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Using lures for improving selectivity of bait intake by red foxes

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Abstract

Context. The use of baits for reducing the populations of harmful animal species, eradicating invasive species, vaccination, contraception or producing conditioned aversion, is widespread worldwide. However, baiting programs are often not successful enough and affect non-target species, requiring new approaches for baiting methods.

Aims. The aim of the present study was to evaluate two attractants used in carnivore studies to improve bait intake probability by red foxes and minimise bait intake by non-target species.

Methods. Non-toxic baits were distributed across 1000 ha, with bait intake monitored by camera traps during 3-week trials. Baits were assigned to two treatments with lures (lynx urine and Fatty Acid Scent – FAS) and one control. Bait intake by red foxes and non-target species was analysed using Generalised Linear Mixed Model (GLMM) and Kaplan–Meier survival analyses.

Key results. Lynx urine significantly increased the bait intake by red foxes (58.8%) compared with control (5.7%) and FAS (16.7%) treatment. However, FAS did not significantly increase the bait intake by red foxes compared with control. Bait intake by non-target species differed significantly between treatments, with lower intake in lynx urine (23.5%) treatment than control (54.7%), but not regarding FAS (36.7%), and neither between FAS and control. The probability of bait persistence after the 3-week trial period differed significantly among treatments, being lower in lynx urine treatment (0.18) than FAS (0.50) and control (0.43). All baits taken by foxes with lynx urine treatment (58.8%) occurred within the first 10 days, whereas intake by non-target species (23.5%) stopped after Day 7.

Conclusions. The use of lynx urine lure increased the proportion of baits consumed by red fox and reduced bait intake by non-target species.

Implications. Lures can serve to optimise bait delivery methods for red foxes in their different applications, such as conditioned aversion studies, vaccination, live trapping or predator control, while minimising risks to non-target species and reducing the costs and application time.

Keywords: baiting, conservation biology, lures, non-target species, predators, selectivity, wildlife management.

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Introduction

Since prehistoric times humans and wildlife have competed for resources (Conover 2002) such as food and shelter, or more recently, for crops, livestock or game species (Reynolds and Tapper 1996; Sillero-Zubiri and Schwitzer 2004). To reduce the threat that wildlife poses to human safety and their resources, humans have controlled populations of species considered harmful or pests (Reynolds and Tapper 1996; Treves and Bruskotter 2014), especially predators. Baiting has been extensively used worldwide for reducing populations of animal species considered harmful, eradicating invasive species, vaccination, contraception or producing conditioned aversion (Bradley *et al.* 1999; Díez-Delgado *et al.* 2018; Ballard *et al.* 2020; Tobajas *et al.* 2020*a*).

Baits are often used with poisons, for example sodium fluoroacetate (1080) and 4’-para-aminopropiophenone (PAPP), both of which are widely employed to control invasive species in Australia and New Zealand (Moseby and Hill 2011; Eason *et al.* 2017; Philip 2020) and are considered a cost-effective method to control invasive and harmful species (Thomson *et al.* 2000; Eason *et al.* 2017; Ballard *et al.* 2020). Baiting programs are also used in contraceptive or vaccination campaigns of several animal species, to regulate fertility or reduce the incidence of diseases that potentially could affect humans or their economy (Bradley *et al.* 1999; Díez-Delgado *et al.* 2018). Other uses of baits in wildlife management include live trapping to attract the animals to the traps (Díaz-Ruiz *et al.* 2016) and Conditioned Food Aversion (CFA) studies, where a chemical compound is added to baits to elicit its rejection after a non-lethal adverse effect induced by the chemical compound (Tobajas *et al.* 2020*b*, 2021*a*). CFA with baits has been used to reduce the bait consumption of poisoned baits (Nielsen *et al.* 2015), for monopolisation of baits in vaccination campaigns (Gentle *et al.* 2004), for livestock predation (Gustavson *et al.* 1976; Tobajas *et al.* 2020*c*) and for post-release predation of prey species (Tobajas *et al.* 2021*a*). Recently, CFA (using artificial nests) has been demonstrated to be effective in reducing nest predation and improving population of ground-nesting birds (Tobajas *et al.* 2020*b*). In this experiment, the authors argue that one of the main problems in the application of this technique is the predation by the non-target species, which makes conditioning of the target species difficult, requiring an increase of nests and the extension of the conditioning period (Indigo *et al.* 2018; Tobajas *et al.* 2020*b*, 2021*a*).

