

1 **Title:** Not such silly sausages: Northern quolls exhibit aversion to toads after training with
2 toad sausages.

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26 **Abstract**

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

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50 **Introduction**

51 Invasive species are a major threat to biodiversity (Reaser *et al.* 2007; Woinarski, Burbidge
52 & Harrison 2014). In Australia, species such as feral cats (*Felis catus*) (Legge *et al.* 2017),
53 domestic dogs (*Canis familiaris*) (Doherty *et al.* 2017), foxes (*Vulpes vulpes*) (Short & Smith
54 1994; Risbey *et al.* 2000) and cane toads (*Rhinella marina*) (Burnett 1997; Letnic, Webb &
55 Shine 2008; Jolly, Shine & Greenlees 2015) all have serious impacts on native species.
56 Controlling these species at a landscape scale, however, has proved extremely difficult
57 (Ziembicki *et al.* 2015). Because of this, increasing attention is being paid to mitigating the
58 impact of invasives, rather than supressing their populations (Simberloff *et al.* 2013).

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

71 Such training can be achieved through conditioned taste aversion (CTA).
72 Conditioned taste aversion is a powerful innate response found across all vertebrates; an
73 evolved defence mechanism against poisoning (Sinclair & Bird 1984; Conover 1995; Cohn &

74 MacPhail 1996; Bernstein 1999; Mappes, Marples & Endler 2005; Page & Ryan 2005;
75 Glendinning 2007). With CTA, animals acquire an aversion to a referent food as a result of a
76 nauseating experience (Gustavson & Nicolaus 1987). Agriculturalists and wildlife managers
77 have used conditioned taste aversion to reduce wildlife damage to crops, industry, or
78 livestock (Gustavson *et al.* 1974; Ellins & Catalano 1980; Avery 1985; Provenza *et al.* 1990;
79 Ternent & Garshelis 1999; Smith *et al.* 2000). CTA has also been used successfully to reduce
80 predation on native or introduced wildlife (Nicolaus & Nellis 1987; Conover 1989; Nicolaus
81 *et al.* 1989; Semel & Nicolaus 1992; Avery *et al.* 1995; Bogliani & Fiorella 1998; Cox *et al.*
82 2004).

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

98 single generation could yield significant conservation benefits. The remaining challenge then
99 is to effectively deliver CTA training to a large number of individuals under field conditions.

100 In captivity, CTA training was achieved by feeding quolls a small non-lethal-sized
101 toad laced with the nausea-inducing chemical thiabendazole. Such a strategy is not feasible at
102 a large scale in a field setting. To achieve in situ training at scale requires use of a
103 manufactured training bait. Any bait, of course, needs to fulfil the criteria we have identified
104 above: elicits aversion to toads, has a high uptake rate; and effectively trains a high enough
105 proportion of the population that population persistence is assured. An additional
106 consideration is whether the bait is taken by non-target species. This is a major concern in
107 lethal baiting campaigns (Sinclair & Bird 1984; Avery *et al.* 1995; Fairbridge *et al.* 2003;
108 Glen & Dickman 2003; Claridge & Mills 2007; Jolley *et al.* 2012), but a smaller
109 consideration in non-lethal baiting such as we envisage here. Non-target uptake remains
110 important, however, because it can reduce target species' access to bait and so significantly
111 increase the cost and complexity of the baiting effort. Because of this, it is important to
112 understand non-target species uptake rates.

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

117 **Methods**

118 *Cane toad sausages*

119 Cane toad sausages were made up of 15g of minced skinned adult cane toad legs, 1 whole
120 cane toad metamorph weighing <2g, and 0.06g of Thiabendazole (per sausage; dose rate less
121 than 300mg/kg adult quoll body weight, determined by the smallest – 200g – adult seen at

122 our study site) packed into a synthetic sausage skin. In our captive trials, we used the same
123 sausage composition, to accurately reflect our field scenario. Thiabendazole is an
124 inexpensive, broad-spectrum anthelmintic and antifungal agent (Robinson, Stoerk & Graessle
125 1965). It is orally-effective and regarded as relatively safe, producing low mammalian
126 mortality: oral LD₅₀ is 2.7g/kg body weight (Dilov *et al.* 1981). It is fast acting and peak
127 concentration occurs in the plasma one hour after consumption (Tocco *et al.* 1966).
128 Thiabendazole has produced strong aversions to treated foods in lab rats (Gill, Whiterow &
129 Cowan 2000; Massei & Cowan 2002), wolves (*Canis lupus*) (Gustavson, Gustavson &
130 Holzer 1983; Ziegler *et al.* 1983), and black bears (*Ursus americanus*) (Ternent & Garshelis
131 1999). Thiabendazole induces a robust CTA after a single oral dose (Nachman & Ashe 1973;
132 O'Donnell, Webb & Shine 2010) and is physically stable at ambient conditions in the bait
133 substrate (Gill, Whiterow & Cowan 2000; Massei, Lyon & Cowan 2003).

134 *Captive trials*

135 The uptake of toad aversion sausages by *D.hallucatus* and their subsequent response to toads
136 was observed in captive northern quolls previously collected from toad-free areas of Astell
137 Island, and then housed at the Territory Wildlife Park, Northern Territory. Animals (9 male
138 and 9 female) were randomly allocated treatment ($n= 9$) or control ($n=9$) sausage groups.
139 Treatment sausages were exactly as described previously. Control sausages were comprised
140 of store purchased beef sausages. These were selected as a control sausage as it was an item
141 that animals are also not familiar with to control for hunger differences and any possible
142 neophobic responses.

143 To measure individual responses to cane toads following ingestion of sausage, each
144 individual was presented with a dead adult cane toad the following evening. The dead adult
145 toad was secured in a wire cage, so that animals could see and smell the prey item but not

146 access it. The experiment was run over 3 nights. Experiments began at sunset and ran for on
147 average 2 hours. The response was filmed using a GoPro Hero 3 White camera (GoPro Inc,
148 San Mateo, California, USA).

