Research Article

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An assessment of seasonal bait uptake by individual grey squirrels to develop a delivery system for oral contraceptives

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Abstract

Globally, human–wildlife conflicts continue to increase, owing to human population growth and expansion. Many of these conflicts concern the impacts of invasive non-native species. In the UK, the invasive, non-native grey squirrel *Sciurus carolinensis* negatively affects tree health and has caused the decline of the native red squirrel *Sciurus vulgaris*. Oral contraceptives are being developed to manage the impacts of the grey squirrel. To be effective, contraceptives will need to be deployed at a landscape scale, and will require a delivery system that is practical and economically viable. Understanding grey squirrel feeding behaviour is important so that delivery methods can be designed so that a sufficient number of target individuals receive an effective contraceptive dose at a time of year that will ensure their infertility throughout peak times of breeding. The main aims of this study were to assess how sex, season, squirrel density and bait point density influenced; (1) the probability of a squirrel visiting a feeder and (2) the amount of bait consumed from feeders. Field trials were conducted on six woodland populations of squirrels in three seasons, with four days of bait deployment via purpose-designed squirrel-specific bait hoppers with integrated PIT-tag readers. It was possible to deliver multiple doses on most days to most male and female grey squirrels, with bait deployment more likely to be effective in spring, immediately before the second annual peak in squirrel breeding, followed by winter, immediately before the first peak in breeding. The results from this study could be used to design methods for delivering oral contraceptive baits to grey squirrels in the future and the methods used could be applied to other small mammal species and other bait delivery systems.

Supporting information may be found in the online version of this article.

Keywords: fertility control; PIT-tag; *Sciurus carolinensis*; feeding behaviour; wildlife management; small mammals

1 INTRODUCTION

Globally, human–wildlife conflicts continue to increase in frequency, scale and severity as a result of human population growth and expansion.[1] Many of these conflicts concern the economic and environmental impacts of non-native and invasive species. Injectable contraceptives are used to reduce local numbers of wildlife.[2–4] To be effective, a critical level of contraceptive coverage is required, and a sufficient proportion of the target population treated.[5,6] Injectable contraceptives in most instances require the capture or restraint of animals, which limit their practical application to relatively small and localised populations.[4,7,8] Oral baits facilitate the deployment of pharmaceuticals on a landscape scale and this has led to their successful utilisation in the control of wildlife disease, as with the rabies vaccine for wild carnivores.[9,10] In recent years, research also has focused on the development of oral contraceptives for population control.[7,8,11–14]

In the UK, oral contraceptives are being developed to control the impact of the invasive non-native grey squirrel *Sciurus carolinensis*.[15] Grey squirrels were introduced into England in the 19th Century and it is estimated that their numbers now exceed 2.5 million, whereas the red squirrel *Sciurus vulgaris*, the only squirrel native to the UK, has declined to <300 000 individuals.[16,17] Grey squirrels can cause considerable damage to trees[18] and a recent report by the Royal Forestry Society[19] estimated that,

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over the next 40 years, this will cost the forestry sector in England and Wales at least £1.1 billion (US$1.4 billion) in damaged timber, lost carbon capture and tree replacements. Additionally, the grey squirrel has been responsible for the decline of the native red squirrel through direct competition and transmission of disease.[20,21] Lethal control has been used to eradicate or reduce numbers of grey squirrels and mitigate their damage at a regional level[22]; on a national scale, however, the impact of this species continues to grow.

The successful management of grey squirrels in the UK requires landscape-level coordinated control. Oral contraceptives deployed on this scale require a delivery system that is effective, practical, targeted and economically viable. The effectiveness of a contraceptive is determined by the number of individuals it is delivered to, together with the proportion of individuals rendered infertile. To maximise effectiveness, oral contraceptive delivery should predominantly target the females in a population and the majority of the females should be rendered infertile for the peak times in breeding.[4,23] Understanding grey squirrel feeding behaviour is important so that delivery methods can be designed to ensure that a sufficient number of individuals receive an effective contraceptive dose. What constitutes an effective contraceptive dose, and the longevity of its effect will be determined in laboratory trials. It is estimated that the development of an oral contraceptive and its registration for use in the UK will take a minimum of six years.[15] Within this time frame, it is important that a suitable bait delivery system is developed for trialling contraceptives in the field to ensure that they are effective and have minimal impact on nontarget animals.

Bait uptake can be affected by several factors including sex, body weight, reproductive status, season, population density and bait point density. For grey squirrels, Beatham *et al.*,[24] found that bait uptake was affected by the number of feeders deployed and the density of grey squirrels. In UK woodlands, the average size of a grey squirrel home range is <5 ha[20,25] and average grey squirrel density is reported to be between 4 and 5 squirrels ha^{-1}, ranging from < 1 to >13 squirrels ha^{-1}.[26,27] In group-living rodents, such as the grey squirrel, high reproductive rates and high population densities may result in intraspecific competition for resources, particularly between individuals of different sex and breeding status. For instance, in California ground squirrels *Spermophilus beechey*, Whisson and Salmon[28] found that a greater proportion of males visited bait stations than females, and that males consumed more bait and made more visits to bait stations than females. Likewise, Beatham *et al.*[29] found that male grey squirrels made more visits to feeders and visited a greater number of feeders than female squirrels. Jacob *et al.*[30] found higher bait uptake in breeding female house mice Mus dometicus *versus* other females, whereas Inglis *et al.*[31] found that female Norway rats *Rattus norvegicus* made more feeding visits to bait than males.

Season also can affect bait uptake; for instance, more grey squirrels were found to take bait from feeders in summer than winter.[24] The choice of season in which to deliver fertility control is particularly important if the duration of the effect of a contraceptive is relatively short, which makes it necessary to treat females immediately before mating. In grey squirrels, there are two main peaks in mating: December to January and April to May, with some females producing two litters within the same year.[32] It may therefore be necessary to deploy a contraceptive in different seasons and it is important that levels of bait uptake are sufficient across these seasons, to ensure that fertility control is effective.

