efficient and fast means of distributing baits over large or otherwise inaccessible tracts of country (Thomson 1986). Success is mostly hampered by the attractiveness and palatability of baits or the willingness of individuals in the population to consume baits. It is increasingly appreciated, for example, that some individuals may be less bait-susceptible, because they are neophobic, bait-shy, or otherwise uninterested when their usual diet is abundant (Birch 1999, Francis, Szegda et al. 2003, Mappes, Marples et al. 2005, Kelly and Phillips 2017).

Estimating the heterogeneity across a population in the propensity to take baits is a useful first step in assessing a baiting program. A useful second step is to optimise bait delivery by determining the fewest baits required to achieve a given proportion of the population baited. These aims require data on bait uptake within the target population and how this changes with density of baits delivered into the landscape. Collection of these data is often logistically difficult and costly. As a consequence, management decisions are often made based on operator experience rather than empirical evidence (Cook, Hockings et al. 2010). Remote monitoring tools (such as camera trapping) offer a cost-effective means to acquire the relevant data, but these data do come with analysis challenges. We seek to estimate key parameters (such as the proportion of baitable individuals, the survival rate of

baits once deployed into the environment, and the effect of bait density on uptake probability) from mark-recapture data acquired from camera traps.

Cane toads were introduced in north-eastern Australia in 1935 and have since rapidly expanded across the north of Australia (Phillips, Brown et al. 2007). The toads carry with them a suite of defensive toxins – Bufadienolides – unlike toxins possessed by native Australian animals. As a result many vertebrate predators, including northern quolls (*Dasyurus hallucatus*), die after attacking or consuming toads (Webb, Shine et al. 2005, Smith and Phillips 2006, Hayes, Crossland et al. 2009, Shine 2010, Webb, Pearson et al. 2011). Northern quolls are now listed as federally endangered as a consequence of the toad invasion. The delivery of conditioned-taste-aversion baits (cane toad sausages) can, however, be used to train individual northern quolls to avoid toxic invasive cane toads (*Rhinella marina*) (Indigo, Smith et al. 2018). Conditioned-taste-aversion (CTA) is a powerful innate response that has evolved as a defence mechanism against poisoning (Conover 1995, Cohn and MacPhail 1996, Mappes et al. 2005, Page and Ryan 2005, Glendinning 2007) and results in an animal acquiring an aversion to a referent food as a result of a nauseating or unpleasant experience (Gustavson, Nicolaus et al. 1987). There is currently intense interest in training native Australian predators to avoid cane toads (O'Donnell, Webb et al. 2010, Webb, Legge et al. 2015, Ward-Fear, Pearson et al. 2016, Jolly, Kelly et al. 2017, Kelly et al. 2017). Previous research by Indigo et al. (2018) showed that quolls consuming a cane toad sausage reduced their attack behaviour towards, and overall interest in, cane toads. Importantly, bait uptake by quolls was also observed when baits were delivered under a realistic field scenario.

Conditioned taste aversion baiting on a landscape scale is a relatively new technology. Thus the importance of developing evidence-based predictions to guide decision makers is imperative (Jackson, Moro et al. 2007). In many ways, though, it is simply a new baiting technology, so methods developed for assessing CTA baits apply equally to any other bait. Here, we develop an analysis using camera-trap data to estimate the proportion of baitable individuals and how uptake probability changes with bait availability.

*Study Area*

The study was conducted in the Artesian Range section (c. 170,000 ha) of Charnley River – Artesian Range Wildlife Sanctuary (16°24'S, 125° 30'E, 300,000 ha), a property managed by the Australian Wildlife Conservancy (AWC) in the Kimberley region of Western Australia. We worked at eleven sites on the property (Figure 5-1); sites were selected based on the detection of quolls in the AWC's fauna surveys (AWC unpublished data, 2017). Each site was separated by at least 5 km to maximise independence between sites. At the time of the study, toads were yet to arrive at any of our sites.
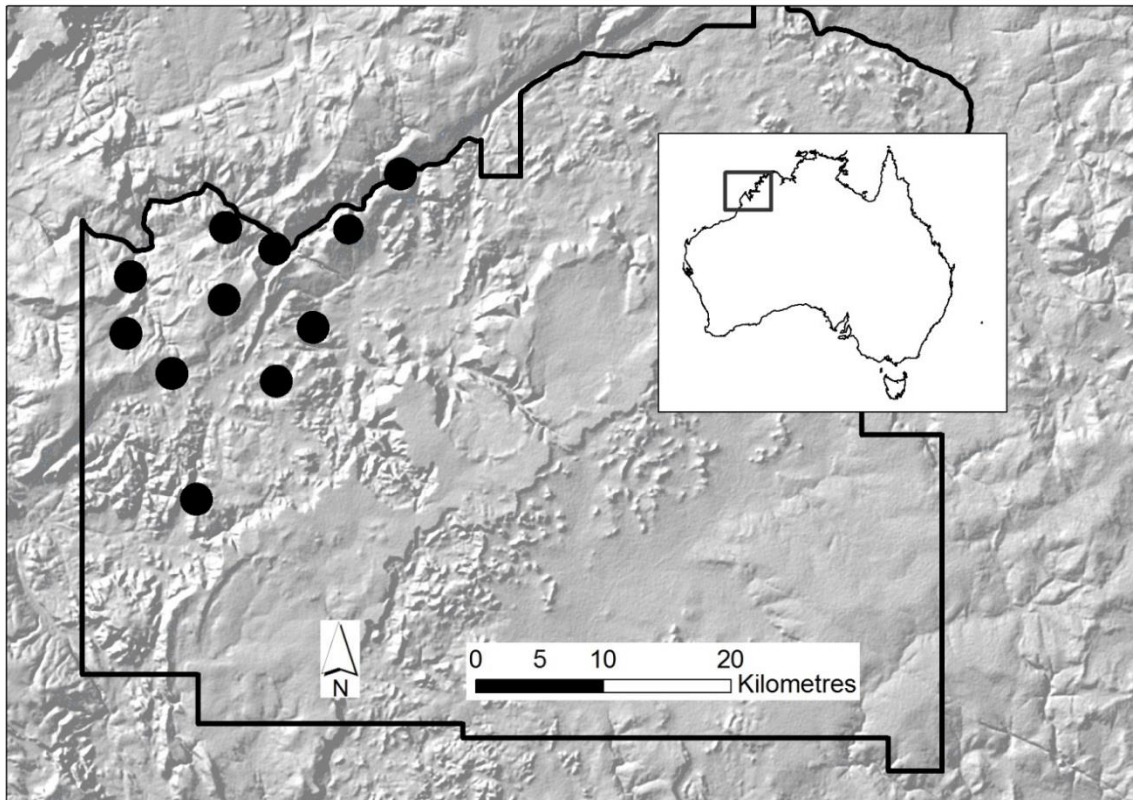
**Figure 5-1:** Location of the eleven sites and broader study area within Australian Wildlife Conservancy's Charnley River – Artesian Range Wildlife Sanctuary, in the Kimberley region, Western Australia.

## Methods

### *Cane Toad Sausages*

Cane toad sausages were made of 15g of minced skinned adult cane toad legs, 1 whole cane toad metamorph (weighing <2g), and 0.06g of Thiabendazole (per sausage; dose rate less than 300mg/kg adult quoll body weight, determined by the smallest – 200g – adult seen at our study site), all packed into a synthetic sausage skin and deployed fresh (Indigo et al. 2018). Thiabendazole is an inexpensive, broad-spectrum anthelmintic and antifungal agent (Robinson, Stoerk et al. 1965). It is orally -effective and regarded as relatively safe, producing low mammalian

mortality: oral $LD_{50}$ is 2.7g/kg body weight (Dilov, Chaleva et al. 1982). Thiabendazole induces a robust CTA after a single oral dose (Nachman and Ashe 1973, O'Donnell et al. 2010) and is physically stable at ambient conditions in the bait substrate (Gill, Whiterow et al. 2000, Massei, Lyon et al. 2003).

*Bait Stations*

In this study, "site" is the location where we deployed an array of bait stations and cameras. "Bait station" is a location within a site where bait was offered. A "session" is a time interval when bait stations were active. A total of one session was conducted over the course of the eight-day period. Each session recorded two "bait events", where new bait was placed unsecured at a bait station and the old bait removed. In this study, there were two baiting events - BE1 and BE2. BE1 (days 0-3) used a highly attractive non-toxic bait and was used to estimate population size at each site.  BE2 (days 4–7) used the bait of interest (a toad sausage intended to make an animal sick), and we used data from this second session to estimate bait uptake probability conditional on the population size estimated in BE1.

Each site contained 12 bait stations placed 100-120 m apart in a 4 × 3 grid plot arrays. A single camera trap (White flash and Infrared Reconyx Motion Activated, HP800, U.S.A) was placed at each bait station. To habituate quolls to the provisioning of bait in the first bait event (BE1), the bait was peanut butter (Kraft, Australia) and mackerel in brine (Homebrand, Australia Ltd, Australia) (Hohnen, Ashby et al. 2013, Austin, Tuft et al. 2017), available for four nights. In the second bait event (BE2), cane toad sausage baits were made available at the bait station for four nights.

Cameras were secured to trees or rocky ledges approximately 1 m from the ground and aligned to face directly downwards (Hohnen et al. 2013, Diete, Meek et al. 2016). Cameras were set to take five consecutive photographs for each trigger with no delay between triggers. Each bait was placed inside a ring of powdered insecticide (Coopex) to protect from ant spoilage. A total of 132 individual cane toad sausages were deployed across the 11 sites over the period of study.

### *Camera Trap Data Collation*

Images from bait stations were collated and for each individual pass we recorded the ID of the individual, and (in BE2) whether the bait was taken. We recorded which species (and individuals in the case of quolls) were attracted to bait, and which species (and individuals) took bait. We identified individual quolls visiting 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 software (Version 1.0, January 2011) (Bolger, Vance et al. 2011), which extracts 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 site, and we treated each site (separated by a minimum of 5km) as independent with regard to quoll ID and behaviour.

