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Acknowledgement

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We are committed to genuinely partnering, and meaningfully engaging, with Victoria's Traditional Owners and Aboriginal communities to support the protection of Country, the maintenance of spiritual and cultural practices and their broader aspirations in the 21st century and beyond.



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Front cover photo: Feral cat consuming non-toxic feral cat bait (DELWP).

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Bushfire Biodiversity Response and Recovery Theme 4 Phase 2—assessing factors affecting the use of feral cat control tools

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Contents

Ackı	nowledgements	ii
Sum	nmary	1
	Context:	1
	Aims:	1
	Methods:	1
	Results:	3
	Conclusions and implications:	4
1	Introduction	5
2	Study areas	7
3	Assessing the target specificity of Felixer feral cat grooming traps	10
3.1	Introduction	10
3.2	Methods	10
	3.2.1 Felixer operations	10
3.3	Data analysis	13
3.4	Results	14
	3.4.1 Target specificity	14
	3.4.2 Felixer establishment in the field	16
3.5	Summary	18
4	The attractiveness of Curiosity feral cat bait under field conditions	20
4.1	Introduction	20
4.2	Methods	20
	4.2.1 Study areas	20
	4.2.2 Bait trials	20
	4.2.3 Data analysis	22
4.3	Results	22
4.4	Summary	23
5	Uptake of Curiosity by non-target species under field conditions	25
5.1	Introduction	25
5.2	Methods	26
	5.2.1 Study site	26
	5.2.2 Aerial baiting operation	26
5.3	Results	28
5.4	Summary	29

6 enco	Probat unter ha	bility of Curiosity bait consumption by feral cats and non-target species, given bait as occurred	31
61	Introdu	ction	31
6.2	Methoo	ls	31
	6.2.1	Data analysis	31
6.3	Results		32
	6.3.1	Feral cat bait consumption given encounter	32
	6.3.2	Simulated encounter and consumption rates	33
	6.3.3	Non-target species removal of Curiosity cat bait	34
6.4	Summa	ary	35
7	Feral c	at density	37
7.1	Introdu	ction	37
7.2	Method	i	37
	7.2.1	Remote cameras	37
	7.2.2	Hair snares	38
	7.2.3	Predator scats	39
7.3	Results	3	41
	7.3.1	Feral cat density—detection of individual feral cats from camera traps	41
	7.3.2	Feral cat density—DNA extracted from hairs collected on hair snares	41
	7.3.3	Feral cat density—DNA extracted from scats	42
7.4	Summa	ary	45
8	Predat	or diet	47
8.1	Introdu	ction	47
8.2	Method	ls	47
	8.2.1	Presence of native species in introduced predator scats	47
8.3	Results		47
	8.3.1	Predator diet	47
8.4	Summa	агу	48
9	Conclu	isions and implications	50
Refer	ences		53

Tables

Table 1. Activities undertaken in each of the six study areas.	. 9
Table 2. Matrix for the Felixer identifying target and non-target species.	14
Table 3. Summary of the numbers of detections of triggering and non-triggering events by Felixers pooled across all locations.	15
Table 4. The capture histories of native and introduced mammals at Tulloch Ard	28
Table 5. The proportion of each species captured that had the RhB biomarker present in whisker samples. 2	29
Table 6. Encounter and consumption of feral cat baits across habitat types.	32
Table 7. Simulated bait take rates based on various encounter scenarios	34
Table 8. A list of all non-target taxa recorded as removing or consuming a non-toxic Curiosity bait	34
Table 9. Estimated feral cat occupancy rates and daily detection rates at the three project locations	41
Table 10. Summary of the species identification results across the various tests and subsampling strategies	s. 44
Table 11. Prey items recorded as less than 3% in fox scats at Tulloch Ard State Forest	48

Figures

Figure 1. Location of activities undertaken as part of the BBRR feral cat management project. NP = National
Park; SF = State Forest
Figure 2. Position of the activation (height 230 mm) and blocking sensors (height 460 mm) designed to
determine target versus non-target activation of the Felixer. A Brush-tailed Bettong (Bettongia
penicillata) is seen passing underneath both activation sensors and hence would not trigger the unit
(from Read et al. 2019)1
Figure 3. Location of Felixer trials in Victoria12
Figure 4. Typical Felixer set-up in the field13
Figure 5. Target and non-target triggering of Felixers by: (a) a feral cat, (b) a fox, (c) a Red-necked Wallaby,
(d) a Superb Lyrebird, (e) a European Hare and (f) a Common Wombat
Figure 6. Setting up a Felixer at Bogong High Plains17
Figure 7. A Felixer washed out after heavy rain impacted its sensor alignment at Bogong High Plains 17
Figure 8. Warning sign posted near each Felixer to alert the public to the potential danger and to deter
interference
Figure 9. Curiosity feral cat bait under a wire cage to exclude animals from accessing baits

Figure 10. Examples of Curiosity feral cat bait identified as 'unattracctive'
Figure 11. The predicted effects on bait survival (attractiveness) at different locations from the 'best' model for the mean levels of temperature and rainfall. The red dotted line indicates the point at which 50% of baits became unattractive
Figure 12. Location of non-toxic Curiosity bait drop points at Tulloch Ard State Forest. Yellow dots are bait drop-points, and orange dots show the locations of dwellings. Purple line = 2-km buffer around the outside of bait drop-points
Figure 13. An example of Rhodamine B marking in the whisker of an Agile Antechinus (<i>Antechinus agilis</i>) collected at Tulloch Ard
Figure 14. The indicative locations of small mammal cage and Elliott trap transects (pink lines), feral cat cage traps (blue squares) and hair snares (yellow dots see section 7)
Figure 15. The probability a feral cat or 'other species' will consume a Curiosity cat bait, having encountered the bait
Figure 16. Examples of features used to identify individual feral cats
Figure 17. Hair snares set at Tulloch Ard were used to collect samples for DNA extraction. Each stake was wrapped in sticky tape staring 10 cm above the ground, and hairs were deposited as animals pushed between the stakes
Figure 18. Canidae Development detector dog working at Tulloch Ard searching for predator scats
Figure 19. DNA concentrations of the 39 extracted hair samples. Colours represent the qPCR test results. 42
Figure 20. qPCR melt-curve analysis of predator scats subsampled using three different methods (outside scraping, cross-sections and swabbing). Coloured bars represent the expected ranges of melt-curves from the five different Australian mammalian predators included in the test. Each point represents an extract, and the lines connect extracts from the same sample
Figure 21. The main food items in (a) Fox ($n = 61$), (b) Dingo ($n = 17$) and (c) feral cat ($n = 4$) scats at Tulloch Ard State Forest. Items with less than 3% occurrence are not shown

Summary

Context:

The 2019/2020 bushfires were exceptional in size and impact and have had a devastating effect on native plants and animals in Victoria. More than 1.5 million ha were burnt in Victoria, and many habitats and threatened species were severely impacted. Post-fire analysis has revealed that intensified and sustained control of introduced pest predators (such as feral cats) and of introduced herbivores is a priority action needed, both immediately and long term, to support the recovery of multiple native species listed as being of particular concern (DELWP 2021).

Over the past few decades, robust evidence has emerged demonstrating the significant impact of feral cats (*Felis catus*) on native wildlife. In Victoria, there are 43 species listed under the *Flora and Fauna Guarantee Act 1988* (Vic.) or the *Environment Protection and Biodiversity Conservation Act 1999* (Cth) as threatened by feral cat predation.

In July 2018, the Victorian Government declared feral cats an established pest on public land under the *Catchment and Land Protection Act 1994* (Vic.) (CaLP Act). This declaration allows more practical application of currently available tools for feral cat control (e.g. confinement traps, shooting) and enables the use of emerging tools (e.g. poison baits, grooming traps) when they become available. With the registration of the Curiosity® feral cat bait by the Australian Pesticides and Veterinary Medicines Authority (APVMA), land managers now have a tool for implementing landscape-scale feral cat control.

We have only a few examples of effective use of Curiosity (providing guidance on timing of baiting, number of repeated applications, spatial scale, and level of population reduction achievable) in south-eastern Australian temperate and wet forests. There is also a limited amount of information about the rate at which baits are removed by non-target animals and the environmental factors that affect the bait's attractiveness and palatability to feral cats, particularly in fire affected areas. Improving our understanding of these factors will help improve the efficacy of the available techniques for managing feral cats.

Aims:

This project aimed to:

- assess the target specificity of Felixer™ grooming traps
- assess the duration of the 'attractiveness' of non-toxic Curiosity baits to feral cats under field conditions
- assess the rates at which target and non-target animals encounter and consume Curiosity feral cat bait
- determine the density of feral cats across a range of habitats
- make this information available to land managers and policymakers to enable informed decisionmaking when planning feral cat control in Victoria and to guide future investment and research.

Methods:

Activities were undertaken across six locations: Barry Mountains (BM, feral cat density), Bogong High Plains (BHP, Felixer grooming traps), Gippsland Lakes Coastal Park (GLCP, Felixer grooming traps, bait attractiveness, encounter and consumption rates, feral cat density), Mount Buffalo National Park (MBNP, Felixer grooming traps), St Helena Spur in the Snowy River National Park (SHS, Felixer grooming traps) and Tulloch Ard State Forest (TA, bait attractiveness, encounter and consumption rates, feral cat density). To increase the geographical spread of the findings, we incorporated results on bait attractiveness, encounter and consumption rates, feral cat density from previous studies at Hattah–Kulkyne National Park (HKNP), Big Desert Wilderness Park (BD) and Wilsons Promontory National Park (WPNP).

Felixer grooming trap trials

We assessed the target specificity of Felixer grooming traps at locations where feral cats were known to be present, representing a diverse set of habitats (including locations impacted by the 2019/2020 fires), and for which knowledge was available regarding the presence of both target and non-target species. We deployed eight Felixers at each of three locations and four at one location. All traps were set in photo-only mode to assess the target specificity of these traps for feral cats and Red Foxes (*Vulpes vulpes;* hereafter 'foxes'), as opposed to non-target species. Deployment times varied from 16 to 33 days. Felixers were set on tracks considered likely pathways of movement by feral cats, foxes, and non-target species. We inspected all images and recorded the number of target and non-target species triggering events for each species at each location. We used this data to determine the probability that the Felixer: correctly identified a target species and was triggered; incorrectly identified a non-target species as a target species and was triggered; incorrectly identified a non-target species and was not triggered; and correctly identified a non-target species and was not triggered; and correctly identified a non-target species and was not triggered; and correctly identified a non-target species and was not triggered; and correctly identified a non-target species and was not triggered; and correctly identified a non-target species and was not triggered.

Attractiveness of Curiosity feral cat bait

We placed 50 non-toxic Curiosity feral cat baits in small wire cages pegged to the ground at three locations. Baits were otherwise open to the elements. Contained within the pellet inside each Curiosity bait was a biomarker, Rhodamine B (RhB). This marker produces a persistent and harmless mark that appears as a distinct fluorescent band in the hair and claws of animals that ingest the dye. At all sites, we inspected baits daily over a 10–14-day period to assess how the attractiveness of baits to feral cats changed over time and at what point they became unattractive. Attractiveness was determined by visual inspection of baits. Photographs were taken of each bait every day, and notes were made about the presence of insects (mainly ants), fungus or mould, and the physical condition of the bait structure (whether nibbled or broken down). We incorporated data from previous studies at two additional locations to increase the sample size and expand the range of habitats and environmental conditions to which the baits were exposed. We used Bayesian binomial regression models to analyse the influence of rainfall and temperature on the exposure duration at which baits became 'unattractive' to feral cats.

Uptake of Curiosity by non-target species under field conditions

We investigated the presence of RhB in the whiskers of animals exposed to non-toxic baits at the operational scale, i.e. baits laid across the landscape at a density of 50 baits/km². We deployed ~2945 non-toxic baits from a helicopter over an area of ~5900 ha at TA in autumn 2022.

To assess the presence of RhB in the whiskers of native animals, we used cage traps placed at each end of 12 transects, and Elliott traps set at 25-m intervals along the same 12 transects, throughout the baited area to live-capture native animals. Traps were checked daily for 17 days. We also established 60 feral cat cage traps baited with raw chicken and tuna oil in the same area. We collected six whiskers from each individual of each species, beginning 10 days following the aerial baiting operation, and examined these for the presence of RhB. We then assessed the proportions of sampled small mammals and of feral cats in which the presence of RhB indicated bait consumption.

Probabilities of consumption of Curiosity bait encountered by feral cats and by non-target species

Surveys were undertaken at three locations, and we incorporated results from three further locations from a previous study. We assessed the fate of baits by placing a single bait on the ground in front of each of a number of heat-in-motion digital cameras and inspecting the resulting images to determine what species took the bait and when. We deployed bait in front of 106 cameras at TA, 49 at BM, 46 at GLCP, 98 at HKNP, 39 at BD, and 90 cameras at WPNP for a total of 536 sampling days. We used Bayesian binomial regression models to determine the probability of consumption by feral cats and by non-target species when they encountered a bait.

Feral cat density

We deployed 106 cameras at TA, 49 at BM, and 46 at GLCP, as used in the encounter and consumption trials. At TA, we also deployed 80 hair snares to obtain DNA for individual animal identification, as an

independent dataset for density estimation and to compare the estimates from the two approaches. We identified individual feral cats from camera images and used repeated detections of these individuals in space and time to assess abundance using spatial mark–recapture models. Where insufficient detections of individuals precluded using these models, we assessed the relationships between occupancy rates and several covariates to determine the drivers of feral cat occurrence across the landscape. We used two separate analytical methods to produce the individual genotypes from DNA extracted from hairs of feral cats collected in the hair snares. The first method used a panel of cat-specific microsatellite markers that would allow individual cats to be identified. The second test was a quantitative PCR multiplex melt-curve analysis designed to distinguish Australian native mammalian predators from introduced mammalian predators.

Results:

Felixer grooming trap trials

The <u>specificity rate</u> (the percentage of events in which the Felixer was correctly not triggered, given the animal was a non-target species) was 92% [95% confidence interval (CI): 89–93%]. The <u>sensitivity</u> or true positive rate (the percentage of events in which the Felixer was correctly triggered, given the animal was a target species), was 41% (95% CI: 31–51%).

The <u>non-target misidentification rate</u> (the percentage of events in which the Felixer was incorrectly triggered, given the animal was a non-target species) was 7% (95% CI: 5–9%). However, when events involving European Hares (*Lepus europaeus*) were removed, the target misidentification rate dropped significantly to 0.12%. The <u>target misidentification rate</u> (the percentage of events in which the Felixer was incorrectly not triggered, given the animal was a target species) was 59% (95% CI: 49–69%).

The <u>target precision rate</u> (the percentage of events in which the Felixer was correctly triggered, i.e. a target species was present, given the Felixer had been triggered) was 47% (95% Cl: 42–64%). The <u>target</u> <u>imprecision rate</u> (the percentage of events in which the Felixer was incorrectly triggered, given the Felixer had been triggered) was 53% (95% Cl: 42–69%).

Attractiveness of Curiosity feral cat bait

Bait survival was primarily impacted by the location and the period of time in which the bait was left out. The effect of location on bait survival suggests that there is an unidentified driver of the bait decay rate. The predicted survival rates for the mean rainfall and temperatures during the times of our study suggest that bait survival was lowest at GLCP, with 50% of baits classed as unattractive by day 7.5; this level of bait decay was not reached till day 10.5 at WPNP and day 11.5 at HKNP. Environmental conditions were relatively stable during the bait survival trials at all three of these sites, which had moderate temperatures and rainfall. Data from previous studies indicate that both rainfall and temperature extremes will shorten the field life of Curiosity.