149 **Field trials**

150 *Study area*

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

157 We worked at four sites on the property; Site 1 (SJ) was at Sir John Gorge
158 (17°31.780S, 126°13.080E) along the Fitzroy River. Site 2 (KP) (17°31'43.032,
159 126°13'11.050) was approximately 2 km upstream from Site 1 in the same gorge. Site 3 (TC)
160 (17° 30' 37.213,126°14'4.092) was 5 km upstream from Site 2 in a narrow rocky gorge that
161 feeds into Sir John Gorge. Site 4 (RP) (17°35'12.119, 126°19'21.959) was a narrowly incised
162 sandstone gorge following a watercourse within rocky range country approximately 9 km
163 north-east of Site 1. Sites were selected based on the detection of quolls in the Australian
164 Wildlife Conservancy's fauna surveys (AWC unpub. data). At the time of the study, toads
165 were yet to arrive at our sites; they subsequently arrived by March 2017.

166 *CTA sausage field trials*

167 In this study, "site" is the location where an experiment took place. "Bait station" is a
168 location within a site where sausage bait was offered. A "session", is a time interval when
169 bait stations were active. A total of four sessions were conducted approximately five months

170 apart. Sessions recorded up to four “bait events”. Bait Events are defined as an occasion
171 when new bait was placed at a bait station and (if still existing) the old bait removed.

172 Each site contained 20 bait stations placed 50-80m apart in a linear transect along a
173 gorge wall where the presence of *D. hallucatus* was previously confirmed (AWC, unpub.
174 data). Bait stations consisted of one cane toad sausage placed under a single camera trap
175 (White flash and Infrared Reconyx Motion Activated, (HP800, U.S.A). Cameras were
176 secured to trees or rocky ledges approximately 1m from the ground and aligned to face
177 directly downwards (Diete *et al.* 2016). Cameras were set to take five consecutive
178 photographs for each trigger with no delay between triggers. Each cane toad sausage was
179 placed inside a ring of powdered insecticide (Coopex) to protect from ant spoilage. Each
180 session’s bait stations were rebaited up to three times (for a maximum of 4 bait events within
181 any given session) whereby bait stations were rebaited with a fresh CTA bait and the old bait
182 removed (Table 1). A total of 513 individual cane toad sausages were deployed over the
183 period of study.

184 **Data analysis**

185 *Captive trials*

186 Videos were scored by the same observer who was blind to the quoll’s treatment or control
187 group. Following Kelly and Phillips (2017) we separated the time that quolls spent
188 exhibiting various predatory behaviours into three categories: “Sniff”, “Investigate” and
189 “Attack”. Sniff was defined as when quolls were visibly twitching their nose in the direction
190 of the toad, “Investigating” behaviour was defined as the quoll being engaged with the cage
191 containing the toad, exhibiting scent marking or digging around the outside of cane toad
192 enclosure and “attack” behaviour was defined as quolls exhibited pawing or licking or biting
193 behaviour to toads cages. We summed all of these to measure the total time spent interacting

194 with a toad. We converted each of these variables to a proportion of time spent in each of
195 these activities, where the denominator was the total time that the animal was observable on
196 camera. These response variables were not normally distributed, and could not be made to
197 conform to normality through transformation. Because of this we used bootstrapping to
198 obtain confidence intervals for the mean time engaged in each behaviour, and to test the null
199 hypothesis that there was no difference between treatments in mean time spent in each
200 activity. The perception that animals exhibit a lower propensity towards attacking a prey item
201 following ingestion and subsequent malaise during CTA training is non-controversial
202 (Gustavson *et al.* 1974; Gustavson *et al.* 1976; Gustavson 1982; Gustavson & Basche 1983;
203 Gustavson, Gustavson & Holzer 1983; Ziegler *et al.* 1983; Gustavson & Nicolaus 1987;
204 Nicolaus 1987; Nicolaus & Nellis 1987; Nicolaus *et al.* 1989; Schneider & Pinnow 1994;
205 Smith *et al.* 2000; Riley & Freeman 2004; Sevelinges *et al.* 2009; O'Donnell, Webb & Shine
206 2010; Thornton & Raihani 2010; Thornton & Clutton-Brock 2011). More relevant to this
207 study is the outcomes of previous trials by O'Donnell, Webb and Shine (2010) and Kelly and
208 Phillips (2017) where quolls exhibited less interest in prey items after consuming a toad
209 metamorph laced with thiabendazole. Based on these previous results, we had a strong *a*
210 *priori* expectation that animals could either be unaffected or only become less interested in
211 toads after ingestion of cane toad sausages. Thus, we employed a one-tailed test, with the
212 alternative hypothesis that the mean time spent investigating and attacking toads will be
213 lower in the treatment group. This analysis was performed using R (R Core Team, 2016).

214 *Field trials*

215 Images from bait stations were collated and tagged by pass, session, site, bait-event, species
216 and activity. A 'pass' was defined as when a new species entered the frame or when images
217 that were at least 5 minutes between when the previous detection of the same species passed.
218 This reduced any likelihood of individuals of the same species being overlooked during

219 analysis. “Activity” was hierarchical, with the highest activity being ‘Bait taken’; this was
220 defined as either photographic evidence of animal eating bait or bait being taken from the bait
221 station. ‘Bait investigated’ was defined as when bait was sniffed but not consumed or taken.
222 ‘Bait area investigated with no bait available’ was defined as when no bait was available at a
223 bait station, but the animal was still visiting or investigating the bait station.

224 We analysed data using two levels of observation to determine 1) which species were
225 attracted to bait, and 2) which species took bait. A frequency distribution (n times each
226 species was recorded) was calculated and the proportion of bait takers in each species was
227 estimated. Passes in which we were unable to identify the species were pooled and removed
228 from further analysis. Additionally, if a species total number of visits was less than 10 we
229 removed that species from the analysis. Additionally *Varanus tristis*, *V. panoptes*, *V. mitchelli*
230 and *V. mertensi* were pooled into ‘*Varanus* other species’ due to small sample sizes.