### Statistical Analysis

*The model*

We assume that there are two classes of individuals: those that will take a bait given the opportunity and those that will not. We denote the number of individuals at site $j$, $N_j$. We are interested in estimating the proportion of $N_j$ that are initially bait-susceptible, $z$, and the proportion of baitable individuals that take a bait under our baiting strategy, $u$. This latter proportion can be made a function of bait longevity, and baiting density (details below).

In our first baiting event (BE1), our observations consist of a sighting history for each observed individual quoll over four nights of camera-trapping at the site. In our second baiting event (BE2) we record when individuals at a site took a bait. To estimate $N_j$ we used a closed population mark-recapture analysis in which each individual (denoted $i$) was either observed or not according to a draw from a Bernoulli distribution:

$$O_{it_1} \sim Bernoulli(d_{t_1})$$

where $t_1$ indexes day within the first baiting session ($t_1 \in \{0,1,2,..3\}$). Previous experience with quolls showed that detection rate of individuals declines over time (Smith, Legge et al. 2017), thus we assumed that detection probability ($d_{t_1}$ driven by attraction to baited stations) declines over time according to:

$$\text{logit}(d_{t_1}) = \mu_d + \beta t_1$$

were $d_{t_1}$ denotes the expected detection probability at time $t_1$, $\mu_d$ is the expected detection probability at $t_1 = 0$, and $\beta$ is the change in log odds of detection over time.

We used the "data augmentation" method (Royle, Dorazio et al. 2007, Kery and Schaub 2011) in combination with this detection probability to estimate $N_j$ for each site. Data augmentation offered a flexible way to model patterns of detection probability in our closed populations (Kery et al. 2011). Under this approach, the data are 'padded' by adding an arbitrary number of zero-only encounter histories of 'potential' unobserved individuals. The augmented dataset is modelled as a zero-inflated version of the complete-data model (Royle et al. 2007) and changes the problem from estimating a count to estimating a proportion (that is, the proportion of the padded number of individuals that are real). This was executed by adding a latent binary indicator variable, $R_i$, to classify each row in the augmented data matrix as a 'real' individual or not, where $R_i \sim \text{Bernoulli}(\omega_j)$. The parameter $\omega_j$ is estimated from the data, and $N_j = \sum\{R_{i(j)}\}$. This process allowed us – using data from the first baiting event (four days) – to estimate population size at each site.

Our second baiting event – in which we deployed toad-sausage baits – was used to estimate the proportion of the population that took baits. The observation type here was an individual taking a bait on a particular day, $T_{i(j),t_2}$. We assumed this variable:

$$T_{i,t_2} \sim \text{Bernoulli}(u_{i,t_2})$$

where $u_{t_2}$ is the mean probability of a quoll taking a bait at each $t_2 \in \{0,1,2,3\}$ within the second baiting event. This probability is made conditional on the quoll being a 'real' individual ($R_i$=1) and being bait susceptible ($Z_i$=1), such that

if either of these conditions are not met, $u_i = 0$. We define a quoll's initial

susceptibility to being baited as a latent indicator variable:

$$Z_i \sim \text{Bernoulli}(z),$$

where $z$ is the probability that an individual is bait susceptible.

Under the condition of an individual being real and susceptible, $u$, is the

probability of two events: a quoll encountering a bait, E, and taking that bait, T.

Thus $u_{i,t_2} = P\left(E_{t_2} \cap T_{t_2} \mid R_i = 1 \cap Z_i = 1\right)$. The probability of event E is

determined by the number of baits in the landscape at a given time. Here we

assume an exponential decay of baits, such that the mean number of baits at t2:

$$B_{t_2} = B_0 s^{t_2}$$

where $B_0$ is the initial number of baits deployed (at $t_2 = 0$; $B_0 = 12$ in our field

trial), and $s$ is the per-day survival probability of baits. Assuming encounters are

independent, the probability that an individual encounter's at least one of these

baits within each time is thus,

$$P(E_{t_2}) = 1 - (1 - \tau)^{B_{t_2}}$$

where $\tau$ is the mean probability of an individual encountering any one of the $B$

baits. Given that we are only considering real, susceptible individuals, the

probability of taking a bait at $t_2 = 0$ is one: $P(T_0) = 1$. At $t_2 > 0$ however, we need

to account for the probability that our individual may have taken a bait in one of

the previous time intervals and so is no longer susceptible. If we assume that all

susceptible individuals encountering a bait take the bait, and avoid baits thereafter

(and dropping individual indices),

$$u_0 = P(E_0)$$

$$u_1 = P(E_1)(1 - u_0)$$

$$u_2 = P(E_1)(1 - (u_0 + u_1))$$

With the general expression:

$$u_{t_2} = P(E_{t_2})(1 - \sum_{k=0}^{t_2-1} u_k)$$

Our model contains parameters for detection probability – $\mu_d$ and $\beta$ – parameters
for estimating population size at each site – $\omega_j$ – and parameters that describe
uptake probability in terms of initial susceptibility to baiting, $z$, survival rate of
baits, $s$, and probability that an individual encounters a bait given it is present in
the environment, $\tau$.

The model described above is summarized in Figure 5-2 by a Directed
Acyclic Graph ($DAG$) (Clark and Gelfand 2006). These DAGs represent relationships
(arrows) between the observed data and the unknown parameters or hypotheses
of the model (nodes). The lines represent the relations and the hierarchy between
nodes. The nodes symbolise the data observed e.g. $O_{i,(j)t}$, parameters to estimate
e.g. s, $Z$, $B_t$. The model was fitted using Bayesian Markov Chain Monte Carlo
(MCMC) methods within the package JAGS (Plummer 2016) using Program R (R
Core Team 2019). We used minimally informative priors (see Table 5-1) except for
$z$. For this parameter we had prior information on the proportion of quolls likely to
take a bait from Indigo et al. (2018). We compared the modelled output with an
identical model, in which we substituted a minimally informative prior for $z$
(Figure 5-3).  Parameter estimates were based on 100,000 iterations with a

thinning interval of 5 following a 10,000-sample burn-in. Three MCMC chains were

run; model convergence was assessed by eye and using the Gelman-Rubin

diagnostic (Gelman and Rubin 1992).
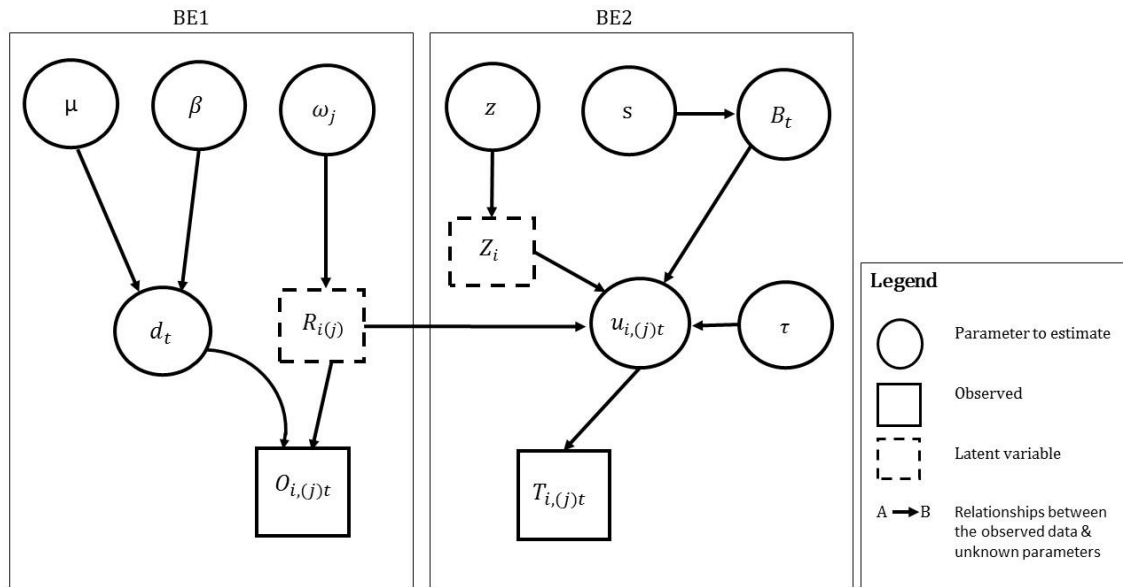


**Figure 5-2**: Directed Acyclic Graph (DAG) representing relationships (arrows)
between the observed data and the unknown parameters or hypotheses of the
model (nodes). The arrows represent the relations and the hierarchy between
nodes. The square nodes symbolise the data observed in our first and second
baiting events: whether and individual at site j was observed or not at time t
(within the first time period), $O_{i,(j)t}$; and whether an individual was observed to
take a bait at time t (within the second time period) $T_{i(j),t}$. Parameters $\mu_d$ and $\beta$ set
the detection probability ($d_t$) driven by attraction to baited stations, and $\omega_j$
estimates the proportion of our augmented dataset that represents real individuals
in the population (real or not indicated by latent variable $R_i$). Our second baiting
event – in which we deployed toad-sausage baits – was used to estimate the
proportion of the population that took baits. Here, the probability of an individual
quoll taking bait $u_{i,t}$ is conditional on the quoll being a 'real' individual $R_i = 1$, and
also being initially susceptible to baiting $Z_i = 1$. The time-dependence of u(t)
results from two processes: 1) the number of baits remaining at time t ($B_t$,
determined by s), and 2) the probability that an individual has already

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encountered a bait, has learned, and so is no longer susceptible (determined by the encounter rate, $\tau$).