The low detectability or probability of encountering the baits by the target species forces the use of extensive methods to deliver the baits (Moseby and Hill 2011; Ballard *et al.* 2020). Increasing the number of baits deployed means increased cost, but also increases the risk of bait intake by non-target species (Dundas *et al.* 2014; Hohnen *et al.* 2020; Smith *et al.* in press). These drawbacks require new approaches to increase the effectivity of baiting on target species (Morgan *et al.* 2015; Heiniger *et al.* 2018; Moseby *et al.* 2020). Thus, optimisation on the application of the baits for the target species must be the focus of managers and researchers (Indigo *et al.* 2018; Smith *et al.* in press).

Several methods have been previously evaluated to reduce the ingestion of baits by non-target species. Repellence or CFA to the baits have been attempted to reduce the ingestion by non-target species (Orr-Walker *et al.* 2012). In a different way, the use of attractants has been tested to improve the baiting effectivity for target species, but in some cases inconsistent results were obtained or the effect of these lures on the non-target species were not addressed (Morgan *et al.* 1995; Moseby *et al.* 2011; Ferreira-Rodríguez and Pombal 2019). However, several attractants have shown their effectiveness in attracting certain species of predators in camera trap studies (Monterroso *et al.* 2011; Ferreras *et al.* 2018), showing potential for their use in improving the effectiveness of baiting campaigns. Thus, the potential of using specific attractants to improve bait intake by the target species while minimising the intake by non-target species should be explored.

The red fox (*Vulpes vulpes*) is a widely distributed opportunistic canid predator highly adaptable to changes in habitat and food resources (Macdonald and Sillero-Zubiri 2004). Throughout its distribution range, red fox populations are commonly controlled for game management to avoid domestic stock losses, particularly in Australia because it is recognised as a devastating invasive pest species (Dickman 1996; Delibes-Mateos *et al.* 2013). Thus, for population control and also in CFA studies for predation control, toxic baits have been employed (Thomson *et al.* 2000; Tobajas *et al.* 2020*b*). Although improvement of baiting effectivity for foxes has often been attempted (Moseby *et al.* 2009, 2011; Towerton *et al.* 2013), more effective methods to improve bait intake by foxes while minimising intake by non-target species are needed. The aim of the present study is to evaluate the performance of two attractants (lynx urine and Fatty Acid Scent – FAS) used in carnivore studies to improve the bait intake probability by red foxes while minimising the intake by non-target species in a short period of bait deployment. The design is focused to increase bait intake in CFA studies to reduce nest predation of ground-nesting birds, but it is widely applicable to other baiting programs with different goals.

Materials and methods

Study area

The study was carried out on a private property within the Ciudad Real province (Central Spain). The study area is a 1000-ha estate of homogeneous habitat covered mainly by cereal fields and secondarily by mediterranean scrubland and sparse patches of holm oak (*Quercus ilex* subsp. *rotundifolia*). The climate is mediterranean, characterised by wet, mild winters and dry, hot summers. During the study period (March to May 2017) the weather was mostly dry, with some days of light rain. The predator community of ground nests is mainly composed by red fox, stone marten (*Martes foina*), European badger (*Meles meles*), Egyptian mongoose (*Herpestes ichneumon*), wild boar (*Sus scrofa*), garden dormouse (*Eliomys quercinus*), black-billed magpie (*Pica pica*), western jackdaw (*Coloeus monedula*) and ocellated lizard (*Timon lepidus*). The European rabbit (*Oryctolagus cuniculus*) is found in high abundance, and is the main prey for foxes in the study area; small mammals and ground-nesting birds such as the red-legged partridge (*Alectoris rufa*) are secondary prey.