Uptake of Curiosity by non-target species under field conditions

We captured 353 individual native and introduced mammals at TA. The most common species caught was Bush Rat (*Rattus fuscipes*), followed by House Mouse (*Mus musculus*) and Agile Antechinus (*Antechinus agilis*). We captured three feral cats from 1635 cage trap nights.

Overall, 4.5% of all individual animals captured had RhB present, and most positive whiskers were detected in the Bush Rats (6.2% of Bush Rats captured and 3.4% of all captured animals). None of the three feral cats captured had RhB detected in their whiskers.

Probabilities of consumption of Curiosity bait encountered by feral cats and by non-target species

Bait take by feral cats was highest at GLCP, with all encountered baits (12 of 46 laid baits) being consumed. Bait consumption at the remaining sites ranged between 2 of 7 at BD (29%) and 0 of 106 and 98 at both TA and HKNP.

The model that best described the data included the number of days for which a bait had been laid, with location and bait station as random effects. The probability a feral cat would have taken an encountered bait by day 14 was 0.09 (95% CI: 0.03–0.25), while the likelihood that a non-target species would have taken a

bait by day 14 was 0.98 (95% CI 0.1–0.99). However, these probabilities are affected by the length of time a bait has been deployed, the feral cat/non-target species encounter rate, and environmental conditions. We simulated plausible encounter rates and showed that the chance a feral cat will consume an encountered bait could range between ~8% and ~27%, and that the chance that a non-target species will consume an encountered bait could range between 64% and 92%.

Feral cat density

The DNA extracted from the collected hair samples was insufficient to yield species identifications or individual genotypes of feral cats to undertaken density estimates. As density estimates were not possible, we used single season occupancy models to assess the occurrence of feral cats. The occupancy rate at TA was 0.41 (95% CI 0.26–0.56), at GLCP 0.74 (95% CI: 0.13–0.98) and at BM was 0.56 (95% CI: 0.25–0.82); however, the 95% CI's were wide.

Feral cat habitat distribution model

As the density estimates were unsuccessful, we built a habitat distribution model for feral cats using presence–absence data derived from detections obtained over 2020–2021 from surveys using 106–120 cameras. We included Landsat composite indices and terrain, soil and climate variables. The model had an average area under the curve of 0.65 in out-of-bag testing, indicating moderate predictive capacity. The habitat model suggests a preference of feral cats for lower-elevation parts of the landscape, e.g. gullies.

Predator diet

We collected 83 predator scats (61 from foxes, 17 from Dingoes, 4 from feral cats and 1 probably from a Spot-tailed Quoll *Dasyurus maculatus*) and determined the frequency of occurrence of the prey items in each scat. The most common prey item in fox scats was Bush Rat (54%); in Dingo scats, Common Wombat (*Vombatus ursinus*) was the most frequently occurring species (29%), followed by a relatively even occurrence of five other species. Bush Rats were the dominant species (53%) in the four feral cat scats collected.

Conclusions and implications:

This project has increased our understanding of the factors that affect the effectiveness of tools for managing feral cats in fire-affected areas of Victoria and beyond. This information will be valuable to land managers and policymakers, aiding in planning and future policy development for controlling feral cats in Victoria.

An increasing number of tools are available to land managers for controlling feral cats. However, at present, in Victoria not all these tools are available or easily implemented. For example, we found the Felixer grooming traps to be highly target specific; however, it is not registered for use in Victoria, requiring further data on possible ingestion of para-aminopropiophenone (PAPP) by Dingoes and Spot-tailed Quoll. Curiosity is also highly target specific, but its effectiveness can be limited by environmental conditions (which can affect the bait's attractiveness) and non-target interference rates (which reduce encounters and consumption by feral cats); similarly, further data is required on possible impacts on Spot-tailed Quoll and small Dingoes. Soft-jawed leg-hold traps are only permitted for use as an additional tool in eradication programs, and only with ministerial approval. Those tools that are permitted for use (baiting, shooting, and confinement trapping) are limited spatially, e.g. cats are a declared pest only on specific land tenure, and shooting and trapping are only usable at small spatial scales.

The outcomes from this and related studies indicate that landscape-scale feral cat control in Victoria, while possible, will be challenging and will require highly flexible resourcing and detailed planning.

1 Introduction

The 2019/2020 bushfires were exceptional in size and impact and have had a devastating effect on native plants and animals in Victoria. More than 1.5 million ha were burnt in Victoria, and many habitats and threatened species have been severely impacted. Post-fire analysis has revealed that intensified and sustained control of introduced pest predators [such as feral cats (*Felis catus*)] and of introduced herbivores is a priority action needed, both immediately and long term, to support the recovery of multiple native species listed as being of particular concern (DELWP 2021).

Over the past few decades, robust evidence has emerged demonstrating the significant impact of feral cats on native wildlife through direct predation (Nogales et al. 2004; Marlow et al. 2015; Jones et al. 2016). Predation by feral cats has been identified as the critical factor contributing to the failure of several reintroduction programs (Moseby et al. 2011, 2015; Hardman et al. 2016). Feral cats preferentially select small mammals as prey (Kutt 2012), and some individual cats can be disproportionately responsible for predation on populations of native species (Moseby et al. 2015). Feral cats are also the main predator of medium-sized mammals in locations where there has been sustained control of Red Foxes (*Vulpes vulpes*; hereafter 'foxes') (Marlow et al. 2015). In Victoria, there are 43 species listed under the *Flora and Fauna Guarantee Act 1988* (Vic.) (the FFG Act) or the *Environment Protection and Biodiversity Conservation Act 1999* (Cth) (the EPBC Act) as threatened by feral cat predation.

In Victoria, feral cats have become established in almost every terrestrial habitat type, although limited data are available on their densities or habitat use. Density estimates range from 0.24 cats/km² in the Mallee in spring (Robley et al. 2020) to 0.98 cats/km² in wet forests of the Otway Ranges (Rees et al. 2019).

Feral cats are obligate carnivores and can obtain their water requirements almost entirely from their food (Duffy and Capece 2011). As a result, feral cats prefer eating live prey such as European Rabbits (*Oryctolagus cuniculus*; hereafter 'rabbits') and other small mammals and lizards (MacDonald et al. 1984; Holden and Mutze 2002) over carrion, or baits deployed during control programs. In addition, feral cats tend to consume baits only when hungry, regardless of their palatability (Algar et al. 2007). In arid environments, the likelihood of feral cats consuming baits is related to the ratio of small mammal prey abundance to feral cat abundance (Algar et al. 2007; Christensen et al. 2013; Read et al. 2015).

In July 2018, the Victorian Government declared feral cats an established pest on public land under the *Catchment and Land Protection Act 1994* (Vic.) (CaLP Act). This declaration allows more practical application of currently available tools for feral cat control (e.g. confinement traps, shooting) and enables the use of emerging tools (e.g. poison baits, grooming traps) when they become available. On Parks Victoria estate, on forested lands managed by the Department of Environment, Land, Water and Planning (DELWP), Phillip Island Nature Parks, and in the areas controlled by the Alpine Resorts Victoria board—which are the land tenures where the declaration applies—land managers may now humanely destroy cats identified as feral and caught in cage traps. In addition, DELWP can undertake spotlight shooting of feral cats without demonstrating first that all reasonable attempts have been made to capture them.

The declaration also enables the use of poison baits for controlling feral cats. With the registration of the Curiosity® feral cat bait by the Australian Pesticides and Veterinary Medicines Authority (APVMA), land managers now have a tool for implementing landscape-scale feral cat control. The APVMA label sets out the conditions for the use of Curiosity. As feral cats rarely exhume buried food, poison baits must be surface laid. For ground baiting, baits must be placed at intervals of a minimum of 100 m, not exceeding 50 baits/km². For aerial baiting, baits must be dispersed at a maximum lay rate of 50 baits/km². The aerial and ground deployment of bait has the potential for non-target species to encounter and consume baits, thus reducing the efficacy of the control program.

The Curiosity bait is a small meat-based sausage prepared from kangaroo meat, chicken fat, and additional flavour enhancers. The toxin is encapsulated in a pellet, known as a hard-shell delivery vehicle (HSDV) (Johnston et al. 2020), formed from a pH-sensitive polymer that encapsulates the toxicant para-aminopropiophenone (PAPP).

The HSDV presentation was developed to reduce the primary hazard to those mammals, birds, reptiles and invertebrates that would be expected to consume the surface-laid meat lure, by exploiting the difference in dentition and feeding behaviours between feral cats and wildlife species (Marks et al. 2006; Buckmaster et al. 2014). Feral cats shear food portions into manageable sizes large enough to still contain the HSDV before swallowing them. In contrast, the size and hardness of the HSDV leads many other species to either eat around it or reject the HSDV during mastication to avoid tooth damage. Several studies have indicated that the HSDV effectively reduces the exposure of non-target species to PAPP (Marks et al. 2006; Hetherington et al. 2007; Forster 2009; Johnston et al. 2020). Buckmaster et al. (2014) assessed the potential for exposure of all Australian reptile, bird and mammal species to the encapsulated toxicant. They recommended further studies evaluating the hazard that the baits present to non-target species, and DELWP has developed a risk assessment strategy for the use of Curiosity in Victoria.

The Chemical Standards Branch, Department of Jobs, Precincts and Regions (DJPR) (Agriculture Victoria), administers the *Agricultural and Veterinary Chemicals (Control of Use) Act 1992* (Vic.), which regulates the use of agricultural and veterinary chemical products in Victoria. Under this Act, a non-target hazard assessment is required in an application to Agriculture Victoria for a permit to use Curiosity bait in Victoria, and this assessment must have the support of DELWP before an application can be submitted to DJPR.

We currently have few examples of appropriate use of Curiosity (including timing, number of repeated applications, spatial scale, and level of population reduction achieved) in south-eastern Australian temperate and wet forests. There is also a limited amount of information about the rate at which baits are removed by non-target animals and the environmental factors that affect the bait's attractiveness and palatability to feral cats. In one study, Johnston (2012) reported that the effectiveness of a control operation was likely reduced by rain, which made the baits unpalatable.

While landscape-scale control of feral cats is required in many parts of Victoria, there are circumstances in which the use of Curiosity can be supplemented or replaced by the application of other tools. Felixer™ grooming traps (hereafter 'Felixers') were developed and patented by Ecological Horizons (with assistance from several non-government organisations and government grants) as a novel, humane and automated tool to help control feral cats and foxes. They use rangefinder sensors to distinguish target cats and foxes from non-target wildlife and humans, and they spray targets with a measured dose of toxic gel containing either 1080 or PAPP and are still in development. The solar-powered Felixer, which can hold 20 sealed cartridges of toxic gel, automatically resets after firing. Felixers photograph all animals detected (including non-targets that are not fired upon) and can be programmed to play a variety of audio lures to attract feral cats and foxes. These traps have been tested, used, and registered as products in South Australia and Western Australia. They have yet to be trialled in mainland Victoria.

This project complements the work on public land funded through the Victorian Government's Bushfire Biodiversity Response and Recovery (BBRR) Program and the Australian Government funding package. The Phase 2 Theme 4 Plan of the BBRR program has been developed to implement a coordinated, strategic, targeted and integrated invasive species management program in response to the 2019/2020 bushfires.

The feral cat management project sits within the Theme 4 plan. The project will be delivered at six locations across eastern and north-eastern Victoria. It will assess a mix of feral cat control techniques, reducing current uncertainties, and ultimately improving both the effectiveness of feral cat control and our understanding of risk to non-target species by:

- increasing capability and management effectiveness for feral cat control in East Gippsland and the Victorian Alps
- filling critical knowledge gaps related to the use of both Curiosity feral cat bait and Felixers in Victoria
- assessing the duration of the 'attractiveness' of non-toxic Curiosity baits to feral cats under field conditions
- assessing the encounter and consumption rates of non-toxic Curiosity feral cat bait by target and non-target animals
- determining the density of feral cats across a range of fire-affected habitats in eastern Victoria.

2 Study areas

Activities were undertaken across six study areas: Barry Mountains (BM), Bogong High Plains (BHP, Alpine National Park), Gippsland Lakes Coastal Park (GLCP), Mount Buffalo National Park (MBNP), St Helena Spur (SHS, Snowy River National Park) and Tulloch Ard State Forest (TA) (Figure 1).



Figure 1. Location of activities undertaken as part of the BBRR feral cat management project. NP = National Park; SF = State Forest.

Barry Mountains

The BM study area is centred on the BM in the Great Dividing Range region of north-east Victoria (Figure 1). It is approximately 250,000 ha in size and encompasses Abbeyard, Mount Selwyn, Mount Cobbler and the old Wonnangatta Station areas. The study area was selected to encompass the locations of known records of Long-footed Potoroos (*Potorous longipes*) from the Great Dividing Range population and the proposed expanded DELWP and Parks Victoria fox control program protecting Long-footed Potoroos.

This area supports a wide range of native animals [32 native mammal species (including 11 native bat species), 224 native bird species and 26 native reptile species], many of which are at risk from feral cat predation. Mammal species of conservation concern include the endangered Long-footed Potoroo and Smoky Mouse (*Pseudomys fumeus*) and the vulnerable Broad-toothed Rat (*Mastacomys fuscus mordicus*). Introduced mammal species include rabbits, foxes and feral cats (Victorian Biodiversity Atlas 2022).

Bogong High Plains

The Bogong High Plains are located in the Victorian Alps (part of the Great Dividing Range) and are within the Alpine National Park and situated south of Mount Bogong (Figure 1).

This alpine and subalpine area supports a diverse mammal fauna including the threatened Mountain Pygmypossum (*Burramys parvus*), the Broad-toothed Rat, the Agile Antechinus (*Antechinus agilis*) and the Mainland Dusky Antechinus (*Antechinus mimetes*). Its insect fauna is diverse, the most notable species being the Bogong Moth (*Agrotis infusa*), which inhabits the area between November and April, as it aestivates away from the heat of the lowlands.

Mammals in the subalpine woodland include those listed as found in the alpine and subalpine open areas above, and also include the Feathertail Glider (*Acrobates pygmaeus*) and the Sugar Glider (*Petaurus breviceps*). Introduced mammal species include rabbits, European Hares (*Lepus europaeus*), foxes and feral cats (Victorian Biodiversity Atlas 2022).

Gippsland Lakes Coastal Park

GLCP is located along a section of the Ninety-mile Beach in East Gippsland, Victoria (Figure 1). This 17,600ha park was established in April 1979. Since 2010, the park has been managed by Parks Victoria jointly with the Gunaikurnai Land and Waters Aboriginal Corporation.

The park supports large populations of Eastern Grey Kangaroos (*Macropus giganteus*), Black Wallabies (*Wallabia bicolor*), Common Brushtail Possums (*Trichosurus vulpecula*) and Common Ringtail Possums (*Pseudocheirus peregrinus*). Less common mammal species present include Sugar Gliders, Eastern Pygmy Possums (*Cercartetus nanus*) and the endangered New Holland Mouse (*Pseudomys novaehollandiae*). Introduced mammal species present include Hog Deer (*Axis porcinus*), foxes and feral cats (Victorian Biodiversity Atlas 2022).

Mount Buffalo National Park

MBNP is located in the alpine region of northern Victoria (Figure 1). The park was established in 1898, expanded in 1908, and then further expanded in 1980 to 31,000 ha.

It supports a wide range of native animals, including 34 species of native marsupial and placental mammals [including Long-footed Potoroo, Southern Long-nosed Bandicoot (*Perameles nasuta*), Agile Antechinus, Mainland Dusky Antechinus, and 2 monotremes], 129 species of native birds and 31 species of native reptiles. Introduced mammal species present include rabbits, hares, foxes and feral cats (Victorian Biodiversity Atlas 2022).