**Table 5-1**: Model parameters and their priors including prior distributions, standard deviation, estimated posterior means and their 95% credible intervals. † denotes empty cells. N denotes normal probability distribution; U denotes uniform probability distribution.

| Name for parameter | Parameter | Distribution (Priors, SD) | Posterior mean | 95% CI |
|---|---|---|---|---|
| Probability of being baitable | z | N (0.63, 0.064) | 0.64 | 0.52, 0.77 |
| Intercept for detection (logit) | $\mu_d$ | N (0,1000) | 0.40 | 0.30, 0.50 |
| Slope of time effect on detection (logit) | $\beta_d$ | N (0,1000) | -0.20 | -0.40, -0.005 |
| Probability of bait being encountered by an individual | $\tau$ | U (0,1) | 0.018 | 0.009, 0.03 |
| Per-day survival probability of bait | $s$ | U (0,1) | 0.52 | 0.29, 0.78 |
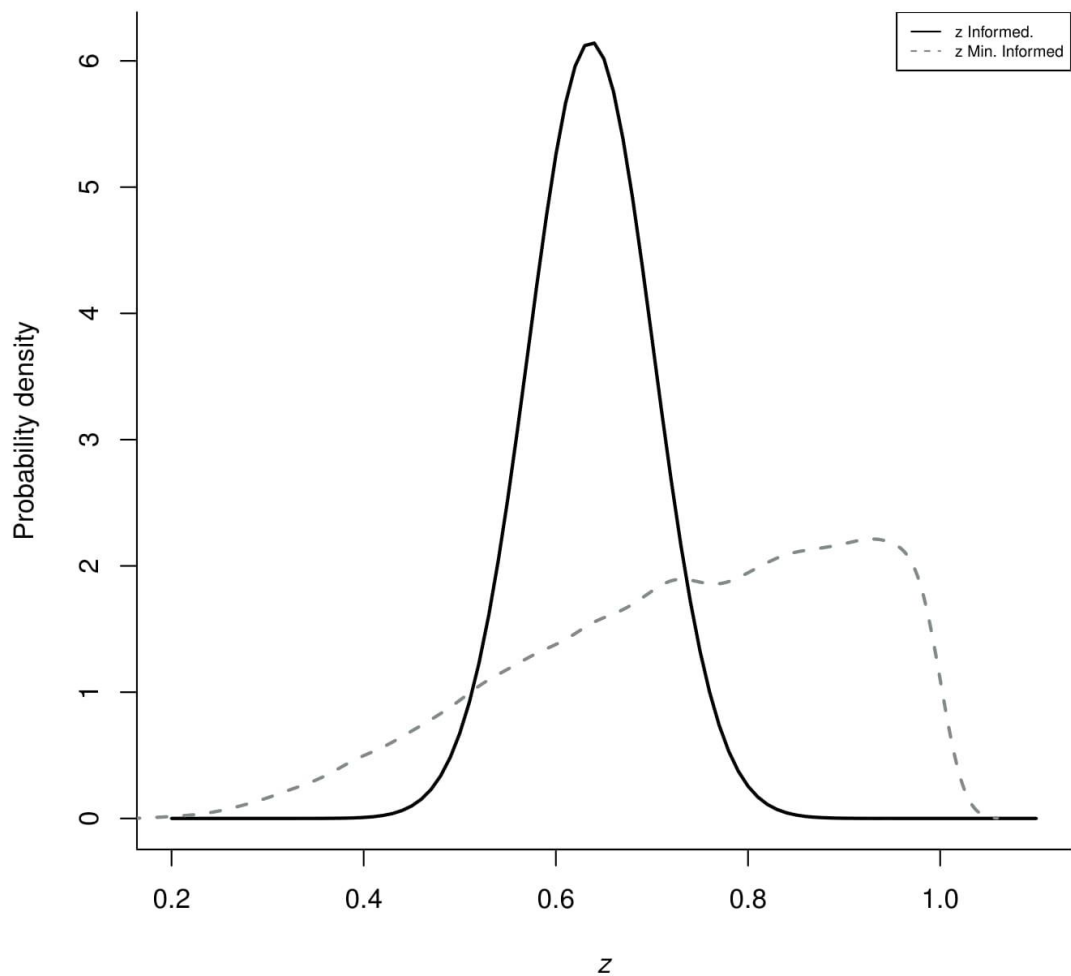
**Figure 5-3:** Posterior density distribution of *z* (probability of an individual being bait-susceptible) when z = minimally informed prior (dashed grey line), versus when z = informed prior (solid black line).

# Results

## *Individual quolls that visited bait stations and bait uptake*

Cameras at bait stations detected a total of 86 individual quolls across our 11 sites. During BE2 (in which sausages were deployed), bait stations were visited by 45

individual quolls. Of these 45 individuals, 21 encountered a cane toad sausage with the remaining 24 animals arriving at the bait station after baits had been taken by other species or other quolls. Of these 21 CTA bait-exposed individuals, 18 individuals took the bait (Table 5-2).

*Non-target CTA bait uptake*

A total of 23 species were identified investigating bait stations in BE2. Of these non-target species, bait was taken by Torresian crows (*Corvus orru; n = 6*), common rock rat (*Zyzomys argurus; n = 4*), northern brown bandicoot (*Isoodon macrourus; n = 4*), skinks (*Ctenotus spp.; n = 3*), golden-backed tree-rat (*Mesembriomys macrurus; n = 1*), and an unknown rodent species (*n = 1*).

*The model*

Bayesian mark-recapture population estimates ($N_j$) and associated credibility intervals (CI = 95%) for each site are listed in Table 5-2. Baiting at the nominal rate of 1 bait per 100m$^2$ resulted in 0.71-4.69 baits available per animal at $t_2 = 0$ (Table 5-2). The probability of an individual encountering an individual bait given that the bait was present in the environment was low ($\tau = 0.018$; 95% CI: 0.009– 0.03). The per-day survival probability of baits was also low, indicating very few baits available after the day of deployment (s = 0.51; 95% CI: 0.29– 0.78).

Overall, baiting at one bait per 100m$^2$ resulted in bait uptake by around 18% of quolls at a site. But the proportion of baitable quolls *z* was estimated to be 0.64 (95% CI: 0.52– 0.77). Thus, our baiting regime delivered baits to less than

30% of the bait-susceptible individuals at each site. Using parameters for bait survival and uptake probability indicated that baiting at the rate 72 baits per 7-ha site (i.e. six times more baits than we used) should result in essentially all baitable individuals taking bait within 5 days of bait deployment (Figure 5-4). By contrast, if we assume that all individuals are baitable, our model suggests we would need to deploy greater than 120 baits (ten times more than we used).

**Table 5-2**: Estimated population size and observed bait take for each population. Posterior mean population sizes ($N_j$ (Site 1: Site 11)) and 95% credible intervals, assuming closure of the population during the time of the study. † denotes empty cells.

| Site | $N_j$ (95% CI) | Baits taken by quolls | Baits taken -non-target spp. |
|------|----------------|------------------------|-------------------------------|
| 1 | 6.12 (5,9) | 1 | 1 |
| 2 | 12.07 (10,16) | 2 | 4 |
| 3 | 16.84 (14,22) | 1 | 3 |
| 4 | 2.57 (2,5) | 2 | 4 |
| 5 | 7.31 (6,10) | 2 | 2 |
| 6 | 15.63 (13,20) | 2 | 0 |
| 7 | 7.31 (6,10) | 3 | 2 |
| 8 | 8.50 (7,12) | 1 | 0 |
| 9 | 8.50 (7,12) | 2 | 1 |
| 10 | 0 | † | 0 |
| 11 | 3.74 (3,6) | 2 | 6 |
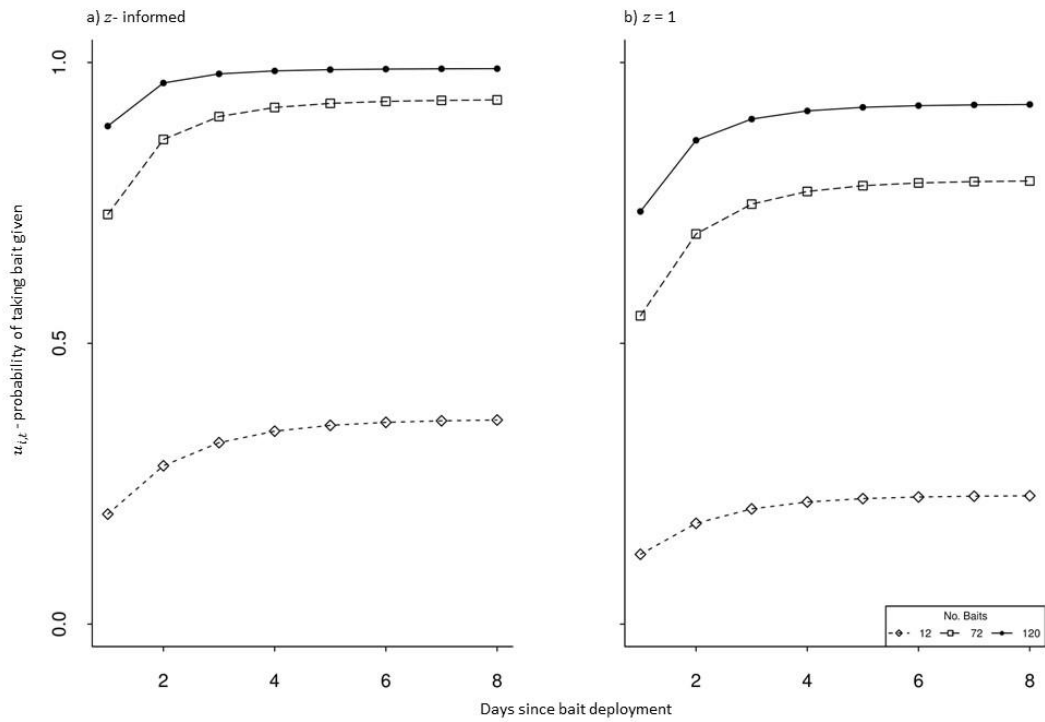
**Figure 5-4**: Modelled probability of bait take ($u_{i,t}$) given an individual is bait susceptible ($Z_i$ = 1). Curves show uptake probability over time (days since bait deployment) comparing two models, in which there is heterogeneity in baitability (a: z ~ 0.64) and no heterogeneity (b: z = 1). Series on each plot represent different initial bait density.