Bait intake monitoring

We compared the performance of two olfactory attractants for carnivores (lynx urine and FAS) with a control group (only bait). FAS was produced in the laboratory by mixing different fatty acids following Roughton (1982). Iberian lynx (*Lynx pardinus*) urine was collected from captive breeding facilities and stored frozen until its use. All the urine used was from the same batch to ensure the same composition during the study. Both lures were placed so as to attract red fox rather than non-target species, but not to produce any type of repellence or avoidance to these species. Lures were sprayed onto a cork piece and placed near the bait (1–2 m) and hidden under a stone. The reason for placing the attractant at a certain distance from the bait was to avoid possible interferences in its use in aversion studies (i.e. to avoid association between odour and the induced aversion by the chemical), but in other types of bait uses the attractant can be introduced into or onto the bait itself. The attractant was renewed every 7 days. Baits consisted of three farm red-legged partridge eggs, simulating a real nest with dry and fresh grass, and were maintained for 3 weeks or until their predation.

To evaluate the effect of lures on the bait intake probability by red fox and by non-target species, we deployed 26–34 baits monitored by motion-triggered digital cameras in four sequential 3-week trials from March to May 2017. The trials sequence was control ($n = 27$), FAS ($n = 30$), lynx urine ($n = 34$), control ($n = 26$). Using this methodology we were able to maximise the number of baits placed in a short period of time, and we achieved a similar bait density as in other studies with the available number of cameras. We assumed that the population of foxes and other predators was closed due to the short study period, but we decided to include a second control round at the end to

Table 1. Bait intake by each species in the control and lure treatment in the study area during the 3-week experimental phase
FAS, Fatty Acid Scent

Species	Control ($n = 53$)		FAS ($n = 30$)		Lynx urine ($n = 34$)	
	n	%	*n*	%	*n*	%
Black-billed magpie (*Pica pica*)	22	41.51	7	23.33	6	17.65
Red fox (*Vulpes vulpes*)	3	5.66	5	16.67	20	58.82
Garden dormouse (*Eliomys quercinus*)	2	3.77	3	10.00	1	2.94
Stone marten (*Martes foina*)	1	1.89	0	0.00	0	0.00
Domestic dog (*Canis lupus familiaris*)	1	1.89	0	0.00	1	2.94
Wild boar (*Sus scrofa*)	1	1.89	0	0.00	0	0.00
Egyptian mongoose (*Herpestes ichneumon*)	1	1.89	0	0.00	0	0.00
Ocellated lizard (*Timon lepidus*)	0	0.00	1	3.33	0	0.00
Unknown	1	1.89	0	0.00	0	0.00
Not predated	21	39.62	14	46.67	6	17.65
Total predated	32	60.38	16	53.33	28	82.35

control for any difference between the beginning and the end of the study. Baits were placed in areas to maximise the probability of bait intake by the red fox, such as hunting areas, rocks suspected as breeding dens or near trails. The density of baits employed was between 2.6 (control) and 3.4 (lynx urine) baits km^{-2}, and distance between neighbouring baits ranged from 170 m to 790 m, depending on landscape features.

One camera trap (Reconyx HC500 Hyperfire Semi-Covert IR; Holmen, Wisconsin, USA or Spartan SR1-BK; HCO Outdoor Products, Norcross, Georgia, USA) was placed in front of each bait (2–3 m) to identify the causative predator species. Camera traps were visited approximately every 7 days for maintenance and data download. Spartan cameras were programmed to record 10-s videos with a minimum time delay (0 s) between consecutive videos, and Reconyx cameras were programmed in RapidFire mode (up to 2 frames per second) in order to maximise the number of photos taken per captured individual. A bait was considered consumed when one or more eggs were damaged or removed. Bait intake was recorded as a binomial variable (consumed versus not consumed).

Statistical analysis

To assess if the lures increased the bait intake by red foxes and by non-target species, we used a Generalised Linear Mixed Model (GLMM) with binomial error and logit link function to analyse the effect of treatment on bait intake probability. Lure treatment was included as a factor and bait location as a random variable to consider differences among locations. Where significant differences were found, pair-wise comparisons were performed using a *t*-test with Bonferroni correction to determine significant differences ($P < 0.05$) among treatments. The two control periods were considered as one in the analyses, since no significant differences among them were observed in a previous analysis following the same procedure. The statistical analyses were carried out using the 'nlme' package with the R software version 4.0.0 (R Core Team 2020).