Tulloch Ard State Forest

TA is an area of approximately 6800 ha of mixed-species forest on the Gelantipy Plateau (elevation 400–800 m) (Figure 1).

This area supports a wide range of native species, including 41 species of native mammals [including Long-footed Potoroo, Spot-tailed Quoll (*Dasyurus maculatus*), Southern Long-nosed Bandicoot, Agile Antechinus, Mainland Dusky Antechinus, White-footed Dunnart (*Sminthopsis leucopus*) and 2 monotremes], 136 species of native birds and 15 species of native reptiles. Introduced mammal species present include rabbits, foxes and feral cats (Victorian Biodiversity Atlas 2022).

St Helena Spur (Snowy River National Park)

The SHS area is in the Snowy River National Park, east of Wulgulmerang and west of the Snowy River (Figure 1). This site was the location of a Felixer trial and covered a small section of the St Helena Spur Track. The general area supports a range of native species, including the endangered Spot-tailed Quoll. Introduced mammal species present include rabbits, foxes and feral cats (Victorian Biodiversity Atlas 2022).

The activities undertaken in each study area are listed in Table 1, and then each activity is discussed in detail in the following sections.

Table 1. Activities undertaken in each of the six study areas.

Activity	Study area	Time period
Non-toxic Felixer trials	Gippsland Lakes Coastal Park	Sept 2021
	St Helena Spur	Dec 2021 – Jan 2022
	Bogong High Plains	Nov – Dec 2021
	Mount Buffalo National Park	Nov 2021
The attractiveness of Curiosity under field conditions	Gippsland Lakes Coastal Park	Sept 2021
Encounter and consumption of	Gippsland Lakes Coastal Park	Sept 2021
Curiosity	Tulloch Ard State Forest	Mar – Jun 2022
Feral cat density estimation	Gippsland Lakes Coastal Park	Sept 2022
	Barry Mountains	Oct 2021
	Tulloch Ard State Forest	Mar – Jun 2022
Uptake of non-toxic Curiosity by non- target species (live trapping)	Tulloch Ard State Forest	Apr 2022

3 Assessing the target specificity of Felixer feral cat grooming traps

3.1 Introduction

The Felixer feral cat grooming traps (hereafter Felixers) are a novel method of controlling feral cats that takes advantage of the compulsive grooming behaviour of cats. The Felixer uses a series of sensors to identify whether an animal passing the device is a feral cat or a non-target species. If the animal is recognised as a target, the Felixer shoots a sticky gel containing 1080 poison (sodium monofluoroacetate) or PAPP onto the animal's fur. When a feral cat grooms and ingests this gel from its fur, it receives a lethal dose of the poison.

We collected information on the target specificity of Felixers across a range of habitat types in eastern and north-eastern Victoria. This project aimed to:

- 1. collect information on the practical application of this tool in the field and increase land managers' awareness of the operation of these devices
- 2. assess the target specificity of Felixers and communicate the findings to land managers and policymakers, thus providing guidance on the likely impact of using this tool under Victorian conditions.

We deployed four to eight Felixers (Felixer 3.1; build release 25-08-2021) in photo-only mode (i.e. without firing the poisoned gel) at each of four locations [GLCP, MBNP, BHP (Alpine National Park) and SHS (Snowy River National Park)] to assess their target specificity.

3.2 Methods

3.2.1 Felixer operations

Felixers use infrared laser–based rangefinder sensors to detect objects moving in front of the trap. Each unit has two blocking sensors and two activation sensors (Figure 2). The unit ejects a dose of poisonous gel when a target animal intercepts both activation sensor beams simultaneously, while not intercepting the blocking beams. Targets are defined by body height >230 mm but <460 mm, body length >250 mm, and a ventral clearance of >60 mm, based on pen trials of precursors to the Felixer (Read et al. 2015). Computer algorithms account for the speed at which passing animals break the sensors and the angle at which they enter the detection zone. Rapid target qualification (150 ms), triggering (<40 ms), and gel ejection speed (60 m/s) ensures the toxin gel strikes a target cat moving at 5 km/h at a maximum 4-m range. Felixers also use a selection of intermittent programmable audio lures to attract feral cats and foxes, capture all sensor activation information, and photograph all triggered events in order to determine the target specificity.



Figure 2. Position of the activation (height 230 mm) and blocking sensors (height 460 mm) designed to determine target versus non-target activation of the Felixer. A Brush-tailed Bettong (*Bettongia penicillata*) is seen passing underneath both activation sensors and hence would not trigger the unit (from Read et al. 2019).

All Felixers were set up as follows:

- The target detection zone was set to the entire width of the track, which ranged between 2 m and 4 m.
- Targeting mode was set to 'Conservative', as recommended in "areas where non-target species are prevalent or of particularly high value, or readily mistaken for a target species".
- Auditory lures were activated to play a combination of sounds (bird and cat calls) at five-minute intervals.
- No olfactory or visual lures were used in association with the Felixers.
- Units were armed in 'photo-only mode'. In this mode, the device acts purely as an infra-red camera trap with a sophisticated laser-based sensor system (Read et al. 2019); the Felixers will not arm the firing mechanism but will otherwise function as when fully armed with respect to sensing and logging data [Felixer 3.1 Operation Manual (10 August 2020)].
- The bait canisters were empty, and therefore no poison was present within the traps or able to be fired.
- The trap has a default 120-s 'cool-down' time between trigger events, meaning that detection events are at least 2 min apart.

Four sites were selected for this trial based on their geographic spread, available knowledge about the presence of both target and non-target species, and to represent a diverse set of habitats (Figure 3).



Figure 3. Location of Felixer trials in Victoria.

We deployed eight Felixers at each site except SHS, where we deployed four traps. All traps were set in photo-only mode to assess the target specificity of these traps for feral cats, foxes, and non-target species. Deployment times varied from 16 to 33 days.

Felixers were set at right angles to tracks considered likely pathways of movement by feral cats and nontarget species (Figure 4). A solid backdrop to the target detection zone was present in each case. This was either a pre-existing structure (e.g. a large tree or log) or locally available material (e.g. coarse woody debris or rocks) was added or enhanced. We also cleared all vegetation from the detection zone and if needed added gravel to level the site.



Figure 4. Typical Felixer set-up in the field.

3.3 Data analysis

Felixers in photo-only mode record an event when an animal or other movement triggers one of the infrared sensors, resulting in a single image being taken. The software classifies each triggering event in the Felixer as either a target event (i.e. the Felixer would have ejected the poison gel if the unit was armed) or not a target event.

We inspected all images and tallied the numbers of target and non-target triggering events for each species at each location. We sorted similar-sized and -shaped species into five groups: medium-sized birds, small mammals, medium-sized mammals, large mammals, and target species (feral cat and fox). The Superb Lyrebird (*Menura novaehollandiae*) and the European Hare (*Lepus europaeus*) made up another two separate single-species 'groups' as there were a large number of detections of these two species, allowing for these to be analysed separately.

We used this data to examine the estimated probabilities that the Felixer:

- correctly identified a target species and was triggered (a 'true-positive' or 'true trigger');
- incorrectly identified a non-target species as a target species and was triggered (a 'false-positive' or 'false trigger');
- incorrectly identified a target species as a non-target species and was not triggered (a 'falsenegative' or 'false non-trigger)'; or
- correctly identified a non-target species and was not triggered ('true-negative' or 'true non-trigger') (Table 2.).

Table 2. Matrix for the Felixer identifying target and non-target species.

		Triggered	Not triggered	
True target	Target species	True trigger	False non-trigger	Total target detections
status	Non-target species	False trigger	True non-trigger	Total non-target detections
		Total triggers	Total non-triggers	

Felixer response

We used a binomial generalised linear model (GLM) to estimate the four probabilities associated with the confusion matrix in Table 2. We then used that model to predict the true and false trigger rates for the Felixer, together with their 95% confidence intervals. The dependent variable was the number of times the Felixer would have been triggered, given the number of detections, and the explanatory variable was whether the detected species was a target or a non-target species. We did not include location, as there was no reason to assume that the triggering rates of the Felixers would differ between locations. To derive the false non-trigger and true non-trigger rates, we subtracted the true trigger and false trigger rates from 1.

We also used the data to assess the specificity of the Felixer [i.e. the true-negative rate, i.e. given the animal was a non-target species, the percentage of events in which it correctly did not trigger the Felixer] and the misidentification rate (the percentage of events in which the Felixer would misidentify a species as a target/non-target species, which is conditional on the true target status).

The misidentification rate for non-target species is thus:

$$Misidentification \ rate_{non-target} = \frac{False \ trigger \ (FP)}{Total \ non-target \ detections} \ x \ 100$$

and the misidentification rate for target species is then:

$$Misidentification \ rate_{target} = \frac{False \ non - trigger \ (FN)}{Total \ target \ detections} \ x \ 100$$

We also determined the imprecision rate (the percentage of events in which the Felixer is triggered, but the detected animal is not actually what the Felixer identifies it to be). The target imprecision rate is then:

$$Imprecision \ rate_{triggered} = \frac{False \ trigger}{Total \ triggers} \ x \ 100$$

The misidentification and imprecision rates can be used for various purposes. The non-target misidentification rate is the percentage of events in which a non-target species detected by the Felixer would erroneously trigger the system, that is, the percentage of events in which non-target individuals would have potentially been exposed to the poison. The target misidentification rate is the percentage of events in which a Felixer is not triggered by a target species. The target imprecision rate is the percentage of events in which the Felixer is triggered erroneously, i.e. triggers are wasted.

3.4 Results

3.4.1 Target specificity

Pooled across locations, Felixers were correctly triggered by feral cats 16 times and by foxes 20 times but failed to be triggered by target species 41 times (feral cat, n = 20; fox, n = 21). Felixers detected non-target species 614 times, correctly not being triggered 562 times, and incorrectly being triggered 52 times (Table 3). In these 52 incorrect triggering events, 47 were caused by European Hares at BHP. Other non-target triggers were caused by Superb Lyrebirds (n = 3), a Black Wallaby (n = 1) and a Common Wombat (n = 1).

Table 3. Summary of the numbers of detections of triggering and non-triggering events by Felixers pooled across all locations.

		Triggered	Not triggered	
True target	Target species	36	41	77
status	Non-target species	5	412	417
	Hare	47	150	197
		88	603	

Felixer response

The <u>specificity rate</u> (the percentage of events in which the Felixer was correctly not triggered, given the animal was a non-target species) was 92% (95% CI: 89–93%). <u>The sensitivity</u> or true positive rate (the percentage of events in which the Felixer was correctly triggered, given the animal was a target species), was 41% (95% CI: 31–51%).

The <u>non-target misidentification rate</u> (the percentage of events in which the Felixer was incorrectly triggered, given the animal was a non-target species) was 7% (95% CI: 5–9%). However, when events involving European Hares were removed, the target misidentification rate dropped significantly to 0.12%. The <u>target misidentification rate</u> (the percentage of events in which the Felixer was incorrectly not triggered, given the animal was a target species) was 59% (95% CI: 49–69%).

The <u>target precision rate</u> (the percentage of events in which the Felixer was correctly triggered, i.e. a target species was present, given the Felixer had been triggered) was 47% (95% CI: 42–64%). The <u>target</u> <u>imprecision rate</u> (the percentage of events in which the Felixer was incorrectly triggered, given the Felixer had been triggered) was 53% (95% CI: 42–69%).

Figure 5 shows examples of target and non-target triggering events.



Figure 5. Target and non-target triggering of Felixers by: (a) a feral cat, (b) a fox, (c) a Red-necked Wallaby, (d) a Superb Lyrebird, (e) a European Hare and (f) a Common Wombat.

3.4.2 Felixer establishment in the field

Felixers require careful attention to set-up to ensure correct triggering. A clear and level site in front of the Felixer is required to a maximum distance of 4 m in front of the unit and 2 m wide. At most sites, this was achieved by placing the Felixer on a low-use vehicle track, either behind closed gates or in locations with minimal or no public access. We also placed them around buildings (a Parks Victoria depot and office). At all locations except GLCP (in sandy coastal habitat), we needed to bring gravel or sand to each site, as the tracks had a compacted gravel road base and wheel ruts making it impossible to level the site (Figure 6). At least at one location, the added substrate was washed away by heavy rainfall (Figure 7) and had to be replaced, because the blocking and activation sensors became incorrectly aligned. Ideally, the location would allow for the securing of the solar panels required to power the unit.



Figure 6. Setting up a Felixer at Bogong High Plains.



Figure 7. A Felixer washed out after heavy rain impacted its sensor alignment at Bogong High Plains.

Closing vehicle access tracks in state forests or on Parks Victoria estate, while possible, presents challenges and is not always successful at preventing access. Units can be locked, cable-locked to trees, have GPS tracking units added and warning signs posted (Figure 8), but concealment of units and restricting access by the public can be challenging when units are placed on public land outside fenced areas. At one location a solar panel was stolen, despite the unit being placed well out of sight for general traffic, and the panel being attached to a tree via a steel cable.

	IMPORTANT INFORMATION						
	Pest animal research for conservation currently underway						
Instru	ments contain and emit potentially hazardous visible and invisible radiation that can damage eyes						
F	OR YOUR SAFETY DO NOT APPROACH OR INTFERFERE WITH DEVICES						
	Project officers will be working in the area at any time (24/7)						
	PENALTIES APPLY for unauthorised use of or interference with devices						
	Planned dates of operation:/ to//						
Arthur Rylah Institute	For further information please contact the Arthur Rylah Research Institute: 03 94508600						

Figure 8. Warning sign posted near each Felixer to alert the public to the potential danger and to deter interference.

3.5 Summary

Our results support the findings of previous studies and extend our knowledge of the effectiveness of Felixers in temperate forested and alpine environments. The target specificity of Felixers has previously been evaluated in other parts of Australia, notably in arid environments, on island systems, and inside fenced enclosures (Dunlop et al. 2019; Read et al. 2019; Moseby et al. 2020). Those trials indicated that Felixers have a high rate of accuracy in both target specificity and non-target identification.

The rates at which Felixers might incorrectly be triggered by non-target species are of particular concern to policymakers and land managers. We recorded very low non-target misidentification rates (5-9%) and high rates of non-target specificity (89–93%). On the few occasions on which Felixers were triggered by nontarget species, they were species that were unlikely to groom the gel [e.g. Superb Lyrebird, Common Wombat (Vombatus ursinus)] or receive a dose of PAPP sufficient to result in death [Red-necked Wallaby (Notamacropus rufogriseus)]. In our study, false triggering may have been related to incorrect set-up of the Felixers. For example, in the case of the Common Wombat and the Superb Lyrebird, the ground between the target and the unit was not completely level, possibly resulting in a misalignment of the triggering sensors. Triggering of the unit by the wallaby was probably a result of its angle of entry and its slow, crouched position as it moved across the sensors. Hares are roughly the same size and shape as feral cats and were incorrectly identified by the Felixer as a target species under the most conservative and low risk setting used in our trials. The then current V3.1 Felixer (as employed in our trials) uses four LiDAR sensors to detect objects, and a software algorithm to discriminate targets (feral cats and foxes) from non-targets. The recent V3.2 incorporates a camera-based artificial intelligence system with a Kendryte processor (https://canaan.io/product/kendryteai) working with the four LiDARs to minimise false-positive triggering. The manufacturers have stated that the newer version reduces false-positives by 8% (down from $\sim 4\%$ to $\sim 12\%$), increases the true-positive rate by 1.6 times (up from ~45% to ~75%) and can compute the likelihood that a passing animal is a target 10 times per second.