## Discussion

Here we have used standardised camera trap arrays across 11 sites to generate data on bait uptake, and how this varies with bait availability. We used these data (with some prior information) to estimate key parameters of interest: the proportion of a population that is baitable, encounter rates, and bait survival rate. These parameter estimates provide valuable information for managers considering a baiting program, allowing them to assess the likely outcome of a baiting program, optimise the baiting rate, and estimate costs. In our case, the

baiting program is to train wild quolls to avoid cane toads immediately prior to the arrival of cane toads in the landscape.

Under the conditions we examined we estimate that the best outcome achievable in our system is for us to deliver baits to 64% of the population. The other 36% of the population appear to not be bait susceptible. Most of our information on this parameter comes from our prior expectation of uptake rate, derived from a combination of field and captive trials by Indigo et al. (2018). We updated our prior value for $z$ – the variable representing the probability of being baitable – with these data, to obtain the posterior value of 64%. We observed only a small shift between prior and posterior for this parameter; thus, our new observations do not provide much additional information on this parameter, suggesting that it may be generally important to estimate this parameter using prior work, as we have done here. Certainly, heterogeneity in baitability should not be unexpected in wild populations. It could arise by innate variation in food preference (Birch 1999), but also through variation in boldness: some individuals may exhibit a neophobic response to bait as a consequence of genetic predisposition (Marples, Quinlan et al. 2007, Hoppitt and Laland 2013, Greggor, Clayton et al. 2014). These predispositions might have developed as a result of early learning, experience, interactions with the environment and the feeding preferences of adults (Birch 1999, Francis et al. 2003, Mappes et al. 2005, Hoppitt et al. 2013).

Irrespective of the mechanism, non-baitable individuals reduce the proportion of the population that are trainable with CTA. While 64% may seem a relatively poor level of maximum bait success, we can reasonably expect that some

fraction of the non-baitable animals may also innately avoid toads. Certainly there is evidence in other taxa that some level of innate avoidance of toads is present in toad-naïve predator populations, e.g., the common planigale (*Planigale maculata*) (Webb, Brown et al. 2008), red-cheeked dunnart (*Sminthopsis virginiae*) (Webb et al. 2011), terrestrial snake species (Phillips, Brown et al. 2003) and varanid lizards (Smith et al. 2006). There is also evidence that such innate avoidance may provide the raw material on which natural selection can act to generate a rapid adaptive response to toads (Phillips and Shine 2006, Smith et al. 2006, Llewelyn, Phillips et al. 2011, Somaweera and Shine 2012, Kelly et al. 2017). Clearly, then, this non-baitable fraction of the population is important, but the fraction of our non-baitable animals that will in fact avoid toads (rather than baits) remains an important avenue for future work.

Our analysis also gives us insight into an optimal baiting rate. We explicitly modelled a link between bait availability and bait uptake probability, an effect that has been demonstrated in lethal baiting programs (Christensen, Ward et al. 2013). Interestingly, some studies suggest that low bait availability can reveal further population heterogeneity; in foxes (*Vulpes vulpes*), it is the dominant individuals that access baits first, consequently reducing access to baits for other individuals within the population (Marks and Bloomfield 1999, Gentle et al. 2004). Our findings suggest that sub-optimal baiting rates can be avoided in our case by increasing baiting density to a minimum of 72 baits per site, resulting in a very high probability of bait take by the baitable fraction of northern quolls. We note, however, that our model treats per-bait encounter rate as independent of bait density. While this is a reasonable simplifying assumption (and was necessary

given we did not impose variation in bait density across sites), where there is competition for baits we might well expect per-bait encounter probabilities to increase with increasing bait density as, for example, dominant individuals are removed from the competition.

Our analysis also gives us insight into the density of quolls and how this varies within the landscape. Baiting at a rate of one bait per hectare in the northern Kimberley, and assuming an average population density, managers may reasonably expect bait uptake from only around 18% of the northern quoll population. Why such a low percentage? At a density of one bait per hectare, many quolls are simply not encountering the bait or arriving at the bait station after baits have been taken or become unpalatable. Deploying 72 baits, however, can be expected to treat almost all of the baitable quolls at a site because encounter probabilities become high enough that most individuals will encounter a bait on the night of deployment. Our parameters (bait survival and encounter rate) can also allow managers to optimise their efforts using not only bait density, but also bait survival. Bait density is clearly the simplest variable to adjust, but in other systems it may also be possible (and possibly more efficient) to adjust bait longevity as well.

An alternative strategy is to bait with a lower bait density, but on multiple occasions. Here, cost considerations suggest that increasing bait density (rather than number of sessions) may often be the optimal strategy. As with many programs, the majority cost associated with our baiting program is transport (helicopter time in our case). Three separate baiting occasions would result in a threefold increase in transport costs. On the other hand, increasing bait density

would generate only a moderate increase in cost (associated with bait production). Increasing baiting density generates an increase in bait production cost, but this is a cost increase whether baits are delivered on one occasion, or two. Alternatively, for a fixed budget, increasing bait density reduces the area that can be completely treated. Clearly, then, given a fixed budget, the optimal baiting strategy in our case will depend upon a trade-off: are we better off to treat a larger area (but fewer animals per area), or a smaller area (but treat almost all the baitable animals in that area)? This is not a trivial problem to solve and requires application of spatially explicit population viability analysis.

Multiple deployments may, however, have other advantages, not captured in cost. For example in our case, training only once prior to toad arrival will need to be delicately timed: too early, and trained animals may lose their aversion before toads arrive (Indigo et al. 2018). Thus, delivering baits over three occasions acts as a bet-hedging strategy against the uncertainty of toad arrival time. Clearly, baiting campaigns will often need to consider such exigencies not captured in budget figures.

## Management Implications

Our results provide important information for designing a baiting program for quolls in the Kimberley. While our results may be used to guide programs elsewhere, this should only be done with caution. Many important variables change between areas. For example, bait uptake may change with patterns of seasonal variation in diet (Oakwood 1997), reproductive status of individuals (Oakwood, Bradley et al. 2001), the suitability and complexity of the target species habitat (Hohnen, Tuft et al. 2016, Hohnen, Tuft et al. 2016); the availability of

preferred/alternative prey (Algar, Angus et al. 2007, Weerakoon and Banks 2011, Christensen et al. 2013) and of course, the extent to which non-target species access baits before the target species. Our results are nonetheless encouraging with regard to the use of toad sausages as a vehicle for large-scale CTA training of quolls. We might only expect to treat around 64% of animals, but we can treat this proportion of the population using a feasible baiting rate: 72 baits per site. The ability to predict the results of baiting provides wildlife managers greater flexibility in optimisation and decision making. From the perspective of developing a working baiting strategy, our results contribute to a growing body of work opening the possibility for broad-scale CTA quoll training using toad aversion sausages: a technique to prevent quolls from attacking cane toads (O'Donnell et al. 2010, Webb et al. 2015, Jolly et al. 2017, Kelly et al. 2017, Indigo et al. 2018).

More broadly, our results speak to the importance of careful monitoring of baiting efficacy, and to assessing population heterogeneity in baitability. There is clear evidence that individuals vary in their food preference and behavioural tendency to accept novel food. If our analysis had not accounted for this heterogeneity, and assumed that all individuals were baitable, we may well have suggested that all individuals could be treated if only we increased our bait density ten-fold (Figure 5-4). By accepting that there is heterogeneity, we do not fall into this trap and, consequently, we are able to design a more efficient baiting program. We achieved our inference using data obtainable from camera trap arrays, and a two-step process in which we use an attractive generic bait to estimate population size, and a second baiting event in which we assess the uptake of the referent bait. It is increasingly feasible to gather the data required informing the design of

baiting programs, and models such as ours allow us to capture the key parameters of interest to managers and decision makers.

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# Chapter 6
# General Discussion

Cane toads occupy more than 1.5 million square kilometres of Australia; their distribution continues to expand and they are extraordinarily difficult to control (Urban, Phillips et al. 2007, Tingley, Phillips et al. 2013).  The cane toads' defensive chemicals (bufadienalides) are highly cardioactive and are unlike toxins possessed by native Australian animals (Hayes, Crossland 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 and Archer 1975, Webb, Shine et al. 2005, Smith and Phillips 2006, Hayes et al. 2009, Shine 2010).

Northern quolls are strongly impacted by cane toads. Toads have caused numerous local extinctions of this native marsupial predator (Burnett 1997, Oakwood and Foster 2008). The distribution of the northern quoll is now restricted to small disjunct populations across northern Australia {Kelly, 2018 #278}. The complex rocky habitats in the Kimberley region of northern Australia is one of the last strongholds for *D. hallucatus* populations (Braithwaite and Griffiths 1994). Furthermore, the population in this region is genetically distinct from the populations of quolls located in other bioregions such as the Pilbara (WA) and NT/Queensland (Westerman and Woolley 2016), and hence should constitute separate management units.

Sadly, the continuous spread of the cane toads across the north of Australia has caused, and is expected to cause, further major declines or local extinctions of northern quoll populations in the Kimberley over the coming decade (Phillips, Chipperfield et al. 2008). The future trajectory of northern quoll populations on the Australian mainland may emulate the decline of its three Australian congeners; the functionally extinct Eastern quoll, the endangered Chuditch and the near threatened Spotted-tailed Quoll (Oakwood 1997, IUCN 2019).

Safeguarding the northern quoll populations in the Kimberley region of WA depended largely upon populations persisting past the cane toad arrival. Conditioned taste aversion was proposed as a strategy to assist with this goal (Webb, Legge et al. 2015). CTA is a powerful form of learning, that can be used to alter an animals predatory behaviour (Garcia, Lasiter et al. 1985). In Australia, there is currently intense interest around using CTA to mitigate the impact of the highly toxic cane toad (O'Donnell, Webb et al. 2010, Ward-Fear, Pearson et al. 2016, Jolly, Kelly et al. 2017, Kelly and Phillips 2017, Kelly, Phillips et al. 2018). Trials on captive quolls confirmed that individuals avoided attacking live toads immediately after CTA training (O'Donnell et al. 2010). The possibility of *in situ* training of northern quolls to avoid cane toads was then raised, but *in situ* training methods had not been developed.  Thus, my thesis addressed that gap.