The main aims of this study were to assess how sex, body weight, season, squirrel density and bait point density influenced; (1) the probability of a squirrel visiting a feeder and (2) the amount of bait consumed from feeders. A squirrel-specific feeder, referred to as a bait hopper, has been developed that can measure individual bait uptake by free-living grey squirrels, based on detections of PIT-tags.[29] The bait hoppers were trialled in two woods in winter 2017/2018 and also have been used in studies measuring population level bait uptake using the bait marker dye Rhodamine B,[24] which can be detected in hair as fluorescence when small amounts are ingested by grey squirrels. For the current study, the bait hopper was upgraded to quantify the weight of bait removed per squirrel visit and additional field trials were conducted in two woods in summer 2022 and in two woods in spring 2023. Over 4 days of bait deployment, using hazelnut paste mixed with Rhodamine B, the duration of visits to hoppers and the amount of bait taken per visit were measured for individual male and female squirrels in different seasons and at different squirrel and hopper densities. The findings from this study could be used to design systems for delivering oral contraceptive baits to grey squirrels in the future and the methods used could be applied to other small mammal species and other bait delivery systems.

2 MATERIALS AND METHODS

2.1 Study sites

Trials were conducted in five independent woods that were located ≥3.5 km apart and within 25 km of York, England (53.96° N, −1.09° E; Fig. 1). Populations of grey squirrels were sampled from woods HW and SC in winter 2017/2018, woods SC and PW in summer 2022, and woods GE and BW in spring 2023. Winter and spring were selected as suitable seasons to deploy a contraceptive, as they coincide with the peaks in grey squirrel breeding[32] and summer was selected as a season when good levels of bait delivery via feeders can be achieved.[24] Autumn was not trialled as a consequence of reduced squirrel breeding activity and high natural food availability, which could potentially deter squirrels from using feeders.[33]

In order to provide two suitable replicates for each season, woods were selected that were of a similar size (between 7 and 8 ha) and structure (mature broadleaf or mature broadleaf–conifer mixed tree species), that had high levels of grey squirrel activity reported by landowners and established squirrel populations, with minimal squirrel control conducted within the previous 12 months. At the end of each trial, the squirrel population was trapped and removed from each wood as part of other related studies.[24] Therefore, woods could not be re-used on trials that were within 12 months of each other. The squirrel population at wood SC was removed in January 2018 and the wood recolonised by squirrels, confirmed by the trap results in this study, so was reused in summer 2022. Thus, it was considered that six independent populations of squirrels were assessed.

All woods had relatively low connectivity to other woodland areas, sharing between 5% and 39% of their boundary with other woodland areas (Fig. 1). Wood selection was dependent on landowner permission and restricted to those that had a good level of access for field operatives but very low levels of public access. Some of the methods employed by this study had to be undertaken by Home Office licensed personnel, and required specialist equipment and significant time and resources. Owing to these limitations, it was not possible to trial more than two woods for each season.

Figure 1. Woods in which field trials were conducted to assess individual bait uptake by grey squirrels in three different seasons. Woods HW and SC were assessed in winter 2017/2018, PW and SC in summer 2022, and GE and BW in spring 2023. The red squares show the locations of bait hoppers deployed in each wood for 4 days and used to monitor the feeding behaviour of squirrels via integrated PIT-tag readers. Woods HW, SC, PW and GE shared <5% of their boundary with other woodland areas, whereas for BW this was 39%. The wooded area to the north of BW (blue outline) had been deforested over a year before the study commenced.

2.2 Hopper field trial

Grey squirrel trapping and sampling methods were approved by the joint Animal and Plant Health Agency (APHA) and Fera Science Ltd Animal Welfare and Ethical Review Body (AWERB). All animal studies were conducted in accordance with the UK Guidance on the operation of the Animals (Scientific Procedures) Act 1986. Field trials were conducted from December 2017 to February 2018 in woods HW and SC, from June to August 2022 in woods PW and SC, and from April to June 2023 in woods BW and GE. Single-catch squirrel cage traps (density 3 ha^{-1}, $n = 21$–24) were deployed across the whole area of each wood on 1-m-high wooden stands and pre-baited with a combination of maize, peanuts, a small number of whole hazelnuts and ≈2 g of 100% hazelnut paste (Bulk™, Colchester, UK) every 2–4 days for between 3 and 13 days, dictated by human resource availability. For animal welfare and health and safety reasons, traps were not set if heavy rain, high winds (>30 mph), or high (>30 °C) or low (<2 °C) temperatures were forecast. Each trap was partly covered with a waterproof sheet to provide animals with shelter. Traps were either set early in the morning and checked in the afternoon, or set late afternoon and checked the following morning.

Trapped squirrels were anaesthetised on site using isoflurane via a mask and a PIT-tag (Identichip®, York, UK) implanted subcutaneously in the scruff of the neck. Squirrels were then sexed, weighed (to the nearest gram) and recorded as adult or subadult (<450 g, slim, soft pelage, body lacking muscle, head large in proportion to body, and female nipples or male testes not visible). Females were assessed for evidence of recent breeding (extended or lactating nipples and palpable foetuses). Hair was clipped from the tail for visual identification. Once recovered from anaesthesia, squirrels were released under a Natural England licence in the location at which they had been trapped. Trapping was conducted for 3–4 days, completed within 18 days, and then the traps removed.

In spring and summer, within 2 days of PIT-tagging, hoppers were deployed at each wood; in winter, hoppers were deployed within 4 weeks of PIT-tagging (Fig. 1). Hoppers were deployed evenly across the whole area of each wood, with locations guided by a 1-ha grid generated in ArcGIS (v10.7.1) overlayed onto a satellite map using the ArcGIS Collector mobile phone application. Locations were, however, adjusted according to accessibility; for example, steep slopes or thick vegetation were avoided. Each hopper was fixed to a 1-m-high wooden stand to reduce nontarget access. In winter and summer, hoppers were deployed at a density of 3 ha^{-1} ($n = 21$–24). Squirrel hopper use data from these studies suggested that lowering the hopper density to 2 ha^{-1} could be more cost-effective for bait delivery in terms of field hours and bait quantity required. This was tested in spring 2023, using a Before-After-Control-Impact (BACI) study design.[34] At woods GE and BW, hoppers were deployed at a density of 2 ha^{-1} ($n = 14$) and baited for four days, then increased to 3 ha^{-1} ($n = 21$) at BW and maintained at 2 ha^{-1} at GE for an additional 4 days each.