We examined differences between treatments in the persistence times of the baits over the monitoring period of 21 days using Kaplan–Meier survival analyses with the 'survival' package of the R software version 4.0.0 (R Core Team 2020). Additionally, we constructed the curves of the proportion of baits not predated by red fox and non-target species to observe how bait intake changes through time, and to determine the best treatment period to maximise the bait intake by the red fox and minimise it by non-target species.

Results

A total of 117 baits were placed in the study area and 67.5% were consumed, mainly by magpie and red fox, accounting for 29.9% and 23.9% of overall consumed baits respectively (Table 1). Non-significant differences were found between the two control periods in the red fox ($F_{1,51} = 0.30$, $P = 0.58$) and non-target species ($F_{1,51} = 0.18$, $P = 0.67$) bait intake. A significant difference in fox intake was observed among treatments ($F_{2,68} = 24.04$, $P < 0.001$) (Fig. 1), with a higher probability of being taken by fox for those baits treated with lynx urine than with FAS ($P < 0.001$) and control baits ($P < 0.001$), but without differences between FAS and control treatment ($P = 0.57$). The probability of bait intake by non-target species differed significantly among treatments ($F_{2,68} = 4.83$, $P = 0.013$) (Fig. 2), with lower intake probability by other species in lynx urine treatment than in control ($P = 0.01$), but with no differences between lynx urine and FAS ($P = 0.829$). Significant differences between control and FAS treatment in bait intake by other species were not found ($P = 0.307$).

During the 3-week monitoring period, bait persistence times differed among treatments ($\chi^2_{2,107} = 17.8$, $P < 0.001$), the persistence times being lower in lynx urine treatment (Fig. 2). The proportion of baits not consumed after 3 weeks was 0.18, 0.5 and 0.43 for lynx urine, FAS and control, respectively (Fig. 2). Data showed that in lynx urine treatment all bait intakes by foxes (58.8%) occurred during the first 10 days, whereas bait intake by non-target species (23.5%) stopped after day 7 (Fig. 3).

Discussion

The present study shows that the use of attractants increased the bait intake by red fox and minimised the intake by non-target species (Fig. 1). Lynx urine has been shown to be the most efficient lure in achieving these objectives, proving to be a good attractant for red foxes. Our results agree with previous studies that showed that lynx urine is a good attractant for foxes

