

Title: Responses of naïve and experienced European rabbits to predator odour.

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Summary

The response of prey species to predator scent has been investigated in many mammalian species. However, there is little information about the responses of European wild rabbits at the population level. Therefore, we conducted a simple experiment to investigate the behavioural response of a rabbit population to native predator cues in the wild. We compared the response to the scent of a predator (red fox) in a wild rabbit population bred in semi-natural conditions and naïve to terrestrial predators with the response of a population in a similar environment where terrestrial predators were present. The response to predators was based on rabbit abundance, inferred from pellet counts and measured by the defecation rate per day (DRD). Our results indicate that rabbits responded to the odour of fox feces in the treatment warrens, resulting in a lower DRD. The main antipredator behaviour observed was spatial avoidance (warren abandonment), which seemed to be more accentuated for rabbits who had not previously had contact with foxes, in the plot where terrestrial predators were excluded. In both the fenced and the unfenced plot the differences in the effect of the predator odour between control and treatment warrens disappeared after cessation of treatment, suggesting a flexible and adaptive behaviour of rabbits to predator cues.

Keywords

artificial warren, behaviour, *Oryctolagus cuniculus*, pellet counts, predation risk

Introduction

Predation is a central feature of ecological communities. Most studies of predator–prey interactions have focused on the capture or consumption of prey by predators. However, predator effects on prey are not limited to predation because it is known that prey can respond to predators by altering phenotypic traits to reduce the risk of mortality (Preisser et al. 2005). Prey responses do not require the presence of predators, they can be induced by cues of their presence, for instance their scent. Many types of responses to predator scent have been investigated among prey species. Spatial avoidance and changes in foraging behaviour have been observed in rodents when a predator cue or scent is present (Wolff and Davis-Born 1997; Ylönen et al. 2006).

Several studies have investigated the responses of European wild rabbit (*Oryctolagus cuniculus*) to predators. Most of these experiments have used individuals under laboratory conditions, and few attempts have been made to validate the results under field conditions (Apfelbach et al. 2005; Monclús et al. 2006). For example, in a laboratory experiment Monclús et al. (2005) demonstrated that wild rabbits recognize predator odours, independently of prior experience. However, the response to fox odour under seminatural conditions was not as pronounced as under laboratory conditions (Monclús et al. 2006). In another experiment under natural conditions grazing spatial behaviour of rabbits was not affected by manipulation of predation risk but it influenced rabbit temporal activity pattern (Bakker et al. 2005).

However, there is little information about the behavioural responses of rabbits at the population level in the wild. Thus, we conducted a simple experiment to investigate the effect of previous experience on the response of a rabbit population to native predator cues in the wild. We expected that both rabbit populations, one experienced

and another naïve to terrestrial predators, will respond to the scent of a predator (red fox, *Vulpes vulpes*) (Monclús et al. 2006; Vitale 1989)..

Materials and methods

Study area and the origin of the animals

The experiment was conducted in two plots (4 ha each, see Rouco et al. 2008) separated by 1 km in Sierra Norte Natural Park of Seville (SW Spain). Eighteen artificial warrens were regularly-distributed on each plot and separated 35-40 m (see Rouco et al. *in press* for further information). Water and food suppliers located close to each warren provided rabbits with water and commercial food pellets *ad libitum*. One of the plots was surrounded by a fence 3 m high that was topped with an electric shepherd to completely exclude terrestrial carnivore predators. Rabbits in this plot were mostly descendants of wild individuals that were translocated during autumn 2002 (Rouco et al. 2010); thus they were therefore largely naïve to direct contact with any terrestrial predator. The second plot was unfenced, so rabbits were in contact with terrestrial predators. Red fox is the main predator in this area (average density 0.79 foxes/km², authors unpublished).

Sampling design

As a source of odour we used fresh red fox feces collected in the study area. To make the odour uniform throughout the experiment, 500 g of feces were stirred with 2 l

of water, and the mixture was left standing for 72 hours. The resulting solution (henceforth referred to as fox feces odour) was filtered and frozen at -20°C until used.

In each plot 3 treatment and 3 control warrens were randomly selected, and 2 odour stations were added to each warren; each station consisted of a chalk tied at approximately 20 cm above ground to a sheaf nailed in the ground. In treatment warrens the chinks were impregnated with 4 ml of fox feces odour every three days during the treatment period (March to August 2004). Odour stations in the control warrens were impregnated with water following the same regime as for the treatment warrens.

To evaluate the presence of predators, and thus the predation risk perceived by the animals in the unfenced plot, counts of scats were performed weekly on a 800 m walked transect within the plot.