For all woods, to simulate the deployment of a contraceptive, each hopper was pre-baited with ≈40 g hazelnut paste every 2–3 days for 6–7 days, with the bait doors propped open, to encourage the squirrels to feed from them. Each hopper was then baited daily with 40 g hazelnut paste mixed with the bait marker dye Rhodamine B **(**Merck Life Sciences UK Ltd, Gillingham, UK) at a concentration of 0.18% for 4 consecutive trial days in winter and summer, and 8 consecutive trials days in spring. The bait was manually weighed in and out of the hoppers each day, and the hopper entrance and stand checked for evidence of bait spillage, easily identified owing to the pink colouration of the bait. Any spillage found was weighed.

Hoppers recorded the date and time at which an individual PIT-tagged squirrel was detected, and the date and time at which the bait door was opened, through the engagement and disengagement of a magnetic door switch.[29] In the summer and spring trials, hoppers also were fitted with a strain gauge, which recorded the weight of the bait taken for each squirrel visit.[29] Hoppers were monitored with HC500 or HS2X remote cameras (Reconyx™, Holmen, WI, USA) and the footage analysed at times when the bait doors were opened, to visually identify animals feeding from them, including any nontarget animals.

2.3 Grey squirrel removal trapping

Within 4 days of hopper deployment in spring and summer, and within 21 days of hopper deployment in winter, squirrel live-capture cage-traps were installed in each wood at a density of 2–3 traps ha^{-1}. Traps were secured to the same 1-m-high wooden stands used in the hopper trials. Trapping was conducted using the same protocol as the trapping for the PIT-tagging, although for this session, traps were set and checked at least once every 24 h where possible. If weather conditions did not permit trapping overnight, traps were set early morning and checked late afternoon the same day. Trapping was conducted within a 4-week period, typically Monday to Friday, for a minimum of 5 days and for a maximum of 14 days, or until squirrel capture rates were reduced to an average of <1 squirrel day^{-1} over 3 consecutive days.

The short trapping period was designed to minimise the movement of grey squirrels into the study woods from other areas once removal began. Trapping effort was high, short-term and conducted across relatively discrete squirrel habitats and across an area larger than the average squirrel home range (<5 ha).[20] A high level of demographic population closure should have therefore been achieved. Lawton and Rochford[25] confirmed through capture–mark–recapture (CMR) that most, if not all, grey squirrels in a population could be trapped within 5 days using a similar trapping regime. The number of squirrels trapped was therefore used as a proxy for the total number of squirrels in each wood that were available to visit hoppers, as per Beatham *et al.*[26] As a comparison, the number of squirrels also was calculated using the closed-population CMR Schnabel method, from the capture records taken from the 3–4 days of PIT-tagging conducted at each wood.[35]

Squirrels that were trapped were humanely dispatched using a UK Home Office approved (Schedule 1) method, by a trained and competent person, and the PIT-tag ID, sex and tail clip (if present) were recorded and ≥20 hairs were taken from the flank, placed in a plastic sample bag, to be later analysed using a DMLB ultraviolet microscope (Leica Microsystems Ltd, Milton Keynes, UK) at ×4 magnification for the presence or absence of RB fluorescence.

2.4 Data analysis

Data management and analysis was conducted using Microsoft Excel and R,[36] with generalised linear models (GLM) built using the *MuMIn* package.[37] The relative significance of GLM fixed effects linked through interactions was assessed through pairwise comparisons of estimated marginal means using the *Emmeans* package.[38] For each GLM, diagnostic checks of residual plots were used to confirm that they were approximately normally distributed and that model assumptions were met. For binomial models, overdispersion was measured using the parameter, theta.

From the PIT-tag data collected by hoppers, for each individual squirrel a dataset was created containing site, season, sex, body weight (g), date, time, PIT-tag ID and hopper ID. Consecutive PIT-tags recorded for the same individual at the same hopper were categorised into visits. The duration of a visit was defined as the time between the first PIT-tag read and the last PIT-tag read for that individual visit. A visit was deemed complete once the PIT-tag of a new individual was recorded, or there was >2-min gap until the next PIT-tag record from the same individual. The 2-min time frame was used to standardise the data, as the hoppers used in different years had different levels of PIT-tag detection sensitivity and squirrels would often enter hoppers multiple times in quick succession during the same visit. When only one PIT-tag read was recorded for an individual, a minimum visit duration of 1 s was applied.

2.5 Effect of hopper density on hopper use and bait uptake by grey squirrels in spring

In order to assess whether reducing the hopper density to 2 ha^{-1} in spring 2023 affected hopper use and bait uptake by individual squirrels at woods GE and BW, the PIT-tag records from squirrels recorded by hoppers deployed at both woods were analysed using a BACI framework, as described in Fenn *et al*.[34] The variable 'Site' represented treatment, with GE assigned as the control site and BW as the treatment site. The variable 'Period' was defined as 1 (before treatment; trial days 1–4, hopper density set at 2 ha^{-1} at GE and BW) and 2 (after treatment; trial days 5–8, hopper density maintained at 2 ha^{-1} at GE and increased to 3 ha^{-1} at BW). Binomial GLMs were used to test how the fixed effects wood, period, sex and number of squirrels per hopper influenced the probability that a PIT-tagged squirrel was recorded visiting a hopper (yes/no) in each time period. Squirrels per hopper was calculated from the total number of squirrels (PIT-tagged and untagged) trapped and removed in each wood divided by the number of hoppers deployed for that time period. It was included in the model as it was hypothesised that there may be increased competition between individual squirrels in higher density populations, potentially resulting in a reduction in hopper use by some squirrels. The inclusion of squirrel ID as a random factor was considered, using a generalised linear mixed model structure, yet it was found that the datasets were too small to support this. The GLM model structure was:

$$\text{glm}\Big(\text{Visited} \sim \text{Period}^{*}\text{Site} + \text{Sex} + \text{squirrels\_hopper},\ \text{family} = {''}\text{binomial}{''}(\text{link} = \text{cloglog}),\ \ \text{data} = \text{dat\_spring},\ \text{na.action} = \text{na.fail}\Big)$$

For PIT-tagged squirrels that were recorded visiting at least one hopper in each of the study woods, a Gaussian GLM with a log link was used to assess the influence of the fixed effects period, sex and site on the quantity of bait uptake by each squirrel, inferred from the total time in minutes (duration) that each squirrel was recorded visiting hoppers. Collinearity between squirrels per hopper and the period/site interaction meant that squirrels per hopper was not included in this model. ANOVAs were used to test whether variation in hopper use or bait uptake was better explained by models that included an interaction term between period and site (indicating a hopper density effect) *versus* those that did not.