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

243 likely, therefore, that any training is forgotten within the 4-5 month window between
244 sessions.

245 **Results**

246 *Captive trials*

247 Of the treatment animals, seven (77%) consumed all or part of a cane toad sausage and eight
248 (88%) control animals consumed beef sausages. Treatment had no significant effect on
249 whether the initial sausage was consumed, ($\chi^2 = 0.0$, $df = 1$, $p = 1$). In our video trials, quolls
250 spent an average of only 0.6% of the total time on camera interacting with the toad. Control
251 animals, however, spent more than twice as much time interacting with the toad than
252 treatment animals (control = 0.95%; treatment = 0.42%, bootstrap p-value = 0.022). When
253 we break this down by specific types of interaction, control animals spend approximately
254 sixty times longer investigating (control = 0.15%; treatment = 0.00024%, bootstrap p-value =
255 0.051); twice as much time sniffing (control = 0.70%; treatment = 0.35%, bootstrap p-value =
256 0.044); and twenty times more time attacking (control = 0.03%; treatment = 0.0015%,
257 bootstrap p-value = 0.036) toads when compared with the control (Figure 2).

258 *Field trials*

259 *Target and non-target uptake*

260 A total of 26 species were captured on camera traps visiting bait stations. For eleven of these
261 species, there were sufficient data to compare their response to bait uptake. The most
262 frequent visitors to the bait stations were quolls, with $n = 345$ passes (Figure 3). Almost all
263 bait removal was executed by quolls that took 65 baits of the 90 baits removed. Other species
264 took far fewer: *Zyomys argurus*, 9; *Ctenotus spp.*, 2; *Pseudantechinus ningbing*, 2; *Varanus*
265 *glauerti*, 2; and *Varanus glebopalma*, 2.

266 *Target uptake and training rates*

267 First pass uptake responses to the bait did not vary systematically across sessions ($\chi^2=1.7$,
268 $df=4$, $p=0.79$; Fig. 4). We thus treated individuals as independent across sessions with regard
269 to behaviour.

270 Following identification of individual quolls within sessions, it became apparent that
271 bait stations were visited by a total of 70 “individual” quolls over the period of the study. Of
272 these individuals, and considering only their first bait encounter, the bait was taken by 28
273 individuals and rejected (bait investigated but not taken) by 18 individuals. Thus the total
274 bait uptake rate at first encounter was 61% ($SE=7.2\%$). A further 24 individuals only visited
275 bait stations when there was no bait available.

276 Across all passes, a total of 31 quolls consumed a bait. Ten of these animals ultimately
277 consumed baits on more than one occasion within a session (32%, $SE=8.3\%$). Clearly, these
278 individuals were not effectively trained, failing to even exhibit aversion to the bait. We have
279 two ways of estimating the conversion rate (from untrained to trained, given bait
280 consumption). Placing an upper bound, we could consider all individuals that took a bait but
281 were not observed to take a second bait (20 of 31 = 68%) as trained. For a lower bound, we
282 could take the conservative approach and consider only those known to have consumed a bait
283 and then seen to approach and reject a bait as trained (7 of 17 = 41%). Thus, somewhere
284 between 41 and 68% of animals consuming a bait appear to have been trained.

285 **Discussion**

286 Our captive trials clearly indicate that training a quoll using a thiabendazole-laced toad
287 sausage changes their behaviour towards toads. Although our sample sizes were modest and
288 not all of our treatment animals fully consumed the bait, it is clear that sausage-trained
289 animals spent substantially less time interacting with a toad —between one half to one
290 sixtieth of the time as control animals. This behavioural shift is reflected across all prey

291 acquisition behaviours: investigating, sniffing, and attacking. Quolls clearly generalise their
292 acquired aversion from the bait to a real toad.

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

303 The TBZ dose of <300mg/kg animal body weight in our cane toad sausages was
304 relatively low compared to earlier work (400mg/kg in (Jolly *et al.* unpublished data;
305 O'Donnell, Webb & Shine 2010; Cremona *et al.* 2017; Kelly & Phillips 2017) but was set
306 low by regulators (Australian Pesticides and Veterinary Medicines Authority- (APVMA)) to
307 allow for potential multiple bait uptake, sub-adult target, and non-target species. Given the
308 LD₅₀ of TBZ is more than nine times higher than our dose rate; the delivered dose is very
309 conservative. Our results suggest, however, that it is still effective. Regulators (APVMA)
310 also limited the number of treatment baits available at a site at any one time to 30 baits per
311 hectare. It is clear from our study that, at this density of baits, many quolls are simply not
312 encountering the bait; arriving at the bait station after baits have been taken; this in a
313 relatively low density quoll population, and despite multiple bait events at each site. Thus, to
314 effectively bait a large proportion of the quolls at a site (particularly a high density site), a
315 greater density of baits will be required.

316 In addition to the high visitation rate of individual quolls to bait stations, some
317 individual quolls took baits on multiple occasions. Of the 70 “individual” quolls that visited
318 bait stations throughout study period, ten individuals consumed a cane toad sausage on more
319 than one occasion within a session. Why did these individuals manifestly fail to train? One
320 possibility is the low dose rate, 0.06g of TBZ in each sausage was calculated to provide
321 300mg/kg to the smallest adult quoll at our site; a 200g female. Long-term trapping at the
322 site (AWC, unpub. data) suggests that adult quolls in this population can reach more than
323 815g in weight. Thus, large animals could receive less than one quarter of the dose ingested
324 by small animals. As a consequence, we could expect larger animals (typically males) to be
325 harder to train with a fixed-dose bait. Another possibility is that these individuals were
326 unhealthy for other reasons (e.g., males in the process of annual die-off) and so were willing
327 to risk poisoning in order to acquire food, although such a mechanism would presumably
328 cause changes in uptake rate across sessions, so seems unlikely.

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

340 the unpredictable timing of the wet season in northern Australia). Thus, any baiting
341 campaign will need to dedicate effort to predicting the date of toad arrival at the site.