While Felixers are effective at reducing feral cat numbers in situations where immigration can be controlled [e.g. within fenced areas (Moseby et al. 2020)], their use in open systems is less straightforward. Modelling of the practical and economic feasibility of using Felixers for population control of free-roaming cats in the Midlands of Tasmania using population viability assessment simulations suggested a clear efficiency advantage in longer-term deployment scenarios (Humphreys 2021). When implemented over periods of >12 months, even small numbers of devices were predicted to successfully reduce a target population of free-roaming cats by >80%. In contrast, short-term scenarios (≤ 6 months) required a four-fold-higher Felixer density to reach maximum (65–80%) population reduction, and eradication of the target population was never achieved.

Theft or damage of wildlife survey equipment (Meek et al. 2019) or control tools (baits and traps) is not uncommon, particularly in state forests, and Felixers are large, heavy and obvious units. However, there are practical considerations to deployment in locations freely accessible by the public. The requirement to place units in locations that maximise passage by feral cats (and foxes) with 2–4 m of clear space in front of the unit means they are more likely to be placed on vehicle tracks or along fences, resulting in exposure to the public and a consequent risk of loss or damage. Gravel or dirt vehicle tracks are often constructed with a firm road base or in locations with naturally rocky substrates. This makes levelling sites problematic; in our study, we used gravel imported to sites via trailer or tip-truck to level the sites, adding to the complexity and to limitations on where units could be deployed.

In Victoria, Felixers would be an ideal additional tool for eradicating feral cats on islands (e.g. Phillip Island and French Island) or within fenced areas (e.g. the proposed Wilsons Promontory Safe Haven or the Trust for Nature property at Neds Corner). Strategic use of Felixers as part of an integrated control operation on Parks Victoria estate is possible but would require careful planning. The use of Felixers in open areas of state forest is unlikely to be practical, as the risk of interference, damage or theft is likely to be high. Currently, the Victorian Government is not supporting the registration of Felixers with 1080 poison as the toxin due to concerns about possible non-target impacts and Felixers are not registered for use with PAPP anywhere in Australia yet. Although at the time of writing, research was underway in Western Australia on incorporating PAPP into a gel formulation with sufficient toxicity to kill feral cats.

4 The attractiveness of Curiosity feral cat bait under field conditions

4.1 Introduction

An effective bait needs to be both attractive and palatable to the target species. Attractiveness refers to the ability of the bait to lure an animal towards it so there is physical contact with the bait. Palatability is the characteristic of the bait that induces the target species to consume it.

There is limited information available about the environmental factors that affect the attractiveness and palatability of Curiosity to feral cats. Research conducted in the arid zone has suggested that the optimum time to conduct baiting programs and maximise their effectiveness is when there are cool, dry conditions, such as in late autumn and winter (Algar et al. 2002). At this time of year, rainfall [which has been reported by Johnston (2012) as causing degradation of Eradicate cat baits] is less likely to occur than during the summer months; in addition, bait degradation due to ants and to hot, dry weather is significantly reduced.

The current APVMA label and Victorian Government permit conditions for Curiosity bait reflect the potential risk to goannas (*Varanus* spp.) and other large reptiles, directing that baiting occur when temperatures are no more than 16°C in the 6 days following bait deployment, to reduce the likelihood of these species encountering and consuming baits. In southern parts of Victoria, where goannas are known to occur, the time of year when this temperature requirement is most likely met coincides with winter, a time of increased and more consistent rainfall. In semi-arid and arid climates, periods of hot daytime temperatures and low humidity may act to rapidly dry baits, which may also result in lowered palatability.

We investigated these factors as part of this study and to increase the sample size and to investigate the attractiveness of Curiosity baits across a broad range of Victorian habitats and environmental conditions, we included data from a previous study (Robley et al. 2020, 2022b). This study was part of the Victorian Government Biodiversity 2037 (*Protecting Victoria's Environment – Biodiversity 2037*) plan to stop the decline of the state's native plants and animals. Funding for implementing actions under the Biodiversity 2037 plan was through the Biodiversity Response Planning (BRP) program. These data were from a semi-arid site in north-west Victoria (Hattah-Kulkyne National Park, HKNP) and a coastal location in southern Victoria (Wilsons Promontory National Park, WPNP).

This current study aimed to assess the change in the attractiveness of Curiosity bait under field conditions. Information from this trial will aid policymakers and provide practical guidance to land managers when planning feral cat bating operations using Curiosity feral cat bait.

4.2 Methods

4.2.1 Study areas

At GLCP, 50 non-toxic Curiosity feral cat baits, each containing an HSDV with 5.5 mg Rhodamine B (RhB) dye in the pellet (manufactured by Scientec Research Pty Ltd, Melbourne, Victoria), in individual small wire cages (12 cm x 3 cm x 8 cm) that were pegged to the ground (Figure 9). Baits were otherwise open to the elements. At HKNP and WPNP, 40-45 feral cat baits were placed similarly.

4.2.2 Bait trials

At GLCP, baits were placed in pairs along a low-use vehicle track, with each of the two baits in a pair set along a transect at 90° to the track and 30 m apart. Pairs were set at 300-m intervals along the track. At WPNP, baits were similarly placed along transects running perpendicular to a low-use vehicle track, but this time baits were spaced at 25-m intervals along each transect and transects were spaced at 50-m intervals. At HKNP, 50 baits were placed at 25-m intervals along 10 transects, spaced at 25-m intervals starting 25-m from a low-use vehicle track.



Figure 9. Curiosity feral cat bait under a wire cage to exclude animals from accessing baits.

Before being laid, Curiosity baits were thawed and placed in direct sunlight for at least 1 hour. This process, termed 'sweating', causes the oils and lipid-soluble digest to exude from the surface of the bait. All Curiosity baits were sprayed during the sweating process with an ant-deterrent compound (Coopex®), the main active constituent being permethrin. This process aims to prevent bait degradation by ant attack; in addition, the physical presence of ants on and around the bait medium may deter bait acceptance by feral cats.

At all sites, we inspected baits daily over a 10–14-day period to assess how the attractiveness of baits to feral cats changed over time and at what point they became unattractive. Bait survival (attractiveness) was determined by daily visual inspection of baits; notes and photographs were taken of each bait on each day, noting the presence of insects (mainly ants), fungus or mould, and the physical condition of the bait (broken down). Our estimates of bait attractiveness were subjective, and observer bias cannot be dismissed entirely. To decrease the risk of this bias, we provided training to personnel involved in the assessment, only one person assessed each location, and a set of reference images of unattractive baits (obtained during previous studies) was provided to aid in standardising classification. Figure 10 provides examples of baits considered to be unattractive to feral cats.





Figure 10. Examples of Curiosity feral cat bait identified as 'unattractive'.

Baits were subjectively scored as either '1' (unattractive) or '0' (attractive). On a few occasions, small mammals were able to remove baits; these baits were known to be 'alive' (attractive) up to the day before they were removed, but their fate remained unknown.

4.2.3 Data analysis

We investigated the effect of several environmental covariates on the survivorship of feral cat baits. For this analysis, we used logistic regression with time-varying components. This approach modelled the likelihood of a bait becoming unattractive as a function of environmental and other covariates, for each particular bait over each period. To help explain what factors might impact bait attractiveness and palatability, we included as covariates: cumulative daily maximum temperature (°C), cumulative 24-hour rainfall (sqrt, mm), 24-hour rainfall (mm) and daily maximum temperature (°C) in the previous 24 hours, time (days since a bait was laid) and location (HKNP, GLCP, WPNP). We looked at the correlations between these variables and found that cumulative temperature and days since bait was laid were correlated, as were both cumulative rain and rainfall in the previous 24 hours; correlated variables were not included in the same models.

We ran several model combinations including the above covariates and compared the model outcomes using leave-one-out cross-validation (LOO-CV) to select the best-performing model (Vehtari et al. 2017; Vehtari et al. 2020). Models were implemented using Bayesian methods with the brms R package (Bürkner 2017, 2018, 2021). We used a weakly informative normal prior with a standard deviation of four. Four chains were run in parallel, with 1000 warm-up iterations and 2000 sampling iterations. We assessed model convergence via inspection of trace plots and Rhat values, which showed good evidence of convergence, with no Rhat values above 1.05 (Gelman et al. 2021).

4.3 Results

We ran eight models with various combinations of location and environmental covariates. Within the selected models, the model that best explained the data included Location * time + temperature in the previous 24 hours + rainfall in the previous 24 hours + a random effect of bait.

Bait survival was primarily impacted by the location of the bait station and the period over which the bait was left out; with some slight evidence (95% CI -0.87 to -0.05) that bait survival was longer with increased rainfall. The effect of location on bait survival suggested that there was unexplained variation driving bait decay, with GLCP appearing to have faster decay rates.

We obtained general survival curves for predicting survival rates from mean rainfall and temperature. These indicated that bait survival was lowest at GLCP, with 50% of baits being classed as unattractive by approximately day 9. At WPNP, 50% of baits were classed as unattractive by approximately day 10, and at HKNP, 50% of baits were classed as unattractive by approximately day 12 (Figure 11).



Figure 11. The predicted effects on bait survival (attractiveness) at different locations from the 'best' model for the mean levels of temperature and rainfall. The red dotted line indicates the point at which 50% of baits became unattractive.

4.4 Summary

We set out to quantify environmental factors that might influence the attractiveness of Curiosity feral cat bait. We expected that increased amounts of rainfall and higher temperatures would affect the rate of decline in bait attractiveness. However, location was the primary factor effecting bait survival with time also having an influence. There was only slight evidence that rainfall was influential.

Similar studies have found that high rainfall and temperatures contributed to decline in bait attractiveness, as indicated by a less-than-expected decline in feral cats following baiting with Curiosity or the similar bait product Eradicat (Algar and Burrows 2004) after rain and high temperatures. Based on the results from the present trial and previous trials at HKNP and WPNP (Robley et al. 2020), there would appear to be a 'Goldilocks zone' in which temperatures are below ~25°C for several days following a deployment, with little increase in temperature over that period, and when rainfall in the 24–48 hours preceding baiting is less than ~20 mm. Curiosity is likely most effective in environments with mild winter temperatures and low to moderate rainfall.

GLCP and the WPNP are both low-lying coastal areas situated in South Gippsland, while HKNP is a semiarid location in northern Victoria. HKNP did experience relatively milder conditions (maximum temperature = 23°C, mean 24-hour rainfall = 1 mm) compared with GLCP and WPNP (19°C and 20°C, and 8 mm and 5 mm, respectively). However, all of the sites' environmental conditions were within the range previously reported as being suitable for baiting, helping confirm that Curiosity is likely to be able to tolerate environmental conditions experienced throughout much of Victoria.

We provided training for observers and written materials, including sets of images of baits with various degrees of 'attractiveness'. Assessment of the 'attractiveness' of baits is subjective, which may have biased the outcomes of the modelling. However, we put in place protocols to minimise potential bias by observers at the different locations, and to standardise the scoring of the state of the bait.

The developers of Curiosity state that selection of the 'initial' moisture content of the bait entailed a trade-off between (a) the bait being sufficiently attractive, palatable and processable by cats for a reasonable period,

and (b) retaining the structural integrity of the poison pellets for that same period. Under ambient conditions, even dry ambient conditions, the attractants in the bait dry out slowly, with a gradual diminution in attractiveness, palatability, and ease of processing. Under optimal conditions, the bait remains effective for 14 days.

However, environmental conditions impact this effective period. Thus, if the temperature is low, there is only slow 'drying' of the bait, and so the moisture content remains 'constant'. Conversely, if the bait is in a 'wet' environment (e.g. there are heavy dews and rain events), the surface, and subsequently the bulk of the bait, becomes soggy. Humid environments promote fungus (mould) growth and microbes (putrefaction). On the other hand, if the bait is in a dry (hot or cold) environment, it becomes desiccated. Desiccation of the attractants in the bait affects the physicochemical properties of the pellet coating matrix. The result is the leaching of one or more of the additives of the coating formulation. Desiccation is accelerated in hot, dry environments.

These results, and those reported previously and elsewhere, indicate that the timing of deploying Curiosity bait must consider the predicted environmental conditions; otherwise, the investment made in a feral cat control operation will be potentially wasted. Aerial baiting operations entail significant planning and preparation time, requiring substantial lead time. Baiting operations must be flexible and incorporate standby time for equipment and staff to accommodate the changes in weather conditions that may negatively impact bait attractiveness and operational effectiveness. This will have implications for budgeting for baiting operations.

5 Uptake of Curiosity by non-target species under field conditions

5.1 Introduction

A key consideration when using poison baits is the risk to non-target species. Potential non-target exposure to PAPP from Curiosity has been investigated and tested against several native species (Marks et al. 2006; Hetherington et al. 2007; Forster 2009; Johnston 2010; Gigliotti 2011). These investigations have shown that encapsulating the toxin in a pellet is effective at reducing exposure.

While this means that a diversity of non-target species may have a significantly reduced risk of being poisoned, these species may still find the bait attractive and remove it, making it unavailable to feral cats. We have less information on the rate at which non-target species remove baits. Algar et al. (2007) reported that native species (mainly varanids) removed up to 80% within 19 h of baits being laid in semi-arid coastal Western Australia.

The declaration of feral cats as an established pest species by the Victorian Government and the registration of Curiosity feral cat bait by the Federal and Victorian Governments have created the possibility for the landscape-scale control of feral cats in Victoria. It is recommended that Curiosity be deployed aerially at a rate of 50 baits/km² to achieve sustained control over a large area. In Victoria, Curiosity was aerially deployed at HKNP and French Island. In neither case were the results as expected (Robley et al. 2022a; M. Johnston pers. comm.); instead, there was well below the expected 70–80% reduction in feral cat occupancy or abundance.

The main aim of the approved project plan was to assess the effectiveness of aerially deployed Curiosity bait in reducing feral cat abundance. Planning and consolation began in October 2021 and the activity was planned for March 2022 at Tulloch Ard in East Gippsland. This included monitoring of feral cat abundance and the potential factors influencing the outcome (such as consumption by non-target native species), with monitoring to be undertaken simultaneously at a non-baited comparison location.

Approval for the use of Curiosity in Victoria is granted by Agriculture Victoria, subject to DELWP approval of an assessment of the Hazard (a measure of the oral toxicity [such as the 'lethal dose' value] and the animal's size) and Exposure (the likelihood of an animal encountering toxic bait(s), how much it is likely to ingest, and whether it is known to [or is able or likely to] consume the HSDV). In addition to this 'hazard + exposure' approach, DELWP, when considering the risk assessment, also considers the conservation status of species in the activity area, the likely impact on statewide populations, the current socio-political environment at the time of the application, and the actual or perceived reputational risk to DELWP.

On evaluation of the risk assessment, the use of toxic baits in this project was not supported by DELWP due to what was considered an unacceptable level of risk to the Dingo (*Canis familiaris*; Jackson et al. 2017). This necessitated a redesign of the project activities while still attempting to achieve the agreed aims of increasing our understanding of the impacts of aerial baiting using Curiosity on both target and non-target species. To do this we simulated an aerial baiting operation at the operational scale using non-toxic baits, focusing on non-target and target risk issues. Each bait contained a biomarker (Rhodamine B, RhB) that can be detected in the whiskers of animals that have consumed a pellet containing the biomarker. This enabled an assessment of the impact on non-target species that might consume bait during an operational baiting.

In this chapter, we report on the investigation into the presence of the biomarker in the whiskers of animals exposed to non-toxic baits at the operational scale, i.e. laid across the landscape at 50 baits/km². We present the probabilities that a feral cat or non-target species will consume a bait, given that a bait has been encountered.