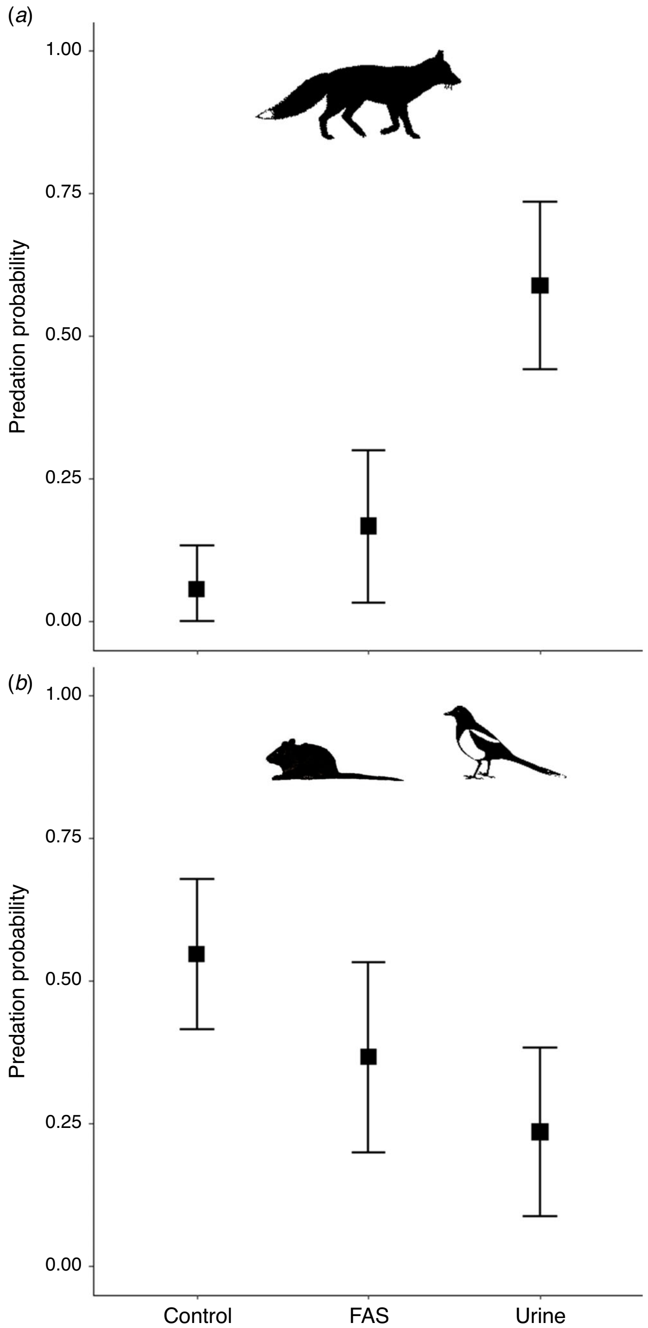

Fig. 1. Bait intake probability (±95% CI) by predator species during the experimental period (3 weeks) using different attractants (FAS, Fatty Acid Scent; urine, lynx urine) and control. (*a*) Target species (red fox *Vulpes vulpes*); (*b*) non-target species (see Table 1).

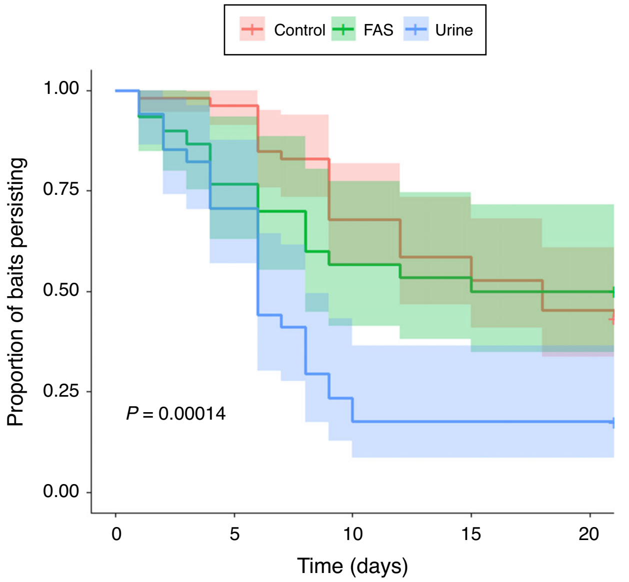

Fig. 2. Kaplan–Meier estimate of the survival function for the proportion of baits persisting during the experimental period (3 weeks) in the treatments using different attractants (FAS, Fatty Acid Scent; urine, lynx urine) and control. Light shadings show the 95% confidence intervals. The *P*-value is from the log-rank test comparing the survival curves.

(Monterroso *et al.* 2011; Díaz-Ruiz *et al.* 2016; Ferreras *et al.* 2018). Conversely, FAS did not significantly increase consumption of baits by red fox compared with control treatment. Although it increased the bait intake by foxes, this intake was significantly lower than that obtained with lynx urine (Fig. 1). Our results do not fully agree with other published studies, which used FAS successfully as an attractant for canids (Roughton and Sweeny 1982; Andelt and Woolley 1996; Suárez-Tangil and Rodríguez 2017), but they agree with studies showing lynx urine is a better attractant for foxes (Monterroso *et al.* 2011). In addition, other studies have shown that certain attractants are better than FAS (Webster and Beasley 2019; Heinlein *et al.* 2020), and that FAS should thus be discarded as a lure to improve bait detectability by the red fox (Fidino *et al.* 2020).

During the control period in our study area, we observed several species consuming baits, but this consumption was dominated by the magpie compared with other predator species (Table 1). Camera trap data show that magpies repeatedly returned to the site where the baits were placed after eating them, showing that the lures did not cause a repellent effect. Due to the poorly developed olfactory sense in birds (Neuhaus 1963), we assume that differences between the control and treatment periods was not due to a repellence effect. The increase in the consumption of baits during control periods by the magpie was probably due to the higher availability of baits not consumed by the other predator species (especially red fox) compared with the treatment periods with lures. This partial compensation of unconsumed baits by other species had been previously observed in the study area (Tobajas *et al.* 2020*b*).