The monitoring of rabbit abundances and trends during the experiment was based on pellet counts (Rouco et al. 2008). Pellets were counted and cleared from 10 fixed circular plots (18 cm diameter) placed randomly at 5m far for each warren. Pellet counts were performed 4 times during the study period; first count in February 2004 (before treatment); second and third counts during May and August, respectively (during treatment), and last count in November (after treatment). To standardize all pellets counts a defecation rate per day (DRD) was calculated for each count. The DRD was obtained by dividing the number of pellets at each counting station by the number of days since the last count at the same station (Cabezas and Moreno 2007).

Data analysis

Pellet abundance data were analyzed using generalized linear models, as implemented in SAS (SAS Institute 2004; Genmod, Glimmix and mixed procedures)

with ‘warren’ and ‘pellet counting station’ as random categorical variables. The dependent variable DRD was modeled with Poisson errors and log link. Three factors were considered in the models of pellet abundance: ‘treatment’ (control and treatment warrens), ‘plot’ (fenced and unfenced) and ‘counts’ (each pellet count in February, May, August and November). Denominator degrees of freedom were estimated using Satterthwaite’s formula (Littell et al. 1996). Nonparametric tests were used to check for differences in pellet abundance between control and treatment warrens.

Results

No differences were found in rabbit pellet abundance between control and treatment warrens before the treatment in either the unfenced (Mann-Whitney U-Test, before: $Z = 0.28$, $p = 0.779$) or the fenced plot (Mann-Whitney U-Test, before: $Z = 0.63$, $p = 0.294$). Rabbit population trends in both plots (unfenced and fenced) were similar (Figure 1), and no significant differences were found in the DRD between the two plots during the study period (Table 1; plot*count). We found a significant effect of treatment (Table 1), with higher DRD in control than in treatment warrens in both plots (Figure 1). The DRD value in control warrens at the end of the experiment was 4.6-times higher in the fenced than the unfenced plot (Mann-Whitney U-Test, $Z = -3.386$, $p < 0.001$). However, after cessation of treatment, the DRD was not different between control and treatment warrens in the unfenced (Mann-Whitney U test: $Z = 0.15$, $p = 0.144$) and the fenced plots ($Z = -0.59$, $p = 0.554$). Predator activity in the unfenced plot increased more than 4-fold during the study, as indicated by the predator scats collected along the walking transects (three in February, four in May, 18 in August, and 23 in November).

Discussion

Consistent with previous studies (Monclús et al. 2005, Vitale 1989), our results indicate that rabbits respond to fox odour, even those rabbits naïve to predator cues. The main antipredator behaviour observed was spatial avoidance, as evidenced by the lower DRD in the treatment warrens. Some animals may have left the treatment warrens due to a greater perceived predation risk. In fact, the effect of intimidation by predators seems to be more important than the consumption of prey during predator–prey interactions (Preisser et al. 2005). The reduced response in the plot with experienced animals (unfenced plot) could be explained by the fact that the control warrens were not an ideal control, because predator scats were naturally occurring nearby. This is not unexpected because fox scent marks were more common in areas of higher rabbit density (Monclús et al. 2009). Thus, the natural presence of odour in both treatment and control warrens in the unfenced plot could reduce the effect of the treatment. For instance, Monclús et al. (2005) suggested that under natural conditions, such as it occurs in our unfenced plot, rabbits can change their use of space in the presence of predator cues. Several studies have confirmed a response of mammalian prey to novel olfactory cues *per se*, but the response is generally stronger with predator scent marks than with non-predator odours (Bakker et al. 2005; Bramley et al. 2000).

In the plot with the predator exclusion fence the effect of the treatment was apparently greater at the beginning of the experiment (Figure 1). After the initial stage, the differences in DRD between the control and treatment warrens decreased. Learnt behaviour could be modulating this response (Griffin et al. 2000), but this was less likely in the unfenced plot owing to the continuous presence of terrestrial predators.

Finally, in both, the fenced and unfenced plots, we observed that the effect of the predator odour between control and treatment warrens apparently disappeared following

cessation of the treatment. Bakker et al. (2005) also manipulated perceived predation risk of a population of rabbits using predator odour, and showed temporal avoidance of predation. It may be that after the treatment ceased and predator cues could no longer be detected, rabbits began re-occupying the warrens, suggesting the involvement of a flexible and adaptive response to predator cues.

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241 **FIGURES CAPTION**

242 **Figure 1** Comparison of DRD (rabbit defecation rate per day) during the four
243 pellet counts (Means \pm Standard Error) on control and treatment warrens in unfenced
244 and fenced plot.