Initially I employed a model based on the data of quoll life history to determine whether CTA would enable quolls to persist following the cane toad invasion (Chapter 2). This study comprised two main components. Firstly, I undertook a demographic analysis of historic mark and recapture records from 2011-2017, in a geographically isolated location on Mornington Wildlife Sanctuary

WA, to answer fundamental questions about northern quoll survival rates in this population. Second, I used a PVA model developed by Kelly and Phillips (2019) to examine the robustness of CTA for preventing population extinction under scenarios in which I varied: 1) the efficacy of the initial CTA training (i.e. the proportion of the population that was successfully trained to avoid toads) and 2) the transmission of the CTA lesson (i.e., the ability of females to pass the lesson on to their offspring.

The work showed that the success of CTA in reducing the probability of extinction was strongly influenced by high transmission and high CTA efficacy. Heritability of the 'toad-smart' phenotype also impacted the success of CTA, with higher heritability leading to a decrease in population extinction probability. Results of the demographic analysis were broadly consistent with previous studies (Cremona, Crowther et al. 2017). For instance, sex-based survival estimates revealed that the annual survival rate of northern quolls in the central Kimberley region of WA; are very similar to those reported from other studies in differing bioregions (Begg 1981, Schmitt, Bradley et al. 1989, Braithwaite et al. 1994, Oakwood 2000). Additionally, results suggested that the quoll population size changes markedly throughout the year; this is consistent with findings recorded elsewhere (Oakwood, Bradley et al. 2001). Data on variation in population size and survival are rarely available for endangered or threatened species. Thus our estimates are valuable for increasing precision when modelling northern quoll populations under different PVA scenarios in the future (White, Franklin et al. 2002, Gerber, Wielgus et al. 2007).

The analysis in this preliminary chapter highlighted that the deployment of toad-aversion baits could help to mitigate the impact of cane toads on northern quolls but would require high cultural transmission and a moderate efficacy of CTA training. Thus, this finding suggested that careful attention should be paid to ensure that high CTA training efficacy (i.e. bait uptake by a high proportion of the population) is possible to achieve, as a first step. By focusing on making the lesson effective, a process researchers can manipulate, CTA management strategies should have a high likelihood of succeeding.

Following this I undertook a small captive trial to assess the value of a manufactured bait ('toad aversion sausages'). I asked whether quolls generalised their CTA from the bait to toads (Chapter 3). Additionally, I assessed whether the bait was taken up by wild quolls and non-target species, and whether it appeared to elicit CTA under field conditions.

Quolls that consumed baits showed reduced interest in toads, indicating generalisation of the aversion response. Individuals that were CTA trained via bait, changed their behaviour towards toads. Although sample sizes were modest, it was apparent that bait trained animals spent less time interacting with a toad. Results of field trials showed that the toad baits are attractive to quolls. Although 26 species encountered the baits, quolls were the most frequent visitors to the bait, and were the most likely species to consume the bait (Indigo, Smith et al. 2018). Thus, non-target uptake was relatively modest, compared with the high level of uptake of baits by non-target animals observed in other lethal-baiting studies (Cowled, Gifford et al. 2006, Dundas, Adams et al. 2014).

In this study, it was more difficult to estimate the rate of successful training in the field. The apparent independence of quoll behaviour to bait uptake across sessions suggested that, in the absence of further reinforcing stimulus (i.e. cane toads), CTA training potentially only elicits aversion for a limited time (<4 months).  Overall, this chapter was encouraging with regard to the use of toad sausages as a vehicle for large-scale CTA training of quolls.  It was inferred 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 opened the possibility for broad scale application of CTA as trial for mitigating the impact of toads on wild quolls. While many questions remained about optimal bait design, delivery, and timing, the experiment suggested that CTA training, using toad sausages, was likely a viable tool for land managers seeking to protect quoll populations.

Subsequently I placed these ideas in a real-world context in Chapter 4. Given the lack of robust long-term study to explore the efficacy of toad aversion training, I executed a robust test of the baiting methodology in a before-after-control-impact (BACI) framework. Given the timing of toad arrival at my site, and the length of time required to run this experiment, the experiment was conducted *before* the optimal baiting design was worked out.  The experiment demonstrated a dramatic decline across seven populations of quolls associated with the arrival of toads. This outcome is consistent with anecdotal reports of quoll extinctions upon toad arrival elsewhere (Burnett 1997), as well as results from several studies showing that quolls typically die within a few days to a few months of sharing habitat with toads (Oakwood 2004, 2008, Woinarski, Oakwood et al. 2008, Jolly, Kelly et al. 2017).

This result also clearly highlighted a failure for CTA baits to reduce the impact of toads upon their arrival.

Why did the CTA baits fail? There are three likely reasons. First, the dose rate of thiabendazole (TBZ) I used may have been too low to produce illness. For field use, a CTA agent should induce a robust CTA after a single oral dose (Nachman and Ashe 1973), and learned aversions may vary with the strength of the nominated CTA agent (Massei, Lyon et al. 2003). Animals receiving a dose that is too small may not have associated the taste or smell of toads with illness (Massei et al. 2003). Unfortunately, the dose rate of TBZ was constrained to a low dose by the government regulators, the Australian Pesticides and Veterinary Medicines Authority (APVMA). Second, it is likely that quolls lost their learned aversion within 120 days of training. Bait uptake rates did not decline across baiting sessions, suggesting lesson decay. Further evidence of decay comes from a small captive trial that due to very small sample sizes – 5 animals – has not been reported to date; whereby the proportion of individuals that remained averse decayed to zero by around 120 days post-training. Finally, it is likely insufficient quantities of baits were deployed to train enough quolls to avoid toads. Kelly et al. (2017) predicted that a minimum of 32% of northern quolls need to be trained to avoid toads, if the population were to persist post cane toad invasion. Our post-hoc estimates of 19-43% of quolls taking baits suggests that our trial may have failed just by not reaching enough animals.

To improve the likelihood of success, I recommend that future baiting programs should offset the above problems by:

(1) increasing the thiabendazole (TBZ) dose rate in each bait from 300mg/kg (this study) to 400mg/kg (O'Donnell et al. 2010, Cremona et al. 2017, Jolly et al. 2017, Kelly et al. 2017)

2) Deliver CTA baits just prior to the arrival of the cane toad front line, ideally, one month before toads invade.

3) Increase the density of baits delivered so that a high proportion of baitable individuals consume a bait (Indigo, Smith et al. 2018).

In the remainder of my thesis, I assessed whether conditioned taste aversion baiting could be a practical and viable management tool. A successful baiting program is one that is cost-effective and results in a large proportion of target individuals taking bait, thus in Chapter 5, I refined the technique of baiting. I aimed to optimise bait delivery by determining the fewest baits required to achieve a given proportion of the population baited. These aims required data on bait uptake within the target population and how this changed with the density of quolls in the landscape. Recent work by my colleagues also showed that a proportion of a naïve quoll population are innately toad averse, and so are unlikely to be baitable in my study (E. Kelly & B. Phillips. Pers comm, 2019). Using this prior information, along with mark-resight data from camera traps, I was able to estimate the survival rate of baits, as well as their uptake rate. These parameters allowed me to design a baiting deployment strategy that maximises delivery of CTA while accounting for the fact that some animals in the population are inherently unbaitable.

The results of this experiment provide important information for designing a baiting program for quolls in the Kimberley. Approximately 64% of individual quolls were bait-susceptible and both encounter rate, and bait survival were very low. Using model parameters, it was estimated that a six-fold increase in bait-density is needed to treat all bait-susceptible individuals in the population. Without accounting for heterogeneity in bait-susceptibility, the model would suggest a ten-fold increase in bait density. Thus, accounting for heterogeneity provides a more efficient baiting design. This result was encouraging with regard to the use of toad sausages as a vehicle for large-scale CTA training of quolls. Land managers might only expect to treat around 64% of animals, but we can treat this proportion of the population using a feasible baiting rate: 72 baits per 7-hectare site. We can also reasonably expect that some fraction of the non-baitable animals may also innately avoid toads (Kelly et al. 2017).

The ability to predict the results of baiting provides wildlife managers greater flexibility in optimisation and decision making. From the perspective of developing a working baiting strategy, the results collected here contribute to a growing body of work; opening the possibility for broad-scale CTA quoll training using toad aversion sausages: a technique to prevent quolls from attacking cane toads (O'Donnell et al. 2010, Webb et al. 2015, Jolly et al. 2017, Kelly et al. 2017, Indigo et al. 2018).

## Future Studies

There are still many valuable avenues for future research on the implementation of CTA to mitigate the impact of cane toads. An implicit assumption of conditioned taste aversion training via baits is that the arrival (exposure) of cane toads to a

population would constitute reinstating the reconditioning process. It was additionally suggested that decay may also occur faster in field situations when food is scarce (Gustavson, Garcia et al. 1974). Decay and eventual extinction of a lesson is defined as decline in the frequency or intensity of a conditioned response following the withdrawal of reinforcement (Pavlov 1927). Such decay is expected and occurs as a result of competition of complex neurobiological mechanisms that control behaviour (Berman, Hazvi et al. 2003). Measuring the length of CTA decay in future studies warrants close and systematic attention.