5.2 Methods

5.2.1 Study site

TA was the location selected for this trial, based on the results of annual camera surveys undertaken by the DELWP East Gippsland Region from 2011 to 2020, which have indicated the presence of increasing populations of Long-footed Potoroo, but few occurrences of other native species, such as Southern Long-nosed Bandicoot and Southern Brown Bandicoot (*Isoodon obesulus*; Robley et al. 2022b). The DELWP surveys also revealed very low occurrences of foxes and Dingoes, but a strong and increasing occurrence of feral cats (35% of sites had feral cats in 2020; Robley et al. 2022b).

5.2.2 Aerial baiting operation

The non-toxic aerial baiting operation at TA and a small adjoining section of the Snowy River National Park was undertaken in the autumn of 2022. A total of ~2945 baits were distributed from a helicopter at 50 baits/km². The aircraft flew 60–80 m above the tree canopy, along 240 km of predetermined transects, covering an area of ~5900 ha. At each point, five baits were dropped, which, as they fell from the moving helicopter, spread over ~10 ha. Bait drop-points were excluded if they were within 2 km of dwellings, within 100 m of permanent or flowing streams and drinking water supply, within 250 m of gazetted public roads, or within 500 m of recreational sites (Figure 12).



Figure 12. Location of non-toxic Curiosity bait drop points at Tulloch Ard State Forest. Yellow dots are bait droppoints, and orange dots show the locations of dwellings. Purple line = 2-km buffer around the outside of bait drop-points.

The biomarker RhB was contained within the HSDV pellet inside each Curiosity bait. This marker produces a persistent and harmless mark that appears as a distinct fluorescent band in the hair and claws of animals that ingest the dye (Figure 13; Fisher 1998). Samples of mystacial vibrissae (whiskers) collected from small mammals at TA were examined under a fluorescent microscope to screen for the presence of RhB. Sample preparation and examination procedures followed those outlined in Fisher (1998). In this study, we used an epifluorescent condenser compound microscope (Zeiss IV FI), incorporating a high-intensity 200-W mercury lamp with a permanent BG38 filter and a green filter combination block (Zeiss I).



Figure 13. An example of Rhodamine B marking in the whisker of an Agile Antechinus (*Antechinus agilis*) collected at Tulloch Ard.

To assess the presence of RhB in whiskers of native animals, we live-captured native animals using cage traps ($610 \times 305 \times 305$ mm, Wiretainers, Melbourne) placed at each end of 12 transects, and Elliott traps ($330 \times 80 \times 90$ mm, Elliott Scientific Equipment, Victoria) set at 25-m intervals along the same 12 transects throughout the baited area (Figure 14). Traps were arranged in rows >50 m from the track and parallel to the track, baited with an ~25-g ball of rolled oats, peanut butter, and honey. Traps were placed under shrubs, lined with bedding material, and wrapped in plastic. Traps were checked daily for 17 days. Captured animals were marked by clipping a small section of fur, so recaptured animals were not processed twice. We also set 60 feral cat cage traps ($810 \times 280 \times 330$ mm, Wiretainers, Melbourne) at 1–2 m off tracks and between the native mammal trap transects. Cage traps were baited with a raw chicken pieces and tuna oil.

We collected six whiskers from each species (three from each side of the face), beginning 10 days after the aerial baiting operation, and examined these for the presence of RhB. The whiskers collected from each animal were placed in individual plastic zip-lock bags, labelled using a unique ID and stored in a fridge at \sim 3°C until processing occurred.



Figure 14. The indicative locations of small mammal cage and Elliott trap transects (pink lines), feral cat cage traps (blue squares) and hair snares (yellow dots see section 7).

5.3 Results

We had 446 captures of 353 individual native and introduced mammals at TA (Table 4). The most common individual species captured was Bush Rat (*Rattus fuscipes*; 55%), followed by House Mouse (*Mus musculus*; 34%) and Agile Antechinus (9%). We captured three feral cats from 1635 cat cage trap nights.

Common name	Scientific name	Total captures	Recaptures	Total individuals	Captures/100 trap nights
Bush Rat	Rattus fuscipes	259	66	193	0.098
House Mouse	Mus musculus	121	0	121	0.061
Agile Antechinus	Antechinus agilis	60	27	33	0.017
Feral cat	Felis catus	3	0	3	0.002
Mountain Brushtail Possum	Trichosurus cunninghami	2	0	2	0.001
White-footed Dunnart	Sminthopsis leucopus	1	0	1	0.001
Total		446	93	353	0.179

Table 4. The capture histories of native and introduced mammals at Tulloch Ard.

Factors affecting use of feral cat control tools

The biomarker RhB was present in two of the six species sampled. Overall, 4.5% of all individual animals captured had RhB present, with the majority of the RhB-positive whiskers detected in Bush Rats (12 individuals from 193 individuals (6.2% of Bush Rats and 3.4% of all captured individuals) (Table 5). None of the three feral cats captured had RhB detected in their whiskers.

Common name	Scientific name	Number of individuals captured	Number of individuals with RhB present	Proportion of RhB detected in each species	Proportion of total captures
Bush Rat	Rattus fuscipes	193	12	0.062	0.034
House Mouse	Mus musculus	121	0	0	0
Agile Antechinus	Antechinus agilis	33	4	0.121	0.011
Feral cat	Felis catus	3	0	0	0
Mountain Brushtail Possum	Trichosurus cunninghami	2	0	0	0
White-footed Dunnart	Sminthopsis leucopus	1	0	0	0
Total		353	16	_	0.045

Table 5. Th	ne proportion of each s	species captured	that had the Rhl	B biomarker	present in
whisker sa	imples.				

5.4 Summary

These results help clarify the overall risk to non-target species by simulating the potential exposure to PAPP by quantifying the presence of RhB in a large sample of potentially at-risk species at the operational scale. The biomarker was detected in a small proportion of the common and abundant species exposed to aerially deployed non-toxic Curiosity bait. This suggests that these species are unlikely to be impacted at a population level by baiting using Curiosity feral cat baits. In a similar study, Fenner et al. (2009) investigated the impact of aerial baiting with 1080 to control wild dogs in north-eastern New South Wales, Australia, on populations of Southern Bush Rats (*Rattus fuscipes assimilis*) and Brown Antechinus (*Antechinus stuartii*). They assessed non-fatal bait consumption with baits containing RhB. Monitoring showed that neither mammal population had decreased in size after baiting; nor was there any increase in the population turnover rates, or any change in the movement patterns of either species. Furthermore, no trapped animal tested positive for RhB, suggesting that these small mammals rarely consume meat baits and that, at the population level, the impact of baiting on them was likely negligible.

RhB presence in the whiskers of an animal indicates that it encountered and fully or partially consumed a Curiosity feral cat bait. However, whether they consumed the HSDV *pellet* is less clear. The design of the Curiosity bait is such that small mammals and rodents like Bush Rats and antechinus species should reject the pellet and thus not be exposed to its contents.

RhB presence in whiskers could have resulted from three possible scenarios: (a) the individuals digested the pellet, (b) the individuals rejected the pellet, but it was cracked and RhB leaked out, or (c) the RhB leaked from the pellet into the surrounding bait matrix before the individual consumed the bait material. The results of the camera trapping (section 6) indicated that, on many occasions, small animals like antechinus took several visits to consume bait material, taking small portions of the bait over multiple nights, thus it may be possible they consumed Rhb that had leached into the bait material.

Consumption of the pellet is unlikely by antechinus species. Previous studies have tested the rejection rate of these and similar species and shown rejection to be reliably consistent (Marks et al. 2006; Hetherington et al. 2007; Forster 2009; Johnston 2010; Gigliotti 2011). Cracking of the pellet (particularly if the pellet has

already been compromised) or leakage of RhB from the pellet into the surrounding bait matrix is, however, possible.

Leakage of RhB from pellets has been noted in previous studies, and the manufacturer is working on improving the pellet. Information provided by Scientec Pty Ltd, the developers of the initial pellet before commercialisation, state that the pellet coating is 'attacked' by the bait material, and that any increase in the moisture content (given 'constant' temperature) or temperature (given 'constant' moisture level) causes an increase in the rate of 'attack'.

The consequence of an increase in either temperature or moisture content is the eventual egress of the drug-core contents (PAPP or RhB) through the coating matrix. Regarding the egress of drug-core materials, it is anticipated that the rate of egress of PAPP and RhB differ. RhB is very water soluble, whereas PAPP is relatively insoluble in water. Thus, the minor leakage is indicated by staining. Analysis of intact pellets that have 'leaked' has found that the PAPP (free base) has been retained within the residual structure (M. O'Donahue pers. comm. Scientec Pty Ltd). At the same time, PAPP that has come into contact with the bait matrix is bound up by and undergoes detoxification (via oxidation) within the bait matrix. Information detailing the environmental fate of PAPP (and the coating matrix components) is contained in the APVMA registration dossier, is owned by the Invasive Animals Cooperative Research Centre, and is not publicly available. Our understanding is that the environmental fate of PAPP is eventual oxidation to carbon dioxide and water via, by way of example, conversion to one or more organic compounds.

That we caught only three feral cats in the cage traps is not surprising, as the trapping was conducted over a relatively short period; the fact that none of those trapped cats had evidence of bait consumption in their whiskers is in line with the results reported in section 6 showing low consumption rates.

The Dingo is a species of concern in relation to aerial baiting. The camera trap data from BM (section 7.3) does indicate that Dingoes consume Curiosity feral cat bait. The toxicity data for PAPP is typically expressed as an oral LD₅₀ value (the amount of ingested toxic agent that is enough to kill 50% of a tested population of animals). The oral LD₅₀ dose of PAPP for Dingoes is 8.5 mg/kg of body weight (APVMA 2015). The average weight of an adult Dingo is 16 kg (APVMA 2015). To receive a lethal dose, an adult Dingo must consume ~136 mg of PAPP. Curiosity contains 78 mg PAPP inside the pellet, which means that a fatal dose for an average-sized Dingo would be ~1.7 Curiosity baits. In addition, PAPP's primary mode of action is the conversion of haemoglobin in an animal's red blood cells to methaemoglobin, which cannot carry oxygen. Increasing levels of methaemoglobin in the blood reduce oxygen transport to the tissues, eventually causing death through oxygen starvation (>80% methaemoglobin concentration) in the brain and other vital organs. To cause mortality in feral cats, the pellet containing the PAPP must be ingested, and within the stomach, the pellet must dissolve to release the PAPP in a single 'pulse', raising the methaemoglobin levels to over 80%. If the lethal peak methaemoglobin elevation (>80%) is not reached, the animal will typically survive the exposure through a range of counteracting physiological stasis mechanisms that restore normal low levels of methaemoglobin, alongside the metabolism of PAPP to less toxic compounds. An adult Dingo will need to eat two or more baits in quick succession for its physiology to be overwhelmed by PAPP.

An alternative feral cat bait (Hisstory) that contains 1080 in the pellet has been trialled and assessed in Western Australia and the Northern Territory (Algar et al. 2020). While this bait may confer a considerably reduced risk to some non-target species, e.g. large reptiles and goannas, it may still pose a risk to the Dingo. One approach that has been considered is the addition of rapid-acting emetics for dogs (agents that cause vomiting in dogs) to the bait. Algar et al. (2020) proposed the use of Apomorphine as a canine emetic. In proof-of-concept trials, however, this agent failed to produce vomiting in dogs, suggesting it was not made available fast enough to induce vomiting. Ongoing development of this alternative approach is planned for 2023 (M. Johnston pers. comm.).

6 Probability of Curiosity bait consumption by feral cats and non-target species, given bait encounter has occurred

6.1 Introduction

For feral cats to be killed in a baiting operation, a cat must (a) encounter the bait, (b) choose to consume the bait, (c) be physically able to access and consume the bait and (d) consume sufficient bait to ingest a lethal dose of toxin (Bengsen et al. 2008). The probability of consuming a bait, given it has been encountered, is an integral part of the equation, and knowing this can help land managers improve baiting operations (i.e. optimise the number of baits used) and reduce non-target risk (i.e. minimise the number of baits deployed). This information can also be incorporated into individual-based spatially explicit population models to assess the likely outcome of various management scenarios (Zurell et al. 2022).

In the previous section, we reported on the presence of a biomarker in the whiskers of animals exposed to non-toxic baits at the operational scale, i.e. baits laid across the landscape at 50 baits/km². In this section, we report the probability that a feral cat or non-target animal will consume a bait, given that it has encountered. We also investigated the rate at which non-target species removed baits, thus reducing the overall availability of bait to feral cats.

To increase the sample size and to investigate the encounter rates and consumption rates of Curiosity baits across a broad range of habitats and environmental conditions, we included data from a previous study (Robley et al. 2022b). This study was part of the Victorian Government Biodiversity 2037 (*Protecting Victoria's Environment – Biodiversity 2037*) plan to stop the decline of the state's native plants and animals. Funding for implementing actions under the Biodiversity 2037 plan was through the BRP process. Data were obtained from two semi-arid sites in north-west Victoria (HKNP and BD) and a coastal location in southern Victoria (WPNP) and were part of the BRP program funded by DELWP.

6.2 Methods

We assessed the fate of baits by placing non-toxic bait on the ground in front of heat-in-motion digital cameras (Reconyx, LLP Wisconsin, USA) and inspecting the resulting images to determine which species take baits and when. Surveys were undertaken at six locations. These were BM, GLCP and TA as part of this study and supplemented with data from the earlier studies in BD, HKNP and WPNP.

We deployed bait in front of 39 cameras at BD over three rounds of camera trapping, each ~16 days long, 49 cameras at BM over one round, 46 cameras at GLCP over one round, 98 cameras at HKNP over two rounds, 106 cameras at TA over three rounds, and 90 cameras at WPNP over two rounds.

Baits were placed on the ground 2 m from the camera, remaining in place for a maximum of 16 days, with a minimum of 7 days between rounds. Each camera was attached to a small wooden stake ~30 cm above the ground and faced south. Vegetation was trimmed to ground level between the camera and the bait and 1 m on either side of the centre-line between the camera and the bait. Cameras were programmed to take five images per trigger, without delay between triggers. No other lures were used to attract animals to the bait sites, and the baits were not tethered.

Before being laid, Curiosity baits were thawed by being placed in direct sunlight for at least 1 h. This process, termed 'sweating', causes the oils and lipid-soluble digest to exude from the surface of the bait. All Curiosity baits were sprayed during the sweating process with an ant-deterrent compound (Coopex, the main active constituent being permethrin). This process aimed to prevent bait degradation by ant attack (the physical presence of ants on and around the bait medium may deter bait acceptance by feral cats).

6.2.1 Data analysis

We modelled the probability of an animal taking the bait, having already encountered it; we did not model factors affecting the encounter rate, which are likely linked to abundance and behaviour. Instead, we modelled the probability an animal will take a bait having encountered it, with a variable being days the bait

has been deployed and a random effect of location and bait station. The interaction between 'days deployed before being taken' and species group (feral cat or other) was also modelled. We implemented a Bayesian binomial regression (logit-link) model with location and bait station as random effects, with just location as a random effect, and a null model. We compared the models using leave-one-out cross-validation (LOO-CV) to select the best-performing model (Vehtari et al. 2017; Vehtari et al. 2020). Models were implemented with the brms R package (Bürkner 2017, 2018, 2021). We used a weakly informative student-*t* prior with three degrees of freedom and four chains run in parallel with 1000 warm-up iterations and 2000 sampling iterations. We assessed model convergence by inspecting trace plots and Rhat values (Brooks and Gelman 1998). Trace plots showed good evidence of convergence, with no Rhat values above 1.05.