Our experiment was based on minimising the persistence time of the baits in the environment by reducing the period when the baits were available to 3 weeks, with the aim of minimising the intake and the potential risks associated for non-target species, as well as minimising the cost of application. Furthermore, the results showed that by using lynx urine the persistence time of baits in the environment could be reduced to 10 days (Fig. 3), which is similar or shorter than the period in which baits are usually applied for population control, vaccination or in CFA with foxes (Steelman *et al.* 2000; Moseby *et al.* 2011; Tobajas *et al.* 2021*a*). However, the persistence time and the effectivity

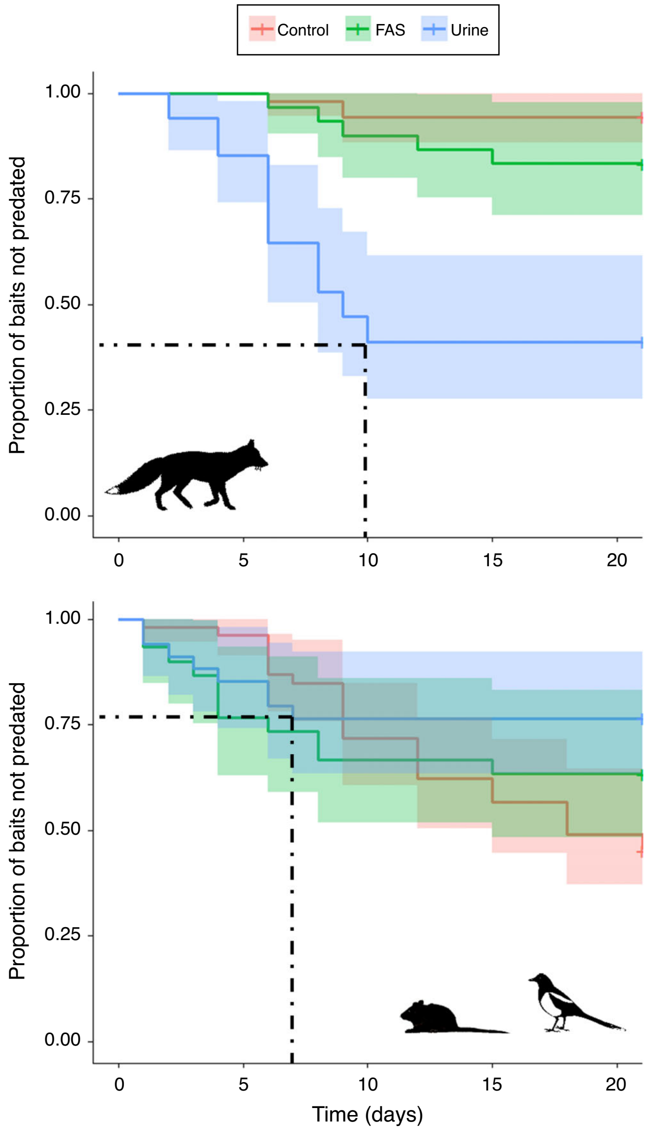

Fig. 3. Kaplan–Meier estimate of the survival function for the proportion of baits not consumed during the experimental period (3 weeks) in the treatments using different attractants (FAS, Fatty Acid Scent; urine, lynx urine) and control. Upper graphs represent the proportion of baits not predated by red fox and lower graphs represent the proportion of baits not predated by non-target species. Light shadings show the 95% confidence intervals. The dotted lines show the time when no further baits were consumed in the urine treatment.

of bait intake could vary depending on the ecosystem and the predator assemblages (Tobajas *et al.* 2021*b*). Studies using baits to control the populations of red foxes and cats in Australia have reported highly variable rates of bait intake (0–70%) and often lower than 20% (Algar *et al.* 2007; Moseby *et al.* 2009; Moseby *et al.* 2011), depending on several characteristics such as bait placement, type of bait, aversion to consuming unfamiliar foods, weather, target and non-target species abundance or alternative live prey availability (van Polanen Petel *et al.* 2001; Algar *et al.* 2007; Moseby *et al.* 2011). In this sense the type of bait (palatability and detectability) and abundance of alternative preys or food resources are the main factors that could modulate the effectiveness of bait intake (Short *et al.* 1997; Algar and Burrows 2004; Moseby *et al.* 2011), so the use of attractants could improve its efficiency in these situations. Similarly, the rate of bait intake in oral vaccination of foxes is highly variable but generally higher than poisoning baits, and the intake by non-target species is one of the major factors determining its efficiency (Steelman *et al.* 2000; Sidwa *et al.* 2005). Importantly, our data are for a specific predator community in mediterranean environments, so it is necessary to test and optimise this methodology for different regions where its application is required (Ferreras *et al.* 2017).