Other avenues of future research include careful assessment of the cultural transmission of foods that are avoided. While it is well known that food preferences are culturally transmitted (i.e. individuals learn what, were and how to eat by observing parents) (Galef and Giraldeau 2001); less is known about the role of parents in teaching offspring to avoid unpalatable or distasteful prey. Previously, studies have demonstrated that red-winged Blackbirds (*Agelaius phoeniceus*) and the house sparrow (*Passer domesticus*) can acquire a learned food avoidance by watching a demonstrator become ill (Mason, Arzt et al. 1984, Fryday and Greig-Smith 1994). Besides this, little evidence exists suggesting animals learn aversions from conspecifics (Jing, Zhou et al. 2014). The failure to obtain evidence for the cultural transmission of a taste aversion is surprising, and my work suggests that this is a major omission in a large majority of CTA studies. Indeed, the success of CTA training hinges on a high transmission of information, so future studies to determine whether there is cultural transmission of information of toxic prey would be a worthwhile. In this respect, small dasyurid marsupials, which, like

quolls, have extended maternal-offspring contact post-weaning, would be ideal models for exploring this question (Strahan and Conder 2007).

Exploring variation in food preferences and the innate ability to avoid toads is an additional avenue for future research. Innate avoidance may provide the raw material on which natural selection can act to generate a rapid adaptive response to toads (Phillips and Shine 2006, Smith et al. 2006, Llewelyn, Phillips et al. 2011, Somaweera and Shine 2012, Kelly et al. 2017). Heritability of toad-smart traits are determined by the animal's genotype and environmental variation, similarly to many behavioural traits, this level likely lies somewhere between 0.1 to 0.3, although the precise level of inheritance is unknown (Roff 2012, Kelly et al. 2019). There is evidence in other taxa that some level of innate avoidance of toads is present in toad-naïve predator populations, e.g., the common planigale (*Planigale maculata*) (Webb, Brown et al. 2008), red-cheeked dunnart (*Sminthopsis virginiae*) (Webb, Pearson et al. 2011), terrestrial snake species (Phillips, Brown et al. 2003) and varanid lizards (Smith et al. 2006). Thus, the non-baitable fraction of the northern quoll population, revealed in Chapter 5, is an intriguing finding with regards to this level. Determining the fraction of our non-baitable animals that will in fact innately avoid toads (rather than baits) is an important avenue for future work.

Finally, the parameters in our model presented in Chapter 5 (bait survival and encounter rate) should allow managers to optimise their efforts using not only bait density, but also bait survival. Bait density is clearly the simplest variable to adjust, but in other systems it may also be possible (and possibly more efficient) to adjust bait longevity as well. Thus, designing a lasting CTA bait for operational use

that is attractive, palatable, shares a close association in taste and odour of the referent food, is safe for non-target species and induces a strong aversion across a range of body sizes after consuming a single portion is, of course, challenging although necessary (Conover 1995, Cagnacci, Massei et al. 2005, Mappes, Marples et al. 2005).

Given that quoll populations in the Kimberley will be completely overrun by toads within the next 5 years, there is an urgent need to address the gaps in my CTA research; as CTA has the potential to be an extremely powerful tool. We proposed 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 apparent short-term nature of aversion training. In addition, to be effective, adaptive management efforts will require incorporated knowledge, support and cooperation among stakeholders.

There is currently intense interest in training native Australian predators (ranging from quolls to reptiles) to avoid cane toads using CTA, (O'Donnell et al. 2010, Webb et al. 2015, Ward-Fear, Pearson et al. 2016, Jolly et al. 2017, Kelly et al. 2017, Tingley, Ward-Fear et al. 2017, Ward-Fear, Thomas et al. 2017), but knowledge about the rate at which CTA training decays is non-existent for most taxa. Ours is the first study to identify decay of aversion as a critical factor in these efforts, and clearly, there is an urgent need for further research to determine dose rates of TBZ (or alternative chemicals) that engender strong, long lasting aversions. This is an area that remains virtually unexplored, as no previous studies have used marsupial models.

# Conclusion

In my thesis I rigorously assessed the utility and applicability of conditioned taste aversion as a tool for preventing population declines in a critically endangered marsupial, the northern quoll. In doing so I have shown how challenging it is to apply CTA in the landscape and have emphasised the importance of developing evidence-based predictions to guide decision makers.  In addition, I have carried out the first replicated field study to attempt to reduce the impact of toads on a critically endangered marsupial. The evidence from my impact-controlled population-level study shows that quolls are seriously impacted by toads.  While my field program did not work out as planned, I provide some clear guidelines for future work, and some suggestions to address gaps in our understanding of the use of CTA in the field. My thesis highlights the challenges faced when testing a new and novel technology, particularly in complex ecological systems. In a world experiencing rapid rates of mammalian extinction, novel conservation strategies such as conditioned taste aversion may help us mitigate threatened populations against the serious impacts of invasive species.

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# Appendix I
# Supplementary material for Chapter 1

## Study Species- Northern Quoll

The northern quoll (*Dasyurus hallucatus*) belongs to the family Dasyuridae and is the smallest of six members of the genus *Dasyurus*. At the time on European settlement the species was widespread across northern Australia (Cardoso, Eldridge et al. 2009), although it is now one of several mammals that has declined over the past decade (Braithwaite and Griffiths 1994). Several factors are attributed to the declines, including predation by introduced carnivores (Woinarski and Fisher 2014) and the arrival of the cane toad (Watson and Woinarski 2003, 2004)

Despite these same threats being considered across the entirety of the northern quolls distribution, the Kimberley region of Australia is thought to be a stronghold for *D. hallucatus* populations (Hohnen, Tuft et al. 2016). Research suggests that this is owing to habitat suitability, whereby rugged rocky areas close to permanent water are likely to provide to most optimal habitat (Schmitt, Bradley et al. 1989, Braithwaite et al. 1994, Oakwood 2000, Woinarski, Oakwood et al. 2008, Hill and Ward 2010). However, the continuous spread of the cane toads into these areas is expected to cause major declines in Kimberley populations of northern quolls over the coming decade (Phillips, Chipperfield et al. 2008).

Northern quolls are predominantly nocturnal and opportunistically omnivorous. Their diet consists of fruits, invertebrates and vertebrate animals such as reptiles, mammals and frogs (Oakwood 1997). For this reason, their susceptibility to poisoning through ingesting cane toads as prey is likely.

## Biology

*D. hallucatus* is a sexually dimorphic species, although their morphological estimates vary throughout populations and locations (Oakwood 2002). Mature adult females weigh between 240-690g while males weigh 340-1120g (Oakwood 1997). The species has a life history strategy characterized by monoestry and pronounced reproductive seasonality (Schmitt et al. 1989). Mating occurs over a period of approximately three weeks in late May to late June (Schmitt et al. 1989, Oakwood 1997). Shortly after, males exhibit semelparity, whereby the population undergoes major decline, usually at one-year of age (Schmitt et al. 1989). Females can live up to three years, although may only breed for two. They produce on average seven young from August-September, following a gestation period of 21-26 days (Oakwood 1997). From eight to nine weeks, young are deposited into dens. At four weeks of age offspring may begin to eat solids such as insects, and at six months are fully weaned. Animals become reproductively mature at 11 months of age (Oakwood 1997).

## Study area

The field component of this study was conducted between November 2015-November 2017 at Mornington Wildlife Sanctuary (c. 300,000-ha, 17°31'S, 126°12'E) and Charnley River Wildlife Sanctuary (c. 170,000-ha , 17°31'S, 126°12'E)  in the Kimberley region of western Australia (Figure A-I-1). These two

properties are separated by approximately 150km and are both managed for conservation by the Australian Wildlife Conservancy (AWC).
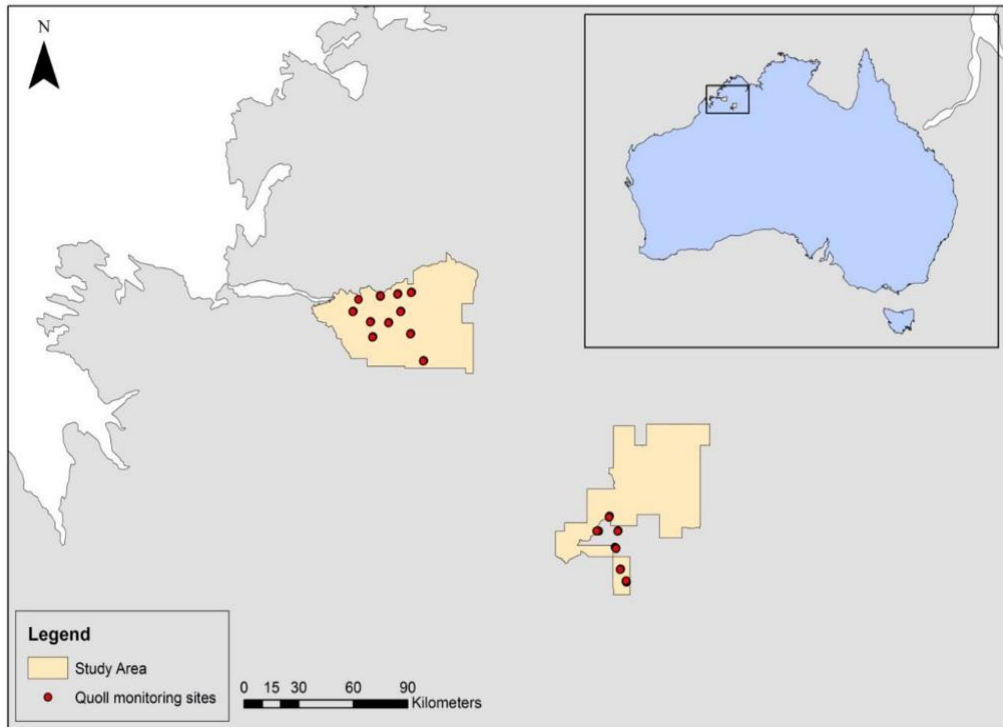


**Figure A-I-1:** Location of Mornington Wildlife Sanctuary (South-east property) and Charnley River Wildlife Sanctuary (North-west property) within Australia, red circles denotes sites selected for the study.