Bait fate was recorded as a '1' when the bait was encountered and consumed by a feral cat or a '0' if the bait was observed to be consumed by a species other than a feral cat [e.g. fox, wild dog, raven (*Corvus* sp.) or other bird species, small mammal (e.g. rodent), or reptile], and was thus subsequently unavailable to feral cats; '0' was also assigned to any bait that was taken from the field of view, its fate being unknown. This occurred on some occasions when the camera failed to trigger when the bait was removed. This may have been due to a small mammal's body temperature being too close to the ambient temperature to trigger the camera's sensor.

6.3 Results

6.3.1 Feral cat bait consumption given encounter

The best-performing model included the time since baits were deployed, with location and bait station as random effects. The probability a feral cat will consume a bait is low unless the feral cat – bait encounter rate is high.

Overall, bait consumption was low, with only 28% of encountered baits being consumed by feral cats. Feral cats consumed more baits at GLCP than at any other site. (Table 6).

Location	Season	Number of baits laid	Number of baits encountered by a feral cat (%)	Number of baits consumed by a feral cat (%)
Coastal				
Gippsland Lakes Coastal Park	Spring	46	12 (26)	12 (100)
Wilsons Promontory NP	Autumn	90	20 (22)	4 (20)
Forested mountains				
Tulloch Ard	Autumn	212 over two rounds	0 (0)	0 (0)
Tulloch Ard	Winter	106	27 (8)	1 (3)
Barry Mountains	Spring	49	10 (20)	1 (1)
Semi-arid				
Big Desert WP	Autumn	117 over three rounds	7 (6)	2 (29)
Hattah–Kulkyne NP	Autumn	196 over two rounds	6 (3)	0 (0)
		816	82 (10)	20 (28)

Table 6. Encounter and consumption of feral cat baits across habitat types.

NP = national park, WP = wilderness park

Based on the model outcome, we generated predictions of the probability a feral cat or other species will take the bait, having encountered it (Figure 15). This probability varies with the number of days since the bait

was deployed. The probability of a feral cat consuming a bait increased the longer the bait had been laid; there was a 50% probability that a bait would be taken by 7 days and a plateau at >90% probability after 10 days. The probability of another species consuming a bait remains relatively constant.



Figure 15. The probability a feral cat or 'other species' will consume a Curiosity cat bait, having encountered the bait.

6.3.2 Simulated encounter and consumption rates

To investigate the various probabilities a feral cat will consume bait under differing encounter rates (by feral cats and by other species), we used the distribution of encounter rates present in the original data to generate some plausible scenarios (Table 7).

If feral cats encounter baits every second day and other species have five encounters a day, there is a \sim 27% chance a feral cat will consume an encountered bait. This decreases to \sim 8% if a feral cat encounter is only every 7 days and 'other species' have five encounters a day.

If the 'other species' encounter rate is high, feral cats are much less likely to encounter and take the bait first. The probability of a feral cat taking the bait and having encountered it is low early in the deployment; even several encounters early on do not ensure a feral cat will consume the bait.

Table 7. Simulated bait take rates based on various encounter scenarios.

Scenario	Species	Probability of consumption
Feral cats encounter bait every 2	Feral cat	0.27
days; other species have 5 encounters per day.	Other species	0.64
Feral cats and other species encounter bait on same day.	-	0.09
Feral cats encounter bait every 2	Feral cat	0.13
days; other species have 10 encounters per day.	Other species	0.78
Feral cats and other species encounter bait on same day.	-	0.08
Feral cats encounter bait every	Feral cat	0.08
7 days; other species have 5 encounters per day.	Other species	0.92
Feral cats and other species encounter bait on same day.	-	0.02

6.3.3 Non-target species removal of Curiosity cat bait

Eighteen non-target taxa removed or consumed 500 (61%) baits across all locations: Table 8 lists all taxa recorded as removing or consuming a bait. Bush Rats (present at four of the six sites) were the most common non-target species that were recorded removing or consuming bait (n = 161; 32%), followed by Ravens. (n = 119; 24%), then unknown species (n = 84; 17%). Foxes were recorded as taking the bait on 26 occasions (5%) and Dingoes (at three of the six sites; on 16 occasions (3%).

Table 8. A list of all non-target taxa recorded as removing or consuming a non-toxic Curiosity bait.

Taxa / Common name	Scientific name	Number of baits consumed or removed	Percentage
Bush Rat	Rattus fuscipes	161	32
Raven	Corvus sp.	119	24
Unknown	-	84	17
Black Wallaby	Wallabia bicolor	33	7
Fox ¹	Vulpes vulpes	26	5
House Mouse	Mus musculus	17	3
Dingo ¹	Canis familiaris	16	3
Rodent	Rattus sp.	14	3
Grey Shrike-thrush	Colluricincla harmonica	9	2
Swamp Rat	Rattus lutreolus	5	1
Black Rat	Rattus rattus	3	1
Small mammal	-	3	1
Stumpy-tailed Lizard ¹	Tiliqua rugosa	2	<1

Factors affecting use of feral cat control tools

Antechinus sp.	Antechinus sp.	2	<1
Laughing Kookaburra	Dacelo novaeguineae	2	<1
White-winged Chough	Corcorax melanorhamphos	2	<1
Grey Butcherbird	Cracticus torquatus	1	<1
Mitchell's Hopping Mouse	Notomys mitchellii	1	<1

¹ Species likely to or known to be able to consume pellets containing PAPP.

6.4 Summary

While non-target species were recorded removing or taking a significant proportion of baits, relatively few captured individuals displayed signs of the biomarker being present (see section 5). This would indicate that the risk to those species from ingesting the toxin in Curiosity is low and that there is unlikely to be any population-level impact from aerial baiting with Curiosity on those species.

The potentially more significant impact of non-target removal of baits is the reduction in the probability of a feral cat encountering a bait (the less bait there is in the environment, the less chance there will be of a feral cat encountering one). This probability will also be affected by underlying feral cat density and behaviour, both of which will be influenced by prey abundance (which affects the home range size), and the density of bait in the environment. Determining this probability was outside the timelines of this project, as it requires data on the movement and activity patterns of a representative sample of feral cats and their encounter rates with baits through time and space.

The probability that a feral cat would consume a bait having encountered it was generally low (i.e. only 28% of feral cat encounters resulted in the bait being taken). While the baiting application rate of 50 baits/km² is designed to allow for significant bait loss, the probability that a feral cat will consume a bait (in our study) was found to be low and decreased with increasing non-target encounters of the bait. Similar low consumption rates by feral cats and high non-target interference were reported by Hohnen et al. (2019) in relation to two trials on Kangaroo Island on the uptake of Eradicat bait (which is the same size as Curiosity but contains 1080 and has no pellet). They reported cats encountering <1% of deployed baits (n = 576). Non-target species accounted for over 99% of identifiable bait takes. In both seasons in their study, >60% of all baits were taken by either Common Brushtail Possums, Bush Rats or Australian Ravens (*Corvus coronoides*). Similarly, Heiniger et al. (2018), in a study of two native mammal species in northern Australia, found that 95% of Curiosity and Hisstory baits (the same size bait as Curiosity but containing 1080 in a pellet) were removed within 5 days. Most of the bait was taken by the Northern Quoll (*Dasyurus hallucatus*; n = 42 of 120 baits) and Northern Brown Bandicoot (*Isoodon macrourus*; n = 17), with only two quolls and one bandicoot consuming the pellet.

The implication is that at locations with relatively high abundance of non-target species capable of taking or removing the bait, the likelihood of a significant knockdown of feral cats is potentially low; when unfavourable environmental conditions are added to the scenario, consumption rates by feral cats fall further. A recent control action associated with the attempt to eradicate feral cats from French Island in Victoria reportedly failed to achieve the estimated 75% initial knockdown (Mays 2021). This was most likely due to abundant small mammals (both by reducing bait availability and as an alternative food source), and a wet environment (Parks Victoria pers. comm.). In toxic trials of an earlier version of the Curiosity bait at WPNP, Johnston (2012) reported that the likely cause of low-level reduction in feral cats was the days of substantial rain following bait deployment. In a toxic trial at HKNP, Robley et al. (2022a) reported that the presence of a substantial House Mouse population contributed to no detectable reduction in feral cats, again both by potentially reducing bait availability and as an alternative food source.

It is worth noting that, while this study has highlighted several issues affecting the likely effectiveness of Curiosity feral cat baiting, both Curiosity and its 1080 counterpart (Eradicat) have reportedly been used successfully to reduce feral cat numbers (Algar and Burrows 2004; Comer et al. 2018; Hohnen et al. 2022).

To improve our understanding of the effectiveness of Curiosity as a control tool in Victoria, we need data on the actual encounter rate of baits by feral cats, and data from toxic trials with treatment/non-treatment comparisons replicated in space and time. These trials need to incorporate monitoring of both feral cat and prey species abundances before and after the control action.

7 Feral cat density

7.1 Introduction

To understand whether a control action has had the desired outcome, managers often require information on operational effectiveness (i.e. has the pest population been reduced) and on outcome effectiveness (i.e. how has the species or community at risk responded to the reduction in the threat). Ideally, managers would assess the total functional relationship between the level of pest species density or abundance and the point at which the various species at risk respond, sometimes called a threshold density or a density–impact curve (Pech et al. 1995; Sinclair et al. 1998; Yokomizo et al. 2009). Knowing this threshold density of predators would allow managers to predict the degree of predator control needed to allow prey to increase and to allocate resources accordingly.

A range of metrics can be derived for assessing change in abundance of the pest species as a result of the control action. Often, due to time and financial constraints, the approach is to calculate a relative index of abundance. Commonly, abundance indices are based on animal signs (i.e. track, vocal, den or faecal counts). Photographs obtained with remote camera traps can also be used. However, it is difficult to validly interpret such indices in terms of actual population abundance or density, particularly if a rigorous assessment of detection probability is not incorporated into the calculation. Index values have been described as intrinsically unreliable (Romesburg 1981) or untrustworthy (Delury 1954) for inferring change in abundance or density (Anderson 2003).

Determining population abundance more directly requires capturing a sample of individuals, marking and releasing them, capturing another sample of individuals, then using the ratio of recaptured marked to unmarked animals to estimate the population size. To estimate the density of animals, the area over which the estimate is required must be defined. This can be problematic, as the animals closer to the traps are more likely to be captured, and the animals far from the traps will certainly not be captured. Spatially explicit mark–recapture (SMR) methods incorporate spatial information by inference (Borchers 2012).

Capture and recapture of individuals can provide population abundance data if identification of individuals is possible from unique coat markings (Rees et al.2019) or from genotyping using DNA collected from (a) samples of hair (Berry et al. 2012; Hanke and Dickman 2013) or (b) scats (Lindsø et al. 2022).

Our aim in this study was to investigate the density of feral cats in various habitat types with and without fire impacts to provide insights into how these factors may affect feral cat density.

7.2 Method

We attempted to assess feral cat density using data collected from camera traps at BM, GLCP and TA, and combining this data with that obtained using DNA extracted from hair samples and scats at TA.

7.2.1 Remote cameras

We used detections of individual feral cats identified from 78 cameras at BM, from 50 cameras at GLCP, and from 106 cameras at TA to determine density using spatially explicit capture-recapture models. At all sites, cameras were spaced at ~300–500-m intervals to allow individual feral cats to be detected at multiple camera trap locations. Detections of individual feral cats at multiple camera sites potentially produce spatially correlated detections which are essential for obtaining unbiased estimates of population density when a population is unmarked (Ramsey et al. 2015).

Images of feral cats were inspected, and if distinctive natural markings could be used to identify the individual (Figure 16), a unique I.D. and corresponding detection history was recorded for that individual. For individuals that could not be uniquely identified, the number of detections of unmarked individuals per camera was recorded.



Figure 16. Examples of features used to identify individual feral cats.

Features included (i) number and position of bands on the tail, (ii) number, shape and position of bands on the forelegs and the hind legs, (iii) pattern of stripes and bands on the body, (iv) shape of ears and (v) colouring, e.g. white or tabby patterns.

We attempted to estimate feral cat density using spatially explicit mark-recapture (SMR) models that utilised both marked and unmarked individuals in the analyses (Royle et al. 2013; Forsyth et al. 2019). SMR models assume that the marked individuals are a random sample from the population and that marking occurs throughout the defined state space (defined below). For the feral cat data, it was assumed that cats with distinctive marks were no more likely to be detected than cats without such markings, and that cats with distinctive markings could be detected on any of the cameras throughout the defined state space. In addition, it was also assumed that all marked individuals were correctly identified, and that no marked individuals were lost or emigrated from the area during the study. Both assumptions appeared to be reasonably well supported by the camera data collected.

The data consisted of an array of J sampling devices having locations at $X = (x_{j1}, x_{j2})$, (j = 1, 2, ..., J) and set for K occasions (k = 1, 2, ..., K) (here J = 55 and K = 21). The detections (*h*) at each device, denoted *hjk*, take binary values, indicating the detection of at least one individual by device *j* at occasion *k*. Hence, *h*1 = (01001) indicates detections on occasions 2 and 5 by device number 1. The resulting data are a J × K matrix of detections.

The encounter histories for the SMR algorithm consist of two parts. The first part consists of the encounter histories *hij* for each marked individual *i* (i = 1 ... m), detected by camera *j* on occasion *k*; the second part relates to the unmarked individuals, for which the full detection histories of each individual by the devices are latent (unknown) and must be estimated. We used the SMR model detailed in Forsyth et al. (2019) to estimate the latter group's latent detection histories and, thus, the population density of feral cats and the structural parameters related to their detection probability and home range utilisation.

The SMR model was fitted using Markov chain Monte Carlo (MCMC) sampling in Nimble (de Valpine et al. 2017). We defined the state space by buffering the locations of the outermost cameras by 2 km in each direction to give a total area (A) of 83 km². We drew 20,000 samples from the MCMC algorithm from each of the three chains, using diffuse initial values and discarding the first 10,000, leaving 10,000 samples from each chain to form the posterior distribution of the parameters. Convergence was assessed using the Brooks–Gelman–Rubin convergence statistic Rhat (Brooks and Gelman 1998).

7.2.2 Hair snares

We deployed hair collection devices, 'hair snares' designed to collect hair samples from feral cats that could be used to extract DNA for identification of individuals, to obtain an independent dataset for density estimation.

Eighty hair snares were deployed across the location. Hair snares consisted of three stakes, set into the ground by 20 cm, each spaced at 7 cm at the bottom, increasing to 12 cm at the top. Each stake was wrapped in sticky tape (Figure 17). Hair snare sets were surrounded by vegetation to obstruct animal entry from the sides. At the centre of each hair snare set, we placed a feral cat scat, and at the base of the stakes, we sprayed a mixture of cat urine and water to entice cats to rub against the stakes. Tapes were checked, hairs removed, and tapes were replaced every second day for 14 days.



Figure 17. Hair snares set at Tulloch Ard were used to collect samples for DNA extraction. Each stake was wrapped in sticky tape staring 10 cm above the ground, and hairs were deposited as animals pushed between the stakes.

Collected hair samples were placed in an individually labelled envelopes and stored in a fridge at ~3°C until analysis. In the lab, the hairs were placed into tubes, and DNA was extracted from them using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions, but with the addition of 20 μ L of 1M dithiothreitol (DTT) to the lysis buffer. The concentration of each extract was checked using a Nanodrop1000 spectrometer.