342 Overall, however, our study is encouraging with regard to the use of toad sausages as
343 a vehicle for large-scale CTA training of quolls. Our results suggest that quolls will consume
344 cane toad sausages in the field and will, as a consequence, be less inclined to attack cane
345 toads. This opens the possibility for broad scale application of CTA as a management
346 technique for mitigating the impact of toads on quolls.

347 While many questions remain about optimal bait design, delivery, and timing, it is
348 clear that CTA training using toad sausages is likely a viable tool for land managers seeking
349 to protect quoll populations. Given that quoll populations in the Kimberley will likely be
350 completely overrun by toads within the next five years, this is a tool that is urgently needed.
351 More generally, however, our work joins a growing list of studies demonstrating that the
352 impact of invasive species can be mitigated not only by controlling the invasive species, but
353 also – or instead - by manipulating its mechanism of impact.

354 **Authors' Contributions**

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

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369 **Data accessibility**

370 Data and scripts are available upon request.

371 **Compliance with Ethical Standards**

372 The study was conducted within Mornington Wildlife Sanctuary in accordance with Wildlife
373 Conservation Regulation 17 (Permit number: SFO10584). The area is jointly managed by
374 traditional land-owners and the Australian Wildlife Conservancy. The research was approved
375 by the University of Melbourne Animal Ethics Committee (Protocol: 1413369.2) and the
376 University of Technology Sydney Animal Care and Ethics Committee (Protocol: 2012-432A)
377 and Department of Parks and Wildlife Animal Ethics Committee (Protocol: DPaW AEC
378 2016_50 and Protocol 2013_37). Additionally this study was conducted in accordance with
379 the approved outline submitted to the AVPMA by the investigating team (Permit to allow the
380 possession and supply for research use of an unregistered Agvet chemical product. Permit
381 number: PER92262).

382

383 **References**

- 384 Avery, M.L. (1985) Application of Mimicry Theory to Bird Damage Control. *The Journal of*
385 *Wildlife Management*, **49**, 1116-1121.
- 386 Avery, M.L., Pavelka, A.A., Bergman, D.L., Decker, D.G., Kinttle, C.E. & Linz, G.M.
387 (1995) Aversive conditioning to reduce raven predation on California least tern eggs.
388 *Journal of the Colonial Waterbird Society*, **18**, 131-245.
- 389 Bernstein, I.L. (1999) Taste aversion learning: a contemporary perspective. *Nutrition*, **15**,
390 229-234.
- 391 Bogliani, G. & Fiorella, B. (1998) Conditioned Aversion as a Tool to Protect Eggs from
392 Avian Predators in Heron Colonies. *Colonial Waterbirds*, **21**, 69-72.
- 393 Bolger, D.T., Vance, B.T., Morrison, T.A. & Farid, H. (2011) Wild-ID. Dartmouth College,
394 Hanover, NH.
- 395 Burnett, S. (1997) Colonizing Cane Toads cause population declines in native predators:
396 reliable anecdotal information and management implications. *Pacific Conservation*
397 *Biology*, **3**, 65-72.
- 398 Claridge, A.W. & Mills, D.J. (2007) Aerial baiting for wild dogs has no observable impact on
399 spotted-tailed quolls (*Dasyurus maculatus*) in a rainshadow woodland. *Wildlife*
400 *Research*, **34**, 116-124.
- 401 Cohn, J. & MacPhail, R.C. (1996) Ethological and Experimental Approaches to Behavior
402 Analysis: Implications for Ecotoxicology. *Environmental Health Perspectives*, **104**,
403 299-305.
- 404 Conover, M.R. (1989) Potential compounds for establishing conditioned food aversions in
405 Racoons. *Wildlife Society Bulletin*, **17**.
- 406 Conover, M.R. (1995) Behavioral Principles Governing Conditioned Food Aversions Based
407 on Deception. *USDA National Wildlife Research Center Symposia National Wildlife*

- 408 *Research Center Repellents Conference* pp. 29-41. DigitalCommons@University of
409 Nebraska - Lincoln, University of Nebraska - Lincoln.
- 410 Covacevich, J. & Archer, M. (1975) The distribution of the cane toad, *Bufo marinus*.
411 *Australia and its effects on indigenous vertebrates. Memoirs of the Queensland*
412 *Museum*, **17**, 305-310.
- 413 Cowled, B.D., Gifford, E., Smith, M., Staples, L. & Lapidge, S.J. (2006) Efficacy of
414 manufactured PIGOUT® baits for localised control of feral pigs in the semi-arid
415 Queensland rangelands. *Wildlife Research*, **33**, 427-437.
- 416 Cox, R., Baker, S.E., Macdonald, D.W. & Berdoy, M. (2004) Protecting egg prey from
417 Carrion Crows: the potential of aversive conditioning. *Applied Animal Behaviour*
418 *Science*, **87**, 325-342.
- 419 Cremona, T., Spencer, P., Shine, R. & Webb, J.K. (2017) Avoiding the last supper: parentage
420 analysis indicates multi-generational survival of re-introduced 'toad-smart' lineage.
421 *Conservation Genetics*, 1-6.
- 422 Diete, R.L., Meek, P.D., Dixon, K.M., Dickman, C.R. & Leung, L.K.-P. (2016) Best bait for
423 your buck: bait preference for camera trapping north Australian mammals. *Australian*
424 *Journal of Zoology*, **63**, 376-382.
- 425 Dilov, P., Chaleva, E., Dzhurov, A., Stoianov, P. & Iotsev, M. (1981) [Toxicological
426 evaluation of "Farmakhim" thiabendazole in mammals and birds]. *Veterinarno-*
427 *meditsinski nauki*, **19**, 92-100.
- 428 Doherty, T.S., Dickman, C.R., Glen, A.S., Newsome, T.M., Nimmo, D.G., Ritchie, E.G.,
429 Vanak, A.T. & Wirsing, A.J. (2017) The global impacts of domestic dogs on
430 threatened vertebrates. *Biological Conservation*, **210, Part A**, 56-59.
- 431 Dundas, S., Adams, P. & Fleming, P. (2014) First in, first served: uptake of 1080 poison fox
432 baits in south-west Western Australia. *Wildlife Research*, **41**, 117-126.