2.6 Factors affecting hopper use and bait uptake by grey squirrels in different seasons

Binomial GLMs were used to test the fixed effects of season, squirrel body weight, sex and number of squirrels per hopper on the probability of a PIT-tagged squirrel entering a hopper (yes or no) during trial days 1–4 in each wood. Body weight was included, as it was hypothesised that larger individuals may outcompete smaller ones when accessing bait. The model structure was:

$$\text{glm}\Big(\text{Visited} \sim \text{Body weight\_g}^{*}\text{Sex} + \text{Body weight\_g}^{*}\text{Season} + \text{Sex}^{*}\text{Season} + \text{squirrels\_hopper},\ \text{family} = {''}\text{binomial}{''}(\text{link} = \text{cloglog}),\ \text{data} = \text{dat\_spring},\ \text{na.action} = \text{na.fail}\Big)$$

Interactions were included for body weight and sex, and body weight and season, as it was hypothesised that body weight may have been influenced by these factors. An interaction also was included between season and sex, based on the evidence that female squirrels may visit hoppers less during periods of breeding when they are less active and are spending more time in dreys with dependent young. Age and female breeding status were not included in the model, owing to seasonal variation in the ability to accurately assess these factors from appearance alone, which might have caused an underestimate in the number of breeding females and subadults. The percentage of trapped squirrels that, through the analysis of their hair samples, tested positive for the bait marker Rhodamine B in each wood was used as a comparative measure of hopper use, as per Beatham *et al*.[24]

For PIT-tagged squirrels that were recorded visiting at least one hopper, a Gaussian GLM with a log link was used to test the effect of season, sex and number of squirrels per hopper on the total amount of time individual squirrels spent visiting hoppers during the four trial days. The data did not support the inclusion of body weight as a fixed effect; however, this was investigated separately. Time spent visiting hoppers was used as a proxy for the amount of bait consumed by squirrels, as bait weight data were only collected in summer and spring.

2.7 Time spent in hoppers *versus* weight of bait taken for individual squirrels

In order to assess whether time spent visiting hoppers by individual squirrels was directly related to amount of bait taken, the weight of bait taken was recorded for each hopper visit. This was calculated from the combined weight of the bait and bait tray recorded at the time of the first PIT-tag read minus the weight recorded at the time of the final PIT-tag read. The weights taken at the precise time of the reads were often unstable, as a consequence of spikes in weight readings caused by squirrels exerting force on the bait trays with their bodies as they fed. Thus, for each visit, the stable weight (at least three repeat values) closest to the time of the first PIT-tag read, or immediately before the first associated spike in weight, was selected as the start weight and the stable weight closest to the time of the final PIT-tag read or immediately after the associated spike in weight, was selected as the final weight. If a stable weight was not recorded within 20 s of a PIT-tag read, a null entry was recorded.

The total weight of bait taken during the 4 trial days by each individual squirrel from the four woods surveyed in summer and spring (PW, SC, GE and BW) was compared with the time each individual spent visiting feeders (the sum of time of last PIT-tag read minus time of first PIT-tag, read across all visits). Data were only included from squirrels if they did not have significant amounts (>10% of values) of weight data missing, as a result of weighing errors or hopper failures. As data were not normally distributed and contained multiple tied observations, a Kendall's Tau correlation analysis was used to assess whether there was a significant relationship between weight of bait taken and time spent visiting hoppers.

3 RESULTS

3.1 Effect of hopper density on hopper use and bait uptake by grey squirrels in spring

In total, 66 squirrels (24 male, 42 female) were PIT-tagged in woods GE and BW in spring 2023 (Table 1). The hoppers in both woods recorded visits by 10 males and 26 females during trial days 1–4, and 14 males and 26 females during trial days 5–8. Two new males recorded in time period 2 came from GE and two came from BW. There was a high resight rate with 94% of the individuals recorded in time period 1 also recorded in time period 2. During trial days 1–4, male grey squirrels at GE and BW spent a median of 39 min (IQR = 33 min) and 50 min (IQR = 45 min) visiting hoppers respectively, compared with 38 min (IQR = 19 min) and 35 min (IQR = 36 min) during trial days 5–8. Female grey squirrels at GE and BW spent a median of 57 min (IQR = 117 min) and 29 min (IQR = 59 min) visiting hoppers during trials days 1–4, compared with 62 min (IQR = 117 min) and 53 min (IQR = 37 min) during trial days 5–8.

There was no evidence that hopper density (2 ha^{-1} *versus* 3 ha^{-1}) influenced the probability that an individual squirrel would visit a hopper or the time they spent visiting hoppers, with no significant difference between GLMs that included an interaction between site and time period and those that did not ($\chi^2_{2,125} = -0.974$, $P = 0.615$) and ($\chi^2_{2,72} = -176.25$, $P = 0.747$) respectively. Site was the only variable found to significantly affect hopper use, with tagged squirrels at GE significantly more likely to enter hoppers than tagged squirrels at BW (Supporting information Data S1; $z_{4,127} = 3.66$, $P = <0.001$). This was supported by the Rhodamine B analysis that showed that 56% of the 41 squirrels trapped at GE and 38% of the 91 squirrels trapped at BW had fed from hoppers (Table 1).

The binomial and Gaussian GLMs performed significantly better than the respective null models (DataS1; $X^2_4 = 21.91$, $P < 0.0001$) and ($X^2_4 = 21.91$, $P < 0.0001$). Residual plots indicated a good model fit for the binomial GLM, whereas the Gaussian GLM data were right-skewed. When the eight highest data points were removed (four from each time period) and the analysis repeated, the residuals were normalised and the initial model results confirmed (Data S1).

3.2 Factors affecting hopper use and bait uptake by grey squirrels in different seasons

In total, 202 squirrels (88 male, 114 female) were PIT-tagged in six woods (Table 1). Of the 202 individuals, seven were assessed as subadult (five in spring, one in summer, one in winter) and 23 females exhibited evidence of recent breeding (16 in spring, four in summer, three in winter). In winter and spring, ≥86% of the squirrels PIT-tagged were recaptured during the removal exercise, compared with 38–52% in summer. After between 7 and 14 days of trap and remove, the capture rate at woods SC (both seasons), HW, GE and BW were reduced to <1 squirrel day^{-1} over 3 consecutive days and 80–98% of the squirrels trapped were caught within the first 5 days. After 14 days of trapping at PW, the capture rate was 1 squirrel day^{-1}, with 51% caught within the first 5 days. Trapping at PW and SC during summer 2022 had to be postponed owing to a heatwave and temperatures were higher than average for UK summer throughout the trapping period. For each wood, the number of squirrels per hopper calculated from the trap records were within 0.7 squirrels per hopper of those calculated from the CMR method (Table 1). Of the 271 squirrels trapped, 56% tested positive for the bait marker Rhodamine B, 38%-66% in each wood (Table 1).