Although the fox density estimated in the study area is relatively high (1.6 ± 0.32 foxes km^{-2}, Jimenez *et al.* 2019), the intake probability by red fox in the control treatment was low, probably due to the high abundance of alternative prey (Moseby *et al.* 2011). Other studies have shown the red fox as the main predator of red-legged partridge nests in some areas (Ferreras *et al.* in press). Our low intake probability compared with other studies may be due to differences in the study design (i.e. artificial versus natural nests), predator species composition, alternative prey abundance and the short period of nests exposure used in the present study. Furthermore, the habitat, bait density and placement can significantly influence the bait intake rate by the target species (Jackson *et al.* 2007; Moseby *et al.* 2011; Ballard *et al.* 2020). Our baiting density with lynx urine treatment (3.4 baits km^{-2}) is similar to or lower than the traditional bait density used in 1080 aerial and ground baiting (Thomson *et al.* 2000; Moseby and Hill 2011; Morgan *et al.* 2015), or oral vaccination (Rosatte *et al.* 1992; Marks and Bloomfield 1999). Using our density of treated baits with lynx urine we obtained a higher bait intake by foxes (58.8%) and a lower bait intake by non-target species (23.5%) than in control nests (red fox 5.7%; non-target species 54.7%) during 10 days (Fig. 3), showing that the use of lynx urine as attractant could improve the cost-effectiveness of baiting campaigns or CFA studies in mediterranean ecosystems. However, the effectivity of lynx urine in other ecosystems or with other species composition should be tested, especially in areas where lynx is not native. It has been observed that the top predator's scent promotes investigative and scent-marking behaviours from other carnivores (Harrington *et al.* 2009; Monterroso *et al.* 2011; Banks *et al.* 2016), suggesting that predator lures other than native predators could be explored if the aim is to improve the baiting methods in different applications and regions (Garvey *et al.* 2016).

One of the main concerns in the use of baiting for population control, CFA or vaccination is the potential effect of baits on non-target species as well as the loss of effectiveness on target species due to the bait intake by non-target species (Steelman *et al.* 2000; Hohnen *et al.* 2020; Tobajas *et al.* 2020*b*). The effects on non-target species are especially important in the case of the use of poison for population control, which could affect threatened species (Dundas *et al.* 2014; Hohnen *et al.* 2020). Similarly, the use of baits in CFA can potentially affect small non-target species through high doses for their body size (Smith *et al.* in press), although advances in the search for safe substances have been made recently (Tobajas *et al.* 2019, 2020*a*). In the case of vaccination, the main problem is the loss of effectiveness in the vaccination of the population of the target species, as well as the increase in costs to achieve a high vaccination rate (Steelman *et al.* 2000; Ballesteros *et al.* 2011). In all these cases, improving the detectability of baits by the target species through the use of lures can reduce the negative impacts on the non-target species, improving the cost-effectiveness of its application. Therefore, the use of lynx urine (and probably other predator scents as lures) could improve the effectiveness and reduce the risks to non-target species in situations where the fox is the target species, and may have utility for other species such as feral cats (Garrard *et al.* 2020). Through its use, the proportion of baits consumed by foxes is increased and that available for non-target species is reduced (Fig. 1); in turn, the persistence time of the baits in the environment can also be reduced (Figs 2, 3), thus minimising the risk of bait consumption by non-target species and lowering the application costs. Therefore, lynx urine or other lures alike can serve to optimise bait delivery methods for red foxes in their different applications, such as CFA studies, vaccination, live trapping or predator control. We urge continued investigation of different specific attractants and delivery methods for target species in the different ecosystems to reduce the impacts of these activities on animal welfare.

Conflict of interest

The authors declare no conflicts of interest.

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