- 433 Ellins, S.R. & Catalano, S.M. (1980) Field application of the conditioned taste aversion
434 paradigm to the control of coyote predation on sheep and turkeys. *Behavioral and*
435 *Neural Biology*, **29**, 532-536.
- 436 Fairbridge, D., Anderson, R., Wilkes, T. & Pell, G. (2003) Bait uptake by free living brush-
437 tailed phascogales *Phascogale tapoatafa* and other non-target mammals during
438 simulated buried fox baiting. *Australian Mammology*, **25**, 31-40.
- 439 Gill, E.L., Whiterow, A. & Cowan, D.P. (2000) A comparative assessment of potential
440 conditioned taste aversion agents for vertebrate management. *Applied Animal*
441 *Behaviour Science*, **67**, 229-240.
- 442 Glen, A.S. & Dickman, C., R (2003) Monitoring bait removal in vertebrate pest control: a
443 comparison using track identification and remote photography. *Wildlife Research*, **30**,
444 29-33.
- 445 Glendinning, J.I. (2007) How Do Predators Cope With Chemically Defended Foods? . *The*
446 *Biological Bulletin*, **213**, 252-266.
- 447 Gustavson, C.R. (1982) An evaluation of taste aversion control of wolf (*Canis lupus*)
448 predation in Northern Minnesota. *Applied Animal Ethology*, **9**, 63-71.
- 449 Gustavson, C.R. & Basche, L.A. (1983) Landrin- and thiabendazole-based conditioned taste
450 aversions in domestic ducks. *Applied Animal Ethology*, **9**, 379-380.
- 451 Gustavson, C.R., Garcia, J., Hankins, W.G. & Rusiniak, K.W. (1974) Coyote Predation
452 Control by Aversive Conditioning. *Science*, **184**, 581-583.
- 453 Gustavson, C.R., Gustavson, J.C. & Holzer, G.A. (1983) Thiabendazole-based taste aversions
454 in dingoes (*Canis familiaris dingo*) and New Guinea wild dogs (*Canis familiaris*
455 *hallstromi*). *Applied Animal Ethology*, **10**, 385-388.
- 456 Gustavson, C.R., Kelly, D.R., Sweeney, M. & Garcia, J. (1976) Prey-Lithium Aversions. I:
457 Coyotes and Wolves. *Behavioral Biology*, **17**, 61-72.

- 458 Gustavson, C.R. & Nicolaus, L.K. (1987) Taste aversion conditioning in wolves, coyotes,
459 and other canids: retrospect and prospect. *Man and wolf*, 169-200.
- 460 Hayes, R.A., Crossland, M.R., Hagman, M., Capon, R.J. & Shine, R. (2009) Ontogenetic
461 Variation in the Chemical Defenses of Cane Toads (*Bufo marinus*): Toxin Profiles
462 and Effects on Predators. *Journal of Chemical Ecology*, **35**, 391-399.
- 463 Hohnen, R., Ashby, J., Tuft, K. & McGregor, H. (2013) Individual identification of northern
464 quolls (*Dasyurus hallucatus*) using remote cameras. *Australian Mammalogy*, **35**, 131-
465 135.
- 466 Jolley, W.J., Campbell, K.J., Holmes, N.D., Garcelon, D.K., Hanson, C.C., Will, D., Keitt,
467 B.S., Smith, G. & Little, A.E. (2012) Reducing the impacts of leg hold trapping on
468 critically endangered foxes by modified traps and conditioned trap aversion on San
469 Nicolas Island, California, USA. *Conservation Evidence*, **9**, 43-49.
- 470 Jolly, C.J., Shine, R. & Greenlees, M.J. (2015) The impact of invasive cane toads on native
471 wildlife in southern Australia. *Ecology and Evolution*, **5**, 3879-3894.
- 472 Jolly et al.unpublished data.
- 473 Kelly, E. & Phillips, B. (2017) Get smart: native mammal develops toad-smart behavior in
474 response to a toxic invader. *Behavioral Ecology*, **28**, 854-858.
- 475 Legge, S., Murphy, B.P., McGregor, H., Woinarski, J.C.Z., Augusteyn, J., Ballard, G.,
476 Baseler, M., Buckmaster, T., Dickman, C.R., Doherty, T., Edwards, G., Eyre, T.,
477 Fancourt, B.A., Ferguson, D., Forsyth, D.M., Geary, W.L., Gentle, M., Gillespie, G.,
478 Greenwood, L., Hohnen, R., Hume, S., Johnson, C.N., Maxwell, M., McDonald, P.J.,
479 Morris, K., Moseby, K., Newsome, T., Nimmo, D., Paltridge, R., Ramsey, D., Read,
480 J., Rendall, A., Rich, M., Ritchie, E., Rowland, J., Short, J., Stokeld, D., Sutherland,
481 D.R., Wayne, A.F., Woodford, L. & Zewe, F. (2017) Enumerating a continental-scale
482 threat: How many feral cats are in Australia? *Biological Conservation*, **206**, 293-303.