Of 121 hoppers deployed for the six field trials, during the 4 trial days, 111 hoppers recorded visits by PIT-tagged squirrels. Of the 10 hoppers that did not record any visits, six hoppers that were deployed in summer did not record any PIT-tags during the trial days, owing to electronic faults. Bait spillage was found on 16% of the 484 checks made to hoppers, with most quantities recorded at <1 g spilled and ≤2 g recorded at any one check. A total of 114 hoppers were analysed for nontarget access to the bait. When the dates and times during which the bait doors were opened were matched to the footage from the associated remote camera, for all six hopper trials, the only nontarget recorded, on three visits to one hopper located at BW, was the wood mouse *Apodemus sylvaticus*. Video footage showed no evidence of the mice taking bait and the door pushed open only temporarily. Other animals recorded in the vicinity of the hoppers included badger *Meles meles*, roe deer *Capreolus capreolus*, pheasant *Phasianus colchicus*, Norway rat, and various species of small rodents and birds.

Overall, 73% of PIT-tagged squirrels visited hoppers during the pre-bait period, compared with 62% (58% PIT-tagged females, 67% PIT-tagged males) during the trial period. The proportion of female squirrels that visited hoppers was much lower in the two woods surveyed in summer (29% and 48%) than those surveyed in winter (86% and 90%) and spring (50% and 87%). A total of 27 individuals visited hoppers during pre-bait only and six

Table 1. Number of grey squirrels PIT-tagged in five woods in three different seasons, including the number of tagged males (M) and females (F), the percentage that entered bait hoppers within a 4-day bait deployment, the number of squirrels that tested positive for the bait marker Rhodamine B, the number that were trapped and removed, and number of squirrels per hopper (CMR Schnabel[35] estimate or number trapped divided by number of hoppers deployed)

		No. PIT-tagged			No. visited hoppers							No./hopper	
Season/year	Wood ID	M	F	Total	M	F	Total	%	No. trapped	%+ve RB	% tagged squirrels trapped	Est.	Trap
Winter 2017/2018	HW	9	12	21	8	10	18	86	31	55	90	0.9	1.3
	SC	15	14	29	14	12	26	90	38	66	86	1.4	1.6
Summer 2022	PW	19	23	42	13	11	24	57	35	57	60	2.4	1.7
	SC	20	24	44	14	7	21	48	32	63	45	2.0	1.3
Spring* 2023	GE	9	15	24	6	13	19	79	41	56	100	2.6	2.9
	BW	16	26	42	4	13	17	40	91	38	90	5.8	6.5
All woods		88	114	202	59	66	125	62	271	56	79	2.4	2.2

*In spring 2023 PIT-tag records were monitored for 4 days; Rhodamine B bait was deployed for 8 days.

individuals visited during the trial period only. Most of the subadult squirrels (six of seven) and breeding females (15 of 23) visited hoppers during the trial period. During the four trial days for all woods, 59 PIT-tagged male squirrels recorded by hoppers made a median of 14 visits (IQR = 12) to five different hoppers (IQR = 3) for a total of 42 min (IQR = 31 min). In comparison, 66 PIT-tagged females recorded by hoppers made a median of 11 visits (IQR = 14) to three different hoppers (IQR = 3) for a total of 31 min (IQR = 47 min). In total 93% of male squirrels and 71% of female squirrels that visited hoppers did so on at least three separate dates during 4 trial days.

Body weight was consistent for PIT-tagged male and female squirrels within the same season that did and did not visit hoppers (Fig. 2). The median body weights for male and female squirrels that entered hoppers during the trial period was 533 g (IQR = 125) and 564 g (IQR = 72), respectively, and 524 g (IQR = 89) and 568 g (IQR =83) for males and females that did not. Binomial GLM analysis found that body weight was not a significant factor determining whether a squirrel visited a hopper (Data S1; $z_{10,191} = 0.540$, $P = 0.589$). There appeared to be sex-specific body weight variation in summer, with most females weighing more than most males and 13 of the 14 heaviest females PIT-tagged not recorded visiting hoppers. The number of squirrels recorded for each sex and season group varied considerably for those that did and did not visit hoppers (2–29 squirrels). As a result, the inclusion of body weight in the model produced much larger standard errors for the sex and season estimates (Data S1). The analysis was therefore repeated with only the fixed factors of season, sex and squirrels per hopper, with an interaction between season and sex. The reduced model did not significantly differ in fit compared with the model including body weight ($\chi^2_{4,195} = -0.263$, $P = 0.621$) and fitted the data significantly more than the null model ($\chi^2_6 = 43.89$, $P < 0.0001$).

The reduced binomial GLM predicted that the probability of a male or female squirrel visiting a hopper would significantly decline, in most scenarios by >25%, with an increase from 2 to 4.5 squirrels per hopper [Data S1; Fig. 3(a), $z_{6,195} = -3.989$, $P < 0.01$]. Spring was the only season in which it was predicted that female squirrels would be more likely to visit hoppers than male squirrels. Squirrels were significantly less likely to visit hoppers in summer than spring ($z_{6,195} = -3.989$, $P < 0.0001$) and this was largely explained by the prediction that females were significantly more likely to visit hoppers than males in spring compared with summer ($z_{6,195} = -1.739$, $P < 0.0001$).

The Gaussian GLM predicted that squirrels would spend significantly more time visiting hoppers in spring compared with summer [Data S1; Fig. 3(b); $t_{6,118} = -3.989$, $P < 0.0001$]. No other variables were predicted to have a significant effect, although time spent visiting hoppers appeared to decrease with increasing numbers of squirrels per hopper. The data could not support the addition of body weight as a variable in the model. When plotted, there was no obvious patterns in the relationship between time spent visiting hoppers and squirrel body weight (Fig. 4), though any trends present are likely to have been masked by between-sex and between-season variation. Only eight squirrels spent longer than 100 min visiting hoppers during the trial days, seven females in spring and one male in winter.