Two separate analyses were undertaken to test whether the hairs were from feral cats and to try to produce individual genotypes from them. The first analysis uses a panel of microsatellite markers specific to cats that allows individual cats to be identified (Cowen et al. 2019). This test was expected to fail if the DNA concentration was too low or the sample was not from a cat. The second analysis is a quantitative PCR multiplex melt-curve analysis designed to distinguish between Australian native and introduced mammal predator species (Berry and Sarre 2007). The qPCR multiplex melt-curve analysis uses primers from multiple species and exploits the difference in the melting temperature of the qPCR products to determine which species' genes have been amplified. The test was expected to fail if the DNA concentration was too low or the sample was not from a mammalian predator [i.e. a feral cat, Dingo, fox, Spot-tailed Quoll or Tasmanian Devil (*Sarcophilus harrisii*)]. Both tests were run with positive (cat tissue extracts) and non-template (water) controls.

7.2.3 Predator scats

Detector dogs (Canidae Development) searched 51 km of tracks for predators scats within the TA site following the deployment of non-toxic aerial baits (Figure 18). Vehicle tracks were searched using two detection dogs in rotation approximately every 2 km over 3 days, surveying ~15–20 km per day.

Collected scats were placed in individual plastic zip-lock bags, labelled using a unique ID and stored in a freezer at approximately –10°C. Scats remained stored in the freezer until analysis for the presence of dietary items. Subjective analysis of the scat age (based on condition, structure, consistency and moisture content of the scat) was made at the time of collection.



Figure 18. Canidae Development detector dog working at Tulloch Ard searching for predator scats.

Each scat was subsampled using three different methods to compare the impact of the subsampling technique on downstream genetic analyses. (i) Scats were first subsampled by swabbing the outside of the scat with a sterile cotton tip, which was then stored in 100 μ L of Longmire's buffer. (ii) A 180–220-mg cross-section was removed from each scat using a sterile, single-use scalpel blade. (iii) Finally, 180–220 mg was scraped from the outside of each scat using a sterile, single-use scalpel blade. When working with herbivore scats, we have found that swab samples often produce the best results. However, outside scrapings are the most common method used in the literature when the aim is to genetically characterise the depositor of a predator scat. Cross-sections are expected to perform poorly for profiling the depositor genetics but are usually preferred for genetic and dietary analyses.

Swabs were extracted using a modified DNeasy Blood & Tissue Kit (Qiagen) protocol, as outlined in Davies et al. (2019). Both outside scrapings and cross-sections were extracted using the QIAamp Stool Mini Kit (QIAGEN) following the manufacturer's protocols.

We trialled three different methods for identifying species from each extract. First, we used a qPCR multiplex melt-curve analysis to distinguish between Australian native and introduced mammalian predator species in a single, economical reaction (Berry and Sarre 2007). When we found that this method gave inconsistent results, we trialled the same markers in single-species responses. Finally, we attempted to amplify and sequence a single 'mini-barcode' marker specific to Australian mammalian predators (Modave et al. 2017). All tests included non-template (water) controls and up to six positive controls in the form of tissue DNA extracts from feral cats, Dingoes and Spot-tailed Quolls. Fox tissue was unavailable at this time.

7.3 Results

7.3.1 Feral cat density—detection of individual feral cats from camera traps

We intended to use the repeated detection of individual feral cats through space and time to estimate feral cat density at each of the three locations. However, this was not possible, as too few repeated detections of individual feral cats occurred. Instead, we applied a single-season occupancy model (MacKenzie et al. 2002) to predict the proportion of sites likely to be occupied by feral cats. Occupancy accounts for the probability that at some camera sites feral cats were present but went undetected.

Tulloch Ard

At TA, cats were detected at 28 sites on 45 occasions from 8590 camera trap nights between February and June 2022.

The occupancy rate at TA was 0.41 (95% CI 0.26–0.56), with a daily detection rate per camera of 0.012 (95% CI: 0.008–0.016) (Table 9). This detection rate is low, with a cumulative detection rate after 35 days of 0.34, or a 34% chance of detecting a cat, given it was present.

Gippsland Lakes Coastal Park

At GLCP, feral cats were detected at 20 sites over 2316 camera trap nights between August and September 2021. Feral cats were distributed broadly across the study area, with a tendency to be detected closer to the lake edge.

The daily detection rate per camera was 0.014 (95% CI: 0.006–0.032). This detection rate is quite low, with a cumulative detection rate after 35 days of 0.39, or a 39% chance of detecting a cat, given it was present. The occupancy rate at GLCP was 0.74 (95% CI: 0.13–0.98). However, the 95% CI was very wide (Table 9).

The minimum number of feral cats identified from coat markings was 10; however, this will be an underestimation, as there were detections of four cats that could not be identified from images captured by cameras. These may or may not have been different individuals.

Barry Mountains

At BM, feral cats were detected at 20 sites over 2831 camera trap nights between October and November 2021.

The daily detection rate per camera was 0.018 (95% CI: 0.006–0.032). This detection rate is low, with a cumulative detection rate after 35 days of 0.47, or a 47% chance of detecting a cat, given it was present. The occupancy rate at BM was 0.56 (95% CI: 0.25–0.82); however, the 95% CI was wide (Table 9).

The minimum number of feral cats identified from coat markings was four; however, this will be an underestimation, as there were detections of black cats at 10 sites. These cats could not be identified from images captured by cameras and may or may not have been different individuals.

Table 9. Estimated feral cat occupancy rates and daily detection rates at the three project locations.

Location	Occupancy rate (95% CI)	Daily detection rate (95% CI)
Tulloch Ard	0.41 (0.26–0.56)	0.012 (0.008–0.012)
Gippsland Lakes Coastal Park	0.74 (0.13–0.98)	0.014 (0.006–0.032)
Barry Mountains	0.56 (0.25–0.82)	0.018 (0.006–0.032)

7.3.2 Feral cat density—DNA extracted from hairs collected on hair snares

The DNA extracted from 10 of the 49 hair samples collected from the 1120 nights of hair snare sampling either had too little DNA to produce species identifications and individual genotypes or were not from cats. Three samples appeared to be from Dingoes, and seven were from non-predator species.

Most extracts had low concentrations of DNA, with only seven extracts having >5 ng/ μ L and most having <1 ng/ μ L (Figure 19). The microsatellite markers failed to amplify for any of the hair samples. The qPCR

analysis failed for all but three hair samples, which the test suggested were from Dingoes. Note that, when using this same test on other project samples, we have found it to be inconsistent when distinguishing between cats and Dingoes; thus, the result from those three hair samples should be interpreted with caution. However, with the results from the microsatellite panel, we can be reasonably confident that these three samples were not from cats. Since the seven samples with higher concentrations of DNA also failed to amplify in the qPCR panel, we can assume that these hairs were not from predators.





7.3.3 Feral cat density—DNA extracted from scats

We collected 85 predator scats from TA. Of these, 45 were labelled as 'fresh' by the collectors, with all others labelled as 'old'. We focused on the 45 fresh samples, which were the most likely to produce good genetic data. From these, we could identify feral cats only nine times, with the remaining scats found to be from either foxes, Dingoes or unknown.

The multiplex melt-curve results were variable across extract types, but relatively consistent across samples (Figure 20). For example, the melting temperature of swab subsamples was consistently lower than that for the other extracts, and cross-section subsamples gave consistently higher melting temperatures. Since the melt-curve test was initially designed to be used with outside scraping samples and the difference between extract type seemed to be relatively consistent, we attempted to correct the swab and cross-section results to make the melt-curve temperatures more comparable. To do so, we estimated the average sample-specific difference between the swab or cross-section extract results, to the outside scraping results (the sample type for which the test was originally designed). We then added or subtracted that difference to the swab/cross section results. Using this approach we found that approximately half the samples were from Dingoes and half were from foxes (Table 10). However, when we included our positive control tissue samples, we found that Dingoes samples overlapped in their melting temperature with feral cat samples and thus, these results were deemed unreliable.

Therefore, we re-ran the qPCR analysis in single-species reactions for each extract. In this test, for each sample, the amplification should fail for all but one reaction (i.e. the reaction including primers for the depositor species). However, this test also returned unreliable results, with most samples amplifying

Factors affecting use of feral cat control tools

successfully using multiple species primers. The Cq results (i.e. the cycle at which the first amplification was detected) did not provide further clarification, as there was a large amount of variability and overlap across subsample types and primers.

Finally, to clarify these results further, we targeted a mitochondrial gene region (i.e. a 'mini-barcode') for sequencing, which was also designed to distinguish between Australian native and introduced mammalian predators. This test had a much higher failure rate than the qPCR analysis and was not predator specific, with some prey species being sequenced (e.g., possums, dunnarts and wallabies; Table 10). Of the 24 samples identified as foxes in the qPCR analysis, 7 could be confirmed through sequencing, with the rest either failing to amplify or returning prey species results. Of the 20 samples identified as Dingoes in the qPCR analysis, 5 could be confirmed as Dingoes, 9 were identified as cats, and 6 failed to sequence or returned a prey species finding. Excluding the prey species results, the sequencing results across subsamples from the same sample were consistent (Table 10).



Figure 20. qPCR melt-curve analysis of predator scats subsampled using three different methods (outside scraping, cross-sections and swabbing). Coloured bars represent the expected ranges of melt-curves from the five different Australian mammalian predators included in the test. Each point represents an extract, and the lines connect extracts from the same sample.

These results revealed considerable variation in the predicted identity of individual samples based on the extraction approach used, and showed that the test was not specific, especially for cats and dingos, as many samples fell outside the individual species Melt temperature ranges.

Table 10. Summary of the species identification results across the various tests and subsampling strategies.

Test	Subsample type	Result	N
qPCR Multiplex melt-curve	Predator scat—outside	Dingo	12
qPCR Multiplex melt-curve	Predator scat—outside	Fox	24
qPCR Multiplex melt-curve	Predator scat—outside	Failed to amplify	9
qPCR Multiplex melt-curve	Predator scat—cross-section	Dingo	18
qPCR Multiplex melt-curve	Predator scat—cross-section	Fox	24
qPCR Multiplex melt-curve	Predator scat—cross-section	Failed to amplify	3
qPCR Multiplex melt-curve	Predator scat—swab	Dingo	20
qPCR Multiplex melt-curve	Predator scat—swab	Fox	23
qPCR Multiplex melt-curve	Predator scat—swab	Failed to amplify	1
Mini-barcode sequencing	Predator scat—cross-section	Dingo	5
Mini-barcode sequencing	Predator scat—cross-section	Fox	8
Mini-barcode sequencing	Predator scat—cross-section	Cat	8
Mini-barcode sequencing	Predator scat—cross-section	Common Brushtail Possum	2
Mini-barcode sequencing	Predator scat—cross-section	Black Wallaby	1
Mini-barcode sequencing	Predator scat—cross-section	Sequence not clean	8
Mini-barcode sequencing	Predator scat—cross-section	Failed to amplify	13
Mini-barcode sequencing	Predator scat—outside	Dingo	1
Mini-barcode sequencing	Predator scat—outside	Fox	2
Mini-barcode sequencing	Predator scat—outside	Cat	2
5		Common Brushtail	2
Mini-barcode sequencing	Predator scat—outside	Possum	
Mini-barcode sequencing	Predator scat—outside	White-tailed Dunnart	2
Mini-barcode sequencing	Predator scat—outside	Sequence not clean	3
Mini-barcode sequencing	Predator scat—outside	Failed to amplify	33
Mini-barcode sequencing	Predator scat—swab	Dingo	1
Mini-barcode sequencing	Predator scat—swab	Sequence not clean	1
Mini-barcode sequencing	Predator scat—swab	Failed to amplify	42

7.4 Summary

Despite these methods having been used successfully elsewhere (Berry et al. 2012; Hanke and Dickman 2013; Rees et al. 2019; Robley et al. 2022a; Glen et al. 2022), we were unable to derive robust estimates of density for feral cats at the three sites using any of our techniques. Our occupancy estimates for feral cats revealed that, while occupancy was highest at GLCP, occupancy was similar and high across all three locations (overlapping 95% CIs). Two of the three study sites were impacted by the large fires in 2019/20 which may have contributed to this outcome. Several studies have looked at the immediate response of feral cats to fire or planned burning activities (e.g. McGregor et al. 2015), but fewer studies have looked at the medium- or longer-term response of feral cats to fire.

To obtain population estimates using the mark recapture approach of recognisable individuals, feral cats must be attracted to the camera stations and be present for a sufficient time to be photographed at various angles to identify individual feral cats. In our study, we did not repeatedly detect enough cats at different camera stations. We believe this was due to the cameras being lured only with the non-toxic Curiosity bait. In previous studies, cameras were lured with various combinations of olfactory, visual, and auditory lures, resulting in a high detection rate. These lures were probably more attractive and held the attention of the feral cats for a longer time. Another factor was likely to have been the spatial arrangement of cameras. Ideally, cameras would be spaced such that 3–4 cameras were within the home range of a particular feral cat; this would allow for multiple detections on different cameras across space and show overlaps in the home range sizes in the literature; however, it may be that the feral cat density at our sites meant that our spacing was too far apart. Another factor is likely to have been the physical landscape: cameras could mainly only be located off existing tracks and roads; even if we had spaced the cameras 200–300 m apart, the sample space would still have been restricted.

Using hair snares to collect genetic material also requires repeated 'captures' of individuals through space and time (Berry and Sarre 2007). In our study, we could not identify individual feral cats from DNA extracted from any hair samples. This apparent failure of cats to leave hair samples in the hair snares could have resulted from a combination of factors, including the presence of foxes affecting how the feral cats used space (Rees 2022), the continuous use of vehicle tracks by project staff and crews for road maintenance operations, and rainfall reducing the 'stickiness' of the tapes and diluting the 'attractiveness' of the lure. In addition, the results from the hair snares suggest that the collected hair samples either had too little DNA to produce species identifications and individual genotypes, or else that they were not from cats.

DNA extracted from scats has been successfully used to enumerate feral cat populations elsewhere (Glen et al. 2022). However, we were not able to collect sufficient material from scats to achieve this in our study. Given the consistent results across subsample types and the relatively significant difference in melt temperatures between fox and Dingo/cat genes, we can be reasonably confident that the 24 samples identified in the qPCR melt-curve analysis as foxes were from foxes. However, the melt-curve study failed to distinguish between cat and Dingo tissue samples; therefore, this test should not be considered reliable for identifying these two species from scat samples. Thus, the remaining 20 samples could have been from cats or Dingoes. The sequencing results suggest that at least 9 of these were from cats and 5 were from Dingoes, with the remaining 6 still in question.

Further investigations are needed to determine how these species identification tests can be improved and what results they would return when one predator has preyed or scavenged on another. For example, determining why there were variable results in the melt temperature across subsampling strategies would be helpful. This may have been due to differences in the elution buffer (i.e. swab extracts were eluted in a different buffer than that used for cross-sections and outside scraping extracts) or different genetic compositions in the extracts (i.e. cross-section samples were expected to contain more non-host DNA).

When comparing subsample types, although the swab samples gave the lowest failure rate for the qPCR analysis, they gave the highest failure rate in the sequencing test. The cross-section extracts were successfully amplified most consistently. This is a surprising result, given we expected these subsamples to perform the worst. These results may be due to the relatively small size of the scats, which led to many cross-section samples containing a significant proportion of the scat surface, and many of the outside

scraping samples containing a substantial proportion of the scat interior. Alternatively, DNA on the outside of the scat is likely to have been more exposed to the environment (i.e. wet weather, UV radiation, etc.), which may lead to accelerated DNA degradation than inside the scat.