- 483 Letnic, M., Webb, J.K. & Shine, R. (2008) Invasive cane toads (*Bufo marinus*) cause mass
484 mortality of freshwater crocodiles (*Crocodylus johnstoni*) in tropical Australia.
485 *Biological Conservation*, **141**, 1773-1782.
- 486 Llewelyn, J., Phillips, B.L., Brown, G.P., Schwarzkopf, L., Alford, R.A. & Shine, R. (2011)
487 Adaptation or preadaptation: why are keelback snakes (*Tropidonophis mairii*) less
488 vulnerable to invasive cane toads (*Bufo marinus*) than are other Australian snakes?
489 *Evolutionary Ecology*, **25**, 13-24.
- 490 Mappes, J., Marples, N. & Endler, J. (2005) The complex business of survival by
491 aposematism. *Trends in Ecology and Evolution*, **20**, 598-603.
- 492 Massei, G. & Cowan, D.P. (2002) Strength and persistence of conditioned taste aversion in
493 rats: evaluation of 11 potential compounds. *Applied Animal Behaviour Science*, **75**,
494 249-260.
- 495 Massei, G., Lyon, A. & Cowan, D.P. (2003) Levamisole can induce conditioned taste
496 aversion in foxes. *Wildlife Research*, **30**, 633-637.
- 497 Nachman, M. & Ashe, J. (1973) Learned Taste Aversions in Rats as a Function of Dosage,
498 Concentration, and Route of Administration of LiCl. *Physiology and Behavior*, **10**,
499 73-78.
- 500 Nicolaus, L.K. (1987) Conditioned Aversions in a guild of egg predators: Implications for
501 aposematism and prey defense mimicry. *American Midland Naturalist*, **117**, 405-419.
- 502 Nicolaus, L.K., Herrera, J., Nicolaus, J.C. & Dimmick, C.R. (1989) Carbachol as a
503 conditioned taste aversion agent to control avian depredation. *Agriculture, Ecosystems
504 and Environment*, **26**, 13-21.
- 505 Nicolaus, L.K. & Nellis, D.W. (1987) The First Evaluation of the Use of Conditioned Taste
506 Aversion to Control Predation by Mongooses upon Eggs. *Applied Animal Behaviour
507 Science*, **17**, 329-346.

- 508 O'Donnell, S., Webb, J.K. & Shine, R. (2010) Conditioned taste aversion enhances the
509 survival of an endangered predator imperilled by a toxic invader. *Journal of Applied*
510 *Ecology*, **47**, 558-565.
- 511 Oakwood, M. & Foster, P. (2008) Monitoring extinction of the northern quoll. *Australian*
512 *Academy of Science Newsletter*, **71**, 6.
- 513 Page, R.A. & Ryan, M.R. (2005) Flexibility in assessment of prey cues: frog-eating bats and
514 frog calls. *Proceedings of the Royal Society*, **272**, 841-847.
- 515 Phillips, B.L. & Shine, R. (2005) The morphology, and hence impact, of an invasive species
516 (the cane toad, *Bufo marinus*): changes with time since colonisation. *Animal*
517 *Conservation*, **8**, 407-413.
- 518 Provenza, F.D., Burritt, E.A., Clausen, T.P., Bryant, J.P., Reichardt, P.B. & Distel, R.A.
519 (1990) Conditioned flavor aversion: a mechanism for goats to avoid condensed
520 tannins in blackbrush. *American Naturalist*, 810-828.
- 521 Reaser, J.K., Meyerson, L.A., Cronk, Q., De Poorter, M., Eldrege, L.G., Green, E., Kairo, M.,
522 Latasi, P., Mack, R.N. & Mauremootoo, J. (2007) Ecological and socioeconomic
523 impacts of invasive alien species in island ecosystems. *Environmental Conservation*,
524 **34**, 98-111.
- 525 Riley, A.L. & Freeman, K.B. (2004) Conditioned taste aversion: a database. *Pharmacology*
526 *Biochemistry and Behavior*, **77**, 655-656.
- 527 Risbey, D.A., Calver, M.C., Short, J., Bradley, J.S. & Wright, I.W. (2000) The impact of cats
528 and foxes on the small vertebrate fauna of Heirisson Prong, Western Australia. II. A
529 field experiment. *Wildlife Research*, **27**, 223-235.
- 530 Robinson, H.J., Stoerk, H.C. & Graessle, O.E. (1965) Studies on the toxicologic and
531 pharmacologic properties of thiabendazole. *Toxicology and applied pharmacology*, **7**,
532 53-63.

- 533 Schneider, K. & Pinnow, M. (1994) Olfactory and Gustatory Stimuli in Food-Aversion
534 Learning of Rats. *The Journal of General Psychology*, **121**, 169-183.
- 535 Semel, B. & Nicolaus, L.K. (1992) Estrogen-Based Aversion to Eggs Among Free-Ranging
536 Raccoons. *Ecological Applications*, **2**, 439-449.
- 537 Sevelinges, Y., Mouly, A.M., Levy, F. & Ferreira, G. (2009) Long-Term Effects of Infant
538 Learning on Adult Conditioned Odor Aversion Are Determined by the Last
539 Prewaning Experience. *Developmental Psychobiology*, 299-398.
- 540 Shine, R. (2010) The ecological impact of invasive cane toads (*Bufo marinus*) in Australia.
541 *The Quarterly Review Of Biology*, **85**, 253-291.
- 542 Short, J. & Smith, A. (1994) Mammal decline and recovery in Australia. *Journal of*
543 *Mammalogy*, **75**, 288-297.
- 544 Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J.,
545 Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R.,
546 Tabacchi, E. & Vilà, M. (2013) Impacts of biological invasions: what's what and the
547 way forward. *Trends in Ecology & Evolution*, **28**, 58-66.
- 548 Sinclair, R.G. & Bird, P.L. (1984) The reaction of *Sminthopsis crassicaudata* meat baits
549 containing 1080: Implications for assessing risk to non-target species. *Australian*
550 *Wildlife Research*, **11**, 501-507.
- 551 Smith, J. & Phillips, B.L. (2006) Toxic tucker: the potential impact of Cane Toads on
552 Australian reptiles. *Pacific Conservation Biology*, **12**, 40-49.
- 553 Smith, M.E., Linnell, J.D.C., Odden, J. & Swenson, J.E. (2000) Review of Methods to
554 Reduce Livestock Depredation II. Aversive conditioning, deterrents and repellents.
555 *Acta Agriculturae Scandinavica, Section A — Animal Science*, **50**, 304-315.