The sites are dominated by sandstone boulders creating varying topographic complexity along the perennial Fitzroy River (Figure. A-I-2 and Figure A-I-3). The rocky habitat is suitable for northern quolls short eared rock wallabies (*Petrogale brachyotis*), common rock rats (*Zyzomys argurus*) and dingoes (*Canis lupus* dingo**)**. Spinifex grass (*Triodia epactia*) and *Eucalyptus brachyandra, Terminalia hadleyana, Vitex acuminata* and C*ochlospermum fraseri* are common along the gorge.   On

average, this area receives 788-900 mm of rain annually, most of which falls from

November to April.



***Figure A-I-2****: Sandstone gorge on Mornington Wildlife Sanctuary. Image highlights topographic complexity of one of the selected study sites.*



***Figure A-I-3****: Comparison of another study site located on Charnley River Wildlife Sanctuary. Image highlights variation in topographic complexity.*

Study sites were selected based on the detection of quolls during AWC fauna surveys (AWC unpub. data, 2017).   At the beginning of the study, toads were yet to arrive at on southern Mornington sites; they subsequently arrived by March 2017. Currently, (June 2019) toads are not present at the sites located in the north-west at Charnley River wildlife sanctuary, although are expected to appear at these locations in the coming 2019/2020 wet-season (personal comm. James Smith AWC 2019).

## Conditioned Taste Aversion Baits- Cane Toad Sausages

Preceding this project, in 2011, a prototype toad aversion bait was tested in captivity at Territory Wildlife Park, NT. Following consumption of the bait, a high proportion of quolls to reduced attack behaviour exhibited towards live toads as prey (Unpublished data, 2011).  Following this, preliminary field baiting trials were then carried out in the field at Mornington Wildlife Sanctuary at Sir John Gorge in October and December 2014. Cane toads had not yet colonised this site.

Researchers trialled two types of bait that were made up of 15g of minced skinned adult cane toad legs and 0.06g of Thiabendazole (per bait) either packed into a synthetic sausage skin (sausage) or without skin (meatballs).  During the trial, 30 cage traps were deployed along a transect and the toad-aversion bait (either meatball or sausage) was randomly allocated and placed inside each trap. Researchers recorded individual quolls that had eaten or partially eaten the bait, this occurred for four consecutive nights in October, and three consecutive nights in December 2014 (Webb, Legge et al. 2015).

It was determined that the bait type with the skin had a longer survival in the field. Additionally, concerns were made that the association between bait and referent food (cane toads) may be too dissimilar. Thus, a <2g toad was also packed into the original matrix.

For the entirely of the current research project, cane toad sausages were made up of 15g of minced skinned adult cane toad legs, 1 whole cane toad metamorph weighing <2g, and 0.06g of Thiabendazole (per sausage; dose rate less than 300mg/kg adult quoll body weight, determined by the smallest – 200g – adult seen at our study site) packed into a synthetic sausage skin (Figure A-I-4). In all trials, I used the same sausage composition, to accurately reflect the field and captive scenario.

We selected thiabendazole as a nausea inducing agent as it was an inexpensive, broad-spectrum anthelmintic and antifungal agent (Robinson, Stoerk et al. 1965). It is orally-effective and regarded as relatively safe, producing low mammalian mortality: oral LD50 is 2.7g/kg body weight (DELVO No date). It is fast acting and peak concentration occurs in the plasma one hour after consumption (Tocco, Rosenblum et al. 1966). Thiabendazole has produced strong aversions to treated foods in lab rats (Gill, Whiterow et al. 2000, Massei and Cowan 2002), wolves (*Canis lupus*) (Gustavson and Basche 1983, Ziegler, Gustavson et al. 1983), and black bears (*Ursus americanus*) (Ternet and Garshelis 1999). Thiabendazole induces a robust CTA after a single oral dose (Nachman and Ashe 1973, O'Donnell, Webb et al. 2010) and is physically stable at ambient conditions in the bait substrate (Gill et al. 2000, Massei, Lyon et al. 2003).
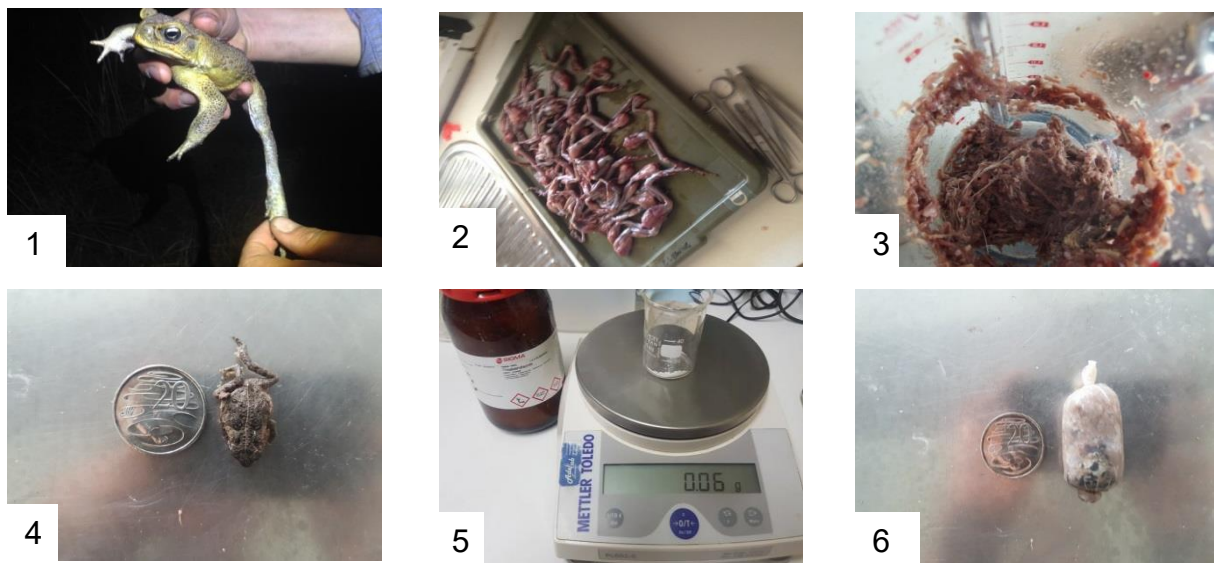
*Figure A-I-4:* Cane toad sausages- 15g of minced & skinned adult cane toad legs (1-3), 1 whole cane toad metamorph (4) & 0.06g of thiabendazole (5), all packed into a synthetic sausage skin (6).

## Data collection

Camera trap methods to estimate bait uptake and quoll population size.  Live trapping was also used to estimate quoll population size at Sir John Gorge (17° 31.78'S, 126° 13.08'E) at Mornington Wildlife Sanctuary in the Kimberley, Western Australia.

Small cage traps (Tomahawk Live Traps, Hazelhurst USA) or camera traps (Reconyx) were baited with universal bait made up of one tablespoon of mackerel in oil, mixed with peanut butter and oats. Coopex (Bayer Environmental Science) was dusted around each cage trap or camera trap to ward off ants. I secured cameras to trees or rocky ledges approximately one metre from the ground facing directly downwards (Figure A-I-5). I set cameras to high sensitivity and programmed them to take three consecutive photographs for each trigger with no

delay between triggers. Using photographs collected from the cameras, I identified individuals from spot patterns (Hohnen, Ashby et al. 2013).



**Figure A-I-5:** *Photographs of two camera stations set up on a rock ledge (left) and in a tree (right).*

## Site selection

Twenty-nine sites were surveyed for northern quolls in 2014 across Australian Wildlife Conservancy Sanctuaries in the central Kimberley, WA. This was to produce baseline distribution data ahead of the arrival of cane toads. At each site, six white-flash camera traps (Reconyx, PC850 Hyperfire White Flash) were set each 100 m apart in a transect following likely quoll habitat (creek line, cliff line, gorge). Cameras were positioned to face down, as described previously.

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# Appendix II
# Supplementary material for Chapter 2.

**Table A-II-1**: Raw survival data from previous mark-recapture studies used to estimate annual sex-based survival rates. † denotes where data was not available.

| Study | sex | age | $\bar{N}$ | surv | die | Survival |
|---|---|---|---|---|---|---|
| Oakwood 2000 | female | 1 | 11 | 3 | 8 | 0.272727 |
| Begg 1980 | female | 1 | 53 | 11 | 42 | 0.207547 |
| Braithwaite and Griffiths 1994 | female | 1 | 150 | 12 | 138 | 0.08 |
| Schmitt 1989 | female | 1 | 16 | 6 | 10 | 0.375 |
| Indigo (current study) | female | 1 | 76 | † | † | 0.248817 |
| Oakwood 2000 | female | 2 | 11 | 0 | 10 | 0 |
| Begg 1980 | female | 2 | 33 | 2 | 31 | 0.060606 |
| Oakwood 2000 | male | 1 | 26 | 0 | 26 | 0 |
| Begg 1980 | male | 1 | 32 | 4 | 28 | 0.125 |
| Braithwaite and Griffiths 1994 | male | 1 | 57 | 0 | 57 | 0 |
| Schmitt 1989 | male | 1 | 46 | 2 | 44 | 0.043478 |
| Indigo (current study) | male | 1 | 75 | † | † | 0.062135 |

**Table A-II-2**: Full set of CJS mark-recapture models compared using program MARK.

| CJS Model | AICc | ΔAICc | $w_i$ | Model Likelihood | N | Deviance |
|-----------|------|-------|-------|------------------|---|----------|
| Φ(sex) p(t) | 524.32 | 0.00 | 0.92 | 1.000 | 25 | 286.42 |
| Φ(sex) p(.) | 530.15 | 5.83 | 0.05 | 0.054 | 3 | 341.85 |
| Φ(sex) p(sex) | 531.81 | 7.49 | 0.02 | 0.024 | 4 | 341.45 |
| Φ(.) p(t) | 533.68 | 9.36 | 0.01 | 0.009 | 24 | 298.24 |
| Φ(.) p(sex) | 538.75 | 14.42 | 0.00 | 0.001 | 3 | 350.45 |
| Φ(.) p(.) | 539.58 | 15.26 | 0.00 | 0.001 | 2 | 353.33 |
| Φ(t) p(.) | 546.67 | 22.35 | 0.00 | 0.000 | 23 | 313.66 |
| Φ(t) p(sex) | 548.04 | 23.72 | 0.00 | 0.000 | 24 | 312.59 |
| Φ(t) p(t) | 554.09 | 29.77 | 0.00 | 0.000 | 43 | 267.87 |
| Φ(sex*t) p(.) | 568.49 | 44.17 | 0.00 | 0.000 | 42 | 285.17 |
| Φ(sex*t) p(sex) | 571.36 | 47.04 | 0.00 | 0.000 | 43 | 285.14 |
| Φ(sex) p(sex*t) | 574.44 | 50.12 | 0.00 | 0.000 | 48 | 273.29 |
| Φ(.) p(sex*t) | 577.55 | 53.23 | 0.00 | 0.000 | 47 | 279.45 |
| Φ(sex*t) p(t) | 587.34 | 63.02 | 0.00 | 0.000 | 62 | 240.23 |
| Φ(t) p(sex*t) | 610.81 | 86.48 | 0.00 | 0.000 | 64 | 256.58 |
| Φ(sex*t) p(sex*t) | 642.25 | 117.93 | 0.00 | 0.000 | 78 | 233.62 |

Φ = survival probability; p = recapture probability; Sex = group (males and females); (.) = constant; t = time; N = number of parameters.