Rees (2022) studied the spatial variation in the diel activity of foxes and feral cats in the Otway Ranges, Victoria. He found that feral cats did not reduce their overall activity in areas with high fox counts but shifted their diel activity patterns to less risky times of the day. In dry habitats of both regions, cats shifted from being nocturnal–crepuscular to primarily diurnal. In wet forest habitats, fox activity was consistent throughout the diel period; but when fox counts were high, cats became more nocturnal, avoiding dawn. Changes in feral cat diel activity patterns may facilitate spatial coexistence between the two predators, potentially shifting impacts onto different native prey species. This possible temporal and spatial differentiation imply that if landscape-scale fox control is implemented, feral cats may be active across a broader temporal period and utilise a wider range of habitats.

There would appear to be a need for integrated invasive predator control that incorporates understanding of the underlying densities and distributions of both foxes and feral cats based on pre-control information. This information would be vital for the planning any such programs and in assessing their effectiveness.

8 Predator diet

8.1 Introduction

Knowledge of the diet of introduced predators is critical information for land managers and is necessary for understanding the likely impact of predators on native prey. It enables insight into the direct impact of introduced predators on threatened species. Knowledge of competition for prey between Foxes and feral cats can indicate likely changes in prey selection by feral cats following fox control actions.

While our study was not explicitly designed to elucidate these aspects of the feral cat diet, we were able to add value to the project by examining the prey items found in the collected scats. To date, there has been a relatively limited number of studies on the diets of feral cats, foxes and Dingoes in eastern Victoria (Coman 1973, Triggs et al. 1984, Davies et al. 2015), and the data provided in this section adds to that store of knowledge.

8.2 Methods

8.2.1 Presence of native species in introduced predator scats

Predator scats collected as part of the assessment of cat density (section 7.4) were also used to assess diet by examining the remains within individual scats and identifying the remains. The frequency of occurrence of a prey item in each scat was presented as a percentage (Lockie 1959), where the number of scats in which a food item occurred was expressed as a percentage of the total number of scats analysed. Although frequency of occurrence methods provides no indication of the importance of prey categories, they can be helpful to our understanding of whether a carnivore is a specialist or a generalist and offer a valuable and consistent measure for comparisons between studies (Klare et al. 2011).

8.3 Results

8.3.1 Predator diet

We collected 82 predator scats (61 fox, 17 Dingo, 4 feral cat and 1 probably Spot-tailed Quoll as determined by visual assessment) and determined the frequency of occurrence of prey items in each scat.

We collected few feral cat scats (n = 4). Bush Rats were found to be the dominant species present (53%). House Mouse (18%), Mainland Dusky Antechinus (13%), unidentified bird (11%) and Brush-tailed Possum (5%) made up the remainder of the items (Figure 21a).

Overall, 16 distinct prey species were identified in fox scats; however, 10 made up less than 3% each. The most common prey item in fox scats was Bush Rat (54%). Figure 21b shows the proportions of prey items in fox scats that comprised \geq 3% of food items. A complete list of prey items is presented in Table 11. Of note is the presence of Long-footed Potoroo, albeit as a small proportion overall. One fox scat showed evidence of consumption of a Curiosity cat bait (i.e. RhB was present).

The variety of prey items in Dingo scats was smaller than in fox scats (n = 7), but the items were more evenly spread in occurrence, with no items being present at less than 3%. Common Wombat was the most frequently occurring species (29%), with a relatively even occurrence of the remaining five species (Figure 21c).



Figure 21. The main food items in (a) feral cat (n=4), (b) Fox (n = 61) and (c) Dingo (n = 17) scats at Tulloch Ard State Forest. Items with less than 3% occurrence are not shown.

Common name	Species	Percentage
European Rabbit	Oryctolagus cuniculus	2
Black Wallaby	Wallabia bicolor	2
Black Rat	Rattus rattus	2
Long-footed Potoroo	Potorous longipes	2
Common Ringtail Possum	Pseudocheirus peregrinus	2
Agile Antechinus	Antechinus agilis	2
Bird	-	1
Beetle/cockroach	_	1
Rat	<i>Rattus</i> sp.	1
Common Wombat	Vombatus ursinus	1

8.4 Summary

These results indicate that feral cats and foxes share similar prey items, but that cats tend to select smaller prey. Prey items found in Dingo scats also showed some overlap with feral cats and foxes, but Dingo scats tended to contain prey items from larger mammals. Of note was the presence of the Long-footed Potoroo in

fox scats; although there was <3% frequency of occurrence in fox scats, this species is listed as endangered (FFG Act 1988). These findings support those of previous studies on feral cat, fox and Dingo diets. Triggs et al. (1984) found that the diet of all three species overlapped in Coopracambra National Park in East Gippsland; while cats tended to take smaller prey, like birds and reptiles, Common Ringtail Possum made up a significant proportion of occurrence. Fox scats and Dingo scats tended to contain medium- to large-sized prey (e.g. Brush-tailed Possum, Common Wombat and Black Wallaby). Buckmaster (2011) reported that the feral cat diet in East Gippsland was dominated by Bush Rats, antechinus species and Black Rats, while Stobo-Wilson et al. (2021), in an Australia-wide review of fox and feral cat diets, found that both foxes and feral cats were most likely to consume medium-sized mammals, with the likelihood of predation of mammals by foxes peaking at ~280 g and predation of mammals by cats peaking at ~130 g.

9 Conclusions and implications

This project contributes substantially to our understanding of the factors that affect the use of tools to manage feral cats in fire-affected areas of Victoria and beyond. The information obtained will be valuable to land managers and policymakers, aiding in planning and future policy development for controlling feral cats in Victoria.

Access to a wide range of tools, and knowledge of the most effective way to use them, increases our ability to manage the impact of feral cats on threatened species in various settings. We found that Felixers were highly target specific. However, using these devices in Victoria when they contain 1080 as the toxin is not permitted, and the development of the use of PAPP as the toxin in gel form is facing significant technical issues. In addition, the units are large, cumbersome, relatively expensive, and require specific settings and careful deployment. Improvements to the unit have been made since we tested these devices. In the right situation, e.g., island eradications or removal of feral cats from enclosed reserves, Felixers are likely to be a very useful addition to the toolbox.

To suppress or eradicate feral cats across larger areas requires a target-specific, surface-laid bait that presents little or no risk to native species, is palatable and attractive to cats for a reasonable period (10–14 days) and is humane. Curiosity feral cat bait was developed to meet this need, and it has been used, along with its close counterpart, Eradicat, to manage feral cats in several locations in Australia (Hohnen et al. 2020; Algar et al. 2020).

Through this project we have increased understanding of the environmental factors that influence Curiosity's attractiveness and likely consumption. Curiosity has been developed to last for ~14 days under optimal conditions (mild, stable temperatures; low humidity). However, these optimal conditions are rarely met in the field. We found that high temperatures and high humidity significantly shorten the bait's field life, a finding supported by previous work (e.g., Johnston 2012; Johnston et al. 2013). This finding has implications for the probability of a feral cat consuming the bait, and thus consequences for the design of feral cat control operations using Curiosity, such as the timing and frequency of baiting operations.

We found that the probability that a feral cat would consume an encountered bait was low under field conditions. This is likely to be a product of bait attractiveness, the amount of available alternative food (or prey), and the rate at which non-target species remove bait. We found most of the bait is consumed or removed by non-target species, such as ravens or small mammals such as Bush Rats or antechinus. This finding is supported by observations from previous studies (Doherty et al. 2021).

While we cannot definitively conclude that no individual non-target small mammal would have consumed PAPP that would have resulted in its death if baits were toxic, the data strongly supports the likelihood that very few individuals would be at risk and that a population-level impact would be improbable. The very low frequencies of RhB detected in a large sample of small mammals, together with the high rate of bait consumption/removal by these species, indicates there is a very low risk of any population-level impact from Curiosity baiting on these species. This outcome is supported by several desktop risk assessments (Buckmaster 2014; DELWP unpublished) and field and pen trials (Marks et al. 2006). We note that as the moisture content of the bait changes and the pellet structure deteriorates, PAPP can leak from the pellet. However, the amount of toxin that would escape is likely to be insignificant, and it would degrade rapidly once exposed to water (M. O'Donoghue pers. comm. Scientec Pty Ltd).

Our findings on the impact of environmental factors and non-target effects on consumption, in part, are the likely explanation for why at least two feral cat control operations in Victoria have failed to produce significant changes in feral cat occurrence or density. An aerial baiting trial at HKNP in autumn 2021 that deployed 3900 baits over ~18,000 ha failed to achieve any detectable change in the occurrence of feral cats (Robley et al. 2018), and the aerial baiting operation associated with an eradication attempt on French Island, Victoria, failed to reduce cats by the predicted amount (Parks Victoria unpublished data). The outcomes of the non-toxic aerial baiting trial undertaken in this current project support this, as the rate of non-target bait take was significant, and the weather conditions were such that it is likely baits became unattractive soon after being deployed.

In the cooler and relatively wet environments of southern Victoria, implementing effective landscape-scale control of feral cats is challenging. The environmental factors identified in this, and previous studies, mean that the window for when baiting can be implemented is limited. Added to the environmental constraints are the spatial limitations on the use of Curiosity, with feral cats declared as a pest species in specific areas of public land; currently, no baiting is permitted in areas with Spot-tailed Quoll or Dingoes. Confounding this further are the current restrictions on the use of some tools available in other states, e.g., soft-jawed leg-hold trap use is only permitted where eradication is possible and only with ministerial approval, and Felixers are not registered for use in Victoria.

These factors reduce the locations in Victoria where broadscale control of feral cats is possible. Areas such as the larger public land blocks in the western and north-western part of the state (which has relatively dry and stable weather), the Otway Ranges and WPNP (where no Spot-tailed Quoll or Dingoes are present) are examples of areas where it may be possible to control feral cats using Curiosity.

During the development of Curiosity and the hard-shelled pellet in which the toxin is encapsulated, pen and field trials assessed the pellet rejection rate by a range of native species (e.g., Marks et al. 2006; Hetherington et al. 2007; Forster 2009; Heiniger et al. 2018; Johnston et al. 2019). There are no published data on this for Dingoes or Spot-tailed Quoll. Current risk assessments are based on the hazard + exposure = risk model. The hazard can be expressed as the LD_{50} (the dose required to kill 50% of tested individuals) of the PAPP concentration housed in the pellet (78 mg) inside the Curiosity bait. For Dingoes, the reported LD₅₀ is 8.5 mg/kg (calculated to produce an 80% increase in methaemoglobin concentration, extrapolated to the LD₅₀ value; Coleman et al. 1960), which equates to the consumption of ~1.74 Curiosity baits by an adult (16 kg) Dingo within a time that will allow the PAPP to increase methaemoglobin to toxic levels. The laboratory and field-based data available about PAPP, generated for registration purposes, are summarised by Eason et al. (2014). In studies with captive dogs (Canis familiaris), sublethal doses of PAPP were rapidly metabolised and excreted, principally through the kidneys, over a period of ~5 days (Eason et al. 2014). Young or light-weight individuals would, however, be at risk from a single dose of the PAPP contained in a Curiosity bait. Spot-tailed Quoll has a reported LD₅₀ = 24.8 mg/kg [NWR 2006 cited by Mcleod and Saunders (2013)], equaling ~1.59 Curiosity baits. Data on bait acceptance and pellet consumption are required before a meaningful risk assessment can be made for Spot-tailed Quoll. Exposure is assessed as the likelihood of encountering and consuming the pellet within a bait. Spot-tailed Quolls and Dingoes have the potential to consume an encountered bait (including the pellet) if they found the bait attractive and palatable.

When feral cat baiting has been successful, using either Curiosity or Eradicat, baiting has occurred when there has been a high predator-to-prey ratio. The ratio is relatively predictable in semi-arid or arid systems, as prey often increase or decrease with favourable or unfavourable environmental conditions, respectively (Dickman et al. 1999; Pavey et al. 2008). This window of baiting opportunity is likely to be less pronounced or absent in more stable and productive environments in Victoria. In addition to the effect of high prey availability on feral cat bait take, there was a high rate of non-target bait interference observed in this and other studies, resulting in lower than necessary bait encounter and consumption rates by feral cats. More information is needed on the relative availability of prey through time to inform managers of when the optimal time of year is for baiting feral cats in Victoria.

Having robust and reliable methods for assessing changes in the abundance of target species and any possible impacts on non-target species is essential if land managers are to measure the effectiveness of their actions and to justify the investment of scarce public funds in feral cat control (Caughley 1980; Hone 1994). The approaches we used in this study to assess abundance and density have been successfully employed in previous studies (Comer et al. 2018, Cowen et al. 2019). However, as we have shown, there are challenges in implementing these methods in the field and in the laboratory. Obtaining reliable estimates of the abundance of a population requires the capturing and identifying of individual animals on multiple occasions. 'Capturing' feral cats on multiple occasions (either physically or by remote camera trapping) is difficult, requiring the use of labour-intensive techniques (e.g., cage trapping, leg-hold trapping, use of scatdetection dogs, or deployment of remote cameras), and identifying individuals during mark-resight analysis is problematic (Sparkes et al. 2021).

Future work

While we have improved our understanding of Felixer traps, Curiosity feral cat baiting, and monitoring techniques, several issues still require resolution. Felixers will not be registered for use in Victoria with 1080 as the poison. Instead, Victoria is waiting on the registration of PAPP as the toxin to be used in these traps. If the current technical issues of infusing PAPP into the gel can be overcome and the device is registered, there are still likely to be residual issues requiring further information. This information gap may include analysis of the risk to Dingo pups. Dingoes are susceptible to PAPP, and young Dingoes may be at risk from baiting. What the actual risk is remains to be quantified. It also remains uncertain whether Spot-tailed Quolls can trigger Felixers and whether they can ingest sufficient PAPP to be poisoned from grooming.

While we now understand the factors that impact the attractiveness and availability of Curiosity, control operations are needed to test how these factors will play out in landscape-scale baiting and how effective the baiting will be. There have been two attempts to use Curiosity in Victoria: one had no detectable impact, and the other resulted in a slight reduction in feral cats. Individual-based spatially explicit population models have been developed that can be used to predict the likely change in feral cat abundance (Hradsky pers. comm.). Future work should be undertaken to refine some of the input parameters, e.g., through a better understanding of feral cat home range size, survival rates, dispersal distances, and bait encounter rates. Future feral cat control operations should take advantage of these models to assess the various alternative management scenarios, e.g., one baiting versus two baiting's, and the timing and frequency of baiting's needed to reduce feral cat abundance to very low levels and maintain it at those levels. A robust monitoring program can test these predicted reductions and the results used to update the model predications. Data can then be fed back into these models to improve their predictive power. This is the essence of an adaptive management approach (Walters and Hiborn 1978; Parkes et al. 2006).

Current management approaches targeting single species, e.g., Red Foxes, or Dingoes bordering private land, may *increase* the negative impacts of feral cats on vulnerable wildlife. While still being debated in the scientific literature, evidence is mounting that a reduction in higher-order predators can lead to either numerical or behavioural changes in feral cats, and that this has the potential to increase feral cat impact on native wildlife (Brook et al. 2012, Castle et al. 2020). Future work should look at approaches for the integration of fox and feral cat control and assessing the relative effects on native species. Complicating this situation is the role of fire, either planned or wild, with research indicating that all three predators (Red Foxes, Dingoes and feral cats) can take advantage of changes in habitat and prey availability immediately following fire (Hradsky B. 2020, Geary et al. 2021).

Effective management of feral cats in fire-affected areas of Victoria is possible with the tools examined in this study. However, it is reliant on (a) a better understanding of the actual level of reduction that can be achieved, considering the limitations described in this report, (b) a better understanding of the optimal timing and frequency of control actions, in particular of aerial baiting, and (c) government policy settings enabling the use of the available tools.

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Factors affecting use of feral cat control tools

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