- 556 Ternent, M.A. & Garshelis, D.L. (1999) Taste-aversion conditioning to reduce nuisance
557 activity by black bears in a Minnesota military reservation. *Wildlife Society Bulletin*,
558 **27**, 720-728.
- 559 Thornton, A. & Clutton-Brock, T. (2011) Social learning and the development of individual
560 and group behaviour in mammal societies. *Philosophical Transactions of the Royal*
561 *Society B: Biological Sciences*, **366**, 978-987.
- 562 Thornton, A. & Raihani, N.J. (2010) Identifying teaching in wild animals. *Learning &*
563 *Behavior*, **38**, 297-309.
- 564 Tocco, D.J., Rosenblum, C., Martin, C.M. & Robinson, H.J. (1966) Absorption, metabolism,
565 and excretion of thiabendazole in man and laboratory animals. *Toxicology and*
566 *applied pharmacology*, **9**, 31-39.
- 567 Urban, M.C., Phillips, B.L., Skelly, D.K. & Shine, R. (2007) The cane toad's (*Chaunus*
568 [*Bufo*] *marinus*) increasing ability to invade Australia is revealed by a dynamically
569 updated range model. *Proceedings of the Royal Society Biological Sciences Series B*,
570 **274**, 1413-1419.
- 571 Webb, J.K., Brown, G.P., Child, T., Greenlees, M.J., Phillips, B.L. & Shine, R. (2008) A
572 native dasyurid predator (common planigale, *Planigale maculata*) rapidly learns to
573 avoid a toxic invader. *Austral Ecology*, **33**, 821-829.
- 574 Webb, J.K., Pearson, D. & Shine, R. (2011) A small dasyurid predator (*Sminthopsis*
575 *virginiae*) rapidly learns to avoid a toxic invader. *Wildlife Research*, **38**, 726-731.
- 576 Webb, J.K., Shine, R. & Christian, K.A. (2005) Does intraspecific niche partitioning in a
577 native predator influence its response to an invasion by a toxic prey species? *Austral*
578 *Ecology*, **30**, 201-209.
- 579 Woinarski, J., Burbidge, A. & Harrison, P. (2014) The action plan for Australian mammals
580 2012.

581 Ziegler, J.M., Gustavson, C.R., Holzer, G.A. & Gruber, D. (1983) Anthelmintic-based taste
582 aversions in wolves (*Canis lupus*). *Applied Animal Ethology*, **9**, 373-377.

583 Ziembicki, M.R., Woinarski, J.C.Z., Webb, J.K., Vanderduys, E., Tuft, K., Smith, J., Ritchie,
584 E.G., Reardon, T.B., Radford, I.J., Preece, N., Perry, J., Murphy, B., P., McGregor,
585 H., Legge, S., Leahy, L., Lawes, M.J., Kanowski, J., Johnson, C.N., James, A.,
586 Griffiths, A.D., Gillespie, G., Frank, A.S.K., Fisher, A. & Burbidge, A.A. (2015)
587 Stemming the tide: progress towards resolving the causes of decline and
588 implementing management responses for the disappearing mammal fauna of northern
589 Australia. *Therya*, **6**, 169-225.

590

591 **Figures**

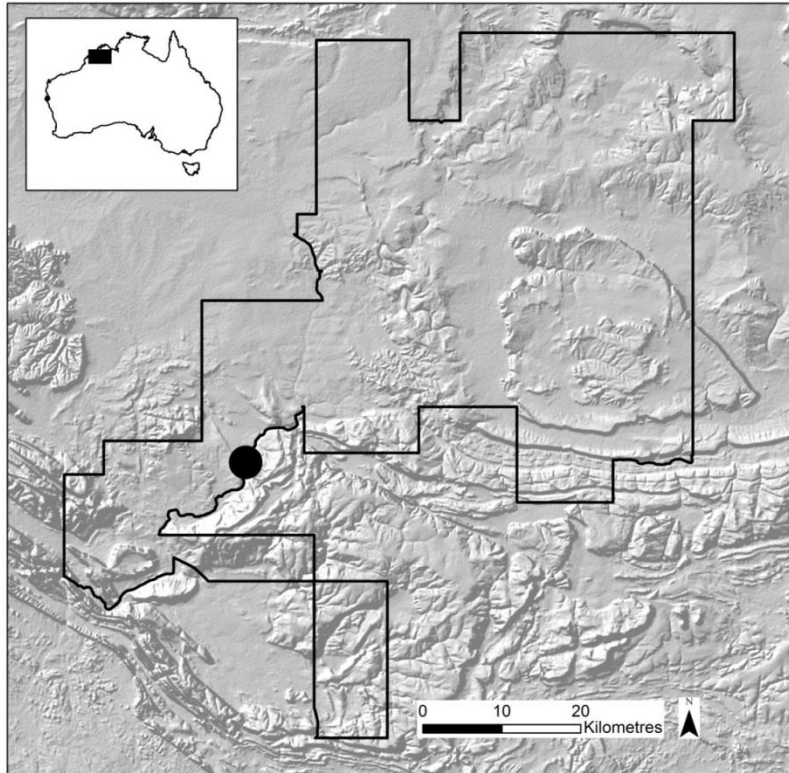


Figure 1: Location of the study area within Australian Wildlife Conservancy's Mornington Wildlife Sanctuary, in the central Kimberley, Western Australia.