**Table A-II-3**: Robust design population sizes ($\bar{N}$ for each sampling session) and 95% credible intervals, assuming closure of the population during primary sessions of the study.  † denotes empty cells.

| Session | Female $\bar{N}$ (95% CI) | Male $\bar{N}$ (95% CI) |
|---|---|---|
| Oct-11 | 5.05 (4.07, 19.06) | 2.53 (2.03, 10.51) |
| Feb-12 | 1.37 (1.03, 6) | 9.62 (7.39, 24.79) |
| Apr-12 | 11.25 (8.6, 25.61) | 12.66 (9.69, 28.29) |
| Jul-12 | 8.14 (8.01, 10.79) | 9.16 (9.01, 11.97) |
| Sep-12 | 3.01 (3, 3.6) | 11.03 (11, 12.24) |
| Mar-13 | 4.09 (4. 6.38) | 7.16 (7.01, 10.37) |
| Mar-13_2 | 1.56 (1.05, 7.03) | 10.91 (7.77, 26.73) |
| Jun-13 | † | 0 (0, 0) |
| Jun-13_2 | 7.5 (5.57,16) | 10.5 (7.91, 20.41) |
| Jun-13_3 | 12.65 (8.33, 30.98) | 12.65 (8.33, 30.98) |
| Sep-13 | 4 (4, 4.18) | 5 (5, 5.21) |
| Sept-13_2 | 6.02 (5.15, 12.14) | 12.04 (10.38, 20.98) |
| Mar-14 | 5.55 (4.23, 14.29) | 16.65 (12.94, 34.99) |
| Jun-14 | 8.37 (7.22, 15.62) | 9.57 (8.26, 17.38) |
| Oct-14 | 3.02 (3, 3.87) | 7.04 (7, 8.39) |
| Oct-14_2 | 14.66 (10.36, 32.52) | 16.28 (11.55, 35.54) |
| Apr-15 | 5.34 (2.62, 20) | 13.36 (7.16, 37.35) |
| Jun-15 | 4.35 (4.03, 8.17) | 8.7 (8.08, 14.1) |
| Oct-15 | 2.11 (1.12, 11.59) | 8.44 (4.73, 30.98) |
| Jan-16 | 10.68 (5.39, 36.12) | 13.35 (6.83, 43.06) |
| Apr-16 | 10.14 (9.16, 16.91) | 7.89 (7.12, 13.78) |
| Jul-16 | 7.06 (7, 8.87) | 5.05 (5, 6.56) |
| May-17 | 6.57 (1.63, 50.61) | 13.14 (3.48, 85.7) |

# Appendix III
# Supplementary materials for Chapter 4. Decay of conditioned taste aversion in captivity.

## Methods

We measured conditioned taste aversion decay in five male captive northern quolls. The quolls were captured from Mareeba, QLD and transported to the Territory Wildlife Park, NT six months prior to the commencement of the experiment. They were housed individually in 2x4m enclosures at the Territory Wildlife Park.

The experiment began by training each individual to avoid a novel bait using thiabendazole (TBZ) to elicit a conditioned taste aversion. We had two types of novel baits 1) pork mince laced with orange essence in a star shape and 2) kangaroo mince laced with vanilla essence in a heart shape. These meat, odour and visual cues were all novel to the quolls. They were randomly allocated a control and treatment bait from the two options. To begin with, we gave each quoll their allocated control bait (5g; not laced with TBZ) for three repeated nights or until the bait was eaten. Then, we presented each quoll with the treatment bait which was laced with chemical TBZ at a dose rate of 400 mg/kg quoll mass in a 5g bait. We repeated the dosed treatment bait until the bait was eaten or for up to three nights. Baits were given instead of the quoll's regular food.

Following the training period, we presented each individual with both their control and treatment bait (without TBZ) daily along with their regular food. Quolls were fed just before dusk and then checked the following morning, when we recorded if the referent bait was eaten or not. This was repeated nightly following the initial training, until an animal left the study, or consumed the referent bait. The experiment was conducted as a pilot on post-breeding males, and the short monitoring period and disparity between individuals was due to the logistical difficulties. One male died of natural causes during the experimental period, and another two were shipped to other zoos in order to make room in the captive breeding facility. These constraints, along with the difficulty of working with an endangered marsupial, have led to the uneven and small sample sizes.

### *Statistical Methods*

The data are classical time to event data (where the event is consuming the referent food item), and they are right censored (animals left the study), so lend themselves to a survival analysis. We fit the simplest, one-parameter, survival model to these data: the exponential (constant hazard) model using the survival package in R (Therneau 2015).

# Results

Four of five individuals consumed the control bait throughout the experiment, bar one individual that did not consume control bait on one night (night six). Two quolls consumed the referent bait on the 15th night post training. The other three animals did not consume the referent bait, and left the study at 11, 13, and 18 nights post training. The estimated per day probability of losing aversion was

estimated as 0.028 (95%CI: 0.0068, 0.11) yielding an expected decay function shown in Figure A-III-1.
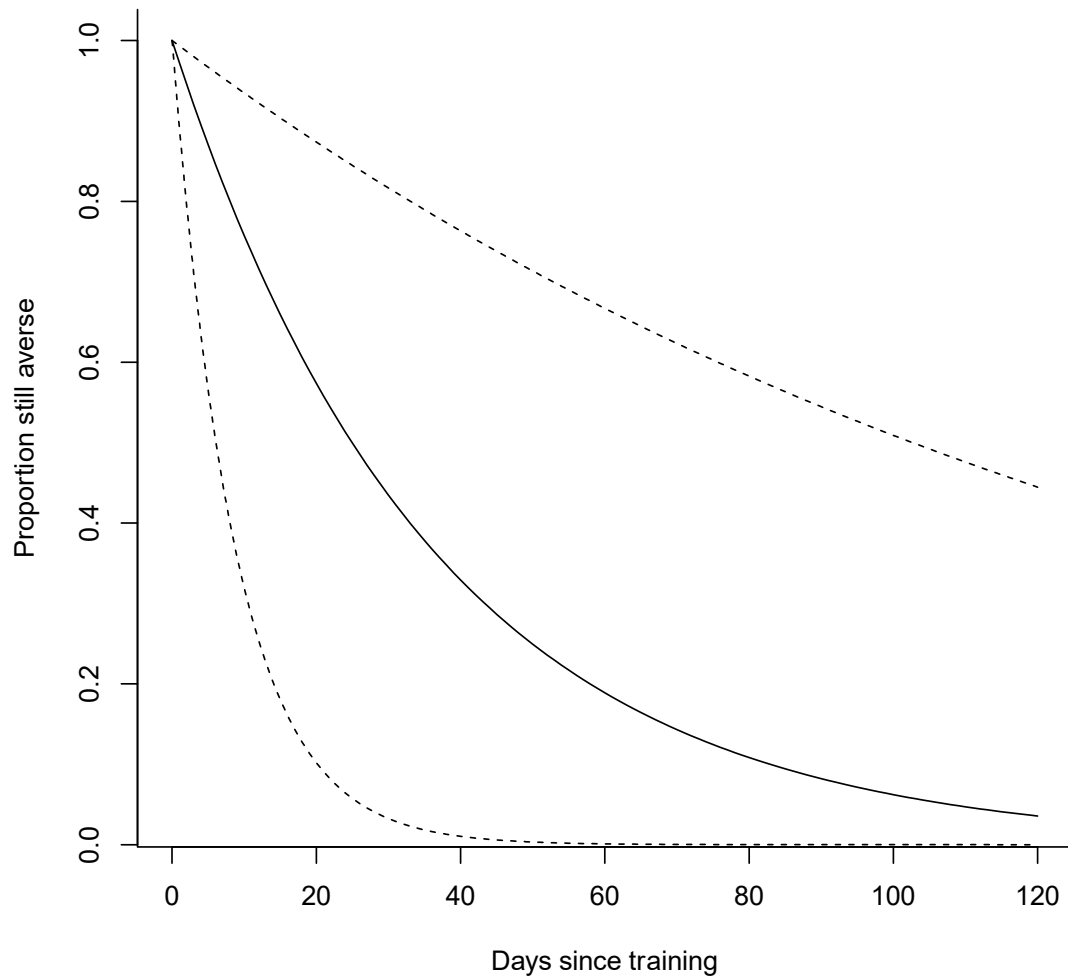


**Figure A-III-1**: Decay of acquired CTA aversion (days since training) (expected value and associated prediction envelope dashed) within 120 days of training. n =5 individual male quolls.

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