3.3 Duration of hopper visits *versus* weight of bait taken

Data for total bait taken in 4 days were recorded for 67 individual squirrels, including 15 females and 23 males monitored in summer 2022, and 19 females and 10 males monitored in spring 2023. There was a strong positive relationship between the number of seconds individuals spent visiting hoppers and the weight of bait taken per visit ($\tau = 0.815$; $P < 0.001$) with individuals taking an average of 1 g bait per minute spent at hoppers (Fig. 5). On average, female squirrels took 46.5 g bait, with a maximum of 173.1 g, whereas male squirrels took an average of 38.8 g bait, with a maximum of 83.2 g.

4 DISCUSSION

This study assessed factors affecting bait uptake by six populations of grey squirrels, by monitoring the feeding behaviour of PIT-tagged squirrels using purpose-designed bait hoppers.[29] We discuss these findings in relation to managing grey squirrel populations in the future using oral contraceptives in baits. The amount of bait taken by squirrels was significantly associated with the time that squirrels spent visiting hoppers in four woods assessed in spring and summer and was therefore used to infer the amount of bait taken from hoppers by squirrels in winter in a comparative analysis. The bait hopper design used in this study was effective at delivering bait to most grey squirrels in the six woodlands trialled, with 58% of PIT-tagged females and 67% of PIT-tagged males recorded visiting hoppers. This is consistent with grey squirrel average bait uptake results measured using the bait marker Rhodamine B in this study (56%) and a previous study conducted in three woods in summer and three woods in winter (67%).[24]

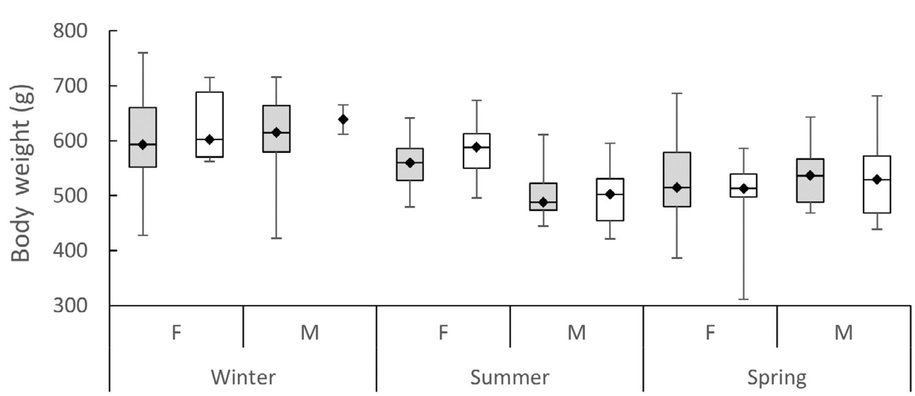

Figure 2. Comparative body weights (g) of 88 male and 114 female PIT-tagged grey squirrels that were (grey boxes) or were not (white boxes) recorded visiting bait hoppers deployed in two woods in winter 2017/2018, two woods in summer 2022, and two woods in spring 2023. Median values are shown by diamonds, interquartile ranges (50% of the records for each group) are shown by boxes and minimum and maximum values are shown by whiskers.

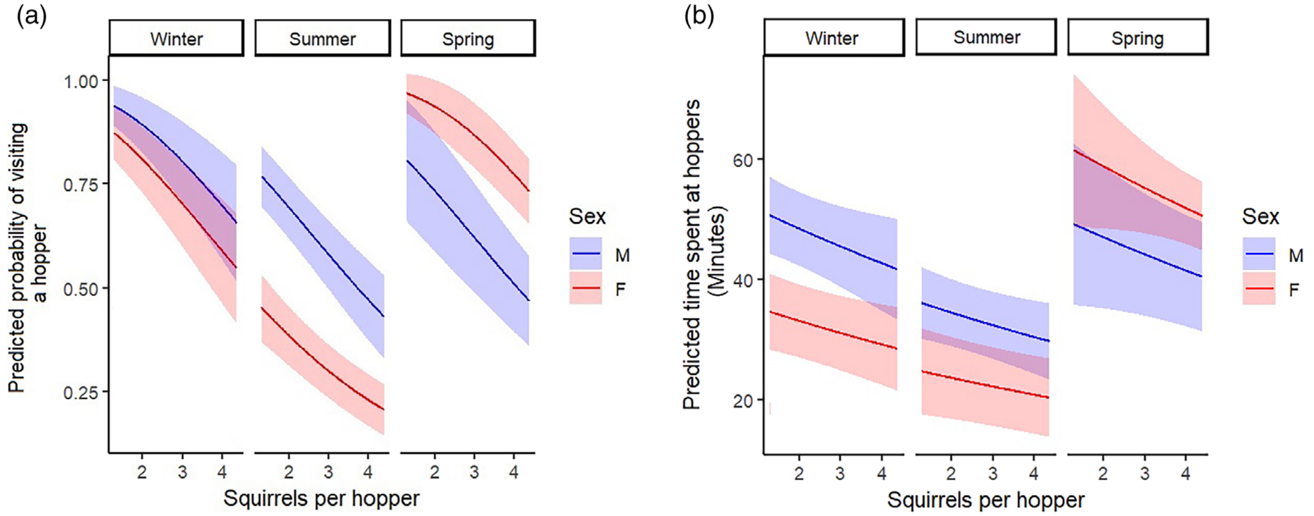

Figure 3. Generalised linear model (GLM) predictions for the average probability (and associated SE) that male and female squirrels will enter a bait hopper (a) and the average time spent visiting bait hoppers (b) within four trial days for different seasons and for increasing numbers of squirrels per hopper. Data were collected in winter 2017/2018, summer 2022 and spring 2023, from 202 grey squirrels PIT-tagged in six independent woodlands (two woods surveyed per season). A total of 21–24 hoppers per wood (3 hoppers ha^{-1}) were deployed in the winter and summer, and 14 hoppers per wood (2 hoppers ha^{-1}) in spring.