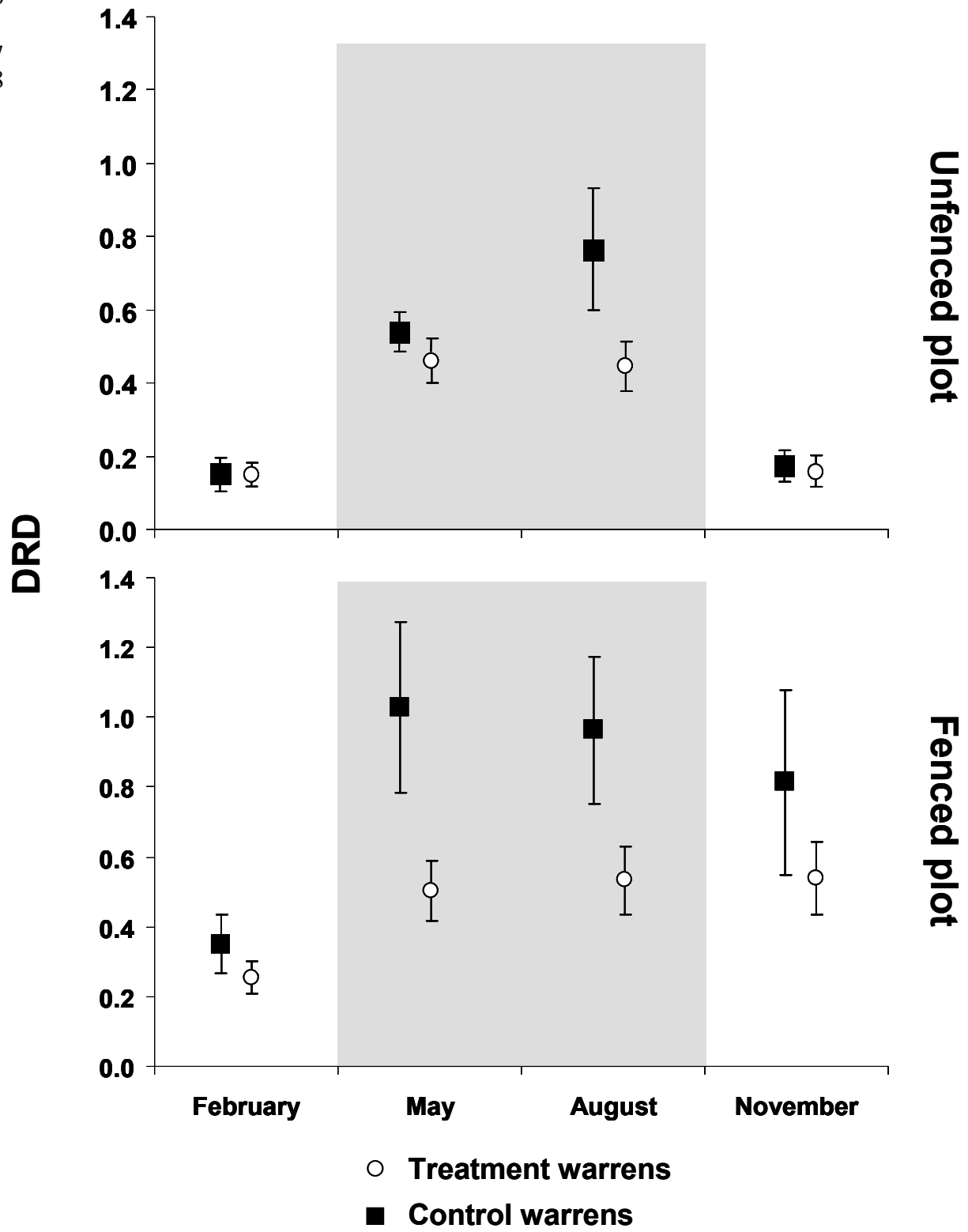
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TABLES

Table 1. F values in GLMMs of the effect of treatment (control warrens *vs.* treatment warrens), plot (unfenced *vs.* fenced) and pellet counts (Counts).

Predictors	DF	F	p
Treatment	1, 358	7.05	0.008
Plot	1, 358	10.80	0.001
Counts	1, 358	4.85	0.028
Plot*Counts	1, 357	0.43	0.514
Plot*Treatment	1, 357	1.29	0.257
Plot*Treatment*Counts	1, 355	0.15	0.696

FIGURE 1



* In shadow is represented treatment period length