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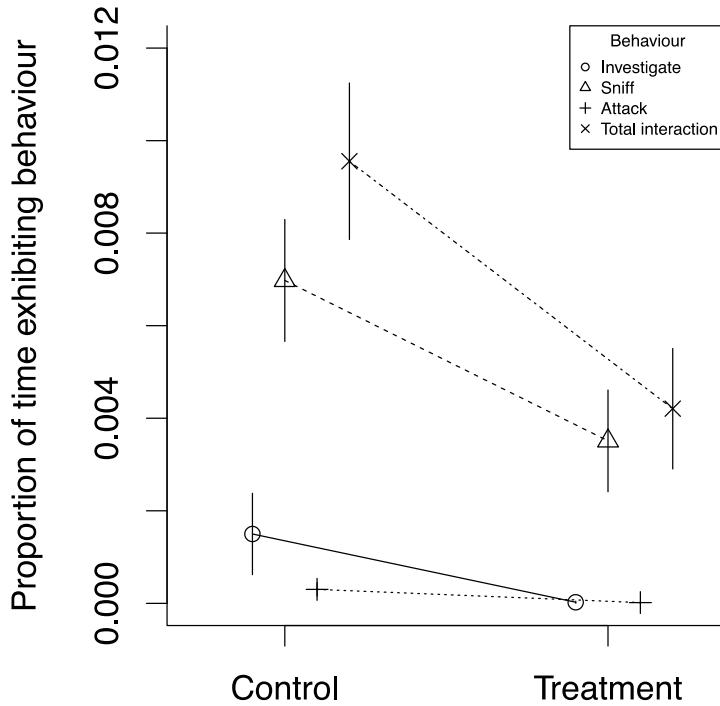


Figure 2: Mean proportion of active time that quolls spent directed towards toads. Behaviours are split into categories and across control and treatment groups. Error bars represent bootstrap standard errors.

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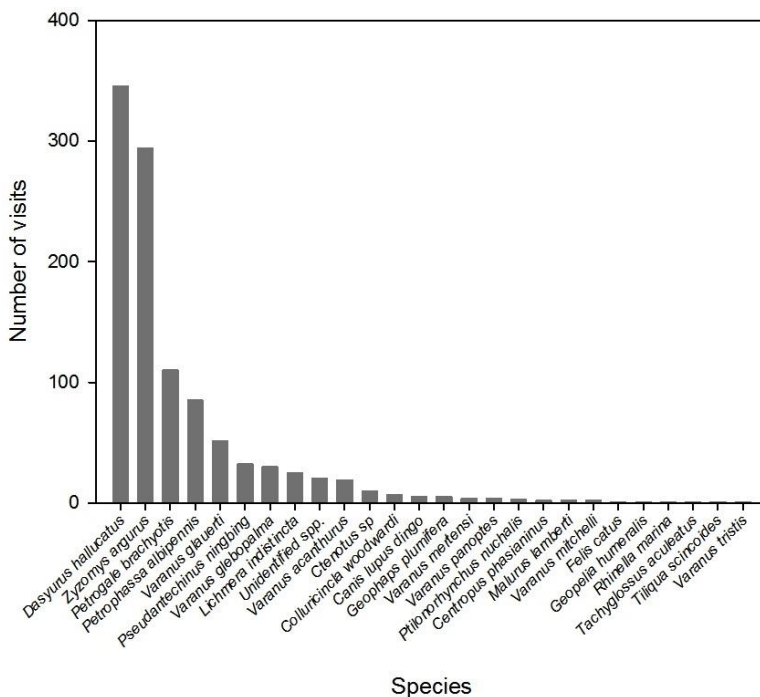


Figure 3: Frequency of visits to CTA bait stations by each species. Unidentified species group comprises unidentified rodents, birds, and frogs.

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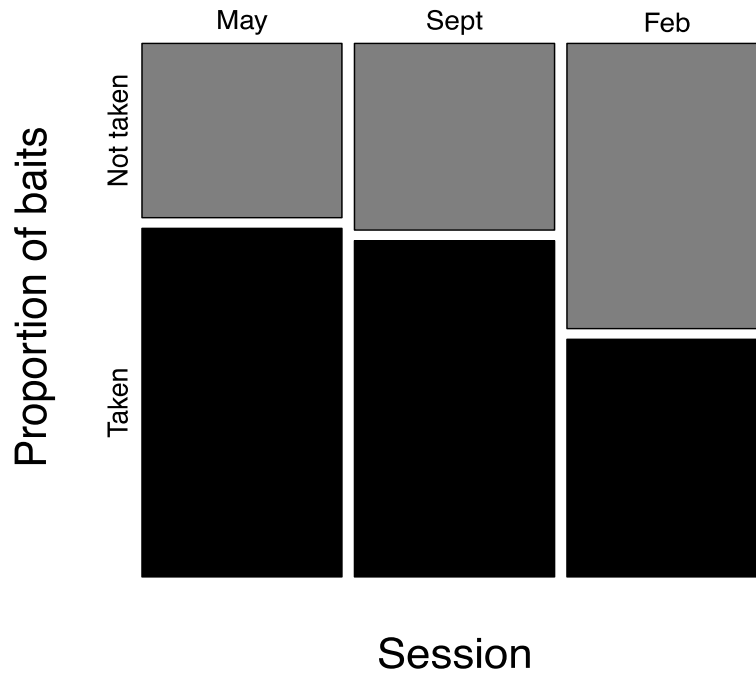


Figure 4: First pass behavioural responses of northern quolls to the bait each session. “Sept” includes the one November session also.

598

599 **Tables**

Site Name	Session Year	Session Month	No. Bait Events (BE)	BE 1-date	BE 2-date	BE 3-date	BE 4-date	No. of bait stations ⁶⁰⁰ ₆₀₁
KP	2016	Nov	1	31/10/16	1/11/16	2/11/16	*	20
RP	2017	Feb	1	3/2/2017	*	*	*	20
RP	2016	May	3	10/5/16	13/5/16	21/5/16	*	20
RP	2016	Sept	3	15/9/16	16/9/16	17/9/16	*	20
SJ	2017	Feb	1	3/2/2017	*	*	*	20
SJ	2016	May	3	10/5/16	13/5/16	21/5/16	*	20
SJ	2016	Sept	4	15/9/16	16/9/16	17/9/16	19/9/16	20
TC	2017	Feb	1	3/2/2017	*	*	*	33
TC	2016	May	3	10/5/16	13/5/16	21/5/16	*	20
TC	2016	Sept	3	15/9/16	16/9/16	17/9/16	*	20

Table 1: CTA sessions and bait events, * denotes empty cells. Bait events occurred at the same time within each site. KP was baited only once in November to expand the sample size and CTA train quolls prior to cane toad arrival.