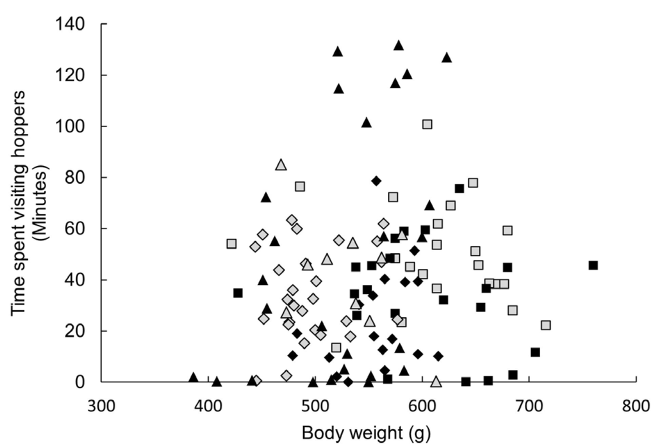

Figure 4. Relationship between body weight (g) and time spent visiting hoppers in 4 days for 59 male (black) and 66 female (grey) PIT-tagged grey squirrels. Data were collected in winter 2017/2018 (square), summer 2022 (diamond) and spring 2023 (triangle).

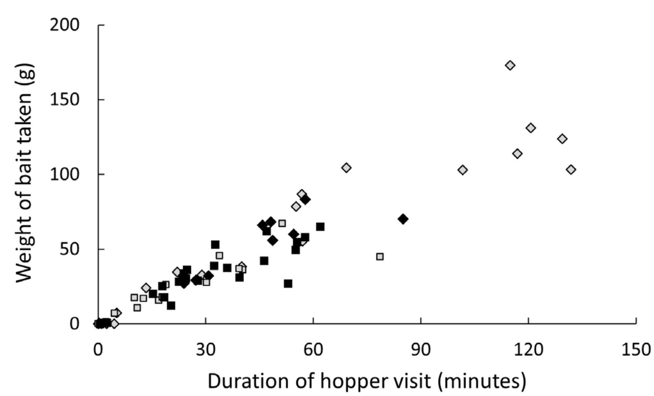

Figure 5. Comparison of the weight of bait taken from hoppers and duration of hopper visits in four trial days for 67 PIT-tagged male (black) and female (grey) grey squirrels. In total, 21–24 hoppers were deployed per wood in two woods in summer 2022 (diamonds) and 14 hoppers per wood were deployed in two woods in spring 2023 (squares). There was a strong positive relationship between the number of seconds individuals spent visiting hoppers and the weight of bait taken per visit ($\tau = 0.815$; $P < 0.001$) with individuals taking an average of 1 g bait per minute spent at hoppers.

Statistical models have suggested that a threshold of 75% contraceptive efficacy is required for effective grey squirrel management,[6,39] although additional empirical data are required on seasonal and annual variations in bait delivery to squirrels to confirm this. Bait delivery to >75% of a squirrel population has been achieved in other Rhodamine B studies,[24] and may be possible with further refinement of the delivery methods. The overall efficacy also will depend on the efficacy of the contraceptive itself.

For a contraceptive to be effective, particularly if the effects on fertility are limited to a few months, ideally it will be delivered to reproductively active female grey squirrels in early December and May, to ensure animals are infertile immediately before the peak mating periods. In the current study, female squirrels were more likely to visit hoppers and male and female squirrels were more likely to consume more bait in spring, followed by winter then summer. This is contrary to previous findings that grey squirrels were more likely to consume bait from hoppers in summer than in winter.[24] This discrepancy may have been caused by differences in sample size between the two studies and annual variation in summer bait uptake. In the current study, two woods were trialled in summer in 2022, whereas Beatham *et al.*[24] trialled six different woods in summer across two different years, 2018 and 2019. Spring was the only season where female squirrels were more likely to visit hoppers and had a higher bait uptake than males, even though a lower density of bait hoppers was used to deliver the bait.

Summer, along with spring, is a peak time for litter production for grey squirrels and the relative proportion of females that produce litters in spring, summer or both will be dependent on food availability related to the seed mast crop of the previous autumn and the severity of the previous winter.[8,32] In good seed years, grey squirrel reproduction will start in winter, in bad seed years, it will be delayed until the following spring.[32] The grey squirrel diet consists of predominantly tree seeds from oak, beech and hazel, and in autumn 2021, the UK based Tree Council[40] reported one of the lowest oak seed (acorn) crops in 7 years. It is therefore likely that squirrels will have cached less food over winter and may have delayed their breeding until summer 2022, when natural food availability improved. At the time of the summer 2022 PIT-tag trial, it is feasible that a greater number of females may have had reduced activity and thus less likely to feed from hoppers, as a result of having dependent young in the drey. This is supported by the fact that the heaviest females that were PIT-tagged at both woods in summer 2022, were not recorded by the hoppers 1 to 2 weeks later. The best female bait uptake results (62–75%) in the bait marker study were found in three woods in the summers of 2018 and 2019, when it is possible that there were fewer females breeding.

The recapture rate of male and female PIT-tagged squirrels in summer 2022 was lower than in winter and spring, whereas the Rhodamine B bait uptake analysis was very similar to the both winter woods and one of the spring woods. This suggests that an increase in movement of squirrels into and out of the study woods may have also contributed to the reduced hopper access by PIT-tagged squirrels. The trapping at both summer woods was delayed due to a heat wave and this may have motivated squirrels to move. Overall, the findings of this study suggest that summer may not be the best time of year for bait deployment, owing to inconsistencies in movement, activity and bait uptake amongst squirrel populations, particularly for female squirrels. Autumn was not included in the study as this is when tree seeds are at their most abundant, so supplementary feeding is unlikely to be successful and trapping rates are typically very low.[32] In winter and early spring, many of the squirrel's main natural food resources are depleted and they are reliant on cached food.[41] This may explain why, in this study, squirrels were highly likely to enter hoppers in winter and spring. With the resource confines of this study, it was only possible to conduct trials on two woods per season and this was conducted in separate years. To confirm these findings, additional trials are required in winter, summer and spring, in order to further understand any annual variation in squirrel feeding behaviour and confirm the best months for bait deployment.

Male and female squirrels consumed very similar amounts of bait and exhibited similar bait uptake behaviour. Variation in bait uptake between individuals of the same sex was greater than the variation seen between sexes. The findings of this study are comparable to Tiberi *et al*. (unpublished data), who also concluded that overall, there was no difference in bait uptake between individuals of different sex, age and reproductive status, yet they found that breeding grey squirrel females made more visits to hoppers than other individuals. Bait uptake has also been found to be similar between male and female house mice and male and female Norway rats, with bodyweight influencing bait uptake by rats of the same sex.[30,31] No evidence was found that body weight influenced bait uptake by squirrels of the same sex, although it is recommended that trials are conducted on additional woods to confirm this. Likewise, additional data are required on the effects of squirrel age or female breeding status on bait uptake, owing to seasonal variations in the difficulty in visually assessing the latter two factors, and a more detailed assessment is required to analyse this.

Male and female squirrels visited hoppers multiple times and on most days of the 4-day bait deployment, thus demonstrating that if oral contraceptives were deployed in baits, individuals that visited hoppers are likely to receive multiple doses within a short space of time. This is important for immunocontraceptive vaccines or synthetic hormones, that require multiple doses to ensure infertility.[12,42,43]

Hopper density is a key factor in determining population level bait uptake in squirrels.[24] Results from the PIT-tag trials conducted in winter and summer found that most squirrels visited multiple hoppers in both seasons. Consequently, it was decided that 2 ha^{-1} should be trialled in spring, as a more efficient deployment, reducing the effort required for bait delivery. Lowering the hopper density did not affect the proportion of squirrels that visited hoppers, or the time squirrels spent feeding from hoppers, although the probability of visiting a hopper was significantly inversely related to the number of squirrels per hopper. Before any bait delivery campaign, it is recommended that an initial squirrel density estimate be obtained using a proven method, such as the camera trap index-based method described in Beatham *et al*.,[26] so that the density of hoppers deployed can be adjusted to ensure that there are fewer than three squirrels per hopper. As this index method requires relatively little time in the field, using the density estimates to minimise the number of hoppers to be deployed would reduce bait waste and the time required to deploy them, bait them and collect them, particularly in larger, less easily accessible woodlands.

Neophobia of rodents towards bait stations has been found to affect bait delivery success.[31] In this study, most PIT-tagged squirrels visited hoppers within the first week, during the pre-bait period, suggesting that neophobia was not an important influence on bait delivery. The proportion of grey squirrels visiting hoppers decreased slightly for the 4-day trial period. This could be because bait marker dye was added to the bait. Rhodamine B has been shown to be unpalatable to rodents at higher concentrations[44] and, although the concentration used in this study was trialled in captive squirrels for its palatability,[24] it is possible that some individuals within the populations may have been more sensitive to its taste, or that the taste may have become stronger under certain environmental conditions.

Another common issue for bait-delivered rodent control campaigns is the impact on nontarget animals. Even when bait boxes are used to target bait delivery to specific species, nontarget small mammals may access bait, which not only wastes bait and reduces cost-effectiveness, but also increases the likelihood of secondary impacts on predators that feed on those small mammals.[45] Ensuring that any bait delivery system is species-specific is therefore important for a viable oral contraceptive delivery campaign. The bait used in this study had a 100% hazelnut butter base which had the consistency of a viscous paste. This bait type was chosen with an oral contraceptive in mind, to ensure that the bait was attractive to squirrels yet difficult to remove from the hoppers. This, together with the use of a weighted door that had to be lifted to access the bait, meant it was more likely to be consumed *in situ*, minimising waste and availability to nontarget species. The lack of spillage recorded at the hoppers during the trial suggested that this was the case, providing reassurance that any bait removed from the hoppers by the squirrels was consumed by them.

The bait door and associated remote camera records suggested that, in all woods studied, on >99% of the times the bait door was opened, it was accessed by grey squirrels. This provides further evidence that the bait delivery system used offered a good level of species selectivity, excluding other common species of UK wildlife such as badger, roe deer, rat, mice, voles and various bird species. This reinforces earlier results, in which only grey squirrels accessed hoppers in 10 woods studied.[24] All of the woodlands included in these studies were located in areas in which grey squirrels were present but red squirrels were absent. This is the case for most of the UK, at present. Nevertheless, more complex feeders, that can exclude red squirrels based on body weight, are currently being developed to facilitate future oral contraceptive delivery in areas in which both squirrel species are present.[15] These feeders also could be adapted to exclude pine martens (*Martes martes*), which, may become a nontarget risk in the future, owing to reintroductions, improved habitat availability and increased protection, leading to an expansion of their range across the UK.[46]

The average amount of bait taken in 4 days for male and female squirrels was 39 g and 47 g, respectively. Oral contraceptives are currently being trialled on captive grey squirrels to determine the contraceptive dose that would be required to render an individual infertile. Once the dose has been determined, data gathered in this study could be used to quantify the frequency of deployment, quantity per deployment and concentration to ensure that most of a squirrel population is treated effectively. It is, however, recommended that additional field trials are required, conducted on additional woodlands in different seasons, different years and using both 2 and 3 hoppers ha^{-1}, to increase the confidence in the findings of this study, which could only account for some factors influencing bait uptake, owing to the small number of sites used. Additional work also is required to assess how reproductive status may affect grey squirrel bait uptake in different seasons, to ensure breeding animals are targeted. Spatial analysis of the PIT-tag data also would provide material on the distances moved by animals between hoppers, informing the minimum spacing distance to use when deploying bait hoppers.

The bait delivery and monitoring system developed and tested for this study could be adapted to design delivery methods for other oral baits and other small mammal species. This could include the delivery of oral vaccines such as the plague vaccine for prairie dogs.[47] Further testing would be required to adapt the hopper to prevent nontarget access by other species relevant to the environment in which the feeders are deployed.

5 CONCLUSION

The bait delivery and monitoring system developed and tested in this study demonstrated that it was possible to deliver multiple doses on most days of a 4-day deployment to both male and female grey squirrels. Bait deployment is likely to be more effective in spring, immediately before the second peak in breeding, followed by winter, immediately before the first breeding season. The findings of this study could be used to design bait delivery methods to deploy oral contraceptives in the future for grey squirrel population management. This system could be adapted to design delivery for other baits and other small mammal species.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.m905qfvb0.

ETHICS STATEMENT

The study was conducted by trained Home Office Personal Licence holders under the advice of the Named Veterinary Surgeon and under a UK Home Office licence, in accordance with the Animals (Scientific Procedures) Act 1986. The study was approved by the joint Animal and Plant Health Agency (APHA) and Fera Science Ltd Animal Welfare and Ethical Review Body.

AUTHOR CONTRIBUTIONS

SB, GM and PS conceived the ideas and designed methodology; SB, IR, ET and JC collected and processed the data; SB analysed the data with input from DG, PS and GM; SB led the writing of the manuscript with input from GM and PS. All authors contributed critically to the drafts and gave final approval for